Reconceiving the Gene

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Seymour Benzer's Adventures in Phage Genetics

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Preface

Molecular biology emerged as a discipline during the 1950s and 1960s and ever since has been dominant among the life sciences. That a new field was constructed to study biological molecules has appeared paradoxical, and to some observers unwarranted, because older fields of biology have been concerned with the molecules of life ever since the chemical definition of a molecule was established in the early nineteenth century. The claims for this investigative domain by those who defined themselves as molecular biologists have been explained in part by contingent personal, sociological, and political circumstances. On intellectual grounds, however, molecular biology has been distinguished from biochemistry most cogently by the fact that it represents a convergence of biochemistry and genetics, another older field, and one that had been pursued successfully for several decades largely independent of questions about the molecular nature of the genetic material.

At the center of the work that brought the language of genetics and the language of the new molecular biology together was an investigative venture carried out by Seymour Benzer, a former physicist who in 1949 had moved into the field of phage biology. Between 1954 and 1961 Benzer mapped the fine structure of the rII region of the genome of the phage T4. By 1957 he had produced strong evidence that the genetic map is linear down to the dimensions of the individual nucleotides as they are aligned, according to the Watson-Crick model, along the DNA molecule. His evidence that the units of recombination, mutation, and function associated with the classical gene had different dimensions led him to propose three new terms to replace the old concept of the gene, but his fine structure maps were broadly similar to those that had been constructed earlier in the century for the locations

of genes on the chromosomes of organisms such as the fruit fly, *Drosophila melanogaster*. The demonstration that the genetic map is linear down to the molecular dimensions provided strong and timely support for the "sequence hypothesis" espoused by Francis Crick in 1958, which claimed that the sequence of base pairs in a nucleic acid is a code specifying the sequence of amino acids in a particular protein. The confirmation that genes can be identified with sequences of DNA nucleotides supplied the chemical basis which some classical geneticists had long sought to give physical meaning to what had been an abstract unit along the genetic map. In this way, Benzer came to stand at the intersection of a newly emerging field with a well-established one and was seen as the person who, more than any other single individual, enabled geneticists to adapt to the molecular age. Moreover, the work itself seemed to contemporaries to be extraordinarily elegant, and the interpretations he drew from it seemed to have been daring. "The feeling of beauty," Raphael Falk told me, "was very strong at the time; it had an important effect."

When he began his project in January 1954, Benzer did not have in mind the goal of building a bridge between molecular biology and classical genetics. According to his own recollection, he knew little about the older mapping tradition when he took up his research on the rII region. Rather, the opportunity to carry the mapping of the bacteriophage itself, which had recently been undertaken by two other phage biologists, to a much finer degree of resolution than had so far been achieved came to him through a fortuitous combination of partly accidental events and an interest in the size of the gene stimulated by reading a paper published in 1952 by the geneticist Guido Pontecorvo.

Benzer has preserved a remarkably complete record of his investigative activity and his interactions with his colleagues before and throughout the period in which he mapped the rII region. I am presently in the early stages of a project to reconstruct from these and published sources a detailed account of how Benzer came to begin and to carry out this landmark investigation. My interest in doing so derives both from the beauty and strategic importance of his research and from the fine opportunity the case affords to continue my quest to understand the nature of creative scientific work at the intimate level of the interplay between daily experimental operations and the reasoning that lies behind them. The course that led Benzer into this project, and that he followed throughout it, is an exemplary illustration of what I have called the "investigative pathways" that scientists typically traverse during their research careers. Like other scientists who attain a

distinct individuality in a field of colleagues doing similar things not only through their initial decisions but through the unforeseen experiences they have along the way, Benzer's pathway did not proceed according to a linear progression marked out in advance. He shifted directions and goals in response to plans that did or did not work out and to unexpected opportunities and encounters that arose in his path. While he did so, however, his pathway maintained continuity in the sense that he moved always from his position at a given time to something nearby; he did not make leaps from one problem to another unrelated either by objectives or by methods. Even in the shift that appears most radical on the surface, the change from physics to biology, he sought bridges that would enable him to carry into his new field experience gained in the field he was leaving. Such patterns of continuity and change are representative of the way the careers of most creative scientists unfold and are embedded deeply in the nature of the scientific enterprise.

The remarks reprinted above are from a lecture delivered in Paris on November 21, 2002, by Frederic L. Holmes, and they form a fitting introduction to the story told in this book. Larry Holmes completed the manuscript only a few days before he died on March 27, 2003. He worked as enthusiastically and as energetically as his waning strength would allow, right up to the end. I had the privilege of enjoying many discussions about this project with Larry from its genesis until its completion in its present form, and when it became clear that he might not have the time to complete this manuscript, he asked me to see it through to completion. I remain honored by the confidence he placed in my skills as a colleague and his affirmation of a long and close friendship. In his usual scholarly approach, he had projected a total of eighteen chapters to carry the story well into the 1960s and the discovery of the genetic code. He saw the present nine chapters, however, as a logically complete part of the overall investigative pathway that is the subject of this book. I have endeavored to use the lightest of editorial touches on the manuscript that Larry managed to complete; the words are entirely his, as are the selections of figures and diagrams. The responsibilities for checking the references and notes against Benzer's research notebooks and for the general tidiness of the manuscript are mine, however.

William C. Summers

Acknowledgments

Because the author did not leave a formal list of the individuals who were helpful in the work that is contained in this book, the editor, with anxious trepidation, has undertaken to acknowledge as many of these individuals as he has been able to identify from the source materials, from the author's correspondence, and from his personal knowledge.

First, the author and the editor acknowledge the invaluable assistance and enthusiastic cooperation of Seymour Benzer. He provided unlimited access to his research notebooks, was generous with his time and recollections, and was especially sensitive to the historian's goal of reconstructing the contexts of past events. The archives of the California Institute of Technology provided much of this context, and we thank Judith Goodstein, Charlotte Irwin, and Bonnie Bludt of the Caltech Archives for their always good-natured assistance.

Interesting and useful perspectives, as well as helpful documents, were provided by Joseph Fruton, Seymour Cohen, and Waclaw Szybalski.

Angela Creager read most of the manuscript and provided very helpful comments, corrections, and criticism.

The support of the author's family, especially Rebecca Holmes, as well has his partner, Petra Werner, has greatly helped the editor in undertaking this task.

Author's Note

Most of the genes that form the subject of the work discussed in this book were named before the standardized conventions were adopted in the 1960s. In order to be congruent with the published work from that time that I cite, and to be consist with the language of Benzer's notebooks, I have generally followed his usage.

Chapter One

Classical Mendelian Genetics

The formation of genetics during the first decades of the twentieth century, following the rediscovery of the long-overlooked paper by Gregor Mendel that provided its fundamental principles, has been the subject of many historical accounts.[1] In this chapter I do not attempt to recapitulate this complex history but only draw attention to certain features of its development that were particularly relevant to what was viewed by mid-century as "classical genetics."

Mendel explained the results of his experiments on the hybridization of pea plants by assuming the presence in the germ cells of *Anlagen* that give life to the individuals that display the particular *Merkmale* by reference to which he differentiated them. When two plants whose Anlagen produced different Merkmale, such as green or yellow seed coats, were mated, the effects of only one of them, which he called the dominant one, were visible in the hybrid progeny. That in the next generation both green- and yellow-coated seeds appeared in definite ratios he attributed to the other, or "recessive," Anlage having remained unaltered during their association, the two types then segregating independently during the formation of the germ cells.[2] At the time there were no structures identified within ordinary or germ cells with which the Anlage could be associated.

Mendel's term *Anlage* was later translated in the English literature as "factor," and *Merkmale* as "character." Whereas the paired German words were suggestive of the relation between an inner predisposition and an outward sign, the words *factor* and *character* lacked these connotations. Because the factors remained abstract entities without known properties of their own, early geneticists often associated them so closely with the "unit characters" they were supposed to produce that some geneticists nearly obliterated the distinction between factor

and character.[3] In an article titled "What Are 'Factors' in Mendelian Explanations?" the American embryologist Thomas Hunt Morgan protested in 1909 against the facile references by some Mendelians to these "hypothetical" factors as the "actual characters themselves." Taking as his illustration the factors for tallness and shortness in Mendel's peas, Morgan asserted that the assumption that two factors can coexist in countless generations of cells without "having produced any influence on each other," then "turn their backs on each other and go their several ways," was a "purely preformation idea" and was only one among various possible explanations. Until all alternate possibilities were examined, it would "be at least judicious to hold the segregation hypothesis, as currently interpreted—a purely formal procedure." While adopting this procedure, it was of "capital importance," according to Morgan, to keep in mind that "the egg need not contain the *characters* of the adult, nor need the sperm. Each contains a particular material which *in the course of the development produces* in some unknown way the character of the adult. Tallness, for example, need not be thought of as represented by that character in the egg, but the material in the egg is such that placed in a favorable medium it continues to develop until a tall plant results. Similarly for shortness."[4]

In the same year Wilhelm Johannsen, who had bred "pure lines" of the bean plant *Phaseolus,* concluded that the concept of unit character did not express the complexity of the relation between the observable characters of his plants and whatever physiological units were involved in forming their overall character. Reviewing the words that others had used to define particulate units of inheritance, Johannsen chose Hugo de Vries's term *pangene* but dropped the first syllable to free the term from its historical associations with particular theories. "The word 'gene,' " he wrote, "is completely free from any hypothesis; it expresses only the evident fact that, in any case, many characteristics of the organism are specified in the gametes by means of special conditions, foundations, and determiners which are present." Elof Carlson has commented that "Johannson's gene was undefined" and, therefore, free to "take on or discard definition."[5] Only gradually, however, did *gene* displace *factor* or *unit character* as the preferred term in the explanation of the results of genetic experiments.

As is well known, Morgan overcame his general reluctance to follow the formal procedures of Mendelism as the result of a chance observation. As he reported it in 1910, "in a pedigree culture of *Drosophila* which had been running for nearly a year through a consider-

able number of generations, a male appeared with white eyes. The normal flies have brilliant red eyes." Breeding this male, to which he referred without comment as a "mutant," with its sisters produced almost entirely red-eyed offspring, but in the generation bred from the hybrids there were red-eyed males and females and white-eyed males. Morgan accounted for these results by the "hypothesis" that the white-eyed male carried the " 'factor' for white eyes" and was heterozygous for a "sex factor." The outcome, he wrote, "is Mendelian in the sense that there are three reds to one white," but the whites were confined to the male sex. Further crosses verified his hypothesis. That Morgan continued to be wary of the idea of "factors," as commonly used, is indicated by the fact that he placed quotation marks around the word. He apparently had no comparable qualms about adopting the word *mutation* to designate a fly that had arisen spontaneously with a character distinctly different from those of its forebears. That word had been coined by Hugo de Vries to describe the apparent sudden origin of a new species of evening primrose in a single generation. Morgan's new usage associated mutations instead with discontinuous but relatively small modifications arising in an existing species or variety.[6]

During the following two years Morgan and the young students who worked with him in the "fly room" at Columbia University found many more spontaneous mutations, including five eye colors and nine wing modifications, and analyzed them according to Mendelian principles. Some but not all of them turned out to be sex-linked. Those that were not, Morgan concluded in 1911, contained the factor for that character in "another part of the hereditary mechanism," perhaps in another chromosome.[7] These rather vague connections between factors, chromosomes, and sex linkage became much clearer later that year, after Morgan encountered another departure from strictly Mendelian principles. When he bred flies differing in two sex-linked characters (white-eyed with long wings and red-eyed with rudimentary wings), he found that the two did not always appear together in the offspring, although they had to be on the same chromosome, according to his theory of sex linkage. Later he noticed that they nevertheless remained together more frequently in the second generation than would be predicted according to the "law" of independent segregation.[8]

In order to explain these anomalies, Morgan referred to a cytological "chiasmatype" theory published in 1909 by the Belgian Frans Alfons Janssens. Having observed that when homologous chromosomes pair together during the reduction division they twist around each

other, and that when they then separate they do so in a single plane, Janssens inferred that this process would result in an exchange of material between the chromosomes. Connecting the cytological behavior of chromosomes with the genetic behavior of the factors, Morgan reasoned, "If the materials that represent these factors are contained in the chromosomes, and if those that 'couple' [are] near together in a linear series, then when the parental pairs (in the heterozygote) conjugate like regions will stand opposed." Citing Janssens's evidence for the manner in which the chromosomes couple and separate, Morgan concluded that "in consequence, the original materials will, for short distances, be more likely to fall on the same side of the split, while remoter regions will be as likely to fall on the same side as the last, as on the opposite side. In consequence, we find coupling in certain characters, and little or no evidence of coupling in other characters, the difference depending on the linear distance apart of the chromosomal materials that represent the factors."[9]

Morgan contrasted his explanation with that of the leading Mendelian of the time, William Bateson, who had accounted for similar observations in chickens by assuming that certain allelomorphic pairs couple with each other and others repel but without being able to offer any underlying mechanism for the process. Morgan believed that his own explanation could account for Bateson's results as well as those seen in *Drosophila.* They are a "simple mechanical result of the location of the materials in the chromosome, and of the method of union of the chromosomes. . . . Instead of random segregation in Mendel's sense we have associations of factors that are located near together in the chromosomes. *Cytology furnishes the mechanism that the experimental evidence demands."*[10]

Morgan made this proposal nine years after Theodor Boveri and William Sutton had independently concluded that the strong parallels between the behavior of chromosomes and that of Mendelian factors suggested that the chromosomes were the physical bearers of the genetic factors. As late as 1910 Morgan had been among those who remained uncommitted with regard to this question.[11] His explanation of coupling now moved him to become a strong advocate for the chromosome theory.

In 1912 Morgan made his first attempt to "analyze the constitution of the chromosome." It was based on the results of his experiments on two classes of sex-linked characters: eye color and wing length. The two principle conclusions to which his studies had led him were that

"sex-limited inheritance is explicable on the assumption that one of the material factors of a sex-limited character is carried by the same chromosomes that carry the material factor for femaleness" and that "the 'association' of certain characters in inheritance is due to the proximity in the chromosomes of the chemical substances (factors) that are essential for the production of these characters."[12] The eye colors that Morgan discussed were the red wild type and the mutants vermilion, pink, and orange. Influenced by Bateson's idea that mutants result from the loss of the factor present in the wild type (known as the "presence and absence theory"), Morgan introduced a symbolism that assumed each of the three mutant eye colors to result from the loss of one of the three factors necessary to produce normal red eyes. This view probably appealed to Morgan in part because it fit with his more general conviction that there is no simple one-to-one relation between factors and characters but that the latter are the products of complex interactions between multiple factors during development. He similarly attributed the mutant short wings to the loss of a factor for normal wings.[13]

Dealing with the fact that in several of his experimental crosses the number of mutants found departed radically from the expected Mendelian ratios, Morgan gave three types of explanation. Some of these "disturbances" probably resulted from mutants' being less fertile or less viable than normal flies. "In some cases," however, "the disturbance can be traced directly to the principle of 'association.' By that I mean that during segregation certain factors are more likely to remain together than to separate, not because of any attraction between them, but because they lie near together in the chromosome."[14]

This was as far as Morgan carried the analysis. Although he stated that "such associations will be more or less common according to the nearness of the associating factors in the chromosome,"[15] he did not attempt to relate the differing degrees of disturbance that he had observed quantitatively to the degree of "nearness" of the factors.

Because Morgan had earlier expressed skepticism about the chromosome theory of inheritance, some historians have inferred that he remained more reluctant than his students to accept that view and was only gradually forced to it by the accumulating evidence that they produced largely between 1911 and 1915.[16] His own writings from this period suggest, on the contrary, an eagerness to pursue the consequences of the view that the factors were material particles embedded in the chromosomes. Not only in the passages quoted above

but throughout this 1912 paper, Morgan referred to factors, which he placed in parentheses, as materials and chemical substances. His theory of eye color in particular seemed to lend itself to a chemical interpretation. Although he acknowledged that he had no facts to offer concerning the chemical nature of the three colors, he appears to have believed that it would be feasible to investigate factors in terms of their chemical properties. He was not, as often portrayed, simply content with an abstract notion of these factors.

When Morgan discussed his theory of "associative inheritance" with the students in his lab, one of them, Alfred H. Sturtevant, immediately thought beyond Morgan's semiquantitative notion of the relation of the interchange of factors between homologous chromosomes to the nearness of their association on the chromosomes. One could use the relative frequency with which two factors separated, Sturtevant saw, to plot their distances from each other on the chromosome. Staying up all night and neglecting his undergraduate homework, he was able to construct such a diagram, which included several sex-linked factors on the basis of experimental results already obtained in the lab.[17]

Following up this quick preliminary effort, Sturtevant studied the frequency of the interchange between homologous chromosomes, which he called "crossing over," among six sex-linked factors. What Morgan had called "association" Sturtevant called "linkage," defining a unit of distance between factors as "a portion of the chromosome of such length that, on the average, one cross-over will occur in it out of every 100 gametes formed. That is, percent of cross-overs is used as an index of distance." If the "hypothesis" were correct, then the sum of the distances calculated between any two factors and a third one situated between them should be equal to the distance between the outside two. Sturtevant found this relation to hold accurately for factors between which the distances were short. For longer distances, the observed distance between the outside factors was somewhat less than that calculated from the sum of the distances of the factors from an intermediate one. Sturtevant explained this discrepancy by assuming that "double crossing over" could in some cases cause the two more distant factors to end up on the same chromosome. "In mapping out the distances between the various factors," therefore, Sturtevant relied "so far as possible on the percent of cross-overs between adjacent points."[18]

In the paper in which he submitted these results for publication in

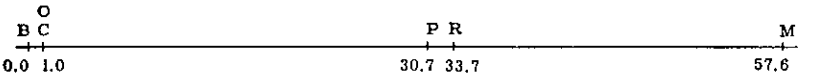


Figure 1.1. Sturtevant's 1912 diagram of distances between factors calculated from crossover frequencies. (From A. H. Sturtevant, "The Linear Arrangement of Six Sex-Linked Factors in *Drosophila,* as Shown by Their Mode of Association," *Journal of Experimental Zoology* 14 [1913]: 70.)

November 1912, Sturtevant labeled the line on which he depicted these distances a "diagram" (fig. 1.1). He assumed that the order in which the factors were shown represented their actual order on the chromosome but acknowledged that "there is no way of knowing whether or not these distances as drawn represent the actual relative distances apart of the factors." If the chromosomes were not of uniform strength, then a break was more likely to come at a weak place than at a stronger one. Such considerations, he thought, "will not detract from its value as a diagram." In his summary, he concluded that his results "form a new argument in favor of the chromosome view of inheritance, since they strongly indicate that the factors are arranged in a linear series, at least mathematically."[19] We may note that, although he referred casually to "mapping out" the distances, Sturtevant did not label the result a genetic map. In the common usage of the time, a map was a two-dimensional representation of a portion of the surface of the earth. Sturtevant's spare linear diagram did not immediately evoke the image of such a map.

In March 1912, while still constructing the linear series of sex-limited factors on the X chromosome, Sturtevant and Calvin Bridges, another undergraduate working in the fly lab, met with Morgan, who agreed to allow them to begin constructing similar diagrams for *Drosophila* chromosomes II and III. The task took them two years. Morgan continued for some time to analyze mutations associated with a particular organ—eye color or wing shape—rather than according to their positions on the chromosomes, but, as Robert Kohler has pointed out, the rapid rate at which *Drosophila* produced mutations gradually undermined his project and diverted the fly lab ever more single-mindedly to the project of his students.[20]

Not long after publishing his paper on sex-linked inheritance in *Drosophila,* Morgan encountered a surprise that forced him to question the theory of presence and absence on which he had based his analysis of the four eye color factors. "A new eye color appeared that was called

eosin. Mated to orange it gave red." To Morgan that result signified that eosin must contain the other two factors necessary to produce red, and he "inferred that it owed its color to the loss of an imaginary O [orange] factor." On reflection, however, he realized that as long as the other two factors were present, any "loss from the germ plasm (giving a new eye color), added to orange, should give red, because orange would contain what the new mutant had lost." The nomenclature he had built around the presence-or-absence theory was, therefore, inconsistent, some of the letters used standing for "different things." It was such double meanings, he believed, that allowed opponents of Mendelian interpretations to "impute to the factorial hypothesis" the view that a "given character, pink color, for instance, is the product of a pink factor alone." Reiterating his long-standing view, he asserted that the factorial hypothesis assumes that each character of the organism is a product of the interaction of factors with one another and that, conversely, any one factor may have "far-reaching consequences" beyond the character most conspicuously affected by the difference between the normal and the mutant type.[21]

A deeper criticism of the presence-or-absence theory was that Morgan had found some evidence of a "backwards" mutation. In a wingless stock that had been bred for many generations, an individual appeared with one normal wing. This "reversion" from the mutant to the normal state must have been somatic rather than in the germ cells, because it affected only one side, but it nonetheless cast doubt on the generalization that mutations are due to the loss of material. Finally, he pointed out that the nomenclature based on the presence-or-absence view made sense only for cases in which "only two changes in the same organ are involved." In the *Drosophila* experiments, however, it was becoming commonplace for three or more mutations to appear in the same location. For all of these reasons, Morgan proposed to replace the common Mendelian symbols, in which an uppercase letter stood for the presence of a factor and the corresponding lowercase letter for its absence, with a scheme in which the mutant was represented by a lowercase letter related to the condition (for example, *b* for *black*) and the normal condition by the same letter in uppercase.[22]

In this paper Morgan did not entirely abandon the presence-or-absence theory. The change in nomenclature, he wrote, "is not of any theoretical importance, but a practical necessity" for all cases in which three or more factors are involved in the same organ. He did, however, distance himself from Bateson's view. He believed it "unwise to com-

mit ourselves any longer to a view that a recessive character is necessarily the result of a loss from the germ cell."[23]

Morgan remained strongly interested in the question what kind of change *is* involved in a mutation:

> If we suppose that a factor is a labile aggregate, and that a rearrangement in it occurs, then the new aggregate in connection with the other parts of the cell produces a character that differs from the old one. Here there need be no loss, but only a change in configuration with a corresponding change in the end product in which the changed part plays a role, along with the other parts of the cell. A factor, in this sense, may exist in two or more forms according to the state of equilibrium; one of its states is dominant-producing, and the other is recessive-producing. Such a view may make it easier for us to appreciate that a mutation need not be a loss, and that a recessive may revert in the sense that it may mutate. In chemical terms, the process is reversible.[24]

While Morgan was busy redefining the factorial hypothesis to fit the new circumstances produced by the flourishing *Drosophila* experiments in the fly lab, his students were silently shifting from use of the word *factor* to Johannsen's *gene*. Thus when Sturtevant reported two mutants that he had shown to be independent of the X chromosome or of chromosome II, he titled his paper "A Third Group of Linked Genes in *Drosophila Ampelophila*." In it he presented "evidence showing the existence of still another group of genes, which are located in the 'third chromosome.' " In the course of the paper he applied the word *gene* twice more but also used the word *factor* twice, apparently without implying any distinction of meaning.[25]

In 1914 Hermann Muller reported his discovery of a gene for the fourth chromosome of *Drosophila*. A mutation had arisen that was constituted of a recessive wing and a leg abnormality that Muller called "bent." He proved that the bent area was not located on any of the three long chromosomes of *Drosophila* by showing that it segregated independently from several markers on each of the three. Because this was the first gene to be found on the fourth chromosome, he could not establish its linkage with any other gene on it, but this condition was in keeping with the fact that the fourth chromosome is much shorter than the other three.[26]

These results showed, Muller concluded, that the genes of *Drosophila* fall into four groups, corresponding to each of the four chromosomes. "Thus the chief gap yet remaining in the series of genetic

phenomena that form a parallel to the known cytological facts in *Drosophila* has now been filled." Moreover, there was a close parallel between the number of genes in each group and the relative sizes of the four chromosomes:

> It is difficult to see why larger groups of genes should follow the distribution of the larger chromosomes unless we conceive the connection between the genes and the chromosomes to be that the genes are material particles actually lying in and forming a part of the chromosomes with which they go. In any case, we must admit that the occurrence of a mutation in a fourth independent group of genes in *Drosophila* forms a further argument, if any more still be needed, in favor of the chromosome theory of heredity.[27]

Muller used the word *factor* several times in his article but clearly favored the word *gene*, which he invoked far more frequently.

During the same year, Calvin Bridges discovered the phenomenon he named "non-disjunction," wherein at the reduction division in the formation of an egg the paired X chromosomes do not separate and pass to opposite poles but move to one pole, leaving the other pole with none. Extensive breeding tests showed that the inheritance of mutant sex-linked characters through several generations fit with this cytological observation, whereas nonsex-linked characters were inherited normally. In a paper titled "Direct Proof Through Non-Disjunction That the Sex-Linked Genes of *Drosophila* Are Borne by the X-Chromosome," Bridges concluded that "there can be no doubt that the complete parallelism between the unique behavior of sex-linked genes and sex in this case means that the sex-linked genes are located in and borne by the X-chromosomes." Bridges made no reference in this paper to factors.[28]

Does this variance between the linguistic preferences of Morgan, who was conspicuously developing his version of the factorial hypothesis in 1913, and of his students, who were shifting at the same time to the gene, suggest a conceptual divergence between the two generations of workers in the fly room? Historians have noted that Muller was highly critical of some of Morgan's reasoning. According to Carlson, Muller believed that Morgan was averse to theory, on one hand, and that Morgan and Sturtevant too easily accepted the ideas of linkage and the linear alignment of factors on chromosomes without providing further rigorous experimental support, on the other. Muller also thought that Morgan's analysis of the sex-linked eye colors was muddled.[29]

According to Kohler, the younger workers had little appreciation for Morgan's long-standing concern to avoid identifying individual factors with characters or for his viewpoint that the factors interacted in a developmental way to produce characters. With their focus on the burgeoning linkage experiments, they more narrowly identified factors with their positions on the chromosomes.[30] Perhaps, whether consciously or not, they came to prefer the otherwise undefined gene to Morgan's factorial hypothesis with its accompanying speculations about the nature of these particles and the nature of mutation itself. As shown above, Muller grounded his further argument for the chromosome theory of heredity solely on the parallels between the four linkage groups and the four chromosomes of *Drosophila.* We cannot reconstruct in chronological detail the development of the different perspectives of those who worked in the fly room, who exchanged ideas so frequently that they sometimes could not recall who originated them. But if this difference in their language preferences circa 1913 and 1914 does reflect some differences in their approach to the chromosome theory of heredity, then there is some irony in the fact that the general direction of the differences between Morgan and Muller, as expressed in their later writings, was nearly the reverse of that at this critical juncture in the evolution of the *Drosophila* group.

Late in 1913 Morgan decided that it was time to put together a book that would summarize the most significant results of the new field of genetics for biologists in general. Morgan wrote the introduction and chapters relating the work of the "Drosophilists" to that of other geneticists.[31] Sturtevant was away on a field trip to collect wild forms of *Drosophila* and contributed little to the volume, whereas Muller composed major sections on the relations between factors and characters, on linkage, and on the association of linkage diagrams with chromosomes. Morgan integrated the volume, which appeared in 1915 under the title *The Mechanism of Mendelian Heredity.*[32] The several authors did not distinguish their contributions, and they appear to have reached a compromise regarding the language to use at points where they differed in their individual research publications. Thus, Morgan accepted the terms *crossover* and *linkage,* which had first been presented in Sturtevant's papers. His own preferred phrase, *association of factors,* did not appear.

Conversely, the volume's first chapter stated that the "Mendelian units" deemed to separate in the germ cells "will be henceforth spoken of as factors."[33] The factorial hypothesis was discussed in several

passages, some of which must have been written by Muller, and, as far as I have been able to find, the word *gene* was nowhere mentioned, not even in passages describing the experiments of the originator of that term, Wilhelm Johannsen.[34] Had Morgan in this case insisted on his preference? Was there any conceptual issue at stake? Nothing in the volume suggests answers to these questions. "The factorial hypothesis," according to the authors, "postulates only three things about the factors with which it works, viz.: (1) that they are constant, (2) that they are usually in duplicate in each cell of the body, and (3) that they usually segregate in the maturing germ cells. But the biologist seldom stops here, for to him the problem involves cells about whose history he has come to know certain facts. Today . . . we . . . may more specifically interpret our numerical results of independent segregation, linkage, and even crossing over on the basis of a chromosome mechanism."[35]

The book did not summarize all of the evidence for this conclusion in any one place, but the various chapters included evidence brought to the question by each of the four principal participants in the activities of the fly room, beginning with Morgan's discussion, first presented in 1911, but also invoking Sturtevant's crossover experiments (showing that the factors can be represented in a linear series), Muller's arguments for the correlation between the size and number of the linkage groups with the size of the four chromosomes, a further argument by Muller based on an extensive series of crosses between flies he had laboriously constructed with multiple recessive mutants, and Bridge's discovery of nondisjunction, which to Morgan provided the most unquestionable proof of the chromosome theory.[36]

In the chapter concerning linkage the book represented crossing over with a diagram (fig. 1.2). Referring back to this diagram in a second discussion of crossing over, the text stated:

> If, as shown in Fig. 24, B, the chromosomes are represented as a linear series of beads (chromomeres), then, when the conjugating chromosomes twist around each other, whole sections of one chain will come to lie, now on one side, now on the other side, in the double chromosome. If, when the two series of beads come to separate from each other, all of the segments that lie on the same side tend to go to one pole, and all of those on the opposite side to the other pole, each series must, in order to separate, break apart between the beads at the crossing point.[37]

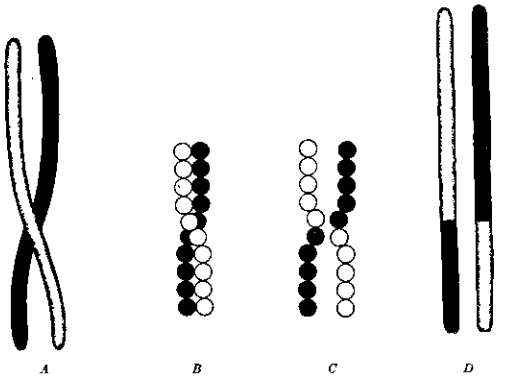
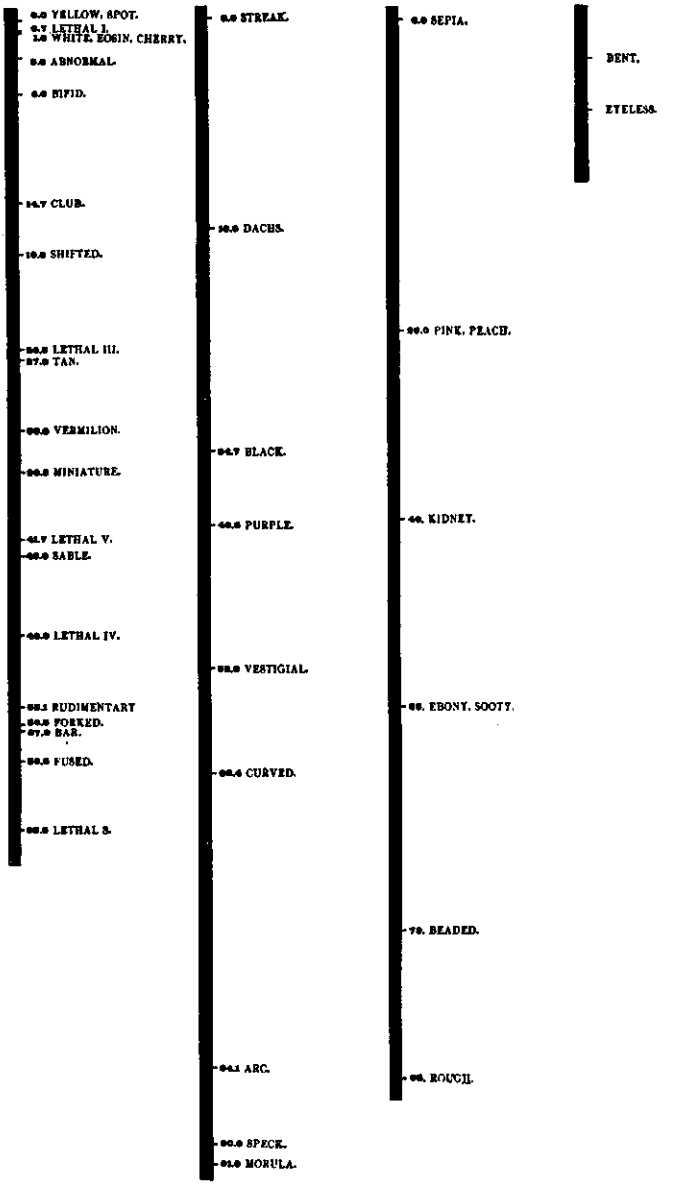


FIG. 24.—Diagram to represent crossing over. At the level where the black and the white rod cross in *A*, they fuse and unite as shown in *D*. The details of the crossing over are shown in *B* and *C*.

Figure 1.2. Morgan's 1915 illustration of crossing over. (From T. H. Morgan, A. H. Sturtevant, H. J. Muller, and C. B. Bridges, *The Mechanism of Mendelian Heredity* [New York: Johnson Reprint Corporation, 1972], p. 60.)

This diagram and its verbal interpretation have probably been largely responsible for the widespread belief among later geneticists as well as historians that the Morgan group in particular, and classical geneticists in general, conceived of genes as beads on a string. There is no indication, however, that the authors intended this to be a literal description of what they still called factors. The final chapter stated, "On the factorial hypothesis the factors are conceived as chemical materials in the egg, which, like all chemical bodies, have definite composition."[38] The text did not elaborate, but obviously it alluded to Morgan's belief that factors were "chemical aggregates" that were of unknown nature but were capable of assuming more than one equilibrium condition—clearly a conception remote from that of a hard, spherical bead. The authors must have intended their diagram and the representation of factors as beads to serve only as a convenient simplification used to render the idea of crossing over more readily. As it happened, the diagram was taken more literally than the authors would have expected by the many who were introduced to the new genetics by this book.

The frontispiece of *The Mechanism of Mendelian Inheritance* was a drawing showing the locations of the factors found by linkage studies to be contained in each of the four *Drosophila* chromosomes (fig. 1.3). In a discussion of linkage, the text referred to the frontispiece once as a



(*Frontispiece.*)

Figure 1.3. Morgan's 1915 chromosome map of *Drosophila* factors based on linkage studies. (From T. H. Morgan, A. H. Sturtevant, H. J. Muller, and C. B. Bridges, *The Mechanism of Mendelian Heredity* [New York: Johnson Reprint Corporation, 1972], frontispiece.)

diagram and several times as a "chromosome map," without making an explicit distinction between the meanings of these two terms. Perhaps the horizontal juxtaposition of the four linear diagrams, giving a two-dimensional array, somehow made the drawing appear more like an ordinary map than the original one-dimensional line in Sturtevant's 1913 paper. In any case, the term *map,* introduced here casually and without exploration of its significance either for conventional ideas about maps or for the relation implied between linkage diagrams and chromosomes, subsequently became the term universally applied to the drawings represented by the activity that similarly came to be known as "genetic mapping."

In 1916 Hermann Muller, who had by this time left the fly room to take up a position at Rice Institute in Houston, Texas, published a series of four articles titled "The Mechanism of Crossing-Over." After reviewing the history of the subject, beginning with Morgan's explanation of the interchange of factors in 1911, Muller introduced his own experiments, in which he had painstakingly constructed *Drosophila* stocks containing nine mutants on the X chromosome and other stocks with seven mutants on chromosome II. With these stocks he was able to show that single crossovers always separated whole groups of factors, confirming that they exist in a fixed linear order on the chromosomes. He was also able to use these results to explore further the phenomenon of "interference" that he had earlier discovered. Double crossovers, he had found, take place less frequently than would be predicted if they were completely independent of one another, and the degree of interference increases with the closeness of the factors.[39]

That Muller, who as early 1914 used the word *gene,* referred throughout these four articles only to factors suggests that he must have been abiding by a convention accepted by the *Drosophila* group at the time of the collective publication of *Mechanism of Mendelian Inheritance.* The following year, however, Morgan switched terms. Responding in an article titled "The Theory of the Gene" to objections that various people had raised to the "method of analysis . . . that has been adopted by those who work in this field," Morgan attempted to "explain what the genetic factor means to those who use it." The Mendelian law stating that when two or more contrasted pairs of characters are involved in a cross the pairs are separated independent of one another in the germ cells means, Morgan asserted, that "the germ plasm must, therefore, be made up of independent elements of some kind. It is these elements that we call genetic factors or more briefly genes."[40]

Morgan made no mention of the fact that up to this point he had always called these elements factors. Nor is there any indication in the remainder of his paper that his conception of the nature or behavior of these elements had changed.[41] Nevertheless, what had been in his former writings the factorial hypothesis now became the "theory of the gene."

Although he gave no reason for changing his language, it is hardly likely that Morgan did not notice this abrupt shift or had no underlying reason for it. We may speculate that one motive was to distance the concept of the gene as an entity from the too-close association that others had made between unit factors and unit characters. He repeated in this paper the argument that each genetic factor has "manifold effects" beyond the formation of the most conspicuous character with which it is associated and that each character is the product of many genes.[42] He had, however, gradually become more pessimistic about the possibility that one could at that time effectively study the complex developmental process by which the factors gave rise to the characters. He now wrote:

> On several occasions I have urged the importance of keeping apart, *for the present at least,* the questions connected with the distribution of genes in succeeding generations from questions connected with the physiological action of the genetic factors during development, because the embryological data have too often been confused in premature attempts to interpret the genetic data. It has been urged that such a procedure limits the legitimate field of heredity to a process no more intellectual than that of a game of cards, for Mendelism becomes nothing but shuffling and dealing out new hands to each successive generation. My plea is, I fear, based largely on expediency, which may only too easily be interpreted as narrow-mindedness; yet I hope to be amongst the first to welcome any real contribution concerning the nature of genes based on the chemical changes that take place in the embryo where the products of the genes show their effects.[43]

When he was invited to present the Silliman Lectures at Yale University in 1925, Morgan gave them the same title as his 1917 paper: "The Theory of the Gene." In the first lecture (corresponding to the first chapter of the published volume that appeared the following year), he summarized the "fundamental principles of genetics," beginning with Mendel's two laws, illustrating them with examples of Mendel's own experiments on peas. He described linkage, crossing over, and the lin-

ear order of the genes primarily by means of individual examples of crosses carried out by his own group on *Drosophila.* At the end of the chapter he declared,

> We are now in a position to formulate the theory of the gene. *The theory states that the characters of the individual are referable to paired elements (genes) in the germinal material that are held together in a definite number of linkage groups; it states that the members of each pair of genes separate when the germ-cells mature in accordance with Mendel's first law, and in consequence each germ-cell comes to contain one set only; it states that the members belonging to different linkage groups assort independently in accordance with Mendel's second law; it states that an orderly interchange—crossing-over—also takes place, at times, between the elements in corresponding linkage groups; and it states that the frequency of crossing-over furnishes evidence of the linear order of the elements in each linkage group and of the relative position of the elements with respect to each other.*

Lindley Darden has treated Morgan's statement as the culmination of the theory of the gene that had been developing since 1900. During this quarter-century the gene had successively taken on additional properties, but they had been expressed largely in the exemplars, or individual experimental results, often depicted diagrammatically. Now, by translating these properties into a verbal statement, Morgan made the theory general and abstract, transcending the exemplars from which it had been drawn.[44]

By 1930, according to Darden, this theory of the gene had reached the form that became standard in textbooks of genetics. Because the statement says nothing about the physical properties of genes, it has also been taken as symbolic of the belief that for classical genetics the gene was a purely abstract entity. It is important, however, to distinguish between Morgan's theory of the gene and the conceptions that he and other classical geneticists held about what the nature of the gene might turn out to be. "The theory of the gene," he wrote at the beginning of the chapter, "differs from earlier biological theories that have also postulated invisible units to which were arbitrarily assigned any desired properties. The theory of the gene reverses this order and derives the properties of the genes, so far as it assigns properties to them, from the numerical data alone."[45] The theory allows geneticists to handle problems on a strictly numerical basis and to predict with precision "what will occur in any given situation. In these respects

the theory fulfills the requirements of a scientific theory in the fullest sense."[46]

Morgan wished, therefore, to limit his theory to what could be rigorously derived from the experimental methods that he and other classical geneticists were using, which were restricted to "questions connected with the distribution of genes within succeeding generations." That he had not lost interest in the eventual search for a material understanding of the gene is again shown by the remarks with which he concluded his lectures, in a section titled "Are Genes of the Order of Organic Molecules?" The only "practical interest" in a discussion of such a topic was, according to Morgan, related to the question of their stability. If the "gene is stable in the sense that an organic molecule is stable, the genetic problem would be simplified." If it is regarded only as a "quantity of so much material," then the question of why it remains constant through the vicissitudes of crossing becomes much more mysterious. But he saw "little hope at present of settling the question." An attempt he had once made to calculate the size of the gene "seemed to show that the order of magnitude of the gene is near to that of the larger sized organic molecules, but further than this we are not justified in going." Nevertheless, he added, it "is difficult to resist the fascinating assumption that the gene is constant because it represents an organic chemical molecule. This is the simplest assumption that one can make at present, and since this view is consistent with all that is known about the stability of the gene it seems, at least, a good working hypothesis."[47]

Whereas Morgan was content to postpone such questions to a time in which embryology was further advanced, by the early 1920s his former student Hermann Muller felt that the structure and nature of the gene had become the "most fundamental question of genetics" itself and that although the "chemical composition of the genes, and the formulae of their reactions, remain as yet quite unknown," he was ready boldly to attack such problems. Whereas Morgan believed that its stability was the property of the gene that called most strongly for chemical explanation, Muller thought that its property of self-propagation—"that characteristic whereby we identify it as a gene"—was the problem that most urgently required solution. "Within the complicated environment of the cell plasm," he wrote in 1922, the gene "reacts in such a way as to convert some of the common surrounding material into an end-product identical in kind with the original gene itself." This property was what

chemists called "autocatalysis," but "the most remarkable feature of the situation is not this oft-noted autocatalytic action itself—it is the fact that, when the structure of the gene becomes changed, through some 'chance variation,' that catalytic property of the gene may become correspondingly changed, in such a way as to still leave it *auto*catalytic. In other words, the change in gene structure—accidental though it was—has somehow resulted in a change of exactly *appropriate* nature in the catalytic reactions, so that the new reactions are now accurately adapted to produce more new material just like that in the changed gene itself." This paradoxical phenomenon underlay the process of mutation, and the central question to be addressed was, "What sort of structure must the gene possess to permit it to mutate in this way?"[48]

Muller's suggestions for approaching this problem were notably general. "A gene cannot be ground in a mortar or distilled in a retort," he noted, and there seemed to be no presently available direct chemical approach to the problem. An indirect approach through the study of chromosome behavior, especially study of the attractive forces that the genes must exert on each other during synapsis, seemed possible, though he admitted that it appeared "very remote from the problem of getting at the structural principle that allows mutability in the gene." A "more direct" method appeared to be the study of the nature of the changes that occur in the chromosomes during breeding and development. Here he gave evidence that mutations are not always losses but drew no concrete conclusions.[49] More suggestive was his treatment of the "localization" of the changes. In all but a few special cases a mutation affects only one kind of gene among the thousands present: "That this specificity is due to a spatial limitation rather than a chemical one is shown by the fact that when the single gene changes the other one, of identical composition, located nearby in the homologous chromosome of the same cell remains unaffected. . . . Hence these mutations are not caused by some general pervasive influence, but are due to 'accidents' occurring on a molecular scale." Muller thought that by study of the distribution of the mutant character of mosaic somatic mutants it might be possible to determine whether the "entire gene changes at once, or whether the gene consists of several molecules or particles, one of which may change at a time."[50]

But he took the most promising approach to be the "experimental one of investigating the conditions under which mutations occur." Up to that point mutations had mostly been taken as "windfalls," but recent experiments had suggested that rates of mutation varied greatly

and might be influenced by controlling the environmental conditions. Finally, Muller pointed out that the invisible, filterable bodies discovered by Félix d'Herelle in 1917 that exerted a lethal action on dysentery bacteria "fulfill[ ] our definition of a gene." They replicate, they mutate, and, in addition, they can be studied in the test tube. If they really were genes, they would "give us an entirely new angle from which to attack the gene problem."[51]

In his conclusion Muller acknowledged that he had painted these possibilities in the "rosiest possible colors," that only meager progress had been made, and that all thinking on the subject was "almost equivalent to speculation."[52] If he could provide only vague leads about how to attack the problem of gene structure, he had at least given the problem more definite shape.

Among the "unusual conditions" that Muller mentioned as possible causes of genetic variations were x-rays, which J. W. Mavor had recently shown to bring about chromosomal abnormalities. In 1923 Muller began to work with x-rays, as well as radium rays, but did not use them to produce mutations. According to Carlson, his reason was that earlier experiments carried out with Edgar Altenberg on the effects of temperature had persuaded him that spontaneous mutations were chemical, not physical, in nature. By 1926, however, he had changed his mind, and after reviewing the literature concerning radiation's effects, he began in November to test various doses of x-rays on some of his mutant stocks, irradiating sometimes the males and sometimes the females.[53] The results were astonishing. Among the progeny of the crosses that he carried out with them he obtained, within a short time, seven hundred mutants, compared to two hundred identified during sixteen years of experimentation with *Drosophila.* "Comparison of the mutation rates . . . showed that the heavy treatment had caused a rise of about fifteen thousand per cent. in the mutation rate over that in the untreated germ cells." The majority of the mutant characters corresponded to those previously observed to arise spontaneously, although there was also a considerable number of new ones. He demonstrated that these were true mutations by breeding them through several generations, during which their stability was equivalent to that of spontaneous mutants.[54]

When news of Muller's achievement was made public, it spread quickly around the world, accompanied by headlines suggesting that mankind was now in a position to control its own hereditary material. Thereafter Muller became a public figure.[55] His own most immediate

concern, however, was to discover what light the effects of x-irradiation shed on the structure of the gene and the nature of mutation. For him the stability of the resultant mutations ruled out the theory that "the gene is compound, consisting of a number of interchangeable molecules or larger members. . . . We may conclude that it is likely that the gene ordinarily consists of not more than one molecule, or at least not of several molecules of the same kind." He now found the maximal estimated size of the gene to be near the size of a single protein molecule. By showing that in cases where a female containing two attached X chromosomes was irradiated, the gene on only one of them was mutated, Muller confirmed that the "transmuting action of the x rays is . . . spatially narrowly circumscribed, being confined to one gene even when there are two identical genes close together. . . . Hence, the accidental position of the gene in the path of the rays, rather than its chemical composition *per se,* may be of major importance in determining whether it happens to become transmuted."[56] In 1928 Muller tested the effects of feeding various chemical substances to his flies, including lead acetate, manganese chloride, and Janus green, and found insignificant effects on the mutation frequencies in their offspring. His collaborator Edgar Altenberg also obtained negative results with ultraviolet rays. These results led Muller to raise the question whether all mutations were "ultimately due to rays of short wave-length and to high speed particles of comparable energy content."[57]

Besides vastly increasing the frequency of genetic mutations, Muller found, x-rays also caused alterations in the structure of chromosomes involving rearrangements of whole blocks of genes. In some cases sections of chromosomes were inverted or removed and reattached to another chromosome, a process he called "translocation." These changes, which he demonstrated mainly by changes in crossover frequencies, he confirmed in a few cases by microscopic observations of the chromosomes: yet further proof, he asserted, "that the genes really do lie in the chromosomes in linear arrangement."[58]

During the next several years, Muller and others confirmed the effects of radiation on *Drosophila* and other animals, including mice. The maize geneticist Lewis Stadler discovered independently in 1928 that irradiating barley seedlings with x-rays or radium induces mutations.[59]

In 1933 Theophilus Painter, a colleague of Muller's at the University of Texas, found that each of the giant chromosomes in the salivary glands of *Drosophila* "has a definite and distinct morphology and is made of

segments, each of which has a characteristic pattern of chromatic lines or broader bands, which appear to run around the achromatic matrix." If the position of these bands is shifted by a translocation, inversion, or deletion, the points of breakage can be determined and the segments identified in their new positions. Consequently it became possible to identify the morphological locations of gene loci produced by rearrangements of a genetic nature and to construct exact chromosomal maps.[60] By the time Painter announced this "sensational finding,"[61] Muller had left for Europe and accepted an invitation from the Russian geneticist Nicolai I. Vavilov to become director of genetics at his Institute of Applied Botany in Leningrad. There, Muller had more ample resources for research and more students than ever before, and in the following years (in Moscow after 1934), he pursued his *Drosophila* experiments vigorously.[62] Painter's discovery, Muller quickly realized, would be particularly valuable in studying the very small rearrangements produced by his own x-ray methods. In March 1934, the Russian geneticist Nikolai Konstantinovich Koltzoff visited the institute where Muller worked and proposed the theory that the giant chromosomes are hollow bundles produced by the proliferation of chromonema (individual chromosome threads), which remain apposed side by side, a view that Muller accepted at once. That year Muller applied Painter's techniques in order to find out the extent to which "the chromosome is subdivisible into its constituent genes (or perhaps still further?), the cytological basis of the constituents thus separated out, and the functional consequences of such separation."[63] For the salivary gland analyses he relied on the skilled Russian cytologist Alexandra A. Prokofyeva.

At one end of the X chromosome Muller was able to produce by x-irradiation two separate breaks, identifiable both by genetic analysis through recombination and by cytological evidence as near to the left and the right side of two genes, respectively—one for a yellow body, one for bristles in the "scute" region—and other double breaks similarly situated with respect to four genes. He reported that the "genetic and cytological maps agreed throughout, and were linear." These convergences led him to speculate about the relation between the number of genes and the number of "chromomeres," identified by John Belling as the cytological units on individual chromonemata in plants corresponding to individual genes or gene clusters. The fact that many experiments produced only a few break points, often repeated, led

Muller to think that genes were discontinuous, with breaks occurring only between them. On the other hand, that some of the rearrangements caused slightly different phenotypical patterns in the offspring resulting from recombination led him to extend the idea of "position effects," first defined by Sturtevant in 1925 for the single case of bar eyes, and to suggest that these effects could extend over several genes. With regard to the position effect, therefore, the chromosome could be considered continuous. Moreover, the similarity of these position effects to ordinary mutations made it appear more difficult than he had earlier assumed to distinguish between "mutations" due to minute rearrangements and a "real intragenic mutation."[64] Meanwhile, Stadler was finding that x-ray–caused mutations in plants were generally attributable to chromosomal changes, casting further doubt on the generality of Muller's original assertion that x-irradiation induced mutations that were identical to spontaneous mutations.[65]

At about the same time, Muller made a new calculation of the dimensions of the gene. He estimated that individual chromonemata had a transverse diameter of not more than 1/50 nor less than 1/250 of a micron. If the genes were as densely spaced throughout the active portion of the chromonema as he and Prokofyeva had found them to be in the band near the end of the X chromosome, then the length of one gene was approximately 1/8 micron: "According to this reckoning, then, the gene length would be between 6 and 30 times as great as its diameter (according to which of the above two estimates of the diameter is chosen). This is in agreement with the fact that proteins and other complex molecules in general are chain-like, being much longer in one dimension than in the other two. We must, however, be careful of accepting these preliminary figures as coming even near to the actualities."[66]

Two comments are pertinent. First, although Muller, too, in writings aimed at a general audience, occasionally described genes as being in "fixed positions in linear order, like beads on a string, within the fine threads that form the skeletal fibres of the chromosomes,"[67] he did not regard them truly as beads but as chainlike. At that time he was entertaining the possibilities that breaks in the string can only come between genes and that genes are divisible. Second, here as elsewhere, he compared the dimensions of the gene to those of a protein molecule. Like his fellow geneticists, he assumed that proteins were the most likely candidates to be the "organic molecules" of which genes are

composed, but he was always careful not to commit himself to that identification. This was not a dogma or a protein paradigm, as some historians have written, but only an expectation.[68]

With Daniel Raffel, a graduate student from Johns Hopkins University who had joined him in the U.S.S.R., Muller continued his study of position effects. In order to make the comparisons of mutants caused by breaks and inversions in nearly the same place on the X chromosome "more exact," they first laboriously constructed stocks, the remainder of whose X chromosome and other two large chromosomes were as nearly as possible identical in their genetic constitutions. Preparation of the "isogenic" X chromosomes alone required twenty-six generations of crossbreeding between carefully selected mutant stocks. The three genes whose phenotypic effects they chose to study, located on a short segment at the end of the X chromosomes, each affected the formation of bristles in the scute region of the abdomens of the flies. In order to detect very subtle differences, they performed statistical analyses of the frequency of the appearance of each of several different types of scute bristles. The most important fact to emerge from this enormous effort was that the scute mutations "phenotypically most closely resembling . . . each other are by far the most like . . . each other in regard to their content and rearrangement of genes in the neighborhood of the scute locus." The "change in expression of these genes in the scute region," they inferred, "must be an effect of the change in positions of the genes relative to one another—the phenomenon known as 'position effect.' "[69]

If the great similarities in the phenotypic effects of these very similar inversions could be ascribed to the position effect, could the slight phenotypic differences observed similarly be caused by slight differences in the arrangements of the genes in the several cases? Muller could not rule out the possibility that genes not yet detected or minute duplications related to differences in the positions of the breaks might also be causal factors. This uncertainty about the meaning of the results drove Muller to take up more general questions about the "degree of divisibility of the genetic material, the criteria by which we recognize the presence of genes and their spatial or numerical limits, and the extent to which the string of genes may be regarded as discontinuous or continuous in structure and functioning."[70]

As he had since 1922, Muller defined the general criterion for distinguishing the gene from all other cellular material as the capacity to

reproduce itself and to do so even after being altered by mutation. Regarding this point, he asserted, there was "little practical disagreement." On the other hand,

> No such understanding has been arrived at concerning the question of how the limits of a gene, as distinguished from its neighbor genes, shall be defined. In genetic theory, genes have been considered as (1) crossover units—hypothetical segments within which crossing over does not occur; (2) breakage units—again hypothetical segments within which chromosome breakage and reattachment do not occur (at any rate, not without destruction of one or both fragments); (3) mutational and functional units—those minute regions of the chromosomes, changes within one part of which may be so connected with changes in the functioning of the rest of that region as to give rise to the phenomenon of (multiple) allelism; or, (4) reproductive units—the smallest blocks into which, theoretically, the gene string could be divided without loss of the power of self-reproduction of any part. A category of auto-attractive units might also be added.
>
> . . . There is as yet no empiric evidence, and only doubtful theoretical ground, for assuming that the lines of demarcation between genes, as defined on any one of these systems, would coincide with those on any of the others, or even for assuming, in the case of any one of these systems, . . . that such lines of demarcation are necessarily invariable, non-overlapping, well defined and absolute.[71]

After discussing the applicability of each of these definitions to the observations that he and Raffel had made of the "genes" of the scute region, Muller concluded that "the gene, as defined by the mutation-allelism test, extends over a larger region than that defined by breakage, crossing over or self-reproducibility, and that, in fact, regions of successive genes, as defined by the mutation-allelism test, do not merely adjoin, but overlap each other." Whether there were finer genetic divisibilities than those found by breakage, Muller wrote, "must still be left an open question."[72] On the basis of similar considerations, including Muller's previous experiments on position effects, the German-born geneticist Richard Goldschmidt asserted in 1937 that there "is no such thing as a gene and certainly no wild type allelomorph." There were only point loci, which must be arranged in a certain order in a chromosome in order to control development.[73] Although he rejected the more radical aspects of Goldschmidt's position,

Muller agreed with him that "the 'gene,' in the rather loose sense in which it has so long been taken for granted by most geneticists, may *perhaps* be genetically further divisible, even into many genetically linearly arranged portions of semi-autonomous character . . . and that, contrariwise, neighboring 'genes' often or usually co-act, in a manner made possible by their juxtaposition, so as to produce character effects that depend upon overlapping regions, somewhat larger than a single 'whole gene' as formerly conceived."[74]

In December 1936, Muller defended the chromosome theory from the attacks of Lysenkoists at the Lenin All-Union Academy of Agricultural Sciences. His exposed position in an increasingly oppressive political situation, however, rapidly made it unworkable for him to remain in the Soviet Union. To protect his workers from retribution, he left in March 1937 by way of Spain, where he took part in an international brigade of Spanish loyalists opposing the Fascist armies of Franco. By November he was in Edinburgh, where a position had been arranged for him at the Institute of Animal Genetics and where he was able to resume his work with a new group of students from Scotland and other countries. He completed the paper just described in 1938, but because Raffel was by then in the United States, its publication was delayed until 1940.[75]

The early negative results of tests to determine whether ultraviolet radiation induces mutations did not discourage geneticists from pursuing the question further. By 1931, Altenberg had shown that ultraviolet light produced mutations in *Drosophila,* but he did not determine whether these involved rearrangements of chromosomal material as the effects of x-rays did. During the following years several geneticists, including Stadler, found that ultraviolet light caused gross translocations in plant chromosomes. At Edinburgh in 1938, Muller, with a Scottish research associate, Kenneth MacKenzie, returned to the question of the effects of ultraviolet light on *Drosophila.* His recent uncertainties about whether minute rearrangements of chromosomes were distinguishable from "gene mutations" or whether the latter were "merely special cases at the extreme lower limits of the former" stimulated him to seek an agent that might discriminate between them. In ultraviolet rays he found part of what he was looking for. Ultraviolet irradiation of male flies produced lethal mutants but no translocations that were visible cytologically. These results suggested that there was a fundamental distinction between gene mutations and rearrangements,

but he could not yet rule out the possibility that ultraviolet radiation produced minute rearrangements too small to be observed.[76]

By 1940 the *Drosophila* group had been pursuing genetic experiments on their favored subject for nearly three decades. As Kohler has shown, during that time the work had grown from the fly room at Columbia to an international network linked by the free exchange of mutant stocks, experimental techniques, students who moved from the core to the peripheries or back, informal newsletters, common assumptions, and certain shared rules of etiquette. After Morgan and his group moved to Caltech in 1928, his laboratory remained the center of the field, a place to which many others came to work for a time, but the Cold Spring Harbor laboratory in New York, under the leadership of Milislav Demerec, emerged as a second major focus. In many other places one or several workers contributed to the collective progress of the mapping project and to other special problems that attracted attention. By the 1930s, however, many of the first- and second-generation *Drosophila* workers were moving beyond the primary project of producing ever more comprehensive maps of the four chromosomes of their reconstructed fly. Morgan, Sturtevant, and others returned, with limited success, to questions about the relations of genes to development that Morgan had put aside in the early days. Others began adapting the results obtained with laboratory flies to the population genetics of wild fruit flies in the field.[77]

The peripatetic Muller was not the only geneticist to concentrate attention on the more fundamental questions about the underlying nature of genes and of the process of mutation, but he was the most prominent and persistent in the pursuit of these questions. The position he had reached by 1940 did not enable him to answer these questions, but it did sharply outline the problems that would need to be solved in order to reach such answers. As Carlson has noted, the intense interest that these questions had aroused during the 1930s diminished during the 1940s. When subsequent generations of biologists returned to them in the 1950s they either had forgotten or had never known the extent of the efforts of their predecessors to elucidate the various meanings of the gene, and they tended to attribute to classical genetics as a whole the simple beads-on-a-string model. Carlson has argued that this representation of the "classical gene concept" was a fiction, one that treated as literal beliefs the analogies and illustrations that had earlier been used in the depiction and popularization of the factorial hypothesis. This concept became a straw man, the "weakest

possible model of the prevailing view" from which a more enlightened cohort of successors had now escaped.[78] Such ways of distinguishing oneself more sharply than is justified from one's intellectual forebears are commonplace, not only in the sciences but in most areas of collective, specialized activity that especially reward real or perceived innovation and originality.

Chapter Two

Genetics and the Phage Biologists

In an address delivered to a session on physics at the Academy of Sciences of the U.S.S.R in March 1936, Hermann Muller made a passionate plea to physicists to "interest themselves more actively" in the problems surrounding the "tiny particles of heredity—the genes—[in which] the chief secrets of living matter as distinguished from lifeless are contained." He focused on three of these problems: the property of "specific auto-attraction" that must somehow exist between corresponding genes on the gene chain—a force that must be unlike the ordinary forces of adsorption known to physicists; the property of "autosynthesis" that makes of the gene a "modeler" which "forms a copy of itself, next to itself"; and, finally, the "very important problem of the way in which changes in genes—mutations—occur." The methods of genetics and physics combined had already shown that the "mutations produced by high frequency irradiations are the results of single ionizations, and that the whole process from ionization to mutation must be rather sharply circumscribed in space." It was with this problem, he added, that the "physicist is to-day most actively and fruitfully helping the geneticist."[1]

When he made this appeal Muller was undoubtedly aware that two physicists in Berlin were engaged in such studies of gene mutation through irradiation. Four years earlier Muller had spent several months there collaborating with the Russian geneticist N. V. Timoféeff-Ressovsky on the genetic changes caused by radiation of varied wavelengths. Timoféeff had been inspired by Muller's discovery of the effects of x-rays on mutations in 1927 and had quickly turned to the study of induced mutations.[2] After Muller left Berlin for the Soviet Union in 1933, Timoféeff enlisted the support of the physicist Karl G. Zimmer, who was particularly interested in the puzzling question of

why the irradiation of biological organisms with increasing doses, in contrast to chemical agents, produced gradually rising effects with no lower limit.[3]

While in Berlin Muller also met a young theoretical physicist named Max Delbrück, a protégé of Niels Bohr who had recently been inspired by a lecture by Bohr to think about moving into biology. Muller later said of Delbrück that "he was a promising young physicist and mathematician. . . . We biologists talked to him with bated breath."[4] Not long afterward, Delbrück came into contact with Timoféeff and Zimmer. They met two or three times per week in the home of the Russian geneticist, where they engaged in very long conversations,[5] the outcome of which was that Delbrück took up the challenge to provide a theoretical quantum interpretation of their experimental studies of the effects of radiation on mutations.

So much lore has come to surround the personality of Max Delbrück, especially in his role as charismatic leader of the loose network of workers from various fields that came to be known after 1945 as the "phage group," that it is difficult to separate the earlier historical figure from the legends that shape his image. Because he sometimes "disparaged biochemistry,"[6] especially in informal remarks, it is maintained that he hoped to unravel the genetics of bacteriophage without recourse to that field. Because as a physicist he hoped initially that he might characterize genes, their mutations, and their reproduction through such indirect physical methods as the "target" theory, it is said that he hoped that he could solve these problems without opening the "black box" of the bacteriophage itself. Because he later recalled that when he began to study *Drosophila* genetics in 1937 he became depressed and felt he could never master this complicated, arcane subject, his studies of phage and bacterial genetics are sometimes seen as disconnected from the long tradition of classical genetics based on the fruit fly. He is thought to have long dismissed the phenomenon of lysogeny in bacteriophage until the discoveries of André Lwoff finally forced him, in 1950, to accept its importance.

None of these images of Delbrück is groundless. Each of them is based on his own contemporary statements or later recollections. Collectively they present, however, a one-sided picture of a complex person who often contradicted his self-portrait. Delbrück expressed his opinions forcefully but playfully, and his attitudes were more mercurial than fixed. Throughout his career he was prepared to modify or

reverse his strongly held views in the face of unexpected results in his own investigations or new developments emerging from elsewhere. He was broadly, though not always deeply, informed about each of the fields of investigation relevant to the problems of the structure, reproduction, and function of genes, as well as their implications for inheritance and evolution.

There is no reason to doubt Delbrück's story that his initial inspiration to move from physics into biology was a lecture titled "Light and Life" that he heard Bohr deliver in Berlin on August 15, 1932, but there is some question about what he heard Bohr say and whether he understood Bohr's views in the same way that Bohr did. In the published version of the lecture Bohr applied his "complementarity principle" to biology in the following manner: If one tried to carry the investigation of the organs of an animal so far as to describe the role played by single atoms in its vital functions, one would undoubtedly kill the animal "In every experiment on living organisms, there must remain an uncertainty as regards the physical conditions to which they are subjected, and the idea suggests itself that the minimal freedom we must allow the organism in this respect is just large enough to permit it, so to say, to hide its ultimate secrets from us." As his biographer, Ernst Peter Fischer, has pointed out, what Delbrück remembered hearing many years later was quite different. Whether Delbrück remembered inaccurately or whether Bohr said something different in his faintly audible lecture than in the written version is impossible to resolve. In any case, Delbrück expressed his own understanding of the application of complementarity to biology not long after the event, in a letter written to Bohr in 1934.

> Our claim: Those assumptions that are necessary for the existence of causality in biological phenomena may partly contradict the laws of physics and chemistry, because experiments with living organisms are certainly complementary to those that determine the physical and chemical events on the atomic level.
>
> Explanation: (1) We do *not* state that the laws of atomic physics can explain the *specific phenomena* of life. On the contrary!
>
> Just as it is no longer believed that chemistry can be reduced to classical physics, because a macroscopic experiment such as the synthesis of a chemical compound is complementary to an experiment to determine the orbit of the electrons of a chemical bond, and quantum mechanics eliminates the possibility for a causal explanation;

> and, since chemical and physical events in organisms are connected down to the atomic level, so physical and chemical notions alone cannot achieve a causal explanation of these connections.
>
> (2) We do *not* state that a biologist in his experiments does kill life or that he has to kill it. On the contrary!
>
> In genetics *and* in development *and* in psychology *and* in biochemistry *and* in biophysics it is characteristic, *and* essential, to investigate processes in the living organism. Because of that, these methods of research cannot gain results about the individual atomic elementary processes. They are far away from it, as everybody agrees here.[7]

Apparently Bohr approved of this version of the application of complementarity to biology. As the last sentence quoted suggests, Delbrück must have formulated these ideas at a time when he was engaged in intense conversations with Timoféeff and Zimmer in Berlin and probably also with Hans Gaffron, a student of Otto Warburg who specialized in photosynthesis, and with the chemist Kurt Wohl,[8] but before he had acquired firsthand experience with any of the fields of biological experimentation to which he alluded. That these views nevertheless became deeply motivating forces driving him toward biology is apparent from his long-standing later preoccupation with these same questions.

In 1935 Timoféeff, Zimmer, and Delbrück jointly published a paper titled "The Nature of the Mutation and Structure of the Gene" in the proceedings of the Scientific Society of Göttingen. The paper was divided into four parts: the first, by Timoféeff, was titled "Some Facts from the Investigation of Mutations"; the second, by Zimmer, was "The Hit Theory and the Origins of Mutations"; the third, by Delbrück, dealt with a "physical atomistic model of gene mutation' "; and the final part, by all three, discussed a "theory of gene mutation and gene structure." It is clear from the contents of these sections and the opening remarks of the paper that the "work presents a cooperation between genetics and physics. It has originated through presentations and discussions in a small private circle of representatives of genetics, biochemistry, physical chemistry, and physics." The three theoretical sections interpreted the experimental results described in the first section according to current theories of physics and physical chemistry. Historians have detected some differences of emphasis between the section by Delbrück and the sections by the other two authors, but there is also a considerable degree of coordination, reflecting the intensity of the interactions to which the paper attests.[9]

Building on Muller's fundamental discovery that the rate of induction of mutations by x-rays is proportional to the overall dose, Timoféeff tested the effects of differing wavelengths as well as different periods of exposure. Through the entire range of x-rays, as well as gamma rays, he found the mutation rate to be independent of wavelength or time; it was a function solely of the total quantity, as expressed in Röntgens. From these results he concluded that there was no minimal threshold dose but that the proportionality could be extrapolated to zero; furthermore, in contrast to many other biological reactions, the process of mutation was not reversible, but the "gene moves from one stable condition to another equally stable condition." Concerning spontaneous mutations, Timoféeff confirmed Muller's observation that the dependence on temperature follows the Van t'Hoff rule with a Q_{10} of about 5 (Q_{10} being the factor by which a biochemical reaction rate increases for every 10°C increase in temperature). He found that the rates of induction of specific mutations differed considerably, an effect he attributed to differences in the structures of the alleles.[10]

From an equation for the curves of Timoféeff's experiments showing the proportionality of mutation rates to dosages, Zimmer readily deduced that a "single hit is sufficient to give rise to a gene mutation." That left open the question of the nature of the hit. Zimmer considered three possibilities. The first of these, that the reaction is due to the absorption of a quantum of energy, he ruled out on the basis that the mutation rates were independent of the wavelength of the radiation. The other possibilities were that the hit consisted of the formation of an ion pair or that a secondary electron arose from a series of hit events. Eliminating the latter by means of more complicated considerations, Zimmer inferred that radiation-induced mutations are caused by single hits consisting in the formation or excitation of an ion pair.[11] It was from this conclusion that Delbrück, in the next section, derived a model for gene mutation.

Delbrück began his discussion by asking whether it was opportune to bring atomistic physical speculations into genetics, which was a logically self-contained, rigorous science. Unlike chemistry, the quantities on which genetics relied were not expressed in absolute units of mass and, therefore, not dependent on more fundamental physical quantities. The development of genetics had, however, through its connections with cytology and the refinements in the analysis of *Drosophila,* led to calculations of the size of the gene comparable to those for

specific structured molecules, and many investigators had inferred that the gene is nothing else but a special sort of molecule whose specific structure was not yet known. He pointed out, however, that normal chemical methods, which depended on the existence of many of a particular kind of molecule, all reacting identically to a given chemical stimulus, could not be applied, even in thought experiments, to the gene, because there was in each living unit only a single representative of one gene, surrounded by a heterogeneous medium.[12]

The main reason for identifying a gene with a molecule was its stability: it remained unchanged in hybridization experiments. As Morgan had many years earlier, Delbrück inferred that "the thought lies near at hand that this stability is immediately connected with the stability of a molecule." Consequently, "when we speak of molecules we do not refer to identical behavior [as in the case of ordinary chemical molecules], but very generally to a well-defined atomic combination, for which we assume that the identity of two genes lies in the fact that in them the same atoms are stably ordered in the same unchangeable way."[13]

Because we cannot establish the atomic identity of genes by means of chemical preparations and know nothing about the way in which genes act as chemical catalysts, Delbrück went on, "we must attack the problem in a more primitive way. We must first investigate the *nature and the limits of the stability* of the gene, and find out whether they fit with what we know from atomic theory about well-defined atomic combinations." In his exploration of these questions he assumed that such atomic combinations have determined mid-positions and that their electronic conditions are determined. Consequently, "changes in the model can take place only by jumps," and they must be composed of "steps of elementary processes," in the sense of quantum theory.[14]

These changes were (1) changes in the condition of vibrations brought about by thermal changes that do not change the chemical character; (2) changes in the electronic condition excited by one or more electrons, after which there is a return to the ground state; and (3) "rearrangements of the atoms to form another equilibrium state." The latter could take place either through changes of temperature sufficient to raise the amplitude of the vibrations of the atomic combination beyond the limits of stability or "through energy delivered to an electron from the outside," as from radiation. In this last case the energy of the electron might be dissipated as it is spread through the atoms or it might, at a certain point, cause a particular atomic rearrangement. Ac-

cording to this view, a "specific mutation is a specific rearrangement within a specific molecule." Showing that these conceptions were compatible with several of the experimental results reported in the first section of the paper, Delbrück inferred that spontaneous mutations were rearrangements brought about by thermal energy, whereas radiation-induced mutations were caused by the energy imparted from ion pairs excited by the radiation, in the manner that Zimmer had proposed.[15]

Delbrück was careful to emphasize the limits of what his model could explain. "The fundamental property of the gene, to duplicate itself exactly in mitosis (whereby this property is convariant with mutation), is not a property of the gene model, but an achievement common to the gene and its surrounding substance." The compatibility of the model with gene reproduction could not, therefore, be tested until this interplay could be incorporated into an extended model.[16]

By 1936, when he accompanied Muller and Timoféeff-Ressovsky to a conference on radiation and genetics in Copenhagen, Delbrück had become deeply enough committed to biology to seek a position in that field. His prospects in Germany were not good, however, because he did not conform to the expected norms of political deportment in the Nazi era. After consultations with Rockefeller Foundation officials who had previously supported him with a fellowship in physics, he obtained a fellowship to study *Drosophila* genetics with Morgan's group at Caltech.[17]

At about this time Delbrück was inspired by the crystallization of tobacco mosaic virus (TMV) by Wendell Stanley[18] to think that such viruses might hold the key to the "riddle of life." In an essay by that name, written just before leaving Germany, Delbrück described viruses both as molecules and as living organisms. Turning to the property that defined it as the latter, "namely its ability to multiply within living plants," he asked himself whether the growth was accomplished by the host or by the virus itself, and he excluded the former as impossible. "Therefore," he wrote, "we will look on virus replication as an autonomous accomplishment of the virus, for the general discussion of which we can ignore the host." Pointing out that replication in higher plants and animals is a highly complex phenomenon and that even "simpler mitotic cell division" is a process modified in various ways to adapt itself to diverse purposes, involving the coordination of the replication of a whole set of genes with the division of the cell, he concluded that

"we want to look upon the replication of viruses as a particular form of a primitive replication of genes, the segregation of which from the nourishment of the host should in principle be possible. In this sense, one should view replication not as complementary to atomic physics but as a particular trick of organic chemistry."[19]

That viruses provided an extraordinary opportunity to study replication in vastly simplified form was not an insight unique to Delbrück. As we have seen, Muller had suggested as early as 1922 that d'Herelle bodies might be essentially like genes, and in his 1936 lecture he had inferred from the crystallization of TMV that a new opportunity had arisen to study the gene. "This material has the properties of a gene," he asserted, "inasmuch as it can reproduce itself . . . and it is probably mutable, since different 'species' of it are known." Because the material could now be obtained in bulk and in pure form, "it will be very important to carry on an active investigation of it."[20] It is not unlikely that conversations with Muller on this topic significantly influenced Delbrück's thinking at this formative time in his transition.

Delbrück arrived at Caltech in October 1937. During his first weeks there he read some reprints given to him by Sturtevant, who suggested that he might occupy himself with some unresolved linkage problems on the fourth chromosome of *Drosophila.* As he recalled much later, Delbrück quickly became disconsolate. He could not understand the arcane language of this complex field and decided that the fruit fly was far too complex to lead him to his goal of understanding the replication of genes.[21] Although there is undoubtedly a core of truth in this memory, it has led to some misunderstandings. As some of his subsequent writings demonstrate, Delbrück came to understand quite well the principles of *Drosophila* genetics. Its complexity should have come as no surprise to him, because he had already encountered the field intensely during his collaboration with Timoféeff-Ressovsky. Nor would he have expected, when he arrived at Caltech, that he would try to unravel the nature of primitive gene replication via *Drosophila* genetics because, as William Summers has pointed out, he had already concluded, in the essay he wrote just before his arrival, that viruses would provide the great simplification necessary to study that question most successfully.[22]

On his return from a camping trip early in 1938, Delbrück learned that he had missed a seminar by Emory Ellis, a young graduate at Caltech, on bacterial viruses. Hired as a postdoctoral research fellow whose work was funded by a grant for basic research on cancer, El-

lis decided to direct his attention to the "filterable viruses," on the grounds that some of them, such as the Rous sarcoma virus, produce tumors. Largely because of time and cost, he chose to work with bacteriophage of the type identified by d'Herelle. When Delbrück looked him up, Ellis was studying the life cycle of a bacteriophage obtained from sewage that infected a strain of the common intestinal bacterium *Escherichia coli* that he had obtained from a friend at Caltech named Carl C. Lindegren.[23]

The nature of bacteriophage had been controversial for two decades. The point of departure for Ellis's study was d'Herelle's observation that the number of corpuscles increased not continuously but by "successive liberations"[24] and his consequent belief that the phage is a particulate body that grows by becoming "attached to a susceptible bacterium, multipl[ying] upon or within it up to a critical time, when the newly formed phage particles are dispersed into the solution."[25] Ellis also accepted d'Herelle's belief that the plaques that form in bacterial cultures infected by phage are empty spots that the phage produce by destroying the bacteria growing within such areas, and that such plaques could be used to count the number of phage released by the bacteria.

Delbrück, who, as a theoretician, had little previous experimental experience, was immediately captivated by the simplicity of the phage experiments. They required only simple equipment, could be completed in one day, and yielded data that were easily amenable to quantification.[26] With Morgan's support, he quickly joined Ellis in a collaborative investigation that resulted, in September 1938, in a joint paper titled "The Growth of Bacteriophage." The major achievement of this paper was to turn d'Herelle's observation of stepwise growth into an experiment that yielded a "single 'cycle' of growth (infection, growth, burst)" by so diluting the mixture of phage and bacteria after the initial adsorption of the phage that little further adsorption took place. They studied the effects on what they called "one-step growth curves" of changes in temperature, the ratio of phage to bacteria, and other factors. Borrowing a method devised by the Australian virologist Frank Macfarlane Burnet, they were able to determine not only the average number of phage released during a burst but the distribution of burst sizes among individual bacteria. First, they tested the notion that a single phage particle was sufficient to give rise to a plaque. In order to do this they withdrew, from a mixture containing many particles, samples small enough to contain on the average one particle or less. Plating

these, they were able to determine, from the fraction that produced zero plaques, one plaque, two plaques, and so on, by means of the Poisson distribution formula, that only one particle was needed to infect a cell. Second, from a culture of cells infected with less than one particle per cell, they withdrew into individual tubes of culture medium samples diluted so that there was an average of one infected cell in each tube. These tubes were incubated until the infected cell burst and released all its intracellular phage. Assay of the entire content of each tube gave a measure of the magnitudes of individual bursts. These ranged from zero to two hundred, a much greater fluctuation than they expected from the differences in sizes of individual bacteria. "The cause of the great fluctuation," they wrote, is "still obscure."[27]

Ellis had already done much of this investigation before Delbrück joined him, and the main contribution of the latter was to provide a statistical analysis of the plating efficiency of the phage. Though Ellis and Delbrück brought similar styles and enthusiasm to the project, their objectives were different. Whereas Ellis examined the life cycle of bacteriophage in the hope of illuminating the life cycles of viruses that cause disease, Delbrück expressed in his report to the Rockefeller Foundation at the end of his fellowship year another view of the object of the work: "The leading idea was . . . that the growth of phage was essentially the same process as the growth of viruses and the reproduction of the gene. Phage . . . seemed to offer the best promise for a deeper understanding of this process through a quantitative experimental approach."[28]

Morgan, who was highly impressed with the work that Ellis and Delbrück were pursuing, recommended that the Rockefeller Foundation renew Delbrück's fellowship for a second year. This was done, but Ellis then found himself constrained by the terms of his fellowship to work more directly on problems related to cancer. Delbrück was left to carry on the investigation of bacteriophage on his own.[29] He focused mainly on more detailed analyses of the growth cycle. Studying the adsorption of the phage on the host bacterium, he found that the rate depended in particular on the physiological state of the bacteria and that under optimal conditions the rate was more than sixty times as great as it was under poor conditions. Examining the nature of lysis more closely, he saw that the bacteria under the microscope seemed simply to "fade out" when the phage were released. He distinguished two types of lysis. When a single phage infected a bacterium the process was quite different from that when a large excess of phage was

present. In the latter case he could see the bacterium swell before bursting. He called the two types "lysis from within" and "lysis from without." He experimented with a second type of phage and a second strain of *E. coli,* comparing the resulting one-step growth curves with those he and Ellis had previously attained. Displaying his outlook as a physicist, he compared the empirical curves he obtained from his results with theoretical curves derived from assumptions about the density distribution of phage particles surrounding a bacterium considered as a resting adsorbing sphere. In the two papers he published early in 1940 about this continued work, Delbrück also compared and contrasted his results with those obtained by the biochemist John Northrop and his assistant, Albert H. Krueger, who had employed different types of phage and different methods of assay.[30]

In these experiments Delbrück was essentially refining an approach that Ellis had already taken up and that derived originally from d'Herelle. Nevertheless, the "single-step growth curve" that emerged from these papers at Caltech came to be associated so closely with Delbrück that he was sometimes thought to have invented it.[31] Moreover, the methods displayed in these papers have been taken to stand for his "black box" approach to replication. As Fischer and Lipson put it, "During these studies, Max did not consider what was happening to the bacteria or how they were destroyed. For his purposes, he regarded the process as a black box, i.e., as a system, for which only the input and the output are analyzed and correlated. A single virus particle serves as the input while the emergence of progeny was the output."[32] Such interpretations mistake views that Delbrück expressed at certain points in his career for fixed positions. That he limited himself in these particular experiments to events at the beginning and the end of the growth cycle does not imply that he was uninterested in the events that took place within the bacterium. As we shall see, his subsequent investigations and writings indicate clearly that he aimed to penetrate eventually to exactly the interior processes where the reproduction of the gene really took place.

At the expiration of the second year of his Rockefeller fellowship, Delbrück was in a difficult personal situation. Conditions in Nazi Germany made it impossible to return to his position in Berlin. Despite his admiration for the work Delbrück had undertaken at Caltech, Morgan did not have the funds to offer him a position there. Morgan did, however, exert his influence on the Rockefeller Foundation to assist in obtaining for Delbrück a position as an instructor in physics at

Vanderbilt University in Nashville, Tennessee, beginning in January 1940.[33] There, Delbrück was able to pursue actively his new venture as an experimental phage biologist. He also continued his earlier role as a "physicist thinking about biology."

At a meeting of the American Association for the Advancement of Science held at Stanford University in 1940, Delbrück gave a talk titled "Radiation and the Hereditary Mechanism" as part of a symposium called "Radiation and Life." In his contribution he reviewed the experiments on radiation with x-rays and other sources of ionizing radiation, as well as ultraviolet light, on both microorganisms and higher organisms. Describing the work of others, for the most part, he mentioned in passing that he himself had irradiated bacteriophage with ultraviolet light. He began the last portion of his paper, concerning the "mutations proper," by noting that it was the "startling announcement by Muller in 1927" and the independent work of Stadler that had initiated the "vast amount of work" he was attempting to survey. In his summary Delbrück was particularly attentive to the recent work of Muller and his students, which aimed at differentiating between chromosome rearrangements and "point-mutations."[34]

Although Delbrück referred briefly to his 1935 paper with Timoféeff-Ressovsky and Zimmer, it is conspicuous that he did not repeat his earlier interpretation of gene mutations as changes in quantum states but alluded instead to chemical categories of change. A "mutation proper," he wrote, is "confined to a simple chemical change affecting only one radical."[35] In his conclusion, he asserted that

> We see here emerging a new field of physiology, beyond that of enzyme chemistry, which at present enjoys such splendid success in the hands of organic chemistry. In this new field a new feature, that of statistical fluctuations always attendant on small numbers of independent entities, must become of paramount importance. At present we do not know how the cell manages to exhibit such outward regularities in spite of the molecular incoherence. This problem will doubtless come into great prominence as we learn more about its details, and may require new conceptual ways of approach.[36]

In its call for new approaches and something beyond enzyme chemistry, this passage superficially resembles views often attributed to Delbrück. But it does not fit the stereotype of someone who wished to

ignore biochemistry. Rather, as his next steps into this arena suggest, he was probably busy familiarizing himself with the current state of this very active field.

During the summer of 1941 Delbrück attended the Cold Spring Harbor symposium "Genes and Chromosomes: Structure and Organization," a meeting specifically intended to bring together "genetics and the borderline fields of physics, chemistry and mathematics." The several sessions, occupying two weeks, ranged from discussions of the cytological structure of chromosomes, the biochemistry of proteins considered pertinent to the problem of the reproduction of genes, and spontaneous and induced mutations to new physical instruments such as the electron microscope that were expected to be important to future studies in the field.[37]

To this wealth of communications Delbrück contributed a short paper titled "A Theory of Autocatalytic Synthesis of Polypeptides and Its Application to the Problem of Chromosome Reproduction." Discussions of chromosome behavior were hampered, he began, "by our lack of knowledge of the chemical reactions in which a gene is involved," especially in the reproduction of the gene. It seemed desirable, he added, to "examine the essential difficulties of a chemical theory in order to get a better understanding of the requirements which such a theory should meet." The first difficulty was the need for a source of energy to supply that needed for peptide synthesis. The second was the autocatalytic nature of the process, the third the fact that reproduction stops after the appearance of only one replica. He proposed a "scheme of synthesis which illustrates the manner in which these difficulties may possibly be overcome." Delbrück took the scheme, in which a peptide bond is formed not from a carboxyl group and an amino group, as in laboratory syntheses of peptides, but from a carbonyl group and an amino group, forming an imid bond that is subsequently oxidized to a peptide bond, almost exactly as outlined in a review of the article "Energetic Coupling in Biological Syntheses" by the Danish biochemist Herman Kalckar that had just appeared in *Chemical Reviews.* Kalckar had, in turn, based his scheme on one proposed in 1939 by Kaj Linderstrøm-Lang in a review of proteolytic enzymes. Delbrück's contribution consisted mainly of several arguments for the applicability of the scheme to the reproduction of genes.[38]

His main argument was that the reaction connecting each amino acid in a peptide bond will be specific, because the catalyst, in this case the appropriate portion of the existing gene, "must fit sterically with

the substrate in order to permit close contact." The oxidation, which Delbrück (following a suggestion by Leonor Michaelis that Kalckar had also discussed) believed to take place in two steps, the intermediary being a semiquinone radical with one unpaired electron, required that the additional electron in the substrate be close in energy to the corresponding state in the catalyst. These conditions are best met by a catalyst with a structure very similar to that of the product of the oxidation, so that the reaction is autocatalytic. The strengths of the scheme, in his view, were that it linked the synthesis with energy-yielding reactions of intermediary metabolism and that it explained autocatalysis not by a "mysterious attraction between like molecules" but by ordinary short-distance interactions to select the correct substrate. By separating the formation of the peptide bond into two oxidation steps, it also suggested why the synthesis normally produces only one replica. The two steps may be separated in time, the first producing a semiquinone double chain, the second a peptide chain. Because these two steps require different redox conditions, each may occur only at a particular phase of the cell cycle and cannot be repeated until the original conditions have been restored.[39]

To judge from the edited discussion, the participants in the symposium took Delbrück's proposal seriously enough to subject it to searching questions. When Dorothy Wrinch criticized it for failing to include the role of the nitrogen component of the amino acids, Delbrück responded that "the scheme even if wholly true will represent only a small part of the whole truth of this matter. For one thing, nucleic acid has not even been mentioned, and it must certainly have an important function. Also, nothing was said about the oxidants concerned. It was not my intention to develop a complete picture, but only to point out an implication of Linderstrøm-Lang's scheme, which seemed hopeful to me."[40]

Hermann Muller asked whether "the molecules come together 'front to front' or 'front to back,' " to which Delbrück replied, "The latter, as in a crystal." When Muller later asked why the back of the structure should fit its front, Delbrück said simply, "We must postulate that they do fit front to back." In response to several questions about how the paired amino acids could come into the necessary close contact, he maintained that diffusion rates could easily account for very fast enzymatic reactions.[41]

At the end of the symposium, Muller provided a résumé of what had been discussed during the two weeks and of the perspectives resulting

therefrom. He treated Delbrück's contribution generously. "Delbrück," he said, "has given us an admitted speculation, but a very ingenious one." In a discussion whose length nearly exceeded Delbrück's paper, Muller spelled out some further implications of the scheme and then raised a number of difficulties, but he added that "it is too much to require any present-day hypothesis . . . to solve at one sroke the whole complicated problem of autocatalysis or self-copying. . . . The scheme . . . has the advantage of being concrete so far as it goes, and it is very nearly the only concrete interpretation of self-duplication that has yet been offered from the side of chemistry or physics. It will have served a highly useful purpose if it brings more chemists and physicists to think and experiment in this direction."[42] Other practicing biochemists at the symposium probably reacted more negatively to an offering from a physicist that must have appeared to them to have been based on no more than a quick reading of several of the latest review articles in their field.

For us, the more interesting question is, what had induced Delbrück, in the space of a few years, to shift from atomic physics to metabolic biochemistry as the source for a theoretical explanation for the behavior of genes? In the first place, the question that Delbrück now sought to examine was quite different from the one he had taken up in 1935. At that time he had restricted himself to an explanation for gene mutations and had explicitly stated that his model could not account for the replication of genes. Now it was exactly the problem of replication to which he addressed himself. The most straightforward reason why he turned to biochemistry for this quest appears to be that his reading of the recent literature showed him that by 1941 biochemistry had reached a stage in which it had much more powerful categories of explanation available than it had had when he first became interested in biological questions. It was, in fact, between 1935 and 1941 that the broad outlines of metabolic pathways, the enzymatic reactions that drove them, and the couplings between such pathways and the storage and release of energy came into sharp focus. An attentive reader of the latest literature could hardly fail to see the potential for extending the accounts already given of the main pathways of anaerobic and aerobic decomposition to an understanding of synthetic reactions, including the synthesis of proteins. Delbrück was probably also stimulated by personal contacts at Caltech, in particular Herman Kalckar, who spent the year 1939 there on a fellowship and who engaged in "lively discussions" with Delbrück, Pauling, and others about

the importance of substrate phosphorylations.[43] Absorbing the contemporary view that chromosomes were nucleoproteins but that the protein component was more likely to be central to the reproduction of the gene, Delbrück was able, with little effort, to skim from recent reviews concerning protein synthesis ideas that he could connect with views expressed by geneticists such as Muller concerning the most cogent features of gene reproduction.

Into these biochemical and genetic concerns Delbrück imported the style that he had grown accustomed to as a theoretical physicist: that is, to think about and give further meaning to information gathered by other experimentalists. Some, such as Muller, who had invited physicists to do just that, and even Morgan, welcomed this activity. Morgan had written the Rockefeller Foundation in 1939 that Delbrück was "quiet, gentlemanly, intensely wide awake to everything going on in the biological field to which he might apply his mathematical physics. He is one of the few men we have known who is a mathematician and to whom we can go with our biological problems and find that he has a real understanding of what we are trying to say."[44] To others who had not come to know him through close contact, Delbrück could appear as an arrogant intruder, offering superficial schemes based on work he had not done.

A little later, Delbrück wrote a general review titled "Bacterial Viruses (Bacteriophages)" for *Advances in Enzymology.* In it he covered the "life cycle" of bacteriophage in sensitive hosts, drawing extensively on his own experiments of the past four years. He discussed also host-virus relationships and their relation to antigen-antibody reactions, as well as virus mutations and the inactivation of virus through radiation. Throughout his review, however, Delbrück stressed the importance of biochemical approaches. Two aspects of the "phage problem" that he singled out for particular interest were the "biochemical basis of the specific relation of the phage to its host" and phage growth. Regarding the latter, he wrote, "it is desired to know the precise relation of the phage growth with the biochemical functions of the host cell, and secondly, one wants to know the nature of the chemical process which secures the accurate reproduction of the virus itself. This has an obvious relation to the problem of the reproduction of the gene."[45] In his concluding paragraph he called for further biochemical investigation: "It is of importance that the dependence of the virus growth on the metabolism of the host cell should be examined more closely." It had sometimes been stated that the host cell provides the

nutrient medium for virus growth, but Delbrück believed that the "dependence of the virus on the cell goes deeper: It seems that in the synthesis of new virus not only short-lived intermediate products of metabolism are employed. The virus makes use of the metabolic machinery of the cell for its own needs. The oxidation-reduction cycle and the phosphorylation cycle of some cell metabolite may be directly involved. Such a study will require the analysis of the growth of host and virus in the presence of a variety of substrates and inhibitors, under aerobic and under anaerobic conditions. In the opinion of the reviewer, the problem of the autocatalytic synthesis in the cell may be approached in this manner with success."[46]

Although his emphasis on biochemistry may have been in part conditioned by the biochemical readership of *Advances in Enzymology,* the fact that Delbrück chose to write for that audience is, in itself, significant. Untrained in biochemistry but increasingly aware from his reading of the relevance of that field for the problems he had first taken up as a physicist, he appeared to be inviting the collaboration, or at least awakening the interest, of those who were in a position to attack these problems with "standard biochemical methods."[47]

Following the symposium on genes and chromosomes, Delbrück stayed at the Cold Spring Harbor laboratories through the summer of 1941 to carry out joint experiments on bacteriophage with Salvador Luria, whom he had met the previous New Year's Day at a meeting of the American Physical Society in Philadelphia. Born in Turin, Italy, Luria, in spite of a strong interest in physics and mathematics, had acceded to his parents' wishes that he go to medical school. Although he did very well as a medical student, he felt inadequate as a doctor and unenthusiastic about practice. With the encouragement of an earlier classmate and friend, he again became interested in the possibility of a career in physics. After a stint in the army he moved to Rome, ostensibly to learn some physics by working as a radiologist, but more important, to take physics courses and to work in the laboratory of the famed physicist Enrico Fermi. After a year, however, he concluded that his mathematical abilities were not strong enough to allow him to become more than an amateur physicist. At about that time someone gave him copies of the articles in which Delbrück had formulated the concept of the gene as a molecule. Immediately inspired by these ideas to go into radiation biology, a little later Luria encountered by chance a bacteriologist named Geo Rita, who was working with bacteriophage and dysentery bacteria. Shown into Rita's laboratory, Luria "played

with test tubes and petri dishes, devising new ways of growing and counting bacteriophage, even using some of the statistical methods [he] had learned in physics—making all possible mistakes but for the first time in [his] life getting excited about research." Bacteriophage appeared to him the perfect object on which to test Delbrück's views about the gene.[48]

In July 1938 the Fascist "Racial Manifesto" suddenly dampened prospects for professional careers for Italian Jews and immediately deprived Luria of a fellowship he had just been awarded for the study of radiation biology in Berkeley. Opting to escape to freedom by leaving Italy for Paris, he had the good luck there to encounter the physicist Fernand Holweck, who obtained fellowship for him from the National Research Fund. Working on the effects of radiation on bacteriophage brought him into contact with Eugène Wollman, the chief of bacteriophage research at the Institut Pasteur.[49]

Holweck and Wollman had already been involved in radiation studies; the latter, together with Antoine Lacassagne, had irradiated bacteriophage of several sizes and found that the "sensitive volumes," as estimated from target theory, correlated with their differences in size, measured using ultracentrifugation techniques. Luria persuaded them to follow the example of Timoféeff-Ressovsky and Delbrück, irradiating the same bacteriophage with different wavelengths of x-rays and with α particles. For each radiation they found that the survival curves for the irradiated phage followed the exponential relation $N/N_0 = e^{-\sigma D}$ (where D = dose, σ = sensitive volume, N_0 = initial number of phage, and N = number of phage after radiation). According to the target theory, such an exponential result indicated that the phage were inactivated by a "single hit," just as Timoféeff's results for the irradiation of *Drosophila* had shown mutations to be the results of single hits.[50]

By the time he and his Parisian mentors had completed this work and sent a note to *Nature,* the Nazi invasion of France forced Luria again to leave quickly. Escaping through southern France by bicycle, he embarked by ship for the United States, arriving in New York in September 1940. There the geneticist Leslie Dunn helped him obtain some small fellowships to enable him to work at the College of Physicians and Surgeons at Columbia University, where he continued his studies of the inactivation of bacteriophage by x-rays.[51]

The aim of the experiments Luria and Delbrück carried out together at Cold Spring Harbor, where Luria was spending the summer, was to learn something about what happens between the time a phage enters a

bacterium and the time its progeny are liberated: that is, they were not content with an input-output analysis of phage growth. As they put it in their first published report of the work: "The growth of a bacterial virus . . . , occurring only in the bacterial cell, may be said to proceed behind a closed door. The experimenter can follow the virus up to the moment it enters the cell, and again after liberation from the cell. There is, as yet, no way of telling what goes on within the cell, except by circumstantial evidence." The plan they devised to try to open the bacterial door was clever: "By the desire to gain more direct insight into the intracellular growth processes, the present authors were led to try the simultaneous action of two different viruses upon the same host cell. There was a possibility that one virus might lyse the cell while the other was still growing. Thus an intermediate state of virus growth might be revealed."[52]

In order to implement this plan they chose two types of bacteriophage that differed in size and other characteristics, sharing only the property of being able to infect a common host, *E. coli* strain B. As indicator strains for each of the viruses, which they rather arbitrarily named α and γ, they chose two other strains of *E. coli,* on one of which only α would grow and on the other of which only γ would grow. They tested the two viruses separately and together in "mixed infections," at various multiplicities, sometimes infecting the bacteria simultaneously with both viruses in equal quantities, sometimes with unequal quantities, sometimes simultaneously, at other times giving one virus varying amounts of head start over the other. In all these experiments, their "expectation did not materialize," because the mixed infections always suppressed the growth of one virus completely, while the other grew normally.[53]

Although blocked in their effort to open the bacterial door, Delbrück and Luria had discovered a striking new phenomenon, which they named "interference." Indirectly, they believed, this effect could yet shed light on the nature of the intracellular growth. They speculated that the bacterial host may contain a "key-enzyme," necessary for the synthesis of virus, that is totally engaged by a single virus particle and thereby made unavailable for the growth of another type of virus.[54] Although they gave no details regarding their key-enzyme, it is notable that they borrowed from biochemistry the language in which to express their hypothesis.

In order to "dissociate the interfering from the reproducing capacity of the virus," Delbrück and Luria next tested whether bacteriophage

α inactivated by ultraviolet light would still be able to suppress the growth of γ. They found that the interfering activity was progressively lost with higher doses of radiation, but not as rapidly as its ability to reproduce. Partially inactivated γ was also able to reduce the growth of the bacteria themselves.[55]

During the following year Luria and Delbrück participated in a landmark event in the study of bacteriophage. Since the time of d'Herelle, phages had been invisible bodies, defined by the fact that they passed through filters that stopped bacteria. The plaques by means of which one could assay the number of phage present in a medium were not phage themselves but merely the empty spaces left where phage had destroyed the bacteria within a limited area of a culture. In 1940 word of the development of electron microscopes that promised to have the resolving power to identify objects in the size range where phage particles were thought to be led Thomas F. Anderson to apply for a fellowship from the National Research Council to employ for this purpose an electron microscope developed by the Radio Corporation of America, which wished to encourage exploration of the application of its new instrument to biological problems. In November 1941 Luria approached Anderson to find out whether the electron microscope could be used to check the sizes of some phage whose cross-section he had estimated from his x-ray studies. By March, Luria had produced high-titer stocks of phage that enabled Anderson and him actually to see particles of uniform size, consisting of "heads" and "tails" that were adsorbed onto the surface of host bacteria and that seemed to be liberated from bacteria when they lysed.[56]

During the summer of 1942 Delbrück joined the project at Woods Hole, Massachusetts, where RCA had installed an electron microscope for the annual summer school course. There, he, Luria, and Anderson studied in particular the two phages, α and γ, that Delbrück and Luria had used the previous summer to study mixed infections. They were able to confirm not only that the two phages had different and characteristic shapes but also each feature of the life cycle of the two phages that they had previously established from their single-step growth experiments. One "unexpected and important fact" that they observed, however, was that the adsorbed phage particles "remain at the surface of the bacterium." On the other hand, it was also obvious that "the new virus is liberated from within the cell." This contrast, they acknowledged, "creates a difficulty in interpreting virus growth." The only preliminary suggestion they could make to resolve the complication

was that perhaps only a single virus particle can penetrate into the cell, just as the penetration of a single sperm into an egg prevents subsequent sperm from entering.[57]

The "shape and structure" of the virus particles as seen by the electron microscope, they wrote, "give the impression of a somewhat more complex organization shown in the pictures of plant viruses" that Anderson had previously taken with Wendell Stanley. Because of this suggestion of complexity, the authors cautioned about the tendency to regard viruses generally as molecules. "While no harm is done by calling viruses 'molecules,' " they wrote, "such a terminology should not prejudice our views regarding the biological status of the viruses, which has yet to be elucidated." From correspondence that Anderson later published, it appears evident that Delbrück had written the discussion portions of the joint paper. That the physicist who had first defined a gene as a molecule and had turned to bacteriophage in the hope that it represented the simplest example of a gene should now warn against omission of the more complex biological aspects of their organization is a measure of the theoretical openness he maintained in the face of unexpected results. Similar flexibility is indicated by the fact that these microscopic observations prompted him and Luria to abandon the key-enzyme hypothesis they had proposed a few months earlier in favor of a vague analogy to the relation between a sperm and an egg (fig. 2.1).[58]

To produce the "indicator strains," on which only one of their two virus strains would grow, in the studies of mixed infections that he and Delbrück had carried out during the summer of 1941, Luria had made use of a technique already used in phage work. "It is a well-known fact," the authors wrote in their first paper on the subject, "that lysis of a bacterial culture is rarely complete. Usually a few hours or days after the first clearing, a secondary growth arises, which can be isolated in pure culture. This new strain is usually resistant to the action of the virus in the presence of which it arose. The sensitivity of such a variant to other viruses may be the same as that of the primary strain."[59] Although they treated the phenomenon as unproblematic, Luria began afterward to wonder how these phage-resistant bacteria originated. One possibility was that some action of the phage on them produced changes in a few of the billions of bacteria present. The other was that the resistant bacteria arose through mutations. Although some investigators had long supported the latter view, the matter remained controversial because of a general opinion at the time that bacteria did

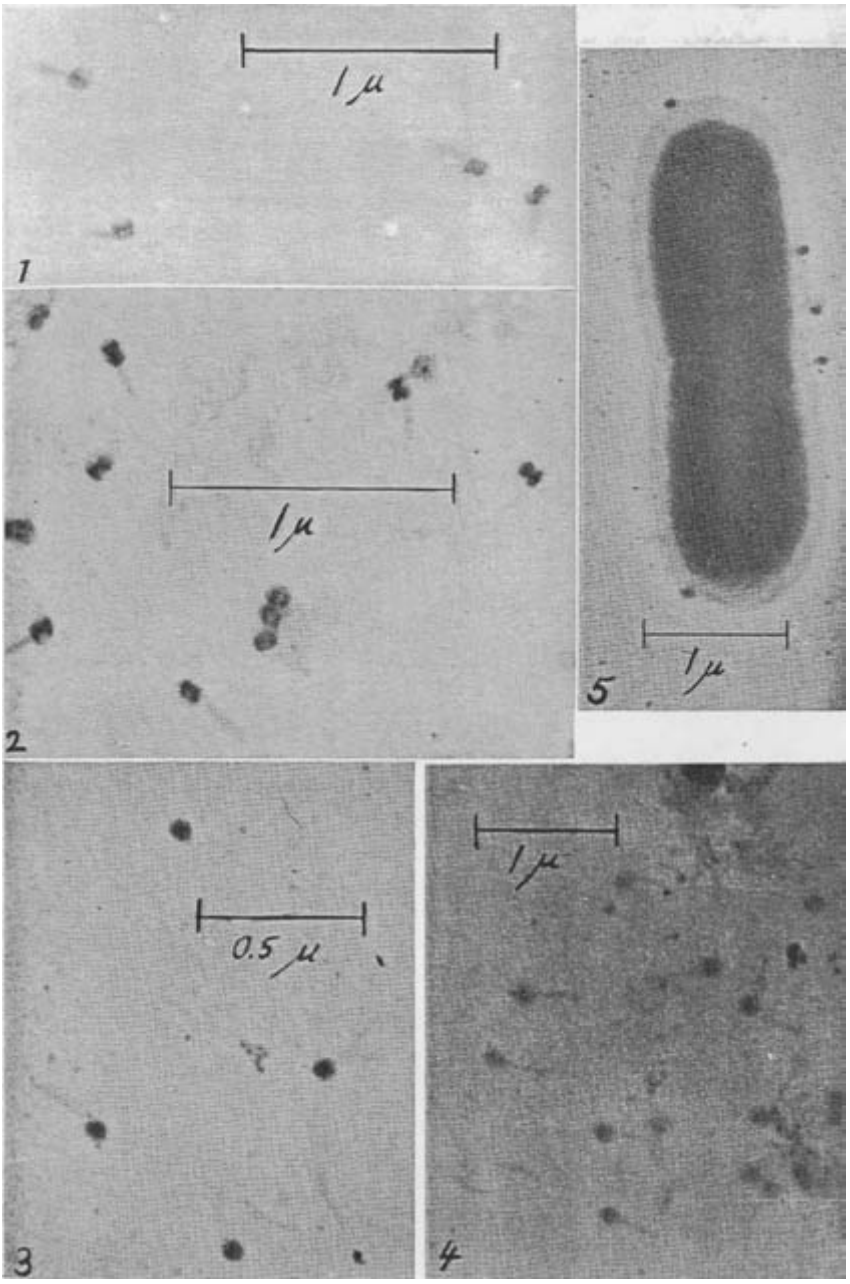


Figure 2.1. Electron microscopic images of bacteriophages obtained in 1942 by Luria, Delbrück, and Anderson. (Reproduced by permission from S. E. Luria, M. Delbrück, and T. F. Anderson, "Electron Micoscope Studies of Bacterial Viruses," *Journal of Bacteriology* 46 [1943]: 57–75.)

not contain genes and that hereditary changes were brought about by changes in their milieu.

In February 1943, a few weeks after arriving at Indiana University, where he had been appointed as an instructor, Luria was watching a colleague playing a slot machine. Although he seemed to be losing repeatedly, the colleague eventually hit the jackpot. While thinking about the mechanism by which a slot machine returns about 90 percent of what is put into it, but in a very uneven distribution, Luria had the idea that this pattern contained something similar to the patterns of bacterial mutation. If the resistant bacteria he encountered had been induced by direct action of the phage, the number should be dependent only on the number exposed to the phage, and in comparable cultures their numbers should be similar. If, on the other hand, they arose through spontaneous mutations, the resistant colonies should be clustered in clones whose size depended on when the mutations had taken place. If mutation had occurred just before the culture was tested, there should be only one colony. If it had taken place several generations before testing, there would be a cluster of two, four, eight, sixteen, or further multiples, depending on the number of generations. The following morning he set up an experiment to test this view and found that the numbers of resistant colonies varied as widely as one would expect according to his mutation theory.[60] Writing a note to Delbrück that described his idea and the first set of experiments, Luria soon received the reply that Delbrück thought the idea had promise and that he was working out the mathematical theory for the case.[61] On this basis they were able to calculate not only the rates of mutation from the further results that Luria gathered but also the ratio of the variance in the numbers of resistant bacteria found in the cultures to their average count. For the case of a direct action of virus on the bacteria these two should have been the same. The experimental results showed an even greater degree of variance than predicted, a degree sufficient to prove that the resistant bacteria in their experiments had originated by mutation.[62]

Thus an investigation of mixed infections initiated in order to illuminate the nature of viral growth within bacteria turned out instead to provide the first persuasive proof that variant bacterial strains can arise by mutation. In view of the prevailing doubts about the genetic nature of bacteria, Luria and Delbrück were cautious in the paper they submitted to *Genetics* in May 1943: "Naming such hereditary changes 'mutations' of course does not imply a detailed similarity with any of the classes of mutations that have been analyzed in terms of genes for

higher organisms. The similarity may be merely a formal one."[63] Nevertheless, the impact of this paper was strong enough so that some observers with a penchant for designating historical landmarks have retrospectively called its appearance "the birth of bacterial genetics."[64]

During the years when Delbrück was becoming attentive to the promise of biochemistry for genetics, others were pursuing investigations intended to advance that promise. Prominent among them was the redoubtable John Burdon Sanderson Haldane. Having been convinced while in the laboratory of Frederick Gowland Hopkins that "biochemical explanations are more fundamental than morphological," Haldane believed that the goal of genetics should not be to find out how genes determine "unit characters" but how they control "unit processes." To illustrate, he drew on the once-neglected but later well-known studies of inborn errors of metabolism by Archibald Garrod. Like several other geneticists, Haldane thought that a favorable domain in which to study genetic control of such processes in plants and animals was that of the various pigments in which mutant forms differed from the wild types, because it might be possible to connect the genetic changes with the metabolic processes through which the pigments are produced. He devoted years to studying the pigments that cause differences in the colors of flowers, but the complexities of both the genetics and the metabolic relationships did not allow him to draw general conclusions.[65]

The many mutations of *Drosophila* involving changes in eye color offered the inviting prospect of applying biochemical methods to the organism with the best-known genetics. In January 1934 the maize geneticist George Beadle, who had come to Caltech in 1931 with a National Research Council Fellowship that required him to familiarize himself with the genetics of another organism, encountered the Russian-born embryologist Boris Ephrussi, who had just arrived at Caltech to study developmental genetics in mice. With strong encouragement from Morgan and Sturtevant, who were at that time especially eager to rejoin classical genetics with embryology, Beadle and Ephrussi undertook a project to connect the genetics and the development of *Drosophila.* The work began in Ephrussi's laboratory in Paris, where Beadle arrived in May 1935 with financial support provided by Morgan. Their first idea was to culture *Drosophila* tissue, but when this turned out to be difficult, Ephrussi suggested that they transplant embryonic imaginal disks (organized groups of cells destined to develop

into specific tissues or organs) from one fruit fly larva into another larva. Within a few weeks they mastered an exacting technique for identifying and extracting the disks (observing the larva together through a pair of binocular microscopes) and inserting them into the abdomen of another larva, where the disks grew into well-defined organs. Soon they were concentrating on optic disks, which they transplanted between larvae differing in their genetic makeup for eye color, where the discs formed complete though morphologically inverted eyes. Because there were twenty-four known mutations for eye color, the plan offered many combinations of donor and implant host interactions. Beadle and Ephrussi found that all but three of the mutant implants developed autonomously within a wild-type host. Of the three that did not, they found that vermilion and cinnabar eyes developed as wild type, whereas claret implants became eyes that were intermediate in character between claret and wild. In all cases but one, wild type implanted in mutant hosts produced eyes with wild-type pigmentation. At the end of 1935 Beadle and Ephrussi returned to Caltech, where they continued this work for another year.[66]

In order to interpret their many results, Beadle and Ephrussi proposed that cinnabar and vermilion eyes lacked a particular substance, that both of these substances were necessary to produce the wild-type pigment, and that these substances could be supplied by the host. A third substance, lacking in claret pigment, they believed to be related to the first two through a scheme that "assumes that the ca^+, v^+, and cn^+ substances are successive products in a chain reaction."[67]

The direct collaboration between the two investigators ended in 1937, when Ephrussi became director of a genetics laboratory in Paris and Beadle became a professor at Stanford University, where he set up a laboratory to continue the *Drosophila* transplantation work. They kept in touch, however, as both pursued these problems in different ways. Each hired a biochemist to help work out the chemistry of the unknown substances they had postulated for the reaction scheme for eye color.[68] In 1938 Ephrussi and Yvonne Khouvine found that under some nutritional conditions, the addition of the amino acid tryptophane partially restored to vermilion-eyed flies a brown pigment otherwise missing in this mutant. Beadle's biochemical collaborator, Edward L. Tatum, then showed that the active substance was not tryptophane itself but a contaminant. Tatum was able to isolated the latter in crystalline form, but before he could identify it, a German group led by Alfred Kuhn and the eminent organic chemist Adolf Butenandt

tested known intermediate products of tryptophane metabolism and showed that kynurenin displayed the activity of substance v^+ both in *Drosophila* and in the meal moth *Ephestia,* in which Kuhn had been studying individuals with mutant eye colors. On this basis Beadle and Tatum suggested in 1941 a scheme for the formation of eye pigment in *Drosophila* in which tryptophane gives rise to kynurenin, which is then oxidized to produce either kynurenic acid, which is inactive, or a mixture of oxidized and reduced pigments that together form the brown pigment that constitutes a component of the normal red eye color.[69]

These developments marked a considerable success in bringing biochemistry to bear on genetics and provided support for the Beadle's and Tatum's assumption that a "given gene has a single primary action," which takes place "through the intermediation of enzymes." There were also, however, many limitations inherent in this approach, among them that the "chemistry of the pigment components concerned . . . is not well understood at present."[70]

While listening to one of Tatum's lectures on biochemical genetics in 1940, Beadle later recalled, "It suddenly occurred to me that it ought to be possible to reverse the procedure we had been following and instead of attempting to work out the chemistry of known genetic differences we should be able to select mutants in which known chemical reactions were blocked."[71] Doing so, however, implied shifting from *Drosophila* to an organism that could be grown in controlled media in which a deficiency in its capacity to synthesize a specific essential metabolite could be detected. Beadle knew immediately that *Neurospora,* a fungus long known as a pest to bakers because it grew prolifically on bread, was ideally suited to the task. He had first heard about *Neurospora* more than a decade earlier, at Cornell University, where Bernard Dodge had given a seminar on its genetics. Dodge had argued to Morgan even then that *Neurospora* was more favorable for genetic studies than the fruit fly, and Morgan had somewhat reluctantly taken some stocks with him to Caltech, where a graduate student named Carl Lindegren was working on its genetics when Beadle arrived there in 1931.[72]

The strategy that Beadle and Tatum devised assumed that x-rays would induce mutations in genes involved in the control of specific known chemical reactions. The organisms so affected would be able to grow in media containing the essential product of the blocked reaction but would not survive in minimal-nutrient media. The only such sub-

stance that wild-type *Neurospora* had been found to require in their media was biotin. By testing irradiated *Neurospora* systematically in various media, each of which contained one essential growth factor such as each of the known vitamins, each of the amino acids, and glucose instead of more complex sources of carbon, they could determine what substances the mutants were unable to synthesize.[73]

Neurospora go through a complex life cycle. Two sexual forms of mycelium that are morphologically indistinguishable fuse, producing a perithecium within which a sequence of four mitotic and meiotic divisions produces eight haploid spores, of which, for each allele, four spores contain the gene contributed by one and four contain the gene from the other sexual parent.[74] It was the growth of these asexual spores that Beadle and Tatum tested in their controlled media. According to Beadle, "There was never the slightest doubt that this approach would be successful," because, as they had put it at the time of their first publication on the subject, it had already been "established that many biochemical reactions are in fact controlled in specific ways by specific genes."[75] Success, in fact, came quickly. At the 1940 meeting of the American Association for the Advancement of Science in Dallas they were already able to report that "three mutants have been found that grow essentially normally on the complete medium and scarcely at all on the minimal medium with sucrose as the carbon source."[76] One of these mutants required vitamin B_6 (pyridoxine) in its medium. Another required vitamin B_1 (thiamine), and the third required para-amino benzoic acid. Genetic analysis showed that these deficiencies were transmitted from generation to generation in the way that would be expected if they were caused by the mutation of a single gene. There was, they noted, certainly "more than one step" in each of these syntheses, and "accordingly the gene differential involved is presumably concerned with only one specific step in the biosynthesis."[77]

Beadle had had this "presumption" in mind ever since he began his work with Ephrussi on *Drosophila* in 1936.[78] In 1945, after having found many additional examples, including separate genes whose mutation blocked each of the steps of the ornithine cycle of urea synthesis, Beadle stated more confidently that "a given enzyme will usually have its final specificity set by one and only one gene." Although he added that "the same is true of other unique proteins, for example those functioning as antigens,"[79] his formulation soon became commonly known as the "one-gene-one-enzyme hypothesis."[80] The hypothesis was not accepted without resistance, and Beadle and Tatum were themselves

uncertain about the relation between gene and enzyme. In their first publication on the subject, in 1941, they had written that "it is entirely tenable to suppose that these genes, which are themselves components of [an integrated system of chemical reactions], control or regulate specific reactions in the system either by acting directly as enzymes or by determining the specificities of enzymes."[81] Nevertheless, by fixing attention on the relation, whatever it was, between a specific gene and a specific reaction that they expected to be controlled by a specific enzyme, rather than on the whole development process resulting in "characters with visible manifestations,"[82] Beadle and Tatum focused the broad problem of gene function more narrowly and made a situation that had seemed too complex to treat effectively far more manageable. The work also stimulated a further convergence between metabolic biochemistry and genetics. It appeared to some, including Beadle, that the new field of "biochemical genetics" was already emerging. So far this field had been "cultivated more by the biologist than by the biochemist," but he hoped that the artificial distinction set up by institutions that separated these fields into different laboratories would soon break down.[83]

During April and May 1944, at the Vanderbilt University School of Medicine, Delbrück gave a series of lectures titled "Problems of Modern Biology in Relation to Atomic Physics." The individual lectures recapitulated in pedagogical form most of the biological questions that had concerned him ever since he had heard Bohr's lecture in 1932. In the first, "Limitations of Atomic Physics as Applied to Biology," Delbrück pointed out that Hermann Helmholtz (the great nineteenth-century physiologist and physicist) had considered the phenomena observed in the living world to be "deducible from the laws of Newton's mechanics as movements of particles due to forces originating in the particles." Delbrück believed this view to still be prevalent among biologists and biochemists, but "the modern physicist may be inclined to take a more liberal view." The reason was that quantum mechanics had abandoned within physics itself the mechanistic aim of determining the motions of the nucleus and electrons under the influence of electric or magnetic forces: "The fine point of quantum mechanics is that we can get away without more detailed description of the motion of the electrons within the atom and still give a perfectly accurate account of the behavior of atoms. Quantum mechanics is essentially the mathematical scheme which enables us to work out the energies

of stationary states and the transition probabilities from one stationary state to another for any system of particles involving any kind of forces."[84]

After explaining succinctly the impossibility of measuring at the same time both the location of an electron and its stationary state, a limitation that had given rise to the "principle of indeterminacy" of Heisenberg and the "principle of complementariness" of Bohr, Delbrück gave reasons for believing that these limitations were permanent, that they should be regarded "not as a loss but as the essential element of our advance," and that they produced no comparable uncertainty for ordinary measurements in physics and chemistry. "But what about the living cell?" he asked. "Could we not, perhaps, consider it a super-molecule, and, ideally, calculate its stationary states as transition probabilities?"[85]

The hitch, Delbrück said, responding to his own question, is that to do so would require precise information about the types, numbers, and positions of each of the atoms making up this supermolecule. The experiments necessary to obtain such detailed knowledge would "blow the living cell to pieces," just as in order to locate an electron in an atom one must "blow it out of its stationary state."

> According to this idea we may expect certain features of the living cell to be complementary to its description in terms of atomic physics. To put it very crudely, we may find out where the atoms in a cell are, but in doing so we kill the cell. We should be prepared, then, to find features of the living cell which are not reducible to atomic physics, just as we find features of the atom, viz., its stability, which are not reducible to mechanics.
>
> This idea puts the relation between physics and biology on a new footing. Instead of aiming at the whole of the phenomena exhibited by the living cell, we may expect to find natural limits and, thereby, implicitly, new virgin territories, in which laws may hold which are independent of those of physics, by virtue of the fact that they relate to phenomena whose appearance is conditioned by *not* making observations of the type needed for applying atomic physics.[86]

This lecture was clearly a further development of the ideas that Delbrück had first expressed in his letter to Bohr in 1934 in response to Bohr's lecture "Light and Life." It appears evident that a decade later the application of Bohr's complementarity principle to biology remained for the younger physicist a strong motivating force. Now it

became clear not only that he acknowledged the recognition of these limitations as a positive step in the relation of physics to biology but that he perhaps hoped that by carrying on research in biology with awareness of these limitations he might be led to "laws independent of those of physics." Were these the same ideas he had already held when he entered biology, or had their meaning for him changed in response to his subsequent experience? It is notable that neither in this lecture nor in the subsequent ones did he refer to the theory of gene mutation based on quantum mechanics that he had proposed in 1935. Did he no longer believe that atomic physics could solve this problem, and was he hoping to find instead a law of gene mutation independent of physics? The abstractness of his discussion leaves us without an answer to this fundamental question.

He introduced his second lecture by asserting that he would now consider applications of the principles outlined in the first lecture to "the living cell," beginning with "some features of metabolism which are classed as oxidation-reductions and as phosphorylations." Despite this claim, he brought no principles from quantum mechanics into the discussion of metabolism. Rather, his lecture was a lucid, straightforward sketch of the elementary principles of the oxido-reduction reactions that were representative of metabolism, illustrated mainly by a partial sequence of the reactions of anaerobic glycolysis and alcoholic fermentation in which he showed how, in the progression from glyceraldehyde to lactic acid or alcohol, two hydrogen atoms are moved from one end of the molecule to the other. He discussed the way in which enzymes enable the reaction to go forward by "lifting" the molecule over the energy hill between the initial and the final state and the fact that the cell "almost never" transfers the hydrogen atoms in the most direct manner: employing a single enzyme on which both substrates are adsorbed. Using two enzymes instead, the cell makes use of a "fairly complicated" carrier molecule, to which enzyme I transfers the hydrogen and from which enzyme II transfers the hydrogen to the acceptor substrate. The sources on which he drew were recent review articles by three prominent biochemists, David Green, Herman Kalckar, and Hans Krebs.[87]

He continued in a similar vein for the next three lectures, the first on energy coupling and the two that followed on photosynthesis. In discussing the latter subject, Delbrück was able to return to quantum physics because the light reaction of photosynthesis was known to involve the absorption of quanta of light energy. He included Einstein's

photoelectric effect and the fact that the "quantum is proportional to the frequency of the light and the constant of proportionality is Planck's quantum of action, h." In short, here was a natural intersection between metabolic biochemistry and quantum physics. This intersection had, however, long been established. The first person to attempt to measure the minimum number of quanta necessary for the reduction of one CO_2 molecule had been the eminent biochemist Otto Warburg in 1926, and Delbrück's most up-to-date sources for his lectures were articles by his friend Hans Gaffron, a former student of Warburg.[88] Thus his selection of the topics for these lectures seems, in spite of their overall theme, to have been derived not from new insights that Delbrück could bring to biology from atomic physics but from his recent recognition of the centrality of metabolism to the problems in biology that most interested him and to which he turned in his sixth lecture.

"Let us now consider," Delbrück stated, "the case of genetics and its actual or potential relation to atomic physics." Despite this declaration, he discussed genetics in this and the next lecture primarily in relation to biochemistry rather than atomic physics. After a brief description of "Mendel's experiment," with which it all started, Delbrück leaped across subsequent history to the very recent experiments of Beadle and Tatum. "Cross, for instance, a wild type mold *Neurospora crassa* with a mutant strain which differs from the wild type in that it needs p-aminobenzoic acid (pab) as a growth factor, because it has lost the ability to synthesize it." The first advantage of this type of experiment was that, because the character is "read from the haploid generation," no complications arose from dominance relations. After several digressions back in time to the discovery of linkage groups and the question whether bacteria have anything analogous to chromosomes, he returned to the genes of higher organisms with the remark that "the geneticists have presented the biochemists with three magnificent problems":

1. What do genes consist of?
2. How do they reproduce?
3. How do they act?

Taking the third problem first, he pointed out that when considering such characters as the size of the wing in *Drosophila,* one runs into the difficulty that "one end of the 'reaction chain,' the character, is expressed in nonchemical terms, while it is attempted to express the other end, the primary gene action, in chemical terms. No progress

here can be made unless one succeeds in tracing back the character to chemical determiner [*sic*] in development." Such an approach had been found in Beadle and Ephrussi's experiments on *Drosophila* eye color, the main results of which Delbrück spent the rest of his lecture summarizing. Superficially, he concluded, it "looks as if the normal gene [is] an enzyme which catalyzes one step in the reaction chain," but there was no assurance that the gene is the enzyme. "The trouble is that there is no criterion which would tell us when we have traced back a chain to the gene itself."[89] These were the same cautions that Beadle, Ephrussi, and Tatum were currently expressing. The main point is that by 1944 these latest developments in the field were persuading Delbrück and others of the rapidly growing importance of biochemistry to genetics.

In his second lecture on genetics Delbrück discussed further the experiments of Beadle and Tatum on the biochemical mutants of *Neurospora,* including the latest results of work by Tatum and David Bonner concerning tryptophane mutants whose growth could be restored by adding to the medium metabolic precursors of that amino acid. He also mentioned briefly similar studies of flower pigments, such as those carried out by Haldane and others. In order to probe more deeply the "primary function of the gene" he turned to newly published "dose effect" experiments by the German geneticist Curt Stern, whom he had met once in Berlin. These involved genes of the fourth chromosome of *Drosophila,* which could relatively easily be produced in a condition of "heteroploidy": that is, with one or three copies of the chromosome instead of the normal two. The effect of the mutant gene *ci*, located on this chromosome, was to interrupt the formation of the fourth wing vein. Two mutants together with one normal gene produced longer interruptions, but three mutants together gave phenotypic distributions approaching the condition of the normal vein. Stern had concluded that in forming the "primary gene product" the mutant allele acted in the same way as the normal gene, only less efficiently. When acting in combination with the normal gene, however, the mutant acted antagonistically, competing for the substrate from which the gene product is formed, and thus reducing the amount available to the normal gene.[90] Delbrück commented:

> We have here the beginnings of a "kinetics of primary gene action." The chief trouble with this approach is that we are, and maybe forever will be, totally ignorant of the nature of the hypothetical

> substrates and products. Yet this approach may teach us something about the nature of the primary gene actions. In saying this we imply that all primary gene actions involve one general mechanism. The assumption of such uniformity is not proved but seems a likely one to us.[91]

Delbrück seemed to revert to the role of the physicist who hoped that a mechanism of gene action could be elucidated without knowing the biochemistry, albeit with *Drosophila* rather than phage genetics. Quickly, however, he reversed himself by stating that it was "obvious we would be much better off" if we knew something about the structure and action of genes. He presented again the theory of autocatalysis of the gene that he had offered as a speculation at the Cold Spring Harbor symposium in 1941. The theory, unchanged, was still based on assumptions first made by Linderstrøm-Lang. One conspicuous change in emphasis that Delbrück now made was to stress that the size of the first step in the removal of two hydrogen atoms would be reduced if the energy of the radical formed in this step could be reduced. When this radical formed a complex with a molecule that had already lost two hydrogen atoms, the complex "would then be a structure which is stabilized by resonance between its two electronic configurations." After describing these hypothetical reactions, he concluded, "The point I want to make is this: Quantum mechanics offers a reason why a two-step oxidation may be catalyzed by a structure which is closely similar to the oxidation product." This was a cogent point to make in a series of lectures about the relation between modern biology and atomic physics, but it should not conceal the fact that Delbrück had originally drawn his theory almost entirely from the biochemical literature.[92]

Delbrück began his eighth lecture, titled "Radiation Effects," by putting in simple language the difference between "threshold effects" and "single hit effects." Threshold effects "do not show up until a minimum dose of radiation has been given, and when this minimum dose is exceeded the effects show up in most of the irradiated individuals. . . . Single hit effects are due to *one* elementary act on an atomic scale which occurs at the right spot. A thousand elementary acts may occur in one organism, 999 of which do nothing while the 1000th hits the right spot and leads to profound effects in the organism, death or mutation. If we irradiate a large number of organisms even a very small dose will produce an effect in a few of them, and the number of

organisms affected will generally be proportional to the dose given. There is no effect of intensity, whether the quanta are given all at once or with intervals between them." Single-hit effects were, he asserted, "proof that the life of the organism depends on atomic structures, each of which is irreplaceable."[93]

After discussing ultraviolet absorption, the several processes that follow the absorption of a quantum of light by a molecule, and the inactivation of enzymes by ultraviolet light, Delbrück turned to the inactivation of viruses by x-rays. Describing the mechanism by which x-rays cause electrons to be ejected from the molecules they strike, the electrons then passing through hundreds of atoms before affecting one of them, where they create little clusters of tertiary ionization, he suggested, "Suppose now we irradiate a suspension of virus particles. It is found that the virus particles are 'killed,' and that the killing is a single hit effect. Will *any* ionization which occurs inside a virus particle inactivate it?" At first that would seem unreasonable, but if one considers the virus to be a big molecule, one should not be surprised if a "change in almost any point of the molecule would throw a monkey wrench into the smooth operation of the reproductive reaction," and experiments had verified that a large part of the virus particle is "sensitive" in the sense that a single ionization will be fatal to its reproductive capacity.[94]

Many of the effects of radiation on higher organisms were also single-hit ones, but the chromosome breaks produced by radiation generally were not. Delbrück spent much of the rest of the lecture on the mechanism of such breaks; his description was for the most part founded on work originated by Hermann Muller. Among the effects of radiation that Delbrück proposed to discuss was "the mutation of genes." In fact, he barely alluded to the subject, noting at the end that in some cases it was possible that "ionization did not 'kill' the gene, but altered it so that it now reproduces in this altered structure. Such 'point mutations' should also be inducible in viruses. Adequate experiments in this field have not yet been done."[95]

Here we see Delbrück seem to approach but then to veer away from the subject that had first drawn him into genetics in 1935. That he did not return to the theory of gene mutation does not necessarily indicate that he no longer believed in it. Perhaps, as his final sentence suggests, he wished to wait until experiments on viruses would provide answers that could not be derived from radiation experiments on *Drosophila.* As we have seen, however, he was not usually reluctant to advance

speculations ahead of adequate experimental support, and his silence on exactly this subject in 1944 is unlikely to have been accidental.

Only in his last lecture, "Bacterial Viruses," did Delbrück finally move from observations drawn mainly from the work of others to the experimental investigations in which he had been engaged for the previous six years. Almost entirely descriptive, this lecture was oriented principally around the "one step growth experiment," which he outlined in language enabling listeners unacquainted with such work to grasp both the basic approach and its essential details. Then he turned to the mixed infections with which he and Luria had recently been occupied. Although he represented these experiments as attempts to get behind the "closed doors" obscuring the growth of viruses within bacterial cells, he indulged in no speculations about what went on there.[96]

Those who heard Delbrück's lectures must have regarded them collectively as an extraordinarily lucid exposition of the actual and potential contributions of atomic physics to problems in biology, because the Panel on Physics of the National Research Council recommended that the texts be reproduced and distributed. They were so well received that a second printing was made in February 1946.[97] The foregoing analysis of their content suggests that Delbrück ranged far more broadly, both in subject matter and in approach, than one might expect from his image as leader of the phage group, generally thought to have focused narrowly on one organism and one problem and to have rejected or ignored much of what had come before. At the end of a short general summary, Delbrück compared the status of biology to that of physics around 1890. The separate branches of physics had seemed then to have reached their "final formulation" just before the discoveries of radioactivity, x-rays, and the electron "completely changed the situation," breaking down the barriers between these branches as well as between physics and chemistry. "Perhaps," he said, "we are approaching a similar phase in biology. Genetics, embryology, biochemistry, and physiology may find a common root in a fundamental theory of the organization of the cell. It would seem that the principles of atomic physics will have a large share in the construction of this 'modern biology.' "[98]

The prediction of a "fundamental theory of the organization of the cell" reflects the temperament of a theoretical physicist thinking about biology, but Delbrück did not claim a hegemonic role for atomic physics; he sought only participation, along with the established biological

disciplines, in a new construction. Like those who followed him from physics into biology, however, Delbrück was not always in such a modest mood.

In their early work on bacteriophage, Delbrück, Luria, and others showed surprisingly little interest in mutations of the virus. In his 1942 review article "Bacterial Viruses (Bacteriophages)" Delbrück noted that "there are two instances of mutations of bacterial viruses recorded in the literature with sufficient detail to justify their inclusion in this review." These had been observed, respectively, by André Gratia and by Macfarlane Burnet, both in 1936. After describing the two cases, Delbrück added that "these findings open up an experimental approach to a wide range of problems."[99] Evidently, however, neither he nor anyone else was engaged at the time in such experimental approaches. In the lectures on atomic physics and the problems of modern biology that he gave two years later, he barely mentioned viral mutations. What he and Luria were primarily interested in was the reproduction of the virus. Soon afterward, however, the situation began to change dramatically because, as with *Drosophila* thirty-five years earlier, the organism itself began to impose its capacity to mutate on the observations of those who were experimenting with bacteriophage. The first to examine in detail an example of bacteriophage mutation was Luria. The observations that drew his attention to the question followed naturally on those that had led him and Delbrück to establish the existence of bacterial mutations. The bacterial mutations had produced strains that were resistant to the adsorption of strains of bacteriophage that reproduced on the strains that had given rise to the mutant bacteria. "We observed, however," Luria wrote in July 1944, "that plating very large amounts of a virus with a resistant bacterial strain occasionally results in the formation of a few clear 'plaques'—that is, of a few virus colonies." The viruses involved were the same two strains—α and γ—that he and Delbrück had earlier used to study mixed infections. He named the mutant viral strains α' and γ'. He demonstrated that these new strains did, in fact, arise through mutation rather than some other process of adaptation in the same way that he had demonstrated the bacterial mutations: by showing that in a series of similar cultures the distribution in the number of plaques formed (presumably by the occurrence of mutants α' and γ') by viruses α and γ when plated, respectively, on the bacteria strains that were resistant to them was far more variable than would be expected from random variation.[100]

In the discussion of his results, Luria commented:

> The changes in virus properties are here called "mutations" because of their apparently spontaneous and random occurrence, of their transmission to the offspring, and of their stability. The same may be said of bacterial mutations affecting virus sensitivity. In making any analogy with the process of gene mutation in plants and animals, we should not forget the lack of any direct evidence of the presence, in bacteria or viruses, of "genes" in the sense of discrete material units, whose existence in higher organisms is proved by linkage studies.[101]

This view helps explain the paradox that, when he and Delbrück turned to phage as the simplest organism in which to study genetics, neither of them emulated classical genetics by studying mutations. The analogy they and others had drawn between viruses and genes likened the whole virus to one gene, whose reproduction, or "autocatalysis," they might be able to elucidate. The comparison was not conducive to imagining that, like higher organisms, the virus itself contains multiple mutable genes.

In 1943 Alfred D. Hershey, an instructor in bacteriology and immunology at Washington University in St. Louis, visited Delbrück at Vanderbilt University and Luria at Indiana University. In collaboration with Jacques Bronfenbrenner, an early researcher in bacteriophage, Hershey had for several years studied immunological phage-antiphage interactions.[102] A taciturn man of penetrating thought and a rigorous experimenter, Hershey impressed Delbrück and Luria so well that he "immediately became a partner" in their phage ventures. On Thanksgiving Day of that year Luria traveled to St. Louis to visit Hershey and told him about the phage mutations he had found. According to Luria, Hershey became "as excited as his nearly silent manner could express and soon took up the study of phage mutation."[103] Within a little more than a year Hershey discovered a second type of mutation.

The properties common to these mutants were that they shortened the time between their adsorption onto the bacteria and the lysis of the bacteria and they produced plaques that were about three times greater in diameter than the ones produced by the wild-type strains. Hershey therefore designated the mutants *r* for *rapid lysis,* the wild type being r^+. Except for their defining character, the mutants appeared in every way to be nearly identical to the wild type. Hershey denoted Luria's mutants *h* for *host range.* Hershey was able to produce double hr mutants, in either order. After several tests he came to the conclusion that

the two classes of mutant represented "independent genetic factors" and separate "loci."[104]

Unlike Luria, Hershey did not hesitate to identify these mutations fully with those of classical genetics:

> The existence of independent genetic factors does not alter the status of the analogy commonly drawn between viruses and genes. The mutational properties of the phage are precisely analogous to those of the R determinants of seed and plant color in maize, which always segregate together, but undergo mutation independently. . . . In either case one has freedom of choice in speaking of multiple genes (that is, mutational units), or of independent functions of a single gene (the crossover unit). Evidently, with viruses as with bacteria . . . the only genetic factors available to study by present methods are the mutational units. For the present, then, no useful distinction can be made between the genetic behavior of a bacterium, a virus, and a crossover unit bearing two or more independently mutating genetic factors.[105]

In March 1945 Hershey communicated his results to Delbrück, who was most struck by the report that mixed infections of an r mutant together with the wild type from which it had been derived gave rise to a mixture of the two types released by lysis. That result appeared to contradict the phenomenon of interference that he and Luria had found three years earlier and that Delbrück, who now called the phenomenon the principle of "mutual exclusion," was continuing to study. With his co-worker W. T. Bailey Jr., he verified that "mutual exclusion breaks down" with several pairs of wild and r mutant phage and suggested the "generalization that mutual exclusion operated the more perfectly the more dissimilar the two infecting viruses." In addition, Delbrück and Bailey found that mixed infections could "induce" further mutations. That is, wild-type particles were released that were different from either infecting particle, representing a "new type, created during the mixed infection." Perhaps "one might dispute the propriety of calling the observed changes 'induced mutations,' " they added. "In some respects they look more like transfers, or even exchanges of genetic materials. We do not pretend to be able to put forward convincing arguments for either point of view."[106]

Meanwhile, Hershey, working partly in collaboration with Luria, had been able to distinguish several host-range and rapid-lysis mutants that arose independently and that differed in the number of hosts they could infect, the strength of lysis inhibition, and the character of

the plaques formed. When Delbrück communicated his observation of induced mutations to him, Hershey saw an important new means of analysis in the method of infecting bacterial cells simultaneously with two types of virus. He carried out similar experiments from which he drew certain conclusions about the independence of the mutations of the two types of infecting phage he used.[107] His experimental reasoning is too complex to summarize here, but more significant than his immediate result was his opinion of the general promise of the method: "A new and promising approach to questions of this kind is by means of the mixed-infection experiments in which one observes an apparent segregation of genetic factors. Whether this phenomenon is related to crossing over, and indeed whether it implies actual exchange of genetic material at all, are pressing and important questions. But whatever the mechanism responsible for the impressed mutations, it is probable that the purely genetic study of their patterns will yield valuable clues to the genetic structure of viruses."[108]

Delbrück, Luria, and Hershey all made significant contributions to the discovery of the first clearly defined bacteriophage mutations. For Delbrück and Luria, however, these rather unexpected observations, like their prior discovery of bacterial mutations, remained subordinate to the central goal: the elucidation of the reproductive cycle of bacteriophage. Hershey, on the other hand, saw in these developments the foundation for a "viral genetics," and he turned his main effort in that direction. As his allusion to a "purely genetic study" of these mutation patterns in the paragraph above implies, in pursuing this goal Hershey opened up a new branch of phage biology more closely aligned to the approach of classical genetics than was the ongoing work of his two colleagues.

According to phage group lore, in the summer of 1944 Delbrück arranged what was later called the "phage treaty." As the number of investigators in the field grew, the danger that each would work on different phages threatened to produce results that would not be comparable. He insisted, therefore, that everyone work with a set of seven phages, designated T1 . . . T7 (where T stood merely for "type"). The supposed reasoning behind these choices was that these phages gave easily countable plaques and that for each there was a phage-resistant strain of *E. coli* B, which could be used to free the plaques from that particular phage.[109] In 1946 Hershey gave a less authoritarian version of this decision: The system of phages "has been developed by a group

of interested workers (M. Demerec, M. Delbrück, S. E. Luria, A. H. Doermann and the writer) in an attempt to combine convenience and descriptiveness within the usual genetic convention." Hershey clearly regarded the plan as a uniform system of notation rather than a limitation of research to these seven types, for he continued to work with another type, formerly designated "AC," that he now called T16.[110]

Contemporary literature suggests also that the choice of the particular organisms designated T1–T7 was less orderly than implied in the conventional story. After Delbrück and Luria reported the bacterial mutations resulting in resistance to one or the other of the two phage strains they were then using, Milislav Demerec at Cold Spring Harbor wished to extend their investigation to "cover resistance to several bacteriophage strains active on the same line of *Escherichia coli*." In order to do so they requested that several workers in the field provide the phage strains they had been using. Luria supplied the α and γ strains, which Demerec renamed T1 and T2. Tony L. Rakieten, formerly d'Herelle's associate at Yale University, sent him a mixture of phages, from which Demerec and his co-worker, the émigré Italian physicist Ugo Fano, isolated four more strains that they called T3, T4, T5, and T6. Ward J. MacNeal of the New York Post-Graduate Medical School prepared for them a standard anticoliphage mixture from which they isolated what they called T7.[111] Perhaps they stopped there because seven phages sufficed for their experimental plan. That these seven became the standard types that the majority of phage workers employed in their investigations in the following years seems, therefore, to be due as much to fortuitous circumstances as to Delbrück's decisions.

Each summer since 1941 Delbrück and Luria had returned to Cold Spring Harbor to continue work on phage. In 1943 Demerec, the director of the laboratories, shifted his research from *Drosophila* to bacterial and phage genetics. In 1944 Luria suggested that they offer a summer school course in bacteriophage research to encourage interest in the field. Demerec supported the idea, and Delbrück agreed to be the instructor. He was assisted by A. H. (Gus) Doermann, who was then working in Delbrück's laboratory at Vanderbilt, and J. Reynolds. The advertisement for the first course, planned to take place between July 23 and August 11, 1945, stated that "the purpose of the course is to acquaint the student with some of the techniques used in bacteriophage research, and with recent results of such work." There were to be nine half-day laboratory periods and nine half-day study periods, and

students were advised that their time would be fully occupied by the course. The laboratory work was strongly oriented around the one-step growth experiment. The six students who enrolled in the first course included the biochemists Rollin Hotchkiss, who was an associate of Oswald Avery at the Rockefeller Institute, and Herman Kalckar, as well as the microbiologist Stuart Mudd from the University of Pennsylvania. Niels Bohr sent T. Sigurgeison from the University of Reykjavik to take the course. Martha Baylor of the University of Illinois also enrolled. The course was successful enough that Delbrück repeated it the following summer.[112]

In 1946 Delbrück received an offer from the University of Manchester to direct the biophysics department there. Considering the offer seriously, he requested a biochemical assistant, giving as his reason the fact that "without a really competent biochemist on hand, not only the phage work, but the entire operations of a biophysics department would be lame." He had already planned to go to Manchester when, in December 1946, George Beadle, the recently appointed head of the Biology Division at Caltech, offered him a professorship. Believing that Caltech was becoming a major center for a new biology, Delbrück quickly decided to go there instead. He urged Beadle to hire Mark Adams, a biochemist who had taken the phage course in the summer of 1946, as well. Adams turned down Beadle's first offer, but Delbrück persisted. In January 1947, he wrote Adams, "I want you . . . to help in the organization of the lab. Your experience with bacteriological and chemical equipment would be very valuable. You know that I am an amateur in both these fields, and always afraid to make a fool of myself." Delbrück added that he thought he had been hired as a general "think-man" because he knew physics, and as a "phagologist," but that he would like Adams to be director of the phage lab.[113] Adams still did not accept, but the effort that Delbrück made to recruit him indicates how strongly he felt about the importance of biochemistry, as well as his understanding that he himself was not qualified to direct the kind of biochemical work he knew to be important to the further development of phage biology.

Three other biochemists enrolled in the phage course in 1946: Earl Evans, Seymour Cohen, and Birgit Vennesland. Evans, at the age of thirty-six, was already chair of biochemistry at the University of Chicago and a well-known pioneer in the use of isotopic tracers to study intermediary metabolism. Cohen had already begun the kind of work on the biochemical aspects of phage growth that Delbrück advocated.[114]

Cohen and Adams were among the eight persons who attended the first organized meeting of the phage group held by Delbrück in Nashville in March 1947. (The others were Anderson, Doermann, Hershey, Luria, and Max Zelle).[115] The phage course at Cold Spring Harbor, which has sometimes been seen in retrospect as Delbrück's "method of bringing physicists into biology,"[116] thus attracted in its first years a significant proportion of workers from biochemistry, the very field that, according to the lore, Delbrück and Luria thought had little to contribute to phage biology.

In 1945 the renowned theoretical physicist Erwin Schrödinger published a ninety-page book titled *What Is Life? The Physical Aspect of the Living Cell.* It was based on a series of lectures that Schrödinger, who immigrated to Ireland in 1939 when the Nazis took over Vienna, had given in February 1943 at the Dublin Institute for Advanced Studies, where he was a senior professor. The general question to which he addressed himself was, How can the events *in space and time* which take place within the spatial boundary of a living organism be accounted for by physics and chemistry? He believed that the inability of "present-day" physics and chemistry to do so was no cause for doubt that they "can be accounted for by those sciences."[117]

In his first chapter Schrödinger raised the question of why organisms, and in particular an organ such as the brain, "of necessity consist of an enormous number of atoms, in order that its physically changing state should be in close and intimate correspondence with a highly developed thought." The reason he gave was that thought is orderly and can, therefore, be applied only to perceptions or experiences that have some degree of orderliness. The events that happen within a brain "must obey strict physical laws, at least to a high degree of accuracy," and the physical interactions between the brain and the outside world must also possess a "degree of physical orderliness." Atoms, however, "perform all the time a completely disorderly heat motion, which . . . opposes itself to their orderly behavior." Events occurring among a small number of atoms do not follow any recognizable laws. Only when enormously large numbers of atoms are involved "do statistical laws begin to operate and control the behavior of these assemblies with an accuracy increasing as the number of atoms involved increases." He illustrated this generalization with three examples: paramagnetism, Brownian motion, and the limits on the reduction in size that can be made in a measuring instrument such as a torsion balance to increase

its sensitivity before irregular motions due to the uncontrollable effect of heat motion begin to interrupt the single deflections through which the instrument is intended to measure an electric, magnetic, or gravitational force.[118]

The "naïve physicist" would conclude, Schrödinger stated in his second chapter, that all biologically relevant processes must have an "extremely 'many atomic' structure" to guard against haphazard events due to single atoms. But he would be wrong because it turns out that, in genetics, "incredibly small groups of atoms, much too small to display exact physical laws, do play a dominating role in the very orderly and lawful events within a living organism."[119] These structures are the chromosomes. In the two chapters that followed Schrödinger provided his readers with a succinct, elementary account of the principles of classical genetics, including cell mitosis, meiosis, the haploid and diploid condition, crossing over, and mutations.[120] From estimates of the maximum size of the gene drawn from the results of *Drosophila* gene mapping, he could conclude that the gene was far too small to "entail an orderly and lawful behavior according to statistical physics."[121]

For his summary of most aspects of the "hereditary mechanism," Schrödinger relied mainly on genetics textbooks by the British cytogeneticist Cyrill D. Darlington, by Edmund W. Sinnott and Leslie C. Dunn, and by J. B. S. Haldane.[122] When he turned to mutations induced by x-rays, however, he drew almost exclusively on the work of Timoféeff-Ressovsky, in particular for two "laws": that "mutation is a single event," inferred from the fact that the amount of mutation is proportional to the dosage of rays, and that mutation is a localized event, inferred from the fact that this proportionality is unchanged by varying the wavelength of the rays.[123] The "general picture of a gene and of mutation" that Schrödinger then depicted was, he wrote, "due mainly to the German physicist M. Delbrück."[124]

"Delbrück's model," according to Schrödinger, showed that the gene is a molecule whose stability cannot be explained by classical physics but is easily understandable in terms of the states of a molecule permitted by quantum mechanics and whose mutations are changes of arrangement made possible by "quantum jumps" from one state to another. To make this clear, he discussed energy levels much as Delbrück had in the paper co-authored with Timoféeff and Zimmer in 1935. His "test[ing]" of the model by comparing it with the genetical "facts" also followed Delbrück's earlier treatment.[125]

Schrödinger asserted that "there is no alternative to the molecular explanation of the hereditary substance. The physical aspect leaves no other possibility to account for its permanence. If the Delbrück picture should fail, we would have to give up further attempts."[126] If he adhered closely to Delbrück's views in most respects, however, Schrödinger added to this picture an original and significant insight. Whereas Delbrück had called the gene a "molecule" or an "atomic assemblage," Schrödinger coined for it the term "aperiodic crystal," by which he meant a solid built up from a small molecule in the same way that an ordinary crystal grows by the aggregation of atoms, but without the "dull device of repetition" that characterizes the crystals with which physicists were generally familiar. Consequently, in the aperiodic crystal "every atom and every group of atoms plays an individual role." Under the heading "The Variety of Contents Compressed in the Miniature Code," he went on: "It has often been asked how this tiny speck of material, the nucleus of the fertilized egg, could contain an elaborate code-script involving all the future development of the organism. A well-ordered association of atoms, endowed with sufficient resistivity to keep its order permanently, appears to be the only conceivable material structure that offers a variety of possible ('isomeric') arrangements, sufficiently large to embody a complicated system of 'determinations' within a small spatial boundary. Indeed, the number of atoms need not be very large to produce an almost unlimited number of possible arrangements." In order to illustrate his contention he pointed out the huge number of possible arrangements derivable from the two different signs of Morse code. "It is no longer inconceivable," he concluded, "that the miniature code should precisely correspond with a highly complicated and specified plan of development and should somehow contain the means to put it into operation."[127]

Schrödinger's viewpoint was more original than he seemed to realize. It had not been "often . . . asked" how the gene might contain a miniature code. Those who had concerned themselves with the problem of how genes control development, from geneticists such as Morgan and Muller to the physicist Delbrück, had thought about the functions of the genes in chemical terms, such as autocatalysis and the actions of an enzyme.[128] As has often been pointed out, Schrödinger's book introduced for the first time a way to think about the relation between genes and development as that between a code as a plan of development and the operations that realize such a plan.

The physicist who had most directly inspired the genetical consid-

erations in *What Is Life?* was relatively uninspired by Schrödinger's little book. In a review published in the *Quarterly Review of Biology* in 1945, Delbrück predicted that the book would "attract many readers" because of the high respect in which the author was held and its "clear, simple, forceful style." It would inspire mainly by "acting as a focus of attention for both physicists and biologists." The latter, however, would not easily appreciate the dilemmas of the "naïve physicists," and both physicists and biologists "may be dissatisfied because Schrödinger does not advance our understanding of cellular mechanisms in any specific respect."[129]

In his extended summary of the contents of the book, Delbrück commented that in Schrödinger's opinion the "dependence of the well determined behavior of living organisms on this new and little studied physical structure, the 'aperiodic crystal,' explains the obvious inability of present day physics and chemistry to account for this behavior." Although this idea would seem "very suggestive to many readers," Delbrück questioned its validity. "One must note that it is based in large measure on a substitution of terms. For the term 'complicated molecule' he substitutes the term 'aperiodic crystal,' the latter term implying a new and unexplored state of matter." There was, Delbrück averred, "nothing new" in Schrödinger's exposition of the nature of the gene, "to which the larger part of the book is devoted, and biological readers will be inclined to skip it."[130]

Nowhere in his review did Delbrück mention that his own discussion of the gene in 1935 had provided the model for Schrödinger's exposition. Once again we see that Delbrück seems deliberately to have passed up an occasion to return to his own earlier views and are left wondering whether he still held them. Did his objection to Schrödinger's substitution of "aperiodic crystal" for "complicated molecule" derive only from his stated reason—that it implied an unexplored state of matter that he considered dubious—or from the fact that since 1935 he had learned to think about the nature of the gene in the terms of the biochemist and not those of the physicist? Finally, that Delbrück did not mention Schrödinger's attribution to the aperiodic crystal of a miniature code should give us pause about the role repeatedly attributed to that move in the light of later events.[131]

That Schrödinger's exposition of the nature of the hereditary mechanism seemed to contain nothing new to another physicist who had been thinking about the same problems for more than ten years is not surprising. Delbrück had formulated many of the same issues, in

greater detail and with more attention to the most recent developments in genetics and in biochemistry. His views were, however, still known to relatively few people. The original paper of 1935, published in an obscure Göttingen journal, had inspired several individuals, the most important of them Luria, but was hardly known to anyone to whom the authors had not sent reprints. The lectures in which Delbrück discussed the relation between physics and biology most broadly had only been circulated in typescript form. Most of those who picked up Schrödinger's book encountered for the first time the bold assertion that quantum physics was the foundation on which the study of the hereditary mechanisms of biology must be based. As Robert Olby has pointed out, "Those who read *What Is Life?* found in it what they were looking for."[132] Not familiar enough with biology to notice its shortcomings, they could be inspired not only by Schrödinger's own case but also by the mysterious figure of the "German physicist M. Delbrück" presented there, the living version of whom could find little to praise in the book.

Chapter Three

The Physicist Becomes a Phage Biologist

Seymour Benzer was born in Bensonhurst, Brooklyn, in 1921, to Polish Jewish immigrants who worked in the needle trades. Seymour was their third child and only son. His sisters were ten and eight years older than he, and a third sister was born ten years after he was. His father often brought bundles of clothes home for his mother to finish on her sewing machine, and Seymour sometimes had to deliver the clothes on the subway. As the only boy in the family, however, he was favored and given much freedom to pursue his own interests and hobbies.[1]

Like many other boys born during the 1920s, young Seymour was inspired by Sinclair Lewis's *Arrowsmith* to aspire to a life in science. He set up a laboratory in the basement of his parents' house where he carried out chemical experiments. At the time of his bar mitzvah, at the age of thirteen, his oldest sister's husband, Harry Lapow, who was like a supportive big brother to him, gave him a microscope instead of the usual ring. With this instrument Seymour examined all manner of animate and inanimate objects.[2]

Entering Brooklyn College with a Regents scholarship in 1938, Benzer intended to major in biology. Because of his high school courses and his microscopic observations he thought he knew enough biology to begin with advanced courses such as microbiology. When he was told that he must first take the prerequisite general biology, which consisted mainly of the taxonomy of plants and animals, he stubbornly refused and instead became a chemistry major. Later he switched to physics.[3]

During his freshman year Benzer met Dorothy (Dotty) Vlosky, a nurse. They often spent time together during Dotty's night duty at the hospital. Dotty and Seymour were married on the very day in 1942 that they had to depart for Lafayette, Indiana, where he had enrolled as a

graduate student in physics at Purdue University. Because it was a Saturday, the wedding ceremony at her home could not begin until after dark. While the guests were still celebrating, the newlyweds dashed to the train that was to take them through the night to the Midwest. The following morning Dotty called the local hospital to see if there were any jobs available for nurses. When the reply was "yes, you can start tomorrow," she protested that she was on her honeymoon, but when asked whether she wanted the job or not, she agreed to begin at once.[4]

Soon after his arrival, Benzer was recruited to take part in a secret wartime project, directed by the Viennese physicist Karl Lark-Horovitz, to examine the semiconducting properties of the element germanium. The aim was to replace the unreliable silicon crystal rectifiers that were used in radar equipment.[5] The group, consisting mainly of graduate students, worked together in a single room. Benzer thrived on the work. He noticed that Lark-Horovitz had the habit of asking another student who had also graduated from Brooklyn College every day what he had done but that he did not pester Benzer that way. Benzer came quickly to appreciate that Lark-Horovitz had confidence that he was making enough progress to be left alone, a situation he preferred.[6]

Seymour's total assets consisted of $100 in wedding gifts from his uncles. His parents, whose finances suffered in the Depression, could not afford to contribute. As a graduate student, Benzer at first received $70 per month, of which he used $35 to pay the rent. Long accustomed to frugality, the couple managed to live within their means. When Benzer's income was raised to $120, he told Dotty that she could go to college. She enrolled at Purdue and finished in three years, then returned to nursing.[7]

In 1946 a fellow member of his research group, Lou Boyarsky, lent Benzer a copy of Erwin Schrödinger's *What Is Life?* What most captivated Benzer about the book was Schrödinger's discussion of Delbrück's model of the gene. The idea that a mutation consisted of a change in energy that raised the molecule of the gene to a metastable state, from which it could also revert to the original state, seemed to connect so directly with the energy level changes he had studied in semiconductors that he imagined immediately that he might be able to apply what he had learned as a physicist to these central unsolved questions.[8] The discussion of x-rays, mutations, and targets also appealed to Benzer as a physicist. Most significant, however, was the

general curiosity that *What Is Life?* aroused in him about how physics can be applied to fundamental biological problems.

Soon afterward, while attending a meeting of the American Physical Society in Bloomington, Indiana, Benzer accompanied a friend who had been invited to a dinner party given by Salvador Luria. When Benzer asked his host about Max Delbrück, Luria urged Benzer to write Delbrück to ask for copies of a set of lectures Delbrück had recently delivered and to attend the summer course at Cold Spring Harbor that Delbrück had established.[9]

Benzer wrote to Cold Spring Harbor inquiring about courses offered in the summer of 1947 but was informed that the only one to be given that year was in bacteriology.[10] From Delbrück, however, he received mimeographed copies of the series of lectures described in Chapter 2 titled "Problems in Biology in Relation to Atomic Physics." The most absorbing of these lectures for Benzer was Delbrück's discussion of the application of Bohr's principle of complementarity to biology. The idea that determinism and life in the living state are complementary in the sense that in order to predict what an organism will do one would have to make measurements that would kill the animal, and the connection between these ideas and quantum mechanics, struck such a deep chord in the young physicist that he thought for a while that he might be able to define the uncertainty principle for biology in quantitative terms. The three lectures on the phage system seemed to him so beautiful that they attracted him powerfully to the idea of someday doing research in that field.[11]

At the end of 1946, while he was finishing the "miscellaneous remaining requirements" for his degree, Benzer seemed to be balancing the powerful incipient appeal of a career in biophysics with the bright and immediate prospects beckoning him toward a career in solid-state physics. The research that he and his associates at Purdue had carried out on the conducting properties of impure germanium crystals had been declassified and could now be communicated freely.[12] For some of the work they were able to file patent applications, six of which were approved. As they began in 1946 to deliver papers at meetings of the American Physics Society and the Institute of Radio Engineers and at other symposia and to publish notes about topics such as germanium high-voltage rectifiers and photocells,[13] the group received quick expressions of interest from departments of physics and from industry, which sensed the possibilities of such devices for the emerging

technology of television. Benzer's reputation as a very able young worker in this group spread, and he began to receive letters inviting him to consider joining the faculties of other institutions. His ambivalence about which way to turn is reflected in his response on November, 27, 1946, to one such letter from the University of Pennsylvania: "So far as my plans for the immediate future are concerned, I hope to continue in solid state, since some of the lines of investigation we have been following seem so promising that it would be foolish to drop them now, just when the picture is beginning to clear up. Later on, however, I would like to have a try at biophysics." In his uncertainty, the University of Pennsylvania seemed "interesting" to him because he had heard that there was some collaboration between the Department of Physics and the Johnson Foundation.[14] It might have been possible, therefore, to pursue both goals there. When he went to Pennsylvania and gave a seminar, however, he found that there was no interest in his plan to enter biophysics.[15]

By the time he received his Ph.D. in February 1947, Benzer was planning to try biophysics sooner rather than later. Perhaps one reason for his move in a direction that seemed to some of his colleagues risky or even "crazy"[16] in the face of the opportunities in solid-state physics was that he thought he saw a chance to apply his experience in semiconductors to a biological problem. Someone had brought to his attention several papers on nerve conduction by Kenneth S. Cole at the Institute of Biophysics at the University of Chicago, and Benzer thought it might be possible to apply principles of solid-state physics to this problem. On March 3, 1947, Benzer wrote Cole:

> It would appear from some of your papers that experience in the semiconductor field might be of some use in attacking membrane problems, although the analogy is probably a superficial one at best.
>
> At any rate, I plan to enter the biological field and would like to visit you and discuss this.[17]

Cole responded that he would be glad to see Benzer, and on March 8 they met in Chicago, along with George Marmont, who had invented the voltage-clamp technique. During lunch at the Quadrangle Club, where, to judge from some sketches that Benzer retained with the correspondence, they discussed such topics as changes of electric potential during the development of a sea urchin egg and impedance diagrams for nerve conduction.[18] This conversation must have strongly encouraged Benzer to believe that he could find ways to apply his

physics background to biophysics. His decision to move more quickly into the new field was probably facilitated most directly, however, by the encouragement he received from Lark-Horovitz to undertake studies in that field at Purdue with the prospect that the department would in the future begin work in biophysics. In April he was promoted to assistant professor of physics[19] with the understanding that he would eventually participate in such a program. To prepare himself for that role, Benzer began seeking possibilities for study in existing programs elsewhere. In July he wrote the executive secretary of the American Institute of Physics about graduate study in biophysics in France. Because he would have to bring his wife, who was expecting, he included questions about travel costs and living expenses there. He received word that opportunities for research existed in university laboratories and at the Institut Pasteur but that salaried positions were available only to French nationals.[20] That restriction appearing to rule out study abroad, Benzer turned to possibilities closer to home. One such opportunity arose in the fall, when he received a letter from Marvin Williams, a member of the Division of Physics and Biophysical Research at the Mayo Clinic, inquiring about the possibility that he might join the research group there. In his reply, in which he welcomed the contact, Benzer wrote: "My work in the past five years has been in the field of the electrical properties of semiconductors, in particular a study of the contact rectification, negative resistance, and photoelectric effects in germanium. The problem of nerve conduction has always seemed intriguing and it appears from the work of E. S. Cole [*sic*] that there are many points of analogy between nerve and semiconductor phenomena. My experience may therefore be useful in that line."[21]

The connection that Benzer had envisioned in March between nerve and semiconductor phenomena, and that he now proposed as a possible basis for his future work, is interesting not only in itself but because it represents a potential pathway that he could follow from the field in which he had been trained to the field he hoped to enter. It could not have been easy to contemplate abandoning a prestigious field in which he had acquired not only experience but success and heady prospects for one in which he was, in most respects, a novice. The two prospects that he had considered by the fall of 1947—to become again essentially a graduate student in France and reeducate himself as a biologist or to begin postdoctoral work in a research group as a physicist, bringing to a new field something of value from his previous achievement—represent the two general strategies that seemed

open to him. The first of these might open up broader possibilities but would come at a financial price that would give the head of a young family pause. The second promised a smoother outward course but depended on the speculative hope that the analogies between semiconductors and nerve phenomena were robust enough to compensate for his lack of experience in the field of neurophysiology.

Neither of these possibilities materialized. In December Benzer received from Williams the disappointing reply, "We are glad to have some information about you in our files," and if "we should ever be in a position to add someone with your training to our staff . . . we would have the necessary information at hand."[22] By then, however, Benzer was probably losing interest in the idea of crossing quickly from physics to biology over the weak bridge of an analogy between semiconductors and nerves. In part because he continued to rely largely on Luria for advice, in part because of its intrinsic interest and because it was a field developed by former physicists, Benzer was most strongly attracted to the study of bacteriophage. He sought further interactions with Luria (who mentioned to him in November the possibility of an opening "for working on phage at Bloomington"),[23] read papers by such leaders in the field as Delbrück and Alfred Hershey, and attended a meeting in Chicago at which papers about phage were presented. Meanwhile, his department chair mentioned Benzer's new direction to Alexander Hollaender, head of the Biology Division at the Oak Ridge Laboratory in Tennessee, where research on just such topics took place, and learned of a possible opening there. On November 13, Benzer wrote Hollaender that he was "interested in basic biological problems, such as gene and virus reproduction and mutation," and asked about the research opportunities and conditions at Oak Ridge.[24]

Perhaps overly eager to show that he had something to contribute, Benzer wrote Luria in January 1948 that from data given in a paper by Delbrück on phage replication he had calculated the relation between the burst size (the number of phage released from a single bacterium) and number of bursts and the relation between the number of infected bacteria and the cube of their length. On a neatly drawn graph he showed that the curves very nearly coincided. He wondered whether these correlations were significant.[25] Luria responded quickly and rather coolly: "As for your calculations of the data on burst size distribution compared with the distribution of the cube of the bacterial length, it seems to me that your assumptions are untenable." He doubted "very much that the correspondence of the two distributions

which you found is anything more than accidental."[26] The tacit message from the co-founder of the phage group seemed to be that, although he welcomed the intention of the young physicist to join his field, Benzer must expect to undergo a new apprenticeship before he would be ready to help advance it.

When he traveled to Oak Ridge to be interviewed, Benzer must also have come across as overconfident about his ability, as a physicist, quickly to solve biological problems. Hollaender called Lark-Horovitz afterward to complain about the "arrogant young man you sent me." Apparently Lark-Horovitz was able to persuade Hollander that his impression was misleading,[27] because in January Benzer received an offer of employment at the Oak Ridge Laboratory. Hoping that he might instead be able to work on phage with Delbrück or Luria, he wrote on January 23 to George Beadle, then head of the Biology Division at Caltech, to see if there were any opportunities there for postdoctoral fellowships in biology, adding, "I am especially interested in the possibility of working with Dr. Delbrück's group."[28] Luria responded to Benzer's inquiries rather guardedly in February, saying that he might be able to find a place for him but that he would not know until sometime in the spring and encouraging him to choose Delbrück's laboratory. Delbrück, Luria wrote Benzer, would "welcome you if you had a fellowship. Personally," he added, "I feel that you would find that atmosphere very stimulating. Delbrück has lots of ideas in real biophysics, besides phage." Luria himself would find it possible to "squeeze in a salary" for Benzer, but he preferred not to, and he "strongly suggested" that Benzer apply for a postdoctoral fellowship from the American Cancer Society. Taking this advice, Benzer made inquiries but was told it was too late to apply, and he apparently heard nothing in response to his previous inquiry to Beadle. With his preferred options provisionally blocked by financial obstacles, he accepted Hollaender's offer at Oak Ridge, but they agreed that he would not come until September so that he could follow Luria's earlier advice to take the summer phage course at Cold Spring Harbor. He thanked Luria for his "interest in helping [him] to get into biophysics."[29] Throughout these negotiations Luria seemed ambivalent about taking on the enthusiastic new convert to biophysics. Despite his own early exposure to physics and his collaboration with the former physicist Max Delbrück, perhaps Luria, like Hollaender, sensed in Benzer too much of the attitude that a well-trained physicist entering biology could solve problems that had eluded those who had long been in that field.

While waiting for these events to take their course, Benzer and his department chair, who also hoped to move in the direction of biophysics, made some efforts to get started on their own at Purdue. In February Lark-Horovitz checked the "inventory of . . . biophysics equipment" and searched for space in which they could locate the equipment. In a letter to a member of the department whom he asked to make space by removing any other equipment "which is not absolutely in use" from the densely packed Room 19, he explained, "Both Dr. Benzer and myself are planning to carry on some biophysics experiments during the next few months."[30] Nothing came of these plans except that they upset the occupant of the room that they cleared out for the intended research.[31]

With Dotty and their baby daughter Barbie, Benzer drove four months later from Indiana to Cold Spring Harbor, where the phage course began on June 28, 1948. Delbrück was in Europe that year, and the course lectures were given by Mark Adams. The thirty young scientists taking the course were divided into groups that carried out the fundamental experimental procedures of phage research. Benzer's lab partner was Margaret (Peggy) Lieb, a graduate student in the Department of Zoology at Barnard College.[32] As the laboratory exercises began, Lieb taught Benzer a key technique: how to hold both a pipette and a cotton plug in one hand, leaving the other hand free to hold the test tube (fig. 3.1).[33]

After learning to distinguish the seven types of phage preferred by the phage group, the students prepared cultures of bacterial *E. Coli* strain B in tubes, observing that the "turbidity [of the culture] becomes notable at 10^7 bacteria/cc." Infecting the bacteria with each of the phage types, they learned the phases of the phage reproductive cycle—adsorption, penetration, multiplication, and lysis. There was, they learned, no visible "evidence that initial particle penetrates, but the progeny are created inside." After a variable latent period in which the phage were assumed to be multiplying, the destruction, or lysis, of the bacteria liberated one hundred to five hundred phage particles, a process that could be followed in time as a curve of decreasing turbidity of the liquid culture medium. To determine the "burst size," or average number of phage released from each bacterium, they plated them on bacteria cultured on "soft agar," using a technique developed by Hershey. Each phage particle produced a clear circular "plaque" by infecting and lysing the bacteria within a given area of the agar plate. Each infected bacterial cell was known as an "infective center." They learned

Figure 3.1. Seymour Benzer at his laboratory bench during the Cold Spring Harbor phage course 1948. (Photo courtesy of Seymour Benzer.)

to recognize the plaque sizes characterizing the different phages. "T1, T3, T7 plaques," they were told, "are very large, . . . so overlapping is serious, and one should use greater dilution." A further complicating factor in the case of the even-numbered phages was "lysis inhibition" due to secondary infection.[34]

Among the results of the first set of experiments, Benzer recorded some of the plaque characteristics he observed. Phage T3 was "large (size of dime)," T4 was "small (2mm. diam.)." Already, however, he noticed that there were differences in the latter due to the formation of mutants. Most of the T4 plaques were "small" with a "fuzzy border," but some were "large" with a "sharp border." The former he designated "r^+," the latter "r mutants." The class was, thus, simultaneously learning to do phage experiments and familiarizing itself with the notation that phage biologists used. Benzer wrote in his notebook that "r stands for rapid lysis (mutant)" and that "r^+ stands for not rapid lysis (wild type)."[35] While Benzer's group carried out these experiments, another group that included Gunther Stent did a tryptophane mutant

experiment, and a third group observed the course of lysis with the microscope. He summarized their results in his notebook as well.

While the experiments were proceeding, Benzer had time to read about recent observations made by established members of the phage group. From a typewritten manuscript by Delbrück he noted that "Delbrück found latent period temperature dependence about the same as the generation time of the bacteria." He learned also that Raymond Latarjet had "irradiated bacteria and found that you can have a lethal mutation where the bacteria grow but cannot divide. These are susceptible to phage." He picked up subtle but important details of technique, for example, "the upper limit of titer is 5×10^{10} in a culture because of absorption on other bacterial cells and lysis inhibition," but "if you plate viruses and bacteria so that you just get confluent lysis, you can get extremely high titer of virus; wash plate in broth, centrifuge; you can get as high as 10^{12}/cc. in a small volume. Can also chop up the agar and centrifuge."[36]

In the third set of experiments the class examined the rate of inactivation of phage by antibodies. Each group worked with a different phage. After using an antiserum for each phage, they tested its action at a series of dilutions. In his notebook Benzer calculated the "K values [that is, the rate constant] for T2 serum" on T2. "It is found," he concluded, "that the rate of inactivation is prop[ortional] to conc[centration] of serum." That is, it had the apparent dynamics of a first-order reaction. It was, however, not a true first-order reaction: "Since there is a large excess of antibody, you get a pseudo–first order reaction. If you try to exhaust the antibody by using very dilute solution, the rate is so slow that it takes too long." He compared the K values obtained in the experiments performed by the class with some of those reported by Delbrück and noted that they varied from one serum sample to another. He discussed the possible causes for the deviations from a straight line obtained in some of the experiments and finally remarked that there was "no detectable immunological diff[erence] between T4r and T4r$^+$."

In the next several experiments, the class tested the ability of each of the phage types to form plaques with various mutants of *E. Coli* strain B. On July 8 Benzer's group plated the "host range mutant" T2h on bacterial strains B, B/2, B/3,4,7, and B/6 "to see if it is really T2h, or a contaminant of T4 or T6." (The notation "/2" or "/3,4,7," read "bar two" or "bar three, four, seven," indicated that the strain was resistant to the lytic action of phage T2 or of phages T3, T4, and T7.) Host-range mutants were defined by their inability to infect and lyse

certain strains of bacteria that normal "wild" types could. If the sample in question was able to form plaques with all four bacterial strains, therefore, there must be a contaminant present. He did not, however, record the outcome of this test. The following day they performed "precision single step growth exp[erimen]ts," the staple fixtures of phage biology research. Here they counted the burst sizes and calculated average burst sizes and the distribution of burst sizes. The last they compared with the predictions of a Poisson distribution, that is, the distribution that would result from statistical chance. In his notebook Benzer remarked on some of the deviations of the actual burst sizes from the "expectancy from Poisson distrib[ution]." The prediction for an average burst size of 125 was that "*one* plate of these will have 2 bacteria." The actual smallest number was one: "Prob[ably] a free phage or a very late-bursting bacteria. Some very large bursts may be due to double bursts (2 bacteria)."

In the ninth series of experiments the class members moved on to the more complicated situation of "mixed multiple infection" and "induced r [rapid lysis] type mutations, using T2r$^+$ and T6r." In the previous experiments they had mixed phage and bacteria in proportions such that statistically one would expect no more than one phage particle to infect one bacterium. Now they increased the proportion of phage to bacteria so as to expect a "multiplicity of infection." That is, there was, on average, more than one phage particle for each bacterium, and when two types of phage were included, some of the bacteria would be infected by both types. Recombination between the two types might be expected also to interchange r and r$^+$ between the two phage types. To determine what had happened, they plated the progeny on *E. coli* B/2 and *E. coli* B/6 and counted the several types of plaques formed. They also plated these phage on mixtures of B/2 and B/6 bacteria to look for infected cells producing both T2 and T6 phage. The results showed some r-type clear plaques, some r$^+$-type clear plaques, and some mottled and turbid plaques. From the total numbers of each, Benzer calculated that 80 percent of the bacteria liberated both T2 and T6, that the "multiplicity of infection comes out 10 for total of the two phages," and that the recombination frequencies were 2.5 percent for T6 and 34 percent for T2.

Some members of the class investigated the "mutual exclusion effect" that Delbrück and Luria had discovered (see Chapter 2). When T1 and T3, T3 and T6, or T1 and T4 were added together to a bacterial strain, in some cases only one type would appear in the progeny. They

found that there was a "depressor effect" but not total exclusion of one by the other. In his notes on these experiments, Benzer commented that "there is no good explanation for mutual exclusion."

The last experimental results that Benzer recorded concerned "u. v. exp[erimen]t (#10—Luria exp[erimen]t." Only a year earlier Luria had found that if one infected bacteria with phage previously inactivated by radiation with ultraviolet light, in proportions large enough to obtain multiple infections, one could get a "reactivation" effect. That is, phage that were no longer capable singly of infecting and lysing bacteria were able to do so in multiple infections, somehow "cooperating" to overcome their individual defects. The class, Benzer wrote, achieved results "demonstrating reactivation."

Adams's lectures were straightforward and not particularly inspiring to Benzer, but the total immersion in phage experimentation immediately enchanted him. The experiments were simple, quantitative, and decisive. They could be finished in one day, and then the group was ready to move on to the next one. The interaction with other students and guest lecturers, who included Bernard Davis on chemotherapy and Renato Dulbecco on the Poisson distribution, was stimulating. Luria himself was among the senior phage scientists present. The students shared a feeling that they were being introduced to something new and beautiful. Before the three-week course came to an end Benzer was certain that he wanted to become a phage biologist.[37] Another student in the course, Gunther Stent, was similarly inspired to make phage biology the beginning of a new scientific career.[38]

Aside from science, the class enjoyed such diversions as clambakes carried out with the aid of an autoclave. The students were put into a kind of a flurry by a lecture by a physicist from the Carnegie Institution on the subject of extrasensory perception who failed to convince most of them that ESP was a scientifically demonstrated phenomenon. The graduation ceremony, following the usual customs of the phage course, included such hilarities as worship of a picture of the absent Max Delbrück.[39] The graduates wore costumes and received a customized degree. A rumor spread that Delbrück had returned just in time for the commencement to play his usual role as the Lord High Inquisitor, but the shadowy figure who walked in covered by a bed sheet and announced that he was Delbrück's ghost turned out to be only a graduate student, Jim Watson.[40]

Benzer drove with Dotty and Barbie in their 1941 Chevrolet to the Oak Ridge National Laboratory, ten miles from Knoxville, in time to

begin his work as a biophysicist in the Biology Division on September 1. Dotty's father joined them there. The Benzers' first contact with their new surroundings was traumatic. The "comfortable two-bedroom house" to which they thought they had been assigned turned out to be a converted army barracks. The ubiquity of cockroaches in the building was only made more evident by the fact that each family was entitled to one free fumigation on moving in.[41] Moreover, the laboratories, in which isotope separations were still being carried out, was run under contract by Union Carbide, and the atmosphere was thoroughly industrial. Benzer was required to show a badge whenever he entered or left the compound and, in a shock to his nocturnal habits, to work daily from nine to five. Nor were he and his family accustomed to the segregation that reigned in this southern location. There were separate toilets and drinking fountains for whites and blacks, and black workers were housed in tents.[42]

In the laboratory Benzer was assigned to a biophysics group led by William Arnold. There were two others in the group who worked on photosynthesis. Benzer did not work with them but moved into the laboratory of the only other phage worker, Thomas Anderson, who provided him with an inexhaustible supply of pipettes. Six weeks later, after Lark-Horovitz had visited, Benzer reported with notably mild enthusiasm that "Oak Ridge is interesting indeed and I am having a worthwhile time, although Dr. Lark-Horovitz' visit did arouse some homesickness in Dotty and myself. Barbie, on the other hand, has had very little difficulty in readaptation."[43]

In his new surroundings Benzer was attracted in particular to the phenomenon of photoreactivation, recently discovered by Luria. Phage that had been inactivated by ultraviolet light after their adsorption on bacteria could be reactivated by exposing the bacteria to visible light. This observation appealed to him because the radiation phenomena were "related to physics,"[44] and their further study might provide another bridge across which he could bring his earlier experience to bear on his new field. Photoreactivation, along with the phenomenon of multiplicity reactivation to which he had been exposed at Cold Spring Harbor, might, he thought, help explain the paradoxical results of a set of experiments just published by Luria and Latarjet. The so-called dark stage between the time at which the phage particle penetrated a bacterium and the release of its progeny through the lysis of the bacterium remained mysterious. As Luria and Latarjet put it, "The process of intracellular phage growth—in particular, of the kinetics of

phage production—has so far escaped every attempt at clarification made either by breaking down infected bacteria or by electron microscopy." Their approach, irradiating the infected bacteria at several time intervals during this phase with three different doses of ultraviolet light in order to determine the changes in the resistance of the infective centers to the radiation during the latent period, again adapted the target theory drawn from radiation physics. They established the survival curves of the infected centers at each of several intervals of time following the infection—that is, the proportion of infected bacteria that were still able to release phage progeny after being irradiated, as a function of the dose of radiation given. According to target theory, if a single hit is able to destroy the infective center, the survival curve should be exponential, and a plot of the logarithm of the proportion of survivors against the dose should give a straight line. If multiple hits are necessary to knock out the infective center, then the curve plotted in this way should at low doses be a concave downward curve, becoming at higher doses a straight line with the same slope as the single-hit curve. Applying this method to *E. coli* B infected by bacteriophage T2, Luria and Latarjet found that in the early stages the curves were single-hit curves, as would be expected if a single virus particle were present. In cases where they produced multiple infections by increasing the ratio of phage to bacteria, they attained some concave downward curves suggestive of multiple hits, but in the experiments in which each bacterium was assumed to be infected by a single phage, the curves became concave upward curves. Luria and Latarjet were unable to interpret these results in a manner that would clarify the intracellular growth process.[45]

In his first months at Oak Ridge, Benzer performed more of the basic phage experiments that he had been taught to do at Cold Spring Harbor, preparing himself to take up the questions left by the Luria-Latarjet experiment. By January 1949 he was ready to draw up a plan of research. After summarizing the results of Luria and Latarjet, he wrote:

> I feel that many aspects of the anomalous results may be explained on the basis of the "reactivation" phenomen[on], which has been studied in detail by Luria, but which seems to have been overlooked in the interpretation of the survival curves. It is not unreasonable to suppose that several intracellular phage particles which are irradiated can subsequently reactivate each other at least as well as if they had been irradiated before infection. The survival (y) expected, therefore, would correspond not to ordinary multiple-hit

> curves, but would be given by curves of y_k vs. r for fixed values of k in the terminology of Luria's paper, where k would be the number of intracellular phages. This is such a powerful effect that a multiplicity value of k = 2 *with* reactivation changes the survival curve as much as would a multiplicity of about 10^3 in the absence of reactivation.[46]

The first prerequisite for independent, original research is to find an opening on the frontier, a place where one can build further on the latest results in the field by identifying some facet of the problem that has so far escaped the notice of its leaders. Benzer had astutely noticed such an opening only a few months after learning the rudiments of phage experimentation. Though a newcomer to the field, he was not a newcomer to scientific research in general, and the similarity between what he had already learned to do as a physicist and the scientific reasoning of the phage biologists, who had also begun in physics, helped illuminate his new pathway. Phage biologists thought in terms of mathematically defined curves and the variables that shape them. They reduced experimental data whenever possible to forms expressible as equations. It was, thus, relatively easy for Benzer to define an interesting research problem in such terms.

In his proposal Benzer predicted the general nature of the results that he would obtain by taking reactivation into consideration in Luria-Latarjet–type experiments: "Reactivation can presumably occur only if a certain minimum time is available after irradiation. Therefore, the apparent resistance of the infection should increase at first, as the number of phages per cell increases, but as the end of the latent period is approached and the time available for reactivation is reduced, the survival curve should approach a multiple-hit curve. Reactivation can therefore explain the observations qualitatively and should be given serious consideration as a possible disturbing factor in these experiments."[47]

Benzer proposed to begin by irradiating the bacteria immediately after they were infected with phage, so that the phage had no opportunity to multiply. By comparing the "killing" results at various multiplicities of infection, he hoped to determine the "manner in which the survival curves shift with multiplicity of infection" and thereby "distinguish between reactivation and the lack of it." He intended to carry out these experiments on T2, as Luria and Latarjet had. If his hypothesis that their anomalous results were due to multiplicity reactivation were correct, however, then one might be able to achieve their original

objective by choosing a phage type in which there was no multiplicity reactivation. Benzer proposed to investigate whether phage T1 would meet this condition. In this case, "a unique opportunity is offered for an experimental check of the target theory for multiple-hit phenomena, since the multiplicity of infection is easily adjustable over a considerable range and the curve for single infection can also be obtained, which makes exact determination of the curvature in the survival curves relatively unimportant." If it turned out that T1 also showed multiplicity reactivation, this result would also be interesting, because it would show that the phenomenon was a general one not restricted to the large-particle phages.[48]

His working conditions at Oak Ridge were more satisfactory than the living conditions. Hollaender was devoted to his division and to staffing it with the best people he could find, and Benzer found him very supportive. Some of the other people around him appeared to Benzer, however, to be disgruntled because they did not have academic positions. A few of them seemed to resent it if he went to his laboratory on a Saturday, and he was never comfortable with the rigid nine-to-five working schedule, the need to show a badge when entering or leaving the compound, and other aspects of the industrial manner in which the laboratories were operated. Once, when he tried to fix an incubator relay that was not working, the union electrician threatened to call for a strike. Outside of work there were few diversions. Sometimes they went all the way to Knoxville for a corned beef sandwich at the nearest Jewish delicatessen or drove up treacherous winding dirt roads into the mountains. One of Benzer's colleagues, who was a pilot, took him on a flight over the Cumberland Mountains, where he allowed him to take the controls. Dotty did not work in Knoxville but devoted all her time to caring for Barbie, the house, and her father, who had come to live with them.[49]

Benzer, who also missed being in an academic environment, had probably planned before he arrived not to stay at Oak Ridge for long if he could arrange to work with Luria or Delbrück. On January 14 he wrote Delbrück that he would be "extremely pleased if it were possible to come to Cal. Tech next year to work in your laboratory after taking [C. B."Kees"] van Niel's [microbiology] course [given at Pacific Grove, California] in the summer." He asked Delbrück's advice concerning application for a fellowship and added, "By now, I have a pretty good idea of what this field is like, and it is becoming steadily more fascinating. I am primarily interested in the mechanism of phage multiplica-

tion and the various approaches to this problem using radiation and genetic techniques."[50] This very general statement suggests little more than that Benzer was attracted to the problems that at the time formed the central interest of Delbrück and Luria. Delbrück replied quickly and briefly, "I would be very glad to have you here next year and suggest that you apply for an AEC post doctoral fellowship in the biological sciences."[51]

Thanking Delbrück for his favorable reply, Benzer wrote that Hollaender had told him he would have a good chance of getting an AEC fellowship and added that he would have time to apply before the February deadline. He enclosed his proposed program for research, asking for Delbrück's reaction and suggestions. He would begin on the program at Oak Ridge but expected that it would need to be altered if he continued it at Caltech.[52] Delbrück returned Benzer's research proposal with a few marginal annotations (replacing Benzer's statement that reactivation "seems to have been overlooked" with the more accurate "which had not yet been discovered when L. and L. published their" interpretation) and with a lengthy comment:

> You may get beautiful curves from these exp[erimen]ts, but I doubt that the curves will be interpretable in as simple a manner as you hope. This does not matter, you can always switch to something more interesting, and your program is a reasonable one as a starter. Dulbecco will be here next year, and we hope to have a good monochromometer for the visible region going, so there will be a good opportunity to pursue photoreactivation studies on UV killed phages. For this your present work and proposed program are a fine introduction.

Finally, Delbrück suggested that if the fellowship came through, Benzer might come to Caltech before starting van Niel's summer course in order to help set up the monochromometer.[53]

Delbrück's response was true to form. Having undoubtedly learned from Luria or others of Benzer's promise as a phage biologist, he welcomed the newly converted physicist readily into his group but did not hesitate to express blunt skepticism about the research plan Benzer proposed. In part the skepticism was based on long experience: Delbrück knew that, however enticingly simple it was to perform experiments on phage compared to work on more complex organisms, and however accessible the results may be to quantitative analysis, meaningful conclusions about the mechanics of phage multiplication were

much harder to attain than a beginner might anticipate. In part, however, it was intrinsic to Delbrück's personality to doubt the validity or practicality of any proposal made to him. It was this combination of his propensity to challenge intellectually those who approached him while warmly welcoming those whom he respected that drew so many young phage investigators to Delbrück and that made his phage group at Caltech a nearly mandatory stopping place along the way to a career in the field.

Obviously excited about his new prospect, Benzer wrote his department chair, Lark-Horovitz, on January 24:

> As for my plans, I have really fallen in love with phage and want to devote a couple of years to it, at least. I plan to go to Pacific Grove next summer and take van Niel's course in general microbiology. He is apparently one of the world's outstanding men in this field and several people have recommended the course as the best training available. It would seem very advantageous to follow this with a year's work with Delbrück. He has said he would be glad to have me and I am applying for an AEC fellowship. I would like to know your reaction and what you think Purdue's reaction would be to a request for extension of leave.[54]

His expression of enthusiasm was conditioned in part by his need for patient approval from Purdue for his plan to stay away for a year longer than he had originally requested, but fortunately for Benzer, his plans fit those of his chief and of Purdue for the future development of biophysics there. In January the dean of the School of Science, W. L. Ayres, asked that Benzer come to Purdue to give a talk about his bacteriophage work, and John Karling, the head of the Department of Biological Sciences, wrote him about plans for the construction of new biophysics laboratories. Lark-Horovitz explained to Benzer that the reason for the second request was that "Dr. Karling does not know you, he wants of course to talk to you to have an idea of what you are doing and what your plans actually are." Benzer replied that he was "involved in a series of bacteriophage experiments which would suffer if interrupted right now" but that he could come late in February. Karling accepted the postponement and wrote Benzer, "While you are here I would like to have you help us in making plans for laboratories, etc. for biophysics in the proposed Life Science building." At the beginning of February Lark-Horovitz came again to Oak Ridge to talk about his work on the permeability of red blood corpuscles. Benzer also took time out

to submit progress reports and publications dating from his earlier work on germanium crystals. Clearly, his absence from Purdue was not viewed by either party as a departure.[55] In February Benzer began to implement the research program he had sent to Delbrück. During the first two weeks he performed preliminary experiments intended to "obtain quantitative data necessary in planning proper timing and dilutions to be used." These experiments included measurements of the growth rate of the bacteria in synthetic media and of the rates of adsorption of the phage and of inactivation by antiserum. Then he planned to produce the first ultraviolet inactivation curves for the phage. By February 15 he expected to be ready to produce the experimental curves for the ultraviolet inactivation of the phage at the beginning of the latent period at various multiplicities and to make "theoretical calculations, taking into account the Poisson distribution of multiplicity of infection." That done, he would spend the month of March gathering a "set of inactivation curves during the latent period." During the next six weeks he would carry out further experiments "depending upon the outcome of previous ones." Optimistically he projected that he would be able to "write up the results for publication" by June 15, before departing for California.[56]

Benzer postponed his trip to Purdue from late February to early March and had to postpone it again when he received word in the last week of February that his father had suffered a heart attack. By the time his only son arrived in New York, his father was in an oxygen tent. He, his sisters, and other members of the family gathered around the senior Benzer, who died soon afterward. Seymour's brother-in-law Harry Lapow took care of arranging the funeral and other urgent matters.[57] Benzer returned to Oak Ridge on March 1. On March 19 he was finally able to go to Purdue, where he spoke to Karling's microbiology group about "recent developments in Bacteriophage research" and discussed "plans for Biophysics in the future" with him. He also went to Bloomington, where his stay was "very informative and stimulating. Luria, Dulbecco, and Watson," he reported back to Lark-Horovitz, "are doing exciting work on photoreactivation and other things."[58] Renato Dulbecco and Jim Watson were, at the time, graduate students of Luria. By the beginning of April Benzer found that he was committed to staying at Oak Ridge until September 1 and could not arrive in California early in the summer, as Delbrück had suggested, or take van Niel's summer course on microbiology. Benzer wrote to the Hopkins Marine Station at Pacific Grove, expressed the hope that he could take the course the

following year, and received back the ten-dollar deposit that he had submitted in order to enroll.[59]

While waiting to hear the result of his application for an AEC fellowship, Benzer continued to negotiate with Luria about going to Bloomington. On April 2 he sent Luria his curriculum vitae, adding, "I look forward to receiving your offer and it should be possible to decide very soon whether I can accept it." A week later he was notified officially that he had been awarded an AEC postdoctoral fellowship to work "under the guidance of Dr. Max Delbrück" on the effect of radiation on bacteriophage and that he could begin any time between July 1 and October 15.[60]

Early in April 1948 the phage group met in Bloomington. Afterward several of its members, including Delbrück, Luria, Dulbecco, and Watson, came to Oak Ridge, where on the fifteenth Dulbecco gave a lecture on the "experiments on photoreactivation with bacteriophage," about which Benzer had learned during his stop in Bloomington in March. Dulbecco had discovered the phenomenon in part by a lucky accident. Having placed a plate over an identical one containing the same number of phage, he noticed that the plate on top produced more plaques and concluded that the reason was that it received more light. Albert Kelner discovered the same effect independently when he found that irradiated bacteria nearer to the light bulb used to heat the water bath had a higher survival rate than those further from it. Benzer was particularly impressed by a comment on these results in which Delbrück invoked the "principle of limited sloppiness." If one controls experiments too rigorously, nothing unexpected is likely to turn up. Too much sloppiness produces nothing at all, but a little bit of it in the design of an experiment is conducive to discovery.[61]

Before or during this meeting Luria offered Benzer a position "at a much higher salary" than he would receive from the AEC fellowship at Caltech, in sharp contrast to the year before, when he had seemed to be stalling by writing that he did not know whether he could find the money. Now it appeared that Luria and Delbrück were bidding for him. When Benzer asked Watson, who had worked with both of them, for advice, Watson told him that Luria would ask him every day what he had done, whereas he might not see Delbrück for weeks at a time. Remembering how much he appreciated not being asked every day by Lark-Horovitz what he had done, Benzer decided in favor of Delbrück. In fact he really needed little convincing, and Watson's comment provided mainly an additional rationalization for the reason he gave in a

letter to Lark-Horovitz on May 25: "I feel that the broader outlook to be acquired from working with Delbrück and van Niel is more desirable."[62] That Luria, who had previously been reluctant to squeeze out a salary for Benzer, now offered him more than Delbrück had speaks to the rapidity with which the physicist had impressed the phage biologists that he had become one of them.

Research programs never go as planned. Within weeks after beginning his, Benzer had to diverge from what he had projected in order to take into account the experiments on photoreactivation about which Dulbecco reported at the Oak Ridge meeting. Dulbecco had found out that phage inactivated by ultraviolet radiation and adsorbed singly into bacteria can be reactivated by exposing them to light in the near-ultraviolet and visible range. This effect was, therefore, independent of the phenomenon of multiplicity reactivation, and Benzer realized that he would have to check to see whether the "peculiar behavior of the survival curve" in the Luria-Latarjet experiments might be due to light rather than to multiple infections.[63]

Reasoning that if the photoreactivation Dulbecco had discovered was limited to the ultraviolet and near-ultraviolet range of the visible spectrum, then light near the other end of the visible spectrum might not have this effect, Benzer found that yellow light "produced very little photoreactivation." He then carried out a Luria-Latarjet experiment using only yellow light for illumination. To avoid multiplicity effects he introduced a sample of phage T2 "containing about 1 phage particle for every hundred bacteria." At intervals of three, six, nine, thirteen, and seventeen minutes he withdrew samples of the infected bacteria, dividing each sample into four parts and irradiating the four parts with four different doses of ultraviolet light shone on them from a germicidal lamp. He then plated each sample on another bacterial culture, on which the surviving infective centers each formed plaques. The curves that resulted from plotting survival against ultraviolet irradiation dose were "very similar" to those that Luria and Latarjet had obtained, and Benzer therefore ruled out the possibility that light was responsible for their anomalous nature.[64]

"In order to specifically test for the effect of light at different stages," Benzer next performed similar experiments in which he illuminated half of the plates with fluorescent light and kept the other half in the dark. He irradiated the phage at intervals of three, six, and fourteen minutes after infection and compared the effects with the effects on phage irradiated before they were adsorbed on the bacteria. The

corresponding curves for the light and dark runs nearly coincided at three and six minutes, but the survival rates were higher for the light experiments at fourteen minutes and for free phage. "Photoreactivation," he concluded, becomes "quite noticeable in later stages," as it is for the free phage, but he was surprised to find that "there is very little photoreactivation when the irradiation is done early in the latent period, since at this time the phage particle would be expected to be not very different from a free phage particle."[65]

Because Dulbecco had found that phage can only be photoreactivated after they are adsorbed into bacteria, Benzer also checked to see whether subjecting the bacteria to ultraviolet radiation prior to their infection by phage would affect their ability to support the photoreactivation of the phage. He found that irradiation "very rapidly reduces their ability to function in the photoreactivation of irradiated phage" and concluded that "this phenomenon is probably responsible for the very small amount of photoreactivation when infected cells are irradiated."[66]

These experiments effectively eliminated photoreactivation as an explanation for Luria and Latarjet's results but left Benzer back where he had begun: examining whether multiplicity reactivation held the answer to the puzzle. He returned to the strategy he had outlined in January, which was to try to irradiate the phage immediately after they had been adsorbed and before they would have had time to multiply. Implementing that plan would not be easy, however, because of the rapidity of the growth of the phage during the latent period. Benzer found a way around this obstacle by infecting the bacteria with phage while the latter were in a medium containing buffer but no nutrients. Under these conditions, "adsorption of the phage occurs, but there is no shift of the survival curve with time."[67] He interpreted this result to mean that the phage were not able to develop within the starved bacteria. That left him free to carry out at his leisure the manipulations necessary to test the effects of various multiplicities on the survival curves under arrested conditions equivalent to those present immediately after adsorption. He could also obtain an entire survival curve from a single experiment, making it unnecessary to group points with similar survival values from several experiments. The curves he obtained, when plotted according to a theoretical equation derived from Luria and Dulbecco, were "not terribly far from straight at fairly low values of x."[68]

"The data obtained for various multiplicities of infection, up to 10

particles per bacterium," he wrote in August in his last quarterly report from Oak Ridge, "are in fair agreement with the theory of Luria and Dulbecco. Due to the multiplicity reactivation, a relatively small multiplicity of infection is sufficient to produce a large increase in resistance to radiation, even without any growth of the phage. This is believed to account for the discrepancy observed by Luria and Latarjet between experiments using multiplicities around 0.01 and those around 0.3."[69] By now, therefore, Benzer had made significant progress toward his initial objective of explaining peculiarities in Luria and Latarjet's experiments through reactivation. That result still fell short, however, of the larger goal: to obtain results that would illuminate the mechanism of phage multiplication. A more promising direction seemed to open up in the pursuit of his other objective, which was to perform the experiment on a phage that did not undergo multiplicity reactivation. He found such an object not in T1, as he had expected, but in T7: "A series of experiments using phage T7," he reported, "reveals marked differences from T2 which are of particular interest, because T7 does not show multiplicity reactivation."[70] He did not, however, give any interpretation of the markedly different survival curves that he had obtained. They struck him as very unusual, because there was no change in the survival times during the first four or five minutes. "This is a very long time," he thought, "because the entire latent period is only 13 minutes." There was also a peculiar "kink" in the curve near the end of the latent period that he thought might be an artifact. The experiments needed to be carried out at a lower temperature, because "things are happening too fast at 37°."[71]

When he examined the burst sizes and the lengths of the latent periods of phages subjected to irradiation during their growth period, Benzer discovered that the effect of the radiation was to "decrease the yield and increase the latent period,"[72] even when the proportion of surviving infective centers was unchanged.

Although photoreactivation did not now seem to be the explanation for the Luria-Latarjet results, Benzer remained puzzled about why he had not observed the phenomenon in T2 early in the latent period. He consulted Dulbecco, who advised him in a letter written on May 31 that "the failure of obtaining PHTR [photoreactivation] in your experiments may be due to insufficient illumination that could not be improved very much in the conditions you have used" and advised him to try some techniques with which he had obtained higher light intensities. When Benzer tried that, he did find that white light produced

photoreactivation in the early stages, but both the amount and the rate of reactivation changed when he applied the ultraviolet irradiation at different times during the latent period. It was, he wrote Dulbecco at the end of July, "difficult to interpret these experiments in a quantitative way because the bacteria are certainly changing a lot as the growth of the phage proceeds and their ability to produce ph[o]t[o]r[reactivation] of phage will change. Furthermore, to get the same survival of infective centers at different stages one must of course use widely different doses of UV, so the competition which you pointed out between the bacteria and the phage for phtr will be different." Mentioning also the "drastic" effects of ultraviolet irradiation on the burst size and latent period that he had observed, he added that "in spite of these difficulties, it may be possible to get some qualitative information, and I would therefore like to do some more experiments."[73]

Benzer did not yet have anything ready to write up for publication in July as he had planned, nor in September when he left Oak Ridge for California. By that time he was probably coming to appreciate the force of Delbrück's comment that he might get beautiful curves but that they would not be as easy to interpret as he expected. He had, however, during these first months on his first research venture into phage biology obtained results of sufficient interest to justify his intention to go on with the project at Caltech. He had been able to explain some of Luria and Latarjet's anomalous results and had devised a significant improvement in the technique for their experiment that offered the prospect of obtaining more rigorous data in future experiments. He must have packed for the long westward journey with some sense of satisfaction in his decision to enter this field.

The Benzer family took two weeks to drive from Tennessee to California. In New Orleans they visited one of Benzer's mathematician friends, who showed them the city's night life. They stopped to see other friends in Houston, made forays into Mexico, and visited the Carlsbad Caverns and the Grand Canyon. It was, he wrote shortly afterward, a "wonderful experience." In Pasadena he rented a six-room house two blocks from Caltech for what seemed to him the unbelievably low rent of $100 per month. Dotty found a job at the local hospital, and her father came along to care for Barbie. Benzer was no less enthusiastic about his new surroundings than about the trip that had taken him there. Two months after settling in, he reported to Lark-Horovitz:

> Caltech is a terriific place. The campus is surprisingly tiny, and there never seem to be many people around, but actually they have collected the best workers in many fields and everything is on a high level of competence and maturity. There are many points of difference from Oak Ridge and I feel the change was advantageous in every respect. Several people (including Hollaender, who was here on a short visit recently) have said that the Biology Dept. here is the best in the country and I find this easy to believe.
>
> Delbrück's biophysics group consists mainly of renegades: 3 physicists, a physical chemist, two M.D.s. and a bacteriologist. It is quite international: German (Delbrück, [Wolfhard] Weidel, Stent), French ([Elie] Wollman), Swiss (Weigle), Italian (Dulbecco), and American (me). My lab roommate is Jean J. Weigle, who recently retired as head of the Physics Dept. at Geneva and is now having himself a very good time observing phage in the dark field microscope. He has already succeeded in demonstrating quite beautifully the emergence of phage in a burst of individual particles and one can see them diffusing apart.
>
> Everyone, including Delbrück, has been most friendly and stimulating, and all are busy and producing good work. [George] Beadle, the head of the department, is really a phenomenon—a first class biologist, a friendly and informal person and a fine administrator.[74]

The only "sour note" Benzer could think of was the climate, which included the smog for which the Pasadena area had become notorious. Even taking into account his need to reassure Lark-Horovitz that he was using his extended leave of absence to best advantage, there is no reason to doubt that Benzer was genuinely delighted with what he found at Caltech. That he was among "renegades" from other fields would have made him feel very much at home, because he felt himself to be in that category. His response to the atmosphere and personalities in Delbrück's phage group and at Caltech in general was similar to those of other young converts to the field who went there in those heady years.

It is not surprising that Benzer should single out Weigle's observations of phage in the dark-field microscope for special mention. For three decades the reproductive cycle of phage had been a series of abstract events, defined indirectly from the traces of their actions on other organisms, including in particular the plaque morphologies, which represented only the characteristic patterns left behind by the way in which they killed bacteria trapped in agar under specified

experimental conditions. Although the electron microscope had provided images of phage particles (see fig. 2.1), these were frozen, dried specimens. Actually to see phage during a phase of their cycle was, therefore, an experience of considerable drama. Being smaller than the resolving power of the light microscope, phages could obviously not "show their shape in the dark field, but appear as luminous points, moving at random according to their Brownian movement." Weigle was able to follow the whole burst phenomenon. "It is of an explosive nature," he reported, "lasting less than a second during which the phage seem to be expelled at a rate apparently larger than diffusion would lead one to expect. The bacterium may swell and take a spherical shape before bursting, or it may simply open up. There is a good possibility that the number of liberated particles may be counted."[75]

At Caltech Benzer continued to build on the promising start he had made at Oak Ridge. The method of mixing phage with bacteria suspended in buffer that he had originally devised to study the effects of multiplicity on the survival curves immediately after adsorption, he now realized, could be extended to improve the resolution of the original Luria-Latarjet experiment. Because the phage were not adsorbed into all the bacteria in a suspension at once, and they developed so rapidly within the individual bacteria that the resistance to ultraviolet radiation changed "from minute to minute," any given sample removed for irradiation and plating would contain phages at different stages of development. By adding the phage to bacteria under conditions in which they did not develop, however, he could allow ample time for a large fraction of the phage to be adsorbed. Then by adding nutrient broth to the medium he would cause the development to begin simultaneously in all the infected cells. The method brought the further advantage that it was no longer necessary go through the usual procedure of adding antiserum to kill the unadsorbed phage.[76]

Further benefits flowed from these changes. Whereas Benzer had formerly used synthetic media because natural nutrient broth absorbed some of the ultraviolet light, he could now use the latter, which gave more reproducible results, and could transfer samples to synthetic medium only when he was ready to irradiate them. Finally, he found a way to overcome another problem due to the rapidity of the growth of the phage. It took a minute or more to irradiate the samples with the required doses of ultraviolet light, during which the resistance of the population of infected cells was changing rapidly, so that the whole dose could not be regarded as administered at the same stage

of the latent period. To ameliorate this problem, he placed the sample to be irradiated in a shallow watch glass chilled from below by ice in a small dish. Under these conditions the resistance remained constant over the period of irradiation. He could remove samples at smaller time intervals than before, quickly chill them, and then take as much time as necessary to irradiate and plate them. The greater precision of the new procedures enabled him to establish survival curves from a single experiment, whereas it had previously been necessary to obtain averages from many experiments.[77] With these technical advances Benzer had essentially overcome the difficulties he had encountered during the previous spring and summer, when everything was "happening too fast." The technique of the Luria-Latarjet experiment had, he wrote Hollaender in January, "been improved to the point where it is possible to work unhurried and quite precisely."[78]

In addition to obtaining more accurate results, Benzer was able to establish six points for each survival curve, whereas Luria and Latarjet had provided only three. The greater range and resolution of results Benzer had attained did not, however, fundamentally change the situation. "The experiment of L. and L. with phage T2 have been repeated with these changes," he wrote in his progress report for December, "and the general trend of the curves confirmed. There are differences in the exact shapes which can be explained by the spread in adsorption and irradiation times in the former experiments."[79]

Thus far Benzer had proved himself a capable phage investigator, able to perform experiments of a quality high enough to improve on the results of the leading figures of the field, but he had not come any closer to the original goal of the Luria-Latarjet experiment. To confirm the general trend of their curves was to reaffirm that with T2 they did not approximate theoretical multiple-hit curves and consequently that target theory could not be directly used to determine the number of phage particles existing at successive stages in the latent period. Benzer looked around, therefore, for other uses to which his refinements in technique could be put. One possibility he saw in December 1949 was that the rapid increase in resistance that he had observed over the course of the latent period was itself an indicator of the development of the phage. In his progress report he wrote:

> After seven minutes in the broth at 37°C, the survival becomes 40 times the initial value. Within this time, [Gus] Doermann, by bursting open infected bacteria, was unable to detect any complete

> phages. Therefore, the radiation tests affords an extremely sensitive index to the progress of intracellular development prior to the appearance of infective particles. . . .
>
> If the logarithm of the survival value is plotted as ordinate as a function of time between addition of broth and irradiation, the curve obtained is approximately linear, after a lag of about one minute.[80]

The "progress of intracellular development" did not necessarily correspond, however, to growth in the numbers of (incomplete) phage particles, and Benzer was not proposing a substitute for Luria and Latarjet's original hope that target theory could provide the latter information. Rather, he saw an opportunity to examine the nature of the nutritive requirements for intracellular development, whatever its nature, to take place:

> It now becomes possible, in principle, using ultraviolet radiation as an analytical tool, to determine the nature of the chemical requirements for synthesis of phage. If infected bacteria are suspended in buffer, there is no change in survival, whereas if all necessary nutrients are supplied there is a rapid linear change. By starting with buffer and adding well-defined nutrients, singly and in combination, one can readily distinguish three cases:
>
> If there is no change in survival value, the nutrients added are not utilizable. If the survival value immediately increases, the compounds are completely adequate in the form supplied. If the survival value begins to increase only after a lag time, then there must be some adaptive enzymes required to utilize the compounds, or they must first be chemically transformed into usable forms. By transferring samples from broth to buffer at intermediate stages, and then adding selected compounds, one could also ascertain whether the nutrient requirements changed over the course of the latent period.[81]

Although he continued to pursue his own research program as he had defined it at Oak Ridge and habitually worked alone, a practice reinforced by his habit of coming into the lab in the early afternoon and working far into the night, Benzer also benefited from his association at Caltech with others who were studying closely related problems. In particular, Renato Dulbecco, who had come to Pasadena with Luria and others of his group for the summer and had been persuaded by Delbrück to stay on as a senior research fellow, was completing his detailed analysis of the phenomena of multiplicity and photoreactiva-

tion. In collaboration with Dulbecco, Benzer continued the experiments on photoreactivation that Dulbecco's discovery of that phenomenon had prompted him to begin the previous spring. Elie Wollman and Gunther Stent were studying the activation of phage by co-factors, an activity that might have encouraged Benzer to think about the chemical requirements for phage development. Wolfhard Weidel was studying an extract containing a high-polymer molecule that inhibited phage development, and Delbrück himself had a project, on which he was not making significant progress, concerning "the phage particles liberated by mixedly infected bacteria displaying transiently characters of both parental phages."[82]

It was Delbrück that Benzer soon found the "most inspiring person to be with." This was "not immediately apparent on short acquaintance," he wrote Lark-Horowitz, "and many people have formed completely distorted opinions of him, but we have begun to understand and share the devotion which the phage people feel toward him." Benzer agreed with Jim Watson's opinion that Max, as everyone near him called him, was "the best there is in phage."[83] People who met Delbrück were seldom neutral about the experience. Some who encountered him only briefly were repelled by what appeared to them to be his arrogant dismissal of views that he questioned. Those who stayed around understood his abrupt criticisms to be a way to challenge those he respected to overcome his objections if they could. A few, like the impulsive Gunther Stent, quickly came to idolize and try to emulate him. The more reticent Benzer learned to admire him without submerging his autonomy in Delbrück's charismatic presence.

There was much more than the work to inspire Benzer in his new surroundings. Participating in the famous camping trips on which Delbrück frequently led his group, he experienced within one hour the contrast between throwing snowballs on Mount Palomar and camping in the desert, and he learned how hard a sleeping bag in the sand can be. When Dulbecco's car got stuck in the sand and Delbrück's car also bogged down trying to haul him out, they had to hike three miles across open desert to a ranch to get help. In November Benzer hiked with other members of the group to the bottom of the Grand Canyon and back to the rim, a round trip of eighteen miles, all in the same day. Wearing a pair of boots that had not been broken in, and never before having climbed much more than two flights of stairs at a time, he emerged totally exhausted and limped for three days afterward. Nevertheless, he thought it a "wonderful" trip, which left a "beautiful

memory." There were also cultural pleasures close at hand in Pasadena. "The concerts, plays, and foreign movies here are naturally overwhelming to one who has come from Oak Ridge," he wrote in November, "and it has been a little hard to keep from going to everything." He especially enjoyed seeing *Don Giovanni* at the San Francisco Opera. He also had opportunities to revive his own skills on the violin. Together with Dulbecco, who played the piano "fairly well," Benzer began playing Handel sonatas. "He is very patient," Benzer reported, "and it has been so much fun that I have taken to practicing with some seriousness."[84]

When he went to Caltech Benzer thought that he would go to Europe the following year. By November, however, Delbrück and Dulbecco had convinced him that he had "more to gain by staying here." By the end of December he was informing Lark-Horovitz and Hollaender that "we are all agreed (Delbrück, Dulbecco, Weigle, and I) that it is essential that I stay here for an additional year, so I plan to apply for other fellowships also and you will undoubtedly be asked for further letters."[85]

In March 1950 Delbrück hosted a conference on viruses to which leading plant, animal, and phage virologists came. After the papers had been presented and discussed in formal sessions, some of the participants continued talking together for three more days "around camp fires in Death Valley." Benzer and the other local phage biologists put together for the occasion the "Syllabus on Procedures, Facts, and Interpretations in Phage," which summarized the methods and lore necessary to do phage research.[86]

Benzer did not pursue his plan to use his improved Luria-Latarjet experiment to examine the nutritive requirements for the synthesis of phage during his first year at Caltech. Was he, perhaps, discouraged from doing so by Delbrück, who regarded this plan as a foray into the "biochemical requirements" for phage growth?[87] According to Benzer's later recollection, "Delbrück deprecated biochemistry, and this influenced some of us to avoid it."[88] During the early 1940s, as we have seen, Delbrück had strongly advocated just such biochemical studies, and it remains an unsolved riddle whether he had completely reversed his attitude by the time Benzer arrived in 1949 or whether the younger scientists who came into his presence mistook some of his typically caustic personal comments about biochemists for a fixed opinion about the field as a whole.

Benzer instead applied his method during the spring of 1950 to

studying the effect of temperature on the development of phage. His shift may have been stimulated or at least reinforced by a letter Delbrück received from Ole Maaløe describing the details of experiments Maaløe had been conducting in Copenhagen on temperature in phage growth. In May Benzer wrote, "Max has suggested that I write you, since some of our experiments, though quite different in kind, are closely related in purpose. We may be able to lend aid and comfort to each other if it is possible to reach similar conclusions by independent means." He pointed out that some difficulties that Maaløe had encountered concerning the length of the latent period and the long adsorption time could be lessened by trying his method of adsorbing the phage while the bacteria are in buffer. "The type of experiment I have been doing, which takes advantage of the Luria-Latarjet discovery," he added, "lends itself readily to studies of the effect of temperature, and I would like to describe some preliminary results."[89]

Benzer described the procedures he had been following, in which he compared the increase in resistance to ultraviolet radiation across the latent period at 20°C with that at 37°C. To accommodate the rapidly increasing resistance over this period, he applied two different standard radiation doses to each of the samples that he took. The change in survival differed at the two temperatures "throughout by a factor of 5 or 6 in time." He commented, "In both our lines of experiment we are assuming a fixed chain of events during the latent period. Your method assumes that the sequence of events is unaffected by temperature changes, although their rates may be altered. My method assumes that the radiation resistance is a proper index to the point reached in the chain at a given time. If we come out with the same answers on temperature dependence of different parts of the chain, it will be very encouraging."[90]

Benzer probably shared the assumption of a fixed chain of events in the synthesis of phage during the latent period with most phage biologists, although no one yet knew what these events were. The assumption seemed to be based not on any specific knowledge of this still dark stage in phage reproduction but on the more fundamental and tacitly teleological biological generalization that all organisms are the products of developmental processes that follow one another in a predetermined order. For Benzer the assumption was critical, however, because he now wished to employ the more precise version of the Luria-Latarjet experiment that he had developed for a variety of purposes in which "the large changes in radiation resistance in T2 with time make

it possible to determine readily the stage in intracellular development (in terms of minutes of growth at 37°C, say) which has been reached at a given time under a given set of conditions."[91] Determining in this way a "stage in intracellular development" was only meaningful on the assumption that the development consisted of something like a "fixed chain of events."

During the first half of 1950 Benzer exploited this method mainly to continue his investigation of the effects of temperature, extending the experiments to a range from 0° to 45°C. At the low end there was "no progress evident" after two hours; at 10°C the rate was about one-thirtieth that at 37°C. At 45°C he expected no development, because Luria had sent him a manuscript titled "A Preliminary Study of the Effect of Temperatures Above Optimum on the Growth of Bacterial Viruses" which stated that that no viruses are liberated from infected cells at temperatures above 43°C and that "the growth of virus is completely suppressed."[92] To his surprise Benzer found that, according to the changes in resistance, the phage started to grow at the same rate as at 37°C but then stopped at a point corresponding to 7.5 minutes' growth at 37°C.[93] Implicit in his assumptions was that the growth stopped at just that point because some particular step in the imagined chain of events could not take place at the elevated temperature.

Showing his growing virtuosity as a phage experimentalist, Benzer worked out a method for performing a Luria-Latarjet experiment on a "bacterium infected with exactly two similar phage particles." He did so by running two identical experiments, in one of which the ratio of phage to bacteria was so low that a negligible number of infective centers contained more than one phage. In the other a multiplicity of 0.1 resulted in about 5 percent of infective centers with two phage and a negligible amount with three. By subtracting a survival curve derived from the first experiment from the equivalent curve derived from the second, he could obtain a survival curve representing doubly infected bacteria. Such results, he thought, would eventually "be useful in considering the relation between multiplicity reactivation and the changes in resistance early in the latent period."[94]

Applying the same method to phage irradiated with ultraviolet light of such a dose that neither particle was active in itself but that two together could produce multiplicity reactivation, Benzer found that the "radiation resistance of such an infective center changes with time, there being a definite change within the first two minutes. However, the further development proceeds more slowly than in the case of

infection with a single unirradiated particle."[95] This experiment was perhaps particularly promising because by reducing the phenomenon of multiplicity to its simplest form, in which only two particles could combine to overcome the defects that made each of them inactive alone, it could perhaps lead toward an understanding of the mechanism. Unless some way were found to identify the unknown chain of events that multiplicity reactivation could restore, however, Benzer's results were still merely increasingly beautiful curves.

During the same period Benzer also pursued his experiments on the phages that did not display multiplicity reactivation. By the middle of June he could report: "A complete series of curves has been obtained at intervals of one minute during the latent period (12 minutes) for T7–infected cells. For the first three minutes, the survival curve remains exponential, with the same slope as for the inactivation of free phage. From four minutes on, there is a shift with time toward curves corresponding to continually increasing multiplicity of hit, with little change in ultimate slope. This is the result originally anticipated by L[uria] and L[atarjet] and suggests that the anomalous behavior of T2 may indeed be related to M[ultiplicity R[eactivation]." Planning to "study the other phages in the same manner," Benzer did some preliminary experiments on T4 in which he found that it "shows MR" but to a much less degree than did T2.[96]

Benzer had now met his original goal of explaining the anomalous results of Luria and Latarjet through reactivation phenomena, but he showed curiously little interest in his achievement. From the increasing multiplicity of the curves he drew no inferences about increasing numbers of phage particles in T7 over the course of the latent period. The center of his attention had shifted to a new opportunity that had arisen unexpectedly during the work. The anomalous results that had once been obstacles to overcome now provided him with a new "tool" to use in the study of phage development.[97] The increasing resistance over time that had once been only puzzling was now his index to something else that was happening along a chain of events.

In thinking about what those events might be, Benzer was strongly influenced by a "model" that Dulbecco was proposing in order to explain the results of his analyses of multiplicity and photoreactivation. In September 1950 Delbrück presented at Cold Spring Harbor a joint paper by Dulbecco and Benzer on ultraviolet experiments on phages that was afterward printed in the informal *Phage Information Service,* a mimeographed document that Delbrück circulated among phage

workers periodically to keep them informed of one others' activities. The paper described Dulbecco's and Benzer's experiments as well as some that they may have carried out together. "As a means of interpreting the foregoing results," the paper concluded, "the following picture is suggested."

> The UV sensitive cross-section of the large phages (e.g. T2) consists of two sectors. One sector is connected with preliminary steps in the latent period; as intracellular development proceeds, those steps that have already passed are no longer susceptible to inhibition by UV and the effect[ive] cross section decreases. This sector can therefore be considered to be connected with the preparation of the bacterial environment for phage reproduction and be called "dispensable." The other sector, whose damage is still inactivating at the moment of the highest UV resistance, should be more directly related with the processes of phage multiplication; it should represent the "core" of the particle, which establishes the genetic continuity and undergoes replication. In the last part of the latent period, adequate dispensable parts would be built again on the newly reproduced cores.[98]

The paper went on to suggest explanations for the phenomena of photo- and multiple reactivation based on this "picture." Although Dulbecco initiated these ideas, they were undoubtedly reinforced and developed by discussions among Dulbecco, Benzer, and Delbrück in the lively atmosphere at Caltech or on the desert expeditions in which they all took part. By the fall of 1950 Weidel and Stent had left, so the three of them, along with Jean Weigle and a graduate student named Bowen, for a time constituted Delbrück's entire group. It appears that all three were enthusiastic about the application of the model to their various research results.

In June and July 1950 Benzer took time out from his work at Caltech to take the course on general microbiology taught by C. B. van Niel at Pacific Grove that he had hoped to attend the previous year. Van Niel's lectures were famous, and Benzer found them as enjoyable as their reputation had led him to expect. The students also did experiments every day, often on bacteria fished from the ocean. Van Niel set up the experiments to illustrate the problems he discussed in lectures, often providing trick problems for them to solve. In one they were given a bacterial colony to purify. When they attempted to do so, they always found two very different types of bacteria under their microscopes. No matter how often they repeated the purification, the same thing hap-

pened. It turned out that the two bacterial types were symbiotic. Some of their observations, such as the growth of luminescent bacteria on a dead fish, were spectacular. Altogether Benzer greatly broadened his knowledge of the practical problems of culturing and performing experiments with bacteria.[99]

Van Niel began his talks with what Benzer called a "beautiful lecture on bacterial variation, taking it up from the historical perspective."[100] In considerable detail, and with deep insight, van Niel followed the long history of the debate between those who believed that bacteria have extraordinary variability, exhibiting everywhere pleomorphisms, and those who maintained that if one achieved pure cultures and well-controlled conditions one would find that bacteria behave like other monomorphic organisms. Using the example of the decomposition of cellulose, he traced the changing views about the degree of specificity of the substrates on which microorganisms can grow. Broadening his discussion to the "general topic of the biochemistry of microorganisms," he traced the history of fermentation from the fundamental work of Antoine Lavoisier at the end of the eighteenth century through the investigations of the chemical nature of the reaction into the twentieth century, treating in a balanced way the debates between Pasteur and the organic chemists about whether organisms are required for fermentation.[101]

Many of the rest of van Niel's lectures dealt with the extension of Pasteur's concept of the physiological importance of fermentation by Winogradsky and others to organisms such as the sulfur bacteria that led to a "new concept of metabolism." Such organisms were found to be able to derive energy from the oxidation of inorganic compounds. In his notes about these lectures Benzer wrote, "At this point vN repeated his famous statement, 'Any reaction which liberates energy can be used by some sort of an organism as an energy supply.' "[102]

In his lecture on growth factors, van Niel described work by the French protozoologist André Lwoff on the catalytic effect of protoporphyrin on the growth of microorganisms. To test the effects of this compound, which he thought might act in the manner of the iron porphyrin compounds of Otto Warburg, Lwoff "went on to perform a series of experiments which are still models."[103]

Benzer typed out a lucid thirty-page summary of van Niel's lectures. The care with which he did so suggests not only his generally meticulous approach but also the great interest with which he had heard the lectures. Having taken no general courses in biology prior to the

summer school at Cold Spring Harbor, which had focused entirely on the life cycle of bacteriophage, he was beginning to make up for the narrowness of his knowledge of the field. Moreover, he was exposed to a type of biology that contrasted strongly with the ethos of the phage group. Whereas they treated bacteria mainly as vehicles for the investigation of bacteriophage, van Niel treated them as fascinating organisms in their own right. Whereas they sought a few standardized organisms on which they could perform quantitatively replicable experiments, he reveled in the great range of variation among the forms, growth cycles, and metabolism of different types of microorganisms. Whereas they often dismissed biochemistry as dull, he focused on the metabolic biochemistry of microorganisms as the center of concern. Whereas they viewed themselves as making a fresh start, with a new style and a break from the past, van Niel saw the present as the continuation of a long historical development that had begun a century and a half earlier, the later stages of which he had lived through. From van Niel Benzer acquired a vision of biology that did not conflict with that of the phage group but could expand his conceptual horizon beyond that of their narrowly focused concerns. A year earlier Benzer had chosen to go to California to obtain a "broader outlook from working with Delbrück and van Niel." Although Delbrück's was the more pervasive influence, van Niel's course was also a great experience for him, and the contrasts between them may have helped nurture in Benzer an outlook less dependent on either of their individual perspectives.

Just as at Cold Spring Harbor, there were many pleasant diversions accompanying the serious work. The Pacific Grove Laboratory was situated next to the Monterey Bay Aquarium, right on the rocky southern California coast. The students regularly ate lunch sitting on the rocks while watching the surf and the cavorting sea lions. At the end of the course there was a lively party, including skits. Benzer impersonated, with appropriate exaggerations, van Niel's demonstration of the way to sterilize an agar tube containing a bacterial culture. The technique required one to push a cotton plug into the tube while rotating the tube in a Bunsen flame, wait until the protruding end of the plug caught fire, and then quickly blow it out.[104]

It may have been the influence of van Niel's concentration on metabolism that encouraged Benzer, after his return to Caltech in September, to implement the plan he had laid out before leaving Oak Ridge to examine the effects of nutritive requirements on the development of phage. In the first of these experiments, probably performed in early

September, he tested the "effect of 5–F-tryptophane on standard experiment of T2r." During the next three months he examined the growth of bacteria in glucose and lactose media and in media "*carencée,*" that is, with progressive dilutions of these nutrient factors. He began to carry out Luria-Latarjet experiments in such media, but he did not include the results of such experiments in his semiannual reports, and it is not evident that he drew any conclusions from them.[105]

In September 1950 André Lwoff came to Caltech for two weeks. Two years earlier he had taken up the investigation of the phenomenon known as lysogeny, in which certain bacteriophage could infect some strains of bacteria and remain within them for generations without destroying them or reproducing themselves. It was first discovered between 1921 and 1925, and its nature and significance had long remained in doubt. In 1946 Delbrück had made the decision to exclude lysogeny from experimental consideration by working only with the virulent T-series of bacteriophage, but at the Institut Pasteur lysogeny remained a major object of investigation. When Lwoff found in 1950 that he could induce lysogenic bacteria to lyse and liberate their phage by irradiating them with ultraviolet light, he quickly converted the skeptics at Caltech.[106] During the summer of 1950 Weigle began to investigate lysogeny in *E. coli* strain K12 in Geneva and continued to do so after returning to Caltech. Lwoff's visit gave a "powerful stimulation" to the interest of the Caltech group in lysogeny, and Delbrück joined Weigle in its study. Two months after the visit, Delbrück wrote in his semiannual report that "the phenomenon of lysogeny, long known to occur in many bacterial strains, has been put on a firm foundation by the splendid work of A. Lwoff and his collaborators." Lwoff had established the "basic facts" that every individual cell of a lysogenic bacterial strain has the potential to liberate phage but that under normal growth conditions only a small proportion of them do so. When they do so, it is by means of an explosive lysis. If they are irradiated by ultraviolet light, a large proportion of them liberate phage by lysis about sixty minutes later, after dividing twice. "To describe these phenomena in a coherent manner," Delbrück went on, "Lwoff has proposed the idea that each individual bacterium of a lysogenic strain carries one or more prophage particles which are propagated with the bacterium like any other sub-cellular unit endowed with genetic continuity; i.e., like a gene or a plastid. The prophage is a nonpathogenic, 'masked' form of the virus." Lwoff's work was of "extraordinary importance," according to Delbrück, because it showed that the alternation

between masked and virulent forms was a universal feature of the life cycle of viruses and that the masked form played a major role in their ecology and evolution. The discovery that ultraviolet radiation can induce lysogenous viruses to lyse was a "tremendous technical advance" because it had transformed a "hitherto highly elusive phenomenon into one open for convenient study."[107]

During his stay at Caltech, Lwoff took part in one of the phage group's camping trips. The participants pitched their tents halfway up Mount San Jacinto and began hiking toward the ten-thousand-foot-high summit. Lwoff and Benzer's wife Dotty tired after a while, however, and decided to turn back, but they wandered off the trail and became lost in the woods. Finally they were rescued by a group of hunters who were annoyed that they had frightened the deer away but nevertheless drove them back. Along the way Dotty mentioned to Lwoff that her husband would very much like to come to Paris but didn't have the courage to ask him about it. When the rest of the group returned from the top of the mountain, Lwoff talked with Benzer about his research using ultraviolet radiation and told him he would see whether he could find space for him to work in his laboratory at the Institut Pasteur.[108] Benzer thought that the space problem was just a polite way to avoid making a commitment, but after Lwoff returned to Paris he wrote to issue a formal invitation:

> I have been very interested by your work on the action of ultraviolet rays on bacteriophage. As you know, our group has been engaged in the study of the inducing effect of ultraviolet on bacteriophage production. In the opinion of many friends—and especially of Dr. Max Delbrück, and also in my opinion, the problem could be attacked with an increased chance of success with your cooperation.
>
> If you would be willing to come to Paris I would offer you the hospitality of my department for a year, say Sept. 51 until Sept. 52.[109]

Benzer needed no persuasion to go to Paris to spend time in one of the leading international centers of the field. The invitation was also a measure of how quickly the newcomer to phage research had impressed its leaders with the quality of his research.

On October 5 Benzer wrote Lark-Horovitz to seek yet another extension of his leave from Purdue: "Dr. Andre Lwoff of the Pasteur Institute has written me the letter of which I enclose a copy. This invitation to spend a year with him in Paris is a very valuable opportunity and I

want to consider it seriously. He is one of the outstanding microbiologists of today, and his recent discoveries are of fundamental importance to all of biology. I feel quite honored by his asking me." Briefly explaining lysogeny and Lwoff's discovery that ultraviolet light can upset the equilibrium, causing the prophage to lyse its bacterial host, Benzer added that "this discovery is important because it indicates a close relation between the phage and the genetic material of the bacteria, suggests the possible origin of phages, and promises to clear up certain mysterious problems in microbiology. The type of experiment I have been doing can be directly applied to Lwoff's problem, and that is his reason for asking me." Aware that he might be pressing his absence from Purdue beyond the limits of patience, Benzer raised the question of another year away cautiously: "Therefore, I wish to apply for still further extension of leave of absence, if this can possibly be arranged with Purdue, since I certainly intend to return if I still can after all this time. It is obvious to me now that I could not have been ready after just a year or two in a new field to assume the job we considered. You seem to have realized this from the beginning and I hope you will be sympathetic to the idea of an additional year of experience abroad."[110]

To let him know what he had been doing at Caltech, Benzer sent Lark-Horovitz a copy of the joint paper with Dulbecco that Delbrück had recently presented. Whether Benzer really needed an additional year to be prepared to teach and work in biophysics at Purdue or whether his enthusiasm for working at the Institut Pasteur for a year only induced him to think so, his diplomatic presentation of the case as being in his and the school's mutual interests had the desired effect. Lark-Horovitz wrote back on November 2, after a trip to Oak Ridge, to say that "both Arnold and Hollaender have expressed their satisfaction that you got the invitation to Paris. They both say that of course you should accept it. Let me know whether this fellowship is going through and if it is perhaps you can also let us know what problems you are going to work on."[111] There was, in other words, a shared belief within the scientific circle in which Benzer worked that a pilgrimage to the Institut Pasteur was an important milestone along the way to maturity as a phage biologist. Purdue generously extended his leave of absence into its fourth year, and the Committee on Growth of the National Academy of Science granted an extension of the fellowship that had supported his second year at Caltech.[112]

Undoubtedly Benzer owed the ready approval of his plans in part to the strong endorsement he received from Delbrück, who wrote

in support of his application to renew his fellowship that his research constituted a "new approach to the study of intra-cellular phage growth" and that the results Benzer had obtained at Caltech were "very valuable." Benzer, he wrote, "is imaginative in his approach to biological problems and in the conception and design of experiments. He is painstaking in the execution of the experiments and careful and critical in their evaluation." He had also "taken every opportunity to widen his background in biology and acquaint himself with other lines of research going on at the Institute."[113]

The sociologist Robert Merton has given a persuasive account of the "cumulative advantage" that can accrue to a talented young person who has attained the support of well-placed seniors in his field at influential institutions. When his sponsors assert that the person has the promise to become outstanding, their recommendations have sufficient force to enable the individual to acquire "successively enlarged opportunities to advance his work," including the "comparatively large resources" that leading institutions can offer, and the time necessary to build toward future success. These advantages can come in the absence of extraordinary completed achievements if his supporters can attest to his "quality of mind as encountered at close range."[114] By late 1950 Benzer had acquired such a position in phage biology. He had yet to publish a paper in the field or to discover anything outstanding. Nevertheless, three of the leading figures in the field had singled him out for the potential they could see in the quality of his work, and their backing assured him of further opportunities to develop that potential, opportunities that might not be open to others of equal talent who happened not to have made such powerful connections so early in their trajectories. These opportunities in turn enabled him to develop his talents in an unhurried manner, without the pressure that less highly favored young investigators often feel to reach quickly for a discovery or a claim dramatic enough to attract attention. In his application for a fellowship to support his year in Paris, Benzer explained the close relation he saw between Lwoff's studies of lysogeny and his own current research. Summarizing Lwoff's discovery of the effects of ultraviolet radiation on lysogenic bacteria and his interpretation that the phage exists in lysogenic strains in the form of a "prophage," Benzer concluded, "This postulated pro-phage condition is analogous to the state of an ordinary phage particle during the dark period of its development." At present, he went on, "I am studying ordinary phage during this dark period, using the approach originated by Luria and

Latarjet." Giving a brief account of this approach, he then proposed to "collaborate with Dr. Lwoff in applying to the case of the lysogenic bacteria the technique I have been using in studying the development of ordinary phage. By using Lwoff's method for inducing phage production, the sequence of events leading from prophage to infective phage can be initiated. From that point on, the analysis of resistance to ultraviolet radiation can be used in the same way as for ordinary phage to analyze the successive development and study the effect of environmental factors upon the individual effects."[115]

If we compare Lwoff's and Benzer's descriptions of the intended purpose of the latter's stay in Paris, it is striking that each saw it as the continuation of his own work. To Lwoff, Benzer's cooperation would increase the chance of success in the study in which his group was engaged to understand how ultraviolet light induces a lysogenic bacterium to liberate its phage. Benzer saw the same collaboration as an extension of his study of the chain of events that take place in the change from ordinary to lysogenic phage. That is how successful research interactions are often initiated.

During the fall of 1950, Benzer pursued mainly the application of his improvement to the Luria-Latarjet experiment to the study of the latent period in bacteria infected with only two identical phage particles, using the method he had developed earlier in the year to subtract curves from an experiment in which the bacteria contained only one particle from curves obtained in otherwise identical experiments in which the bacteria contained either one or two particles. By December he had obtained survival curves for both "dicomplexes" and "monocomplexes" at "various times during the latent period" and was ready, in his semiannual progress report, to draw some conclusions from their respective characters. "At time zero . . . the survival curve for a *monocomplex* is approximately exponential and has the shape characteristic of a free phage. When broth is added, development starts. The curve retains its 'one-hit' character, but the resistance increases with time, reaching a value about ten times the original at a time halfway through the latent period. The shape of the survival curve for small doses remains at this value until almost the end of the latent period: the shape of the curve at higher UV doses indicates that part of the population requires two hits for inactivation." Benzer noted that these results for the second half of the curve differed from those of Luria and Latarjet and claimed that the improvements he had made in the accuracy of the experiment made his results more reliable. "For *dicomplexes,*" he went on,

> the curve is of a multiple-hit character, but the survival is much higher than the two hit curve to be expected if the two phage particles were independent. This is the phenomenon of "multiplicity reactivation" of Luria and Dulbecco. . . . Our curve for dicomplexes at time zero corresponds to their limiting curve for low multiplicity, where nearly all multiply infected bacteria are infected with exactly two particles.
>
> As the development of a dicomplex progresses, the survival curves obtained are always of a multiple-hit character. The resistance increases in time until seven minutes, at which time a maximum is reached. The curve of maximum resistance, when interpreted as a multiple-hit curve *has a multiplicity of two and a final slope similar to that of a monocomplex at the time of maximum resistance.*[116]

Although he was returning to the target theory to interpret his latest results, Benzer did not go back to Luria and Latarjet's original objective, which was to link the number of hits with the number of phage particles present at the various stages in the latent period. He showed himself instead to be increasingly oriented toward Dulbecco's model:

> This result is in accord with the new explanation of "multiplicity reactivation" proposed by Dulbecco and may be interpreted as follows. In a monocomplex, the small cross section for inactivation observed midway in the latent period corresponds to the "core" of the phage particle, the progressive decrease of cross section up to this time corresponding to the passing of steps (inhibitable by UV) concerned with the preparation of the system for phage reproduction. Since the initial slope becomes constant midway in the latent period, it would appear that in most cells the "core" does not multiply, as such, to produce other cores with the same properties. The appearance of a few mature phage particles would not be reflected in the survival curves, since a complete phage is much less resistant to UV than a core. In the case where there are two infecting particles, two "cores" are observed after the preliminary steps are passed.[117]

In this abbreviated discussion, which did not attempt to justify his interpreting his results in terms of this model rather than some other picture of phage growth, Benzer was strongly influenced by his local environment. Working in close proximity to Dulbecco, who was at the same time building his model of phage as composed of an "indispensable" core and a "dispensable" part, Benzer fit his own work into that

context. In his general report on the activities of the phage group, Delbrück wrote that Benzer's results "lend themselves readily to an interpretation in terms of Dulbecco's model."[118] Much more of the report was given over to the evidence that Dulbecco had amassed from his studies of multiplicity and photoreactivation to support his model. As the most junior member of this trio in terms of phage research, Benzer was not yet ready to launch himself along independent theoretical pathways but still played a supporting role in the elaboration and experimental exploration of theories devised by his more experienced colleagues.

When Luria saw Benzer's report, which stated that his results for the second half of the phage survival curves differed from those he himself had obtained with Latarjet, he wrote to ask Benzer about his procedures, suggesting among other things that dirty glassware might have been involved. After comparing his results again with those of Luria and Latarjet, Benzer replied on February 12, 1951: "On closer examination of your paper in J. Bact., there seems to be very little difference in our data. The misunderstanding has come, I think, from the fact that we were looking for different things. You were looking for survival curve shapes corresponding to multiple targets with the sensitivity of a phage and I was looking at the 'core.' If you examine your Fig. 3b, you will see that the *initial slopes* of the 12 through 18 minute curves are pretty constant. A true multiple hit curve should have an initial slope of zero." After summarizing his procedures, Benzer reported that he had started working with synthetic media and would be able to compare the results with those obtained using broth. "My biggest news at the moment," he finished, "is that I got the fellowship to spend next year in Paris with Lwoff. I hope to be able to visit you and your group . . . on the way east next summer."[119]

As Benzer continued to do new experiments through the spring of 1951 while making preparations for travel, Delbrück became concerned lest Benzer escape from his watchful eye at Caltech without publishing anything on the work he had already done. By June, when Benzer had still not produced a paper and his departure was becoming imminent, Delbrück took steps to assure that this would not happen. As Benzer recollected in 1965:

> The urge to do experiments was so strong that we could not get ourselves to sit down and write up the results. Delbrück had a solution for this. He assembled all who had papers to write and

> whisked us off to Caltech's Marine Biological Station at Corona del Mar. There we were locked up for three days and ordered to write. Delbrück's wife, Manny, typed as rapidly as we could spew the stuff out; we mercilessly criticized each other's drafts, and in three days everyone had a completed paper. That was how my paper on UV irradiation of intracellular phage came to be written.[120]

That Benzer had preferred to keep performing experiments might well have reflected not merely the ethos in which he participated but also the fact that his results had not reached a natural point of closure. His use of the radiation resistance measured by the improved Luria-Latarjet experiment was still only a potentially significant indicator of what was happening during the dark period of phage development; it had yet to reveal anything directly about the chain of events involved. Perhaps Benzer delayed writing in part in the hope of making some further advance until Delbrück left him with no choice but to put together what he had for publication. The title of his paper—"Resistance to Ultraviolet Light as an Index to the Reproduction of Bacteriophage"—was a direct reflection of his understanding that what he had acquired after two and a half years of research was only a tool that "offers promise . . . for studying phage growth."[121]

Benzer began his paper, as he had begun all his progress reports at Oak Ridge and Caltech, with a description of the original Luria-Latarjet experiment. Going into considerable detail, he illustrated the theoretical survival curves from which Luria and Latarjet had hoped to "perform a target-theory determination of the number of intracellular phage particles" and discussed the deviations from these curves that had prevented them from doing so. Then he described in detail the improvements he had made in the method that enabled him to start and stop the growth in all cells simultaneously and to control the timing "to within a few seconds."[122]

In accordance with his correspondence with Luria in February, Benzer no longer claimed that his experiments with T2 and its mutant T2r, which had the same sensitivity to ultraviolet radiation, differed from those of Luria and Latarjet on T2. Instead, he wrote that the curves he had obtained "qualitatively confirm the observations of Luria and Latarjet with T2." His result with T7 stood, he wrote, "in marked contrast to T2r and resembles that predicted by target theory." The "exact shapes of the curves" were not consistent with an assumption that all of the complexes had equal multiplicities at a particular time, but the deviations could be explained by differences in the rate of multiplica-

tion of the phage in different bacterial cells. Once again, however, Benzer passed quickly over the achievement with T7 of the original goal of Luria and Latarjet's experiment on T2, remarking only that it "enables us to discern the existence of multiple intracellular entities at a time when fully infective phages are not yet detectable with the Doermann technique." Again he focused his attention on his belief that "the anomalous results with T2r may be of far greater interest." The "enormous changes" in the resistance to ultraviolet radiation in these experiments "may bear a close relation to the phenomenon of multiplicity reactivation. While the resistance changes do not tell us what is actually happening, they at least give us something easy to measure which serves as an empirical *index* to development."[123]

To have only an index to what was happening was not entirely satisfying, and Benzer allowed himself to speculate in print about what might actually be happening:

> A plausible interpretation of the increase in resistance of T2r complexes during the first half of the latent period may be the following: a T2r particle after adsorption to a sensitive bacterium must undergo a series of successive steps A B C D, etc. in the course of reproduction. Each of these steps has a certain cross section for being blocked by ultraviolet (e.g., by inactivation of an enzyme which is concerned with the step). Blockade of any one of these steps prevents normal development and causes inactivation of the phage. At time zero, the total cross section of the phage is therefore the sum of these individual cross sections, and the survival curve is exponential. As development proceeds, the steps which have already been passed are no longer needed, and the effective cross section decreases progressively while inactivation of the remaining steps retains the characteristics of a one-hit phenomenon.[124]

A comparison of this interpretation with the one Benzer had given in his progress report in December is instructive. From that earlier interpretation he retained only the view that a progressive decrease in cross-section can be attributed to the successive passing of steps that afterward are no longer vulnerable to ultraviolet radiation. The rest of the interpretation, previously fit into Dulbecco's model of a core and a dispensable part of the phage, Benzer had now dropped, along with his comparison of the results of experiments with dicomplexes and monocomplexes.

What had caused Benzer to withdraw from his earlier interpretation, which had been "in accord with" the Dulbecco model, and to

suppress discussion of his own experiments on singly and doubly infected bacteria? One clue to the latter may be that the fit of his curves for dicomplexes to "a theoretical curve for a double target," which had then led him to postulate a "double core," now appeared to be perhaps "fortuitous," the result of "slight inhomogeneities in the stage of development."[125] Had he come to recognize his former adoption of Dulbecco's model as an overly enthusiastic response to the views of his somewhat more senior colleague? Or had he merely wished to be more cautious in a formal publication than he had been in an informal progress report? In either case, he clearly retained vestiges of those views in a less specific form. His first publication in the field of phage biology was itself a report of work in progress. He must have hoped that his sensitive index would allow him to replace this proposed interpretation with a fuller account of the chain of events whose existence he confidently assumed.

On June 23, while his manuscript was being typed, Benzer wrote Luria that he would send him a copy as soon as it was ready. "You know from Max, of course, in respect to my results which no longer disagree with yours."[126] To Lark-Horovitz he also wrote on the same day that he would send the manuscript, "which you may consider as a progress report, and would appreciate any comments you might have." About his current situation, he wrote: "As you can imagine, I am now feverishly occupied with working (just a *few* more experiments), writing papers, packing, learning French, and running to the mailbox to see if the passport has arrived."[127]

During the three years that Benzer spent at Oak Ridge and at Caltech, he was able to embark on the early stages of what began to look increasingly like a distinctive individual research pathway. Initiated into phage research at Cold Spring Harbor, he then identified a problem that he could work on by reflecting on something that he thought two of the leading investigators in the field had overlooked in the design and interpretation of an experiment that seemed to have considerable unfulfilled promise to reveal details of the mysterious dark period in the development of bacteriophage. He quickly learned from Delbrück that it was not a matter of Luria and Delbrück's missing something but of developments that had taken place after they had published their experiment. Believing at first that he could within several months examine the effects of multiplicity reactivation on the experiment and write up his results, Benzer soon had to change directions to take into

account the newly discovered factor of photoreactivation. After discounting photoreactivation as a factor, he returned to his original direction. A technical improvement that he devised for the special purpose of studying the effects of multiplicity immediately after infection turned out to be more broadly applicable to the performance of the whole Luria-Latarjet experiment. After he had made a second simple but crucial change that enabled him to increase the accuracy of the determination of the stages at which he radiated the phage, he had sufficiently improved the original Luria-Latarjet experiment so that what had been a problem to solve became a tool for the exploration of various problems connected with the intracellular growth of bacteriophage. He chose at first to apply this tool to the study of nutritional requirements but soon shifted to other factors such as temperature and then refined the methods so as to study effectively the particular cases of bacteria infected by only one and only two phage particles. When he left Caltech for Paris he thought he could further extend the use of the tool to study phage growth in lysogeny.

Benzer's trajectory exhibits the characteristic properties of an individual research pathway, shifting directions frequently as new opportunities arise or obstacles block initial goals but moving always from where the investigator has arrived to another nearby point. That is not only the way much effective scientific research is done, enabling the investigator to build on skills and experience already acquired while taking advantage of the unpredictable events that he encounters along the way, but is also how the successful investigator gradually defines himself within the crowd of others engaged in the same field. When he switched from physics to phage biology Benzer brought no deep and original insights into his new field. Entering an arena already cultivated largely by former physicists, he thought very much the way they did and in general followed the leads already present in this small but lively subspecialty. By building on his technical success in making the Luria-Latarjet experiment more reliable and more precise than it had been in the hands of its originators, he was at the same time marking out among researchers who were working on very similar problems a small piece of personal territory within which he could achieve a special degree of mastery and with which he could hope to attain a distinctive position along the shared research frontier.

Chapter Four

To Paris and Back

At the beginning of August 1951, the Benzer family embarked on the long sea voyage from New York to Paris. For most of the nine days spent aboard the ship they were seasick. They arrived in Paris on August 13, a day that turned out to be a semi-holiday (the Feast of the Assumption), and none of Benzer's contacts at the Institut Pasteur was present. He went to the Fulbright office, where he learned that housing was in very short supply, but there was one listing for an apartment belonging to a well-known American sculptress and her husband. The apartment consisted of two stories, the upper being an open loft, and the only heat came from a wood-burning stove. In the hallway was a primitive communal Turkish toilet. Their new landlords showed them how to light the stove, then left town.[1]

When it quickly turned cold, Benzer started the stove, only to find that the apartment filled with smoke. The distant location of the toilet became a major problem when digestive difficulties developed. Barbie was bitten by bedbugs. To make matters still worse, Benzer was informed by the Fulbright office that it had not received final authorization from the U.S. State Department to begin paying his stipend. Thoroughly miserable by the time Benzer took delivery of a new English car, a Hillman Minx, that he had purchased on the advice of Elie Wollman, the family set out for Geneva, where they met Jean Weigle, the Swiss molecular biologist with whom Benzer had shared laboratory space and some experiments at Caltech.[2]

Noticing at once how depressed his visitors were, the irrepressibly outgoing Weigle suggested that they take a drive together into the Alps. Along the way he asked that they stop, walked up to a farmhouse, and returned with a famous kind of cheese. The family found the cheese to

be "absolutely heavenly." Weigle later told the story that Benzer then smiled for the first time since his arrival.

From that point onward their situation improved. After touring several other countries, the family returned to Paris. François Jacob, who had been looking for them without success because the note that Benzer had left had his apartment's address incorrectly written (he had forgotten to cross the 7s in European style), helped them find an apartment in the country that proved entirely comfortable.

When he arrived at the Institut Pasteur to begin work on September 15, Benzer saw immediately why space had been a problem for Lwoff, whose Service de physiologie microbienne was squeezed into a long hallway on the second floor of the old biological chemistry building. Because the outer walls of the rooms sloped inward behind the mansard roof and had large garret windows, the laboratory was often called the *grenier,* or loft. At one end of the hallway were the rooms used by the group led by Jacques Monod, at the other those used by Lwoff's group. There were relatively few permanent workers, but there was a lively stream of visitors who came for one or two years. Among the long-term workers on Lwoff's team were his wife and collaborator Marguerite Lwoff, as well as Elie Wollman, the son of two eminent phage workers who had been deported by the Nazis during World War II. Wollman had spent two years working in Delbrück's group at Caltech, where his tenure overlapped with Benzer's. The other permanent associate of Lwoff was Jacob, who had earned his medical degree before the war and served in Africa with the resistance forces of Charles DeGaulle. His wartime wounds making it impossible to carry out his earlier plans to enter surgery, Jacob had decided at the age of thirty, after some hesitation about whether he could be good enough, to enter science. After turning him down several times because there was no space in his laboratory, Lwoff finally invited Jacob to join his group in September 1950.[3]

Lwoff installed Jacob in a room that he shared with two Americans, Francis and Betty Ryan, near the center of the corridor of the loft. The room had the disadvantage of being the only one large enough for the workers of the two groups to gather for lunch. Jacob therefore incurred the problem of having to interrupt his experimental operations for two hours each day and participate in the lively discussions that always ensued there. When Benzer joined Lwoff's group, he, too, was crammed into the room occupied by Jacob.[4]

Despite its crowded quarters, Lwoff's Service was a vibrant center of biological research and intellectual intensity. Protected by the long Pastorian tradition and its independence as a private institution from the rigidity of the French university system, its scientists were free to pursue their individual research interests. Lwoff's international reputation enabled him to obtain strong financial support from American sources, including the Rockefeller Foundation and the National Institutes of Health, so that he was able to keep his laboratory well-equipped and filled to its limited capacity with talented young foreign visitors.[5]

Although his style was quite different from that of Delbrück, his was an equally magnetic presence among his co-workers. Formal in dress and manner, Lwoff was reticent, sometimes aloof, but he warmly supported those whom he brought into the Service. Trained as a protozoologist, he had moved into bacteriophage research after the war. Known as a consummate experimentalist, an artist at his craft, he was able to produce illuminating discoveries in a variety of special fields. At the weekly seminars during which visiting scientists were often vigorously challenged, Lwoff was clearly the presiding figure, without attempting, as Delbrück often did, to dominate discussion.[6]

The leading figure at the other end of the hallway was Jacques Monod. Intense in manner and belief, as passionately devoted to music and to the political causes in which he believed as to his science, Monod had worked before the war at the Sorbonne on the metabolism of bacteria. His most interesting observation had been that bacteria grown on lactose and transferred to a glucose medium continued to grow without uninterrupted but that those grown on glucose and then transferred to a lactose medium ceased to grow for a certain latent period, then resumed growth. Lwoff had told him that this was an example of "enzymatic adaptation."[7]

Late in the 1930s Monod had spent time in Morgan's laboratory at Caltech learning classical genetics. During the war he disappeared into clandestine resistance activity in France. Afterward Lwoff, who had also been deeply interested in the metabolism of microorganisms, brought Monod into the Service at the Institut Pasteur, where Monod devoted himself entirely to the further study of enzymatic adaptation.[8]

The excitement about lysogeny that rippled through Delbrück's group after Lwoff's visit in 1950 was well justified. His investigation had not only put the "phenomenon" itself on a "firm foundation" but soon opened the way toward the resolution of fundamental questions

about the general nature of bacteriophages that had been unsettled for more than three decades—questions in which scientists at the Institut Pasteur had been involved since 1917, when Félix d'Herelle first discovered them while working there.[9]

D'Herelle firmly insisted that bacteriophages were obligatory parasites that invaded bacteria from the outside and multiplied at the expense of their hosts. Although they could not be cultivated in any other medium, they were microbes, antagonists of the bacteria that they destroyed. Because the bacteriophage escaped microscopic observation, however, their presence independent of the bacteria they lysed could not be proved. At the Institut Pasteur in Brussels, on the other hand, Jules Bordet and Mihai Ciuca interpreted bacteriophage as an "autogenous" and "active principle" produced by the bacteria themselves. Other interpretations—for instance, that bacteriophage were autolytic enzymes or secretions of the bacteria—were also suggested. Eugène Wollman, who began to work on bacteriophage at the Institut Pasteur in Paris in 1919 and pursued the problem with his wife Elizabeth for the next two decades, gradually developed the idea that bacteriophage were hereditary "corpuscles." As we saw in Chapter 1, some geneticists, most prominently Hermann Muller, suggested during this period that bacteriophage might be like genes. If so, however, the fact that they spent part of their time outside the organisms of which it was speculated that they constitute genetic material stretched the meaning of the classical gene.[10]

Between 1921 and 1925 Bordet and Ciuca found that a culture of bacteria *E. coli* (strain 88 of Gildmeister and Herzberg) that had been purified of bacteriophage by various methods such as dilution or the addition of antiphage serum could lyse spontaneously, giving rise to bacteriophage. Bordet called the phenomenon "lysogeny." Other types of bacteria were soon found with similar properties. Because it was never possible to exclude the possibility that undetected external sources of the phage were present, the reality of lysogeny, despite the work of the Wollmans, of Macfarlane Burnet in Australia, and others, remained in doubt through the 1920s and 1930s. Moreover, that it was a spontaneous process that the experimenter could not control at will made the nature of the process still more uncertain. Delbrück was not alone when he cast doubt on the claims for lysogeny in 1946 and preferred to exclude it from consideration by limiting experiments to the virulent strains of the T series. As Charles Galperin has put it, the "phenomenon of lysogeny itself underwent an eclipse until 1949."[11]

The precise reasons for which Lwoff took up the study of lysogeny in 1949 are uncertain. As Galperin has shown, he had followed the research on the problem by his close friends the Wollmans and had exhibited a sustained interest in the broad question of "genetic continuity" among the various components of cells. Discussions with Monod and Elie Wollman when the latter began in 1947 to examine the effects of viral infection on enzymatic adaptation in bacteria may also have played a part.[12]

"We call lysogenic bacteria," Lwoff and Gutmann wrote in 1950, "those bacteria in which the capacity to form bacteriophage perpetuates itself without the intervention of exogenous bacteria." Citing the conclusions of Bordet and of Burnet that this faculty implies the presence of bacteriophage as a "part of the hereditary constitution" of such bacterial strains, Lwoff acknowledged that the experiments on which this conclusion was founded were "suggestive" but did not "carry certitude."[13] To bring such certitude to the question was among his central motivations for undertaking new research. There were, however, a number of subsidiary questions, the first of which he took up with his co-worker Antoinette Gutmann: "How are bacteriophages liberated by lysogenic bacteria?" Are they continuously secreted or "liberated only at certain stages of the bacterial cycle?" It was not possible to answer such questions, he noted, by means of the numerical, statistical experiments on large bacterial cultures customarily performed by members of the American phage group. Following instead a procedure initiated by the Wollmans in 1932, Lwoff studied isolated or small numbers of bacteria dividing in a few drops of medium under the microscope.[14] As some historians have pointed out, this was the experimental style of a microbiologist, and it contrasted sharply with the style that the former physicists had brought to phage biology.[15]

With a micropipette Lwoff and Antoinette Gutmann were able to take up individual bacteria from very small water drops floating on oil, then successively transfer them through several such drops to ensure that all phage were removed. By means of a "micromanipulator" they then inserted the tip of the micropipette under the microscope into that of a normal pipette, into which the bacteria were placed along with five drops of water. There they were able to watch successive divisions of the bacteria, count their numbers, and afterwards determine with certitude whether any bacteriophage were present in the medium. They carried out all these operations in an oven kept at a constant temperature and under aseptic conditions. In many cases

they found that the bacteria lysed without producing bacteriophage, but in some cases one or a few bacteria did release from one to several hundred phage. They carried out these processes using different media. They followed one of the bacteria through nineteen divisions. All but two of them produced no phage, and when isolated and resown, they turned out to be still lysogenic. This "conservation of lysogenicity" under conditions in which they could be certain that no free phage had appeared could be explained in no other way, Lwoff concluded, than by the "hypothesis that lysogeny perpetuates itself by endomicrobienne ways, without the necessary intervention of exogenous phages." Having thus provided firm support for the picture of lysogeny that had been open to question for so long, he went on to examine the processes of lysis and the liberation of phage more closely. From these observations he concluded that there is first a rapid lysis, followed by the appearance of the bacteriophage, and that the latter are never liberated without the lysis.[16]

On the basis of these results Lwoff proposed the hypothesis that lysogenic bacteria exist in a condition of equilibrium that can be disturbed by various factors, including the history of the bacterium or conditions in the medium. During the former condition the bacteriophage is maintained in a state that he called a "probacteriophage" (soon afterward shortened to "prophage"). The bacteria thus perpetuate "a potentially lethal character," being condemned to death whenever they begin to produce bacteriophage. This view Lwoff named the "induction hypothesis."[17] The next problem he took up was finding out what factors "induce the production of bacteriophage in a population of potentially lysogenic bacteria."[18]

In the search that Lwoff and his co-workers Louis Siminovitch and Niels Kjeldgaard carried out, it became apparent that external factors, such as oxygenation and the constitution of the medium, sometimes acting in conjunction, played an important role in inducing lysogenic bacteria to produce bacteriophage. The effects were, however, irregular and impossible fully to control. He decided, therefore, to look for another means to "induce the lysis of the total population of a potential lysogen." After many negative experiments he was able, using ultraviolet radiation of wavelength 2537Å, to achieve his aim. Preliminary experiments with cultures in petri dishes showed that, according to changes in the optical density of the medium, the radiation caused all of the organisms to lyse, releasing 70–150 bacteriophage. With his micro-drop method he then demonstrated more rigorously that

nearly every bacterium in a group of bacteria in such a drop lysed and produced bacteriophage. Following the irradiation, sixty to eighty minutes elapsed before the lysis began. During this latent period, he showed, the bacteria divided twice.[19] Elaborating slightly on his previous statements, Lwoff wrote that "in a potentially lysogenic bacterium, the probacteriophage behaves itself, so far as we know, as a normal particle endowed with genetic continuity." It is only when the bacterium has been induced to produce bacteriophages that the latter become pathogenic. He believed that this model was "capable of clarifying some obscure points also in the biology of the pathogenic viruses of animals and plants."[20]

Ultraviolet induction of lysogeny provided not only additional evidence for the reality of the phenomenon but also a powerful new tool for its experimental study. For the first time, investigators could instigate the process at will and under well-controlled conditions. Up to that point, however, Lwoff had carried out his experiments only on a single lysogenic strain of *Bacterium megatherium* isolated originally by the Dutch virologist den Dooren de Jong and later studied by Eugène Wollman. In order to establish which of his observations were peculiar to that species and which were more general, it would be necessary to "explore . . . the comparative physiology of lysogeny."[21] It was as part of that project that he gave to François Jacob, when he joined the group in September 1950, the task of analyzing lysogeny in another organism, *Pseudomonas pyocyanea*.[22]

Jacob procured thirty strains of the bacterium from the collection of the Institut and learned how to culture them, to distinguish one culture from another, and to inoculate one culture with a drop of culture from another strain. The first step in his analysis was to pair the cultures two by two in every possible combination in order to see which of them could produce a phage that would act on the other. Within a few weeks he was able to induce lysis by ultraviolet irradiation in some of the strains and to separate the inducible from the noninducible ones. He tested the effects of varying the medium, of exposing the bacteria to ordinary light after the ultraviolet irradiation, and of imposing other conditions similar to those Lwoff had used with *B. megatherium*. Examining whether it was the nature of the bacterium or of the probacteriophage that made some of the pairs inducible, he concluded that the latter was the determining factor. This was Jacob's first experience with experimental research, but he learned his craft rapidly. On December 18, just three months after his arrival, his first paper,

summarizing these results, was presented to a meeting of the Académie des Sciences.[23]

As he continued his research in 1951 Jacob became intently interested in the question, what is the nature of the prophage? Others in the laboratory who were also studying lysogeny were similarly oriented, and there was much speculation in the corridors, at the blackboard, and at lunch about what prophage were composed of, whether they were spread through the cytoplasm of the bacteria or attached to specific structures, what prevents them from being activated, and what the mechanism by which radiation induces the change to an active state might be.[24] In the grenier and in other laboratories where lysogeny was becoming a focus of attention, there gradually emerged a conception that lysogeny took place in three stages: the *prophage* state, a *vegetative* state in which phage particles are being reproduced but are not yet infective, and a *mature* state in which the phages are capable, when released, of infecting other bacteria. The growing belief that the last two states were the same in lysogenous as in "sensitive" bacteria, the former differing only by possessing the inactive prophage state,[25] was now transforming the phenomenon of lysogeny from the obscure, uncertain status it had held before Lwoff took up the problem into a focal point for phage research in general. The long but still unsuccessful quest to uncover the stages of the latent period, often called for that reason the dark period, was increasingly concentrated on the particular event by means of which a prophage could be induced to change to the vegetative state.

When Benzer arrived in Lwoff's laboratory, these questions were animating the whole group. Besides Lwoff and Jacob, Elie Wollman and Louis Siminovitch were strongly engaged in research on lysogeny, and other occupants of the grenier also sometimes joined in. The potential capacity of Benzer's method (based on the Luria-Latarjet experiment) to cast light on the critical event by which prophage is activated would appear to have made his planned participation in this research particularly attractive just then. Instead of pursuing the problem that was Lwoff's reason for asking him to come, however, he took up a problem framed within the long-standing endeavor of Monod to elucidate enzymatic adaptation.

Monod had encountered enzymatic adaptation before the war during the course of his investigation of bacterial metabolism, and he continued to pursue the problem mainly in that context. After entering Lwoff's laboratory he joined in 1947 with Elie Wollman in a study of

the inhibition of growth and of enzymatic adaptation in bacteria infected by bacteriophage. They adopted methods "introduced by Delbrück and his collaborators," including especially the technique of the "one-step growth" experiment, and utilized also the *E. coli* strain B of the "American authors." Departing from the practices of the phage group in one respect, however, they employed a phage discovered by Wollman that did not fit within the T series of phages prescribed by Delbrück for such work.[26]

The objective of Monod and Wollman's investigation was to "study the effect of bacterial infection no longer only globally on growth, but on the formation and activity of a particular constituent of the cell, such as an adaptive enzyme." Monod's previous work had shown that, when nourished on glucose as their only source of carbon, strains of *E. coli* "attack" the substance with a very active constitutive enzyme—that is, one that is present whether or not the medium has previously contained glucose. When fed lactose, however, the same organisms use a "strictly adaptive enzyme," one that is absent in bacteria cultivated without lactose.[27]

Measuring the "growth" of infected bacteria by means of changes in both optical density and rates of respiration, they found that those nourished with glucose continued to grow and then to lyse, but those fed lactose did not, unless the adaptation had already taken place at the time of infection. From these contrasting results they concluded that infection by bacteriophage does not prevent existing respiratory enzymes from oxidizing the glucides, but it does inhibit the formation of "new" enzymes. The broader significance of the result, they thought, was that the multiplication of virus during the latent period does not generally inactivate the whole system of oxidative enzymes but that the "activity of phage must be narrowly localized."[28]

By 1951 the study of adaptive enzymes had broadened to include other enzyme systems, and several investigators, including Sol Spiegelman in the United States and Martin Pollack in England, were contributing new evidence, as well as competing theories. Monod, however, remained the dominant figure in this small but strategic field, and his galactosidase system was the best-characterized example. At about that time he decided that the term *adaptive* was ill-chosen for the situation because of its association with the concept of the selection of favorable mutations in evolutionary theory. In its place he proposed the phrase *induced biosynthesis of enzymes* and called the substances supposed to cause the synthesis of such enzymes *inducers.* He made

no connection between this usage of *inducer* and the usage that Lwoff had introduced two years earlier for the agents that activate lysogeny.[29]

In 1951 the effects of bacterial infection on the induced synthesis of enzymes continued to be a lively topic of investigation within the groups in Lwoff's Service. Moreover, some of the research undertaken there combined Monod's pursuit of induced synthesis with Lwoff's work on lysogeny. At the time of Benzer's arrival Jacob was engaged in just such a study, comparing the effects on the multiplication of virulent and of temperate phage in his *pyocyanea* strain of bacteria when lactose and glucose, respectively, were used as the source of carbon.[30] Because the experimental system Monod had devised to study induced synthesis in infected bacteria relied on the methods of Delbrück, with which he was thoroughly familiar, Benzer would have lost no time learning to use them in order to begin his study of the induced synthesis of enzymes in Paris. Nonetheless, as soon as he arrived there must have been a strong incentive for him to put aside the planned project on lysogeny in favor of work on enzyme adaptation. According to his later testimony, the immediate stimulus to this departure from his intended investigative pathway was his attraction to the possibility that he could apply his experimental method to solve a problem arising in the kinetics of induced enzyme synthesis that Monod believed to pose great difficulties and that another biologist present in the laboratory had declared in print to be "impossible" to treat experimentally.

"In principle," Monod and Melvin Cohn wrote in a review of the topic published in 1952, "the measurement of the synthesis of the enzyme as a function of time during the course of induction could furnish indications about the mechanism of the process of synthesis. In fact, the technical difficulties and the uncertainties of interpretation are considerable." One of the difficulties was that interpretation of such results was impossible if the synthesis of the enzyme in question influenced the overall metabolism of the cell. Only cases in which the enzyme and its inducer played no appreciable part in the metabolism, a condition they called "gratuity," could evade this difficulty. A second requirement for successful measurement was that the overall growth rate be in a state of equilibrium such that the synthesis of the enzyme introduced only the specific supplementary process under study. Up to that point the galactosidase on which Monod had long based his work was the only enzyme to satisfy these conditions. Recently he and Germaine Cohen-Bazire had carried out a study of the kinetics of the enzyme which showed that the "law of the increase in the enzymatic

activity as a function of time was essentially hyperbolic, without an appreciable initial phase of acceleration." This result was suggestive because it contrasted with the usual S-shaped bacterial growth curves, but its interpretation remained doubtful, because "a scheme for the 'synthesis reaction' has no significance except for a cell considered in isolation. The *observed* kinetics concerns the population, it is the *sum* of individual kinetics, but it is not necessarily equivalent to their average." The global measure can represent this average only if the increase in activity in each cell takes place, on the average, over the same interval of time as the global phenomenon. If the rate of synthesis in individual cells is large with respect to the overall phenomenon, then there may be no relation between the observed kinetics and that of the molecular synthesis.[31]

According to his retrospective account, Benzer was "inspired" to take up this problem by reading a similar argument in a review of "Enzymatic Adaptation in Bacteria" by Roger Stanier, a Canadian who was also working in Monod's group at the time Benzer arrived. Discussing the induction of the synthesis of enzymes in microorganisms by the substrates on which they act, Stanier noted, "If the rate of specific [enzyme] activity during adaptation is plotted as a function of time, the curve obtained usually has a very characteristic sigmoid form." A little further on he added, however, "The argument that a sigmoid curve would be obtained if the time required for adaptation by individual cells in the population varied according to a normal frequency distribution is sound, and almost impossible to test experimentally." The reason for his skepticism was the same as that of Monod and Cohn: that the kinetics had been studied by way of the rates of formation of an immediate product or a gaseous exchange that gave only the average rate for the whole population of the organism examined.[32]

The statement that the argument for a sigmoid curve is "almost impossible" to test, Benzer has written,

> was a challenge for me, since the Luria-Latarjet experiment of UV radiation of vegetative phage, on which I had been working at Caltech, made it possible to follow the progressive intracellular development of phage from minute to minute and also the distribution of rates of development among the various cells of a phage-infected population. In those experiments I had shown that in bacteria starved before infection, development of phage is arrested at a very early stage. I thought, therefore, that it should be possible to test the simultaneity of adaptation in a bacterial culture by making

> the metabolism of the cells, and hence intracellular phage development, dependent upon the presence of an inducible enzyme. . . . If starved cells were infected with phage and placed in a medium in which lactose was the only carbon source, only those cells having galactosidase could support phage growth, heterogeneity in enzyme level in the bacterial population would then show up in the Luria-Latarjet experiment as dispersion in the sensitivity of infective centers to UV irradiation.[33]

Like most recollections of the origin of an investigation, Benzer's succinct reconstruction leaves as much unexamined as it explains. Much depends on the question whether the reasoning he summarized here happened all or nearly all at once or emerged in stages over some extended period of time, and, if the former, on when the "flash of insight" that it would in that case represent may have occurred to him.

We may note that his plan followed a pattern already established at Caltech, in which he had looked for opportunities to apply the Luria-Latarjet experiment, as he had by then refined it, to a variety of problems connected with the growth of phage during the latent period. To the extent that the presence of an inducible enzyme could be considered a "chemical requirement" for the synthesis of phage, the planned investigation can be viewed as an extension of the project he had outlined in a progress report at Caltech as far back as December 1949 in which he had already mentioned adaptive enzymes[34]

On the other hand, the experiment to which he planned to apply the Luria-Latarjet method was clearly derived from the experiment on the inhibition of enzymatic adaptation in bacteria infected by bacteriophage that Monod and Wollman had originated in 1947 and of which variations were being performed in the grenier when Benzer began his work there. It is hard to avoid inferring that part of his inspiration came from connecting what he was learning about the experiments going on around him with the problem that Stanier raised for him. A further question then arises: Did he read Stanier's review because of the current work on adaptive enzymes in the laboratory, or did he turn his attention to these experiments as a consequence of reading the review?

To begin, Benzer chose a bacterial strain of *E. coli* K12 then in use in the laboratory and known to be lysogenic for phage lambda and inducible for galactosidase. He chose a T2r rapid lysis phage mutant in order to preclude complications from reinfection by phages released early in a normal lysis. He found, however, that the infected cells

would not lyse, even when previously induced by feeding them lactose. To check whether there was something wrong with the phage stock, he tried it with *E. coli* strain B, for which it worked normally. Rather than pursue further the question why the T2r phage did not work with his original choice of bacteria, he proceeded with the combination of T2r with strain B.[35]

As a preliminary test of his Luria-Latarjet ultraviolet resistance method, Benzer grew the *E. coli* in broth, washed and incubated them for an hour in buffer, then added the phage. Irradiating the infected bacteria in the usual way with six different doses taken at six successive time intervals, he found that the survival curves were nearly linear. Because the theoretical survival curves for a population of bacteria that were heterogeneous in their rate of metabolism would be "bent," he concluded that this population had a uniform rate of metabolism. With bacteria growing on a synthetic medium containing glucose as its carbon source, on the other hand, he did obtain a bent curve, indicating an uneven distribution of utilizable nutrient sources.[36]

Before getting much further with the investigation, Benzer realized that, for the particular problem of the distribution among the infected cells of the induced enzyme galactosidase, a more direct approach was possible. Several year earlier Monod and Wollman had found that when bacteria induced prior to infection lysed, they released the galactosidase into the medium, and Monod and Melvin Cohn had devised a method to assay the activity of the enzyme quantitatively. If cells with a higher content of the enzyme lysed sooner than those with less, Benzer reasoned, and if the enzyme remained active in solution, then by measuring the enzyme present in the medium at various times while the culture is lysing, he ought to be able to determine its distribution. If the population were homogeneous, a plot of the amount of enzyme released over time should give a straight line, if heterogeneous, a curve, with the initial slope corresponding to the cells with the largest amounts of enzyme.[37]

While shifting his approach to try out this method, Benzer switched also to the phage ϕII that Monod and Wollman had used with *E. coli* B in their original experiments in 1947.[38] In order to measure the enzyme activity he used the same method, the rate of hydrolysis of o-nitrophenyl β-D-galactoside as determined by the increase in optical density at a wavelength of 420 mμ, that Cohn and Monod had developed. Following current practice in Monod's group, he measured the growth of the bacteria by the increase in optical density of the culture, as determined by a Meusnier electrophotometer. He defined the *specific*

activity of the enzyme, as they did, by the ratio of the total measured activity to the optical density—that is, the activity per unit of bacterial mass.[39] Instead of using the method he had devised in America, therefore, Benzer was now working entirely with methods already in use in Paris, contributing instead a bright new idea for their application.

In a preliminary experiment, Benzer showed that in bacteria grown first on glucose and then shifted to lactose, the amount of induced enzyme increased over time. When he added phage at three different time intervals following the switch to lactose, the enzyme appeared at three different levels but was afterward "frozen" at that level. He had, essentially, confirmed the result of Monod and Wollman that infection by the phage inhibits the further induction of the enzyme.[40]

To test the assumption on which his whole investigative plan rested—that in bacteria that depend on the substrate for the inducible enzyme as their sole source of carbon and of energy the time taken to reach the stage of lysis is dependent on the quantity of the enzyme present in the cells—Benzer infected cultures with the phage at three different time intervals after inducing them with lactose. The longer the time between induction and infection, the greater the amount of enzyme that ought to be produced before the phage inhibited its formation. As controls, he added a culture grown on glucose and, therefore, not dependent on the content of galactosidase, and one that he had not induced, containing, therefore, no galactosidase. Measuring the optical densities at various time intervals after he had added the lactose gave him curves that demonstrated that the higher the specific activity of the enzyme the more rapidly the bacteria lysed. Finally he verified that enzyme is liberated into the medium over the same time interval in which the cells lyse.[41]

Ready now to begin his analysis of the distribution of the enzyme in the bacterial cells, Benzer settled (perhaps after some trials) on the following procedure. For thirty minutes he agitated a culture in which he had previously induced the enzyme with lactose in a synthetic medium lacking a source of carbon and energy, to starve the cells. He then added phage, which under these conditions was adsorbed but did not reproduce. After fifteen minutes he again added lactose. While the culture lysed he removed successive samples, chilling them with ice to slow their further lysis while he carried out additional operations on them. Next he measured the optical density with half of each sample and, after sedimenting the unlysed bacteria, the enzymatic activity of the other half. Finally he plotted the fraction of cells lysed as determined by the optical density against the fraction of the enzyme liberated.

Later Benzer illustrated the relation he would expect to find between the resulting curves and the enzyme distribution with diagrams representing three hypothetical cases (fig. 4.1). Curve I illustrates the situation if each cell contains either a full amount of enzyme or none. The curve follows a straight line to the point at which all the cells containing enzyme have lysed, then becomes horizontal. If the enzyme is distributed as in case II, there will be successive changes in the slope, as first the cells with most enzyme lyse, followed in turn by those with successively lesser amounts. In case III, where all cells have an equal amount, the curve will be linear from start to finish.

In order to test the capacity of his method to distinguish such differences, he made artificial mixtures of cells containing, respectively, no enzyme and enough enzyme not to be a limiting factor. The former he produced by growing cultures on glucose, which does not induce the enzyme, the latter by inducing them with lactose. The optical density curve for 100 percent lactose cultures turned out, as expected, to be a straight line. Those for mixtures of 50 percent glucose with 50 percent lactose grown, and of 86 percent glucose with 14 percent lactose produced curves rising at first in a straight line, then breaking in a short curve to a horizontal line. The method worked. That the break was not completely sharp suggested that there were limits on the degree of resolution he could attain.[42]

Ready now for the principal experiments, he examined two of the conditions under which Monod and his group customarily induced galactosidase. In the first of them, the lactose that acted as inducer was also the sole source of carbon and energy. Under these conditions the curves he obtained differed according to the length of time that had been allowed for induction before the phage were added. For the shortest time the curve broke sharply after a short linear ascent. As the times became progressively longer, the ascending branch of the curve lengthened, and as it approached the condition in which the cells had been fully induced, it formed a straight line. Benzer concluded that under these conditions a few cells at first contained most of the enzyme. As time went on, the fraction containing the enzyme increased, and the culture became more homogeneous. Under these conditions, consequently, "the average kinetics for the culture *does not* apply at the cellular level."[43]

Benzer tested similarly what Monod called "conditions of gratuity": when the inducer is not metabolized by the cells and the carbon source is not an inducer. To achieve these conditions Benzer used

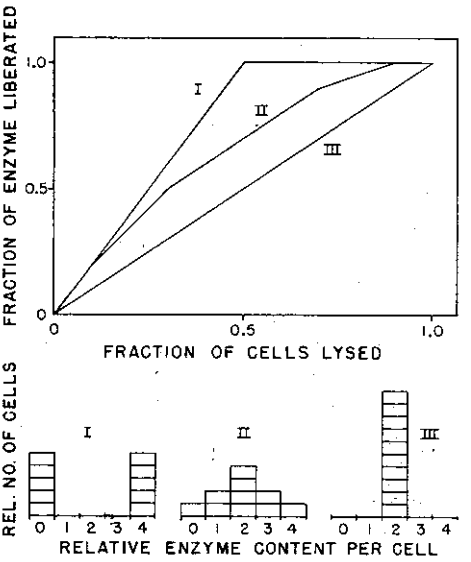


Fig. 4. Below: three hypothetical distributions of enzyme in populations of bacteria. Above: The curves expected from analysis of these populations by the phage procedure.

Figure 4.1. Benzer's illustration of the expected result for adaptive enzyme formation in the Luria-Latarjet test. (From Seymour Benzer, "Induced Synthesis of Enzymes in Bacteria Analyzed at the Cellular Level," *Biochimica et Biophysica Acta* 11 [1953]: 393.)

β-methyl galactoside as the inducer—the same compound that Monod his collaborators used for that purpose—and lactate as the carbon source. Now when he plotted the decrease in optical density against the enzyme liberated, for four different levels of induction, all four produced lines that were, within the range of experimental error, straight. This result told him that for conditions of gratuity, "all cells participate at comparable rates in the formation of enzyme, and the average kinetics gives a legitimate representation of the events in each cell."[44]

Benzer's analysis of the distribution of the enzyme at the cellular level had an immediate impact on the work of Monod and his group. Because Monod already regarded conditions of gratuity as the only situation in which one could interpret studies of the kinetics of induction, the important result for him was that Benzer had found homogeneity under these circumstances. Along with A. M. Pappenheimer and Germaine Cohen-Bazire, Monod was, at this very time, engaged in an

investigation of the "kinetics of the biosynthesis of galactosidase considered as a function of growth." When they used unmetabolizable galactosides as inducers with sources of carbon that do not induce and plotted the formation of enzyme against the growth of the bacteria as measured by optical density, they obtained under varied other conditions an "exactly linear relationship . . . remarkable for its simplicity and regularity." From these and other considerations they were "invited to think that as a general rule enzymatic adaptation corresponds with a complete synthesis of new protein."[45]

At the end of the paper reporting these results, which they submitted for publication in June 1952, Monod, Pappenheimer, and Cohen-Bazaire wrote, "In all the discussions which precede, we have accepted that the biosynthesis of galactosidase is a continuous phenomenon, and that the kinetics observed for the population corresponds approximately to the kinetics at the level of individual cells. . . . We are authorized for that by the recent observations of Benzer, carried out on the same material, which for the first time justifies by experiment these hypotheses, which have generally been implicitly assumed."[46]

Jacob has written that Lwoff and Monod "had developed an atmosphere that blended enthusiasm, intellectual lucidity, non-conformity, and friendliness." In such an atmosphere, one that Benzer, too, perceived that way,[47] he thrived as fully as he had at Caltech. In no other laboratory in France would his own nonconformities, such as working from the afternoon to the middle of the night, have been accepted without question. (When he asked for a key to use the library of the Institut at night, the librarian was dismayed.) The cramming in French that he had done beforehand was sufficient to enable him to participate actively in the "franglais" conversation that pervaded a group nearly equally divided between French and foreign workers and to get along outside the laboratory, where it was essential to speak French.[48] The daily group lunches in the laboratory that he shared with Jacob provided him with a fine opportunity to indulge in his taste for exotic foods, which he picked up at local markets in various parts of Paris on his way to the laboratory and heated over a Bunsen burner.[49] In a letter to Lark-Horovitz in March 1952, he described his experiences there:

> The lab here is really outstanding. This does not apply to the entire Pasteur Institute, and certainly not to French laboratories in general, but the "service" of Lwoff is exceptional. I think one can fairly

> well judge a laboratory by the number of foreign workers it attracts, and here we have about 50%—Stanier of Berkeley, Pappenheimer and Cohn of NYU, Stent of Caltech, and myself, a Canadian, an Australian, and an Italian. Three of us are alumni from Delbrück's lab. The variety in background of the people working together—physical chemists, physicists, biochemists, immunologists, protozoologists, geneticists—gives the group an enormous strength. The lab is very well equipped, by virtue of American grants, is crowded to capacity, and buzzes with activity, arguments, and discussions. The two leading personalities are Lwoff, a fountainhead of biological insight and intuition, and Monod, a fountainhead of logic. One is qualitative, the other quantitative, and they make a wonderful team. Lwoff's group works on phage, while Monod's is the world's foremost in the field of enzymatic adaptation. The work I am doing combines both fields, since I am using phage as a tool for studying enzymatic adaptation at the level of the single bacterial cell. The usual techniques are limited in that one is forced to measure an average effect over very large numbers of cells and cannot distinguish between all-or-none and distributed effects. However, this can be accomplished by using phage in an analytical way.[50]

At first Benzer and Jacob had spoken little to each other. Jacob began early in the morning, interrupted his work for the lunch invasion, and then resumed. Benzer came in late, immersed himself in his cultures, and uttered only the rare grunt until Jacob bid him au revoir when he left at 7 P.M. In time, however, they became very good friends. Jacob found that under his impassive exterior Benzer concealed "much charm and warmth." Both deliberate and penetrating, he often gave no immediate response to a question put to him but came back after a few days with a profound answer.[51]

Now happily installed in a "lovely" apartment within walking distance of the laboratory, as well as such focal points as the Louvre, Montparnasse, and St. Germain de Pres, Benzer and his family took full advantage of the culture of Paris. Enrolled in a school that permitted no English to be spoken, Barbie quickly learned French so well that she began to correct her parents' mistakes. Dotty got along very well in the shops with improvised French. They took in many plays, films, and art exhibits and explored the "inexhaustible" streets and alleys of Paris. Occasionally Benzer played trios with Monod and the wife of Gunther Stent.[52]

By the end of February Lark-Horovitz, who had not heard from Benzer since the latter had left for Paris, was pressing him to reveal

when he planned to return to Purdue and what research he intended to do there.[53] In reply Benzer wrote on March 7 that he expected to arrive in Lafayette by September 15 and drew up a list of equipment that he would require in order to begin phage research there. He also sent a research proposal.

As the title of his proposal—"A Study of the Reproduction of Bacterial Viruses Within Their Host Cells, Using Resistance to Ultraviolet Radiation as an Index to Development"—suggests, Benzer intended to continue the investigative pathway based on the Luria-Latarjet experiment that he had interrupted when he left Caltech. Hoping that further work would lead him beyond only an index of phage development toward an inquiry into "the mechanism underlying the resistance itself," he thought that he could move in that direction by blocking the development with various inhibitors. The ultraviolet radiation method could determine which stage was blocked, because the resistance would cease to change at that point. "By observing which stage is blocked by a particular inhibitor," he wrote,

> for example, 2,4 dinitrophenol, which prevents assimilation, cyanide, which interferes with respiration, or proflavin, which affects nucleic acid synthesis—one may gain insight into the nature of the biochemical processes associated with that stage.
>
> In like manner, it is possible to determine whether a specific substance, such as a chosen amino acid, for example, is required for a particular stage in phage development by employing as host a bacterial mutant unable to synthesize that substance.[54]

It may well be that conversations with the geneticists and biochemists in the grenier had helped Benzer identify such examples for his further work. Monod and his co-workers, for instance, had already performed studies with mutant strains each of which unable to grow without a particular amino acid in its medium.[55] Benzer's research plan, however, incorporated no elements of the investigation of the induced synthesis of enzymes that he was conducting in Paris. That work had already yielded a more decisive result than what he had been able to obtain with his previous applications of ultraviolet radiation to the reproduction of bacteriophage. It was, however, mainly a contribution to the ongoing research program of Monod, which he did not see as the opening of a new fork in his own research trail. For the time being he still regarded his expertise with the Luria-Latarjet experiment as the key to his scientific future.

In April 1952 Benzer, Jacob, and Stent traveled to England together to attend a conference on virus reproduction at Oxford University sponsored by two senior scientists, Frederick W. Bawden and Norman W. Pirie, who during the 1930s had played a major role in the discovery that tobacco mosaic virus is composed of nucleic acids as well as protein. The participants were housed in the tiny rooms and cold corridors of one of the colleges that was emptied of students for the vacation. As Benzer wrote afterward, "Luria was expected to be the star performer, but he was prevented from leaving the United States"[56] by the State Department, which at the height of the McCarthy anti-Communist crusade refused him a visa because of suspicions about his youthful Marxist leanings. Jim Watson read Luria's paper, which proposed that the genetic material of bacteriophage was protein. Watson had, however, just received a letter from Hershey outlining an experiment performed with a Waring blender involving the infection of bacteria by phage labeled, respectively, with radioactive ^{35}S and ^{32}P. After the phage had been adsorbed, he and his assistant, Martha Chase, had sheared off the phage bodies from the external surface of the bacteria by placing the solutions in the blender. Afterward they found 80 percent of the activity of the sulfur in the medium but only 40 percent of the activity of the phosphorus. From this result Hershey inferred that the bulk of the protein remains on the surface, playing no part in the reproduction of the phage, while the bulk of the phage DNA enters the cell. After he had read the letter, Watson commented that the result, together with some supporting evidence that he could adduce from his own work on tobacco mosaic virus, made it "tempting to conclude that the virus protein functions largely as a protective coat for the DNA and that the perpetuation of genetic specificity is largely or entirely a function of the DNA."[57]

The Oxford meeting has become a landmark in the history of molecular biology: the first announcement of the Hershey-Chase experiment, which persuaded the members of the phage group that the genetic material is DNA, rather than protein or nucleoprotein. According to Watson, few of the participants showed much interest at the time. Benzer must have been among those who fully appreciated the significance of the result, especially because he went afterward to visit Watson at Cambridge, where they must have discussed its implications further.[58]

When Benzer returned to Paris he found that Dotty had contracted German measles, and they decided that they must terminate

her pregnancy immediately. They rushed to Switzerland, where the procedure could be performed legally. There were no bad effects, and they were back in Paris by mid-May.[59]

By late spring Benzer had probably completed his experiments and was beginning to write a paper about the use of bacteriophage in the determination of the distribution of enzyme content over individual bacterial cells during enzymatic adaptation. He was ready, therefore, to begin something else during the summer that remained to him in Paris. Earlier in the year Jacob had taken up the project for which Benzer had originally been brought there, studying "a lysogenic pseudomonas phage system by the Luria-Latarjet technique, using UV." Jacob had already found "features which have not been observed with virulent phages." He and Benzer decided to collaborate on a further series of experiments.[60]

Jacob had used strains of *pyocyanea,* the same organism with which he had worked from the beginning in his studies of lysogeny, with phage λ, the temperate phage studied extensively by Jean Weigle. Because this phage, like T7, which Benzer had previously studied, did not show multiplicity reactivation, Jacob could expect the resistance curves plotted from the doses given at successive time intervals during the latent period to show increasing multiplicity without interference. He compared the curves produced when the temperate phage λ infected a strain of the bacteria that was lysogenic to it with those produced when the same phage infected a strain that was sensitive to it. In the latter case the bacteria lysed spontaneously; in the former case he induced the lysis with a dose of ultraviolet light given before the series of doses intended to measure changes in resistance. In general the series of curves was similar in both cases, progressing with the time of exposure from a straight line representing single hits toward increasing multiplicity. The main difference Jacob observed between the two cases was that in the lysogenic strain the resistance decreased somewhat during the first half of the latent period, then increased, whereas in the sensitive strain it increased progressively throughout the latent period.[61]

During the last week of June and the first week of July Benzer and Jacob together performed similar comparative experiments using a sensitive and a lysogenic strain of *E. coli* C. The resulting curves were comparable to those that Benzer had earlier obtained with T7, except that during the first half of the latent period the multiplicity first decreased, then increased again. In this case they found "no important

difference between the sensible infected bacteria and lysogenic induced bacteria in their resistance to U.V."[62]

For Jacob the main goal of these experiments was to shed some light on the nature of the prophage in lysogenic bacteria: "If the sequence of changes is identical in the two types of complexes [of phage with sensitive and lysogenic bacteria], it is probable that the prophage corresponds to one of the states through which the phage passes successively during its development in infected sensitive bacteria. If, on the contrary, the resistance of the lysogenic complex at time zero is different from the whole series of resistances observed in the infected complex, or else the succession of changes is different after infection from after induction, then one could conclude that the prophage is in a state different from the state of the phage during its development in sensitive bacteria." These hopes were not fulfilled. "Unfortunately," he and Benzer wrote afterward in the paper presenting these results, "neither of the two lysogenic systems studied permits the confirmation of one or the other of these hypotheses." In the case of *pyocyanea* it was clear that prophage was not in the same state as the phage infecting at time zero, but the curve of resistance of bacteria irradiated before infection, which they called its "capacity" to produce phage afterward, "masked" the early phage resistance curves, making it impossible to know the resistance of the prophage at time zero. In the case of *E. coli* C, the changes in resistance were "too weak to permit a conclusion."[63]

For Jacob this disappointing outcome was compensated for by the "enrichment and pleasure" it gave him to work with so "acute and meticulous a mind" as Benzer's.[64] For Benzer, once again the great potential he had found in the Luria-Latarjet experiment remained to be justified in the future. Perhaps he was glad that he had not spent his whole year in Paris on this project but had diverted himself from the original plan to take up a different challenge that had proven more immediately rewarding.

In July 1952 Benzer and Jacob took part in the First International Congress on Bacteriophage, organized by Lwoff, in a Cistercian abbey at Royaumont (see fig. 4.2). Almost all of the leading phage researchers, including Delbrück, Luria, and Hershey, were there, and the week-long meeting was full of intense discussion. According to Jacob, as the details of the experiments of Hershey and Chase were disclosed, "DNA became increasingly important, and no one there doubted any longer that it was the substance of the genes." Another outcome of the meeting was a general consensus on the "doctrine of the trinity of virus": that is,

Figure 4.2. ***Left to right:*** **Alfred Hershey, Jacques Monod, Elie Wollman, Gunther Stent, and Max Delbrück, attending the meeting at Royaumont, 1952. (Photo courtesy of Seymour Benzer.)**

the recognition of the three phases of infective virus, vegetative virus, and prophage. Despite the ambiguous outcome of their experiments on lysogeny, Jacob and Benzer presented their results at the meeting.[65]

His time at the Institut Pasteur having run out, Benzer and his family set sail from Europe at the beginning of September.[66] After four years of freedom to learn his craft, from the masters in the field and by his own research efforts, he was finally heading back to take up the responsibilities as a member of Purdue University's Department of Physics that he had been able so long and productively to defer.

Benzer's experience in Lwoff's laboratory was representative of those of the young American phage workers of that time, most of whom went there during their apprenticeship years. There was a steady interchange of information between Paris and the American centers, in particular Caltech, which both Lwoff and Monod visited, though not to work in the laboratory. Contacts established through personal encounters were maintained through active correspondence in an era when letter-writing was still an important means of informal scientific com-

munication. The Parisian phage program borrowed methods from Delbrück and Luria but did not become an extension of the American school. The tradition of phage research was deeply embedded in the Institut Pasteur, and those who pursued it there when Benzer appeared were carrying on the pre-war work of the elder Wollmans as fully as they were incorporating that of the Americans. Moreover, the style of the work reflected a greater diversity of backgrounds, with Lwoff providing deep biological insight and Monod biochemical foundations that were missing in the phage group at Caltech and in Benzer's prior training.

Benzer benefited in multiple ways from this sojourn. In scientific terms he not only expanded the repertoire of methods with which he was familiar and practiced but enjoyed the first triumph of an investigation begun and completed within a few months that fully achieved what he had set out to do. He impressed his hosts with his experimental skills and the depth and rigor of his reasoning. He made lasting professional friendships. In cultural terms he expanded his horizons, becoming more cosmopolitan in his outlook. He found Europe "an intense experience, at once inspiring, discouraging, delightful, disgusting, exhilarating and depressing."[67] Undoubtedly he returned changed and matured by this plethora of experiences, his confidence increased by an auspicious success, his scientific viewpoint enriched by new perspectives and less dependent on the particular views or attitudes of any one of his successive mentors.

Chapter Five

Teaching and Research at Purdue

When Benzer returned to Purdue in September 1952, the Biophysics Laboratory had been completed. Supported jointly by the Departments of Physics and of Biological Sciences, the new enterprise was intended to apply "the point of view of the physicist to the understanding of basic problems in biology." Such programs were founded in other American universities during this period as part of a general movement whose aim was to apply the concepts and methods of physics to biology. There was, however, no widespread agreement about the domain of biophysics, and the subjects studied differed according to the interests of the particular faculty members recruited to the various institutions. At Purdue the research interests followed "two lines, one directed at understanding interface phenomena at the cell membrane (such as the propagation of impulses along a nerve fiber), the other concentrating upon 'nuclear problems' (such as the mechanism of self-reproduction of genes)." The first of these lines represented the interests of Lorin J. Mullins, the second those of Benzer; between the two of them the direction of the laboratory was to be divided.[1]

Benzer found the new laboratory an "elegant place with air conditioning" but not yet equipped for phage experiments. He saw that he would have to improvise with pressure cookers and egg incubators until the autoclave and other pieces of equipment that he had ordered were delivered.

At Caltech and at the Institut Pasteur Benzer had flourished working in close proximity with other phage researchers. Despite his proclivity for performing experiments alone in the laboratory late at night, where he could concentrate without interruption, he also relied heavily on frequent interactions with his colleagues. Delbrück cap-

tured this duality in Benzer's personality perceptively in a recommendation that he wrote for him several years later:

> He is singularly friendly and warm-hearted, entirely free of the gnawing resentments blighting so many gifted scientists. In the laboratory he prefers to work by himself, not collaboratively, and with a minimum of technical assistance.
>
> . . . This does not mean that he does not welcome discussion of his "work-in-progress" with others, or that he closes his eyes to the work of others. On the contrary, he knows how to get the greatest good from the advice of others and his influence in any laboratory is most beneficial. It merely means that he prefers to plan and execute his own work completely and be responsible for every phase of it.[2]

The "vital" atmosphere of discussion, mutual criticism, and advice exchanged among people engaged in similar work that he experienced in Delbrück's and Lwoff's groups had become so important to Benzer that, as the time approached to return to Purdue, he became concerned about how he would manage in its absence. In a letter to Lark-Horovitz in March, after describing the space and equipment he would need in order to begin phage research, he had added, "Something which disturbs me is the relative isolation at Purdue, so far as phage research is concerned. In a field which moves so rapidly, it is essential to have competent people with whom to discuss results. I hope it will be possible to invite others to come there." Noting that visa problems made it difficult to bring in Europeans, he wrote that he hoped to attract one of Hershey's graduate students and inquired about the availability of fellowships for that purpose.[3]

In his first weeks at Purdue, Benzer experienced what he had feared. "I beg you to send me phage and other news," he wrote Delbrück on September 26. "After Paris, the local phage isolation is almost unbearable." Attempting to stir up at long range what he missed so intensely locally, Benzer sent Delbrück a copy of the paper he and Jacob had given at Royaumont, saying that he "would like to have the criticisms of you and Renato [Dulbecco]. . . . There are some ideas in the discussion, vaguely descended from arms-and-legs, your reaction to which we would especially like. The point of vue [*sic*] can explain the 'paradoxes' of ph[oto] R[eactivation], the general ideas can be tested by experiments combining L[uria] L[atarjet] and ph R under precise blocking conditions (such as cyanide, rather than starvation)."

Knowing Delbrück's propensity to wager token stakes on the outcome of experiments, Benzer bet him a milkshake over a prediction about the differences between photoreactivation effects in T2 and T4, based on his belief that T2 has a "UV sensitive *early* reaction" absent from T4.[4]

The discussion to which he referred in the Royaumont paper bears sufficient resemblance to his discussions of phage development in his earlier paper, progress reports, and research proposals to imply that Benzer probably contributed this section to the paper (whereas Jacob is more likely to have written the section discussing prophage). In it he again conceived of the formation of an infectious center as the "end of a series of reactions requiring the participation of the infecting phage and the bacterium. Irradiation . . . can destroy an element necessary to the further achievement of one or more of these reactions. . . . The complex evolves only to a stage corresponding to the first reaction inhibited. If these reactions present different resistances to U.V., the sensitivity of the complex will be the sum of the individual sensitivities." If the radiation is given after certain sensitive reactions take place, then their sensitivity no longer affects that of the complex. He then applied these ideas to the resistances observed with T7 and T2. The pattern for T7 suggested a single reaction most sensitive to the radiation, whereas the fact that the resistance progressively increased during the first half of the latent period for T2 indicated that there "exist several reactions sensitive to U.V." He explained multiplicity reactivation using the same scheme. If a bacterium were infected by two T2 phage particles inactivated in such a manner that the reactions that one of them had lost were still intact in the other, then the products of these reactions could still be used by both phages, and they could reach maturation. That T7 does not show multiplicity reaction could then be explained by the fact "if all the phages inactivated by U.V. lose the power to participate in the same reaction," then one cannot supply what the other had lost.[5]

Delbrück quickly replied that he had read the paper but was too busy preparing for a course to comment in detail. "Your paper reads very well indeed," he wrote, but "the part in the discussion descended from 'arms and legs' philosophy I believe not; at least not the way it starts. I believe: (1) DNA is injected, (2) it unfolds[,] (3) as it unfolds it becomes progressively sensitive to restoring reactions with bacterial components[,] (4) some of these proceed in the dark, are strongly temperature sensitive, and are promoted by the metabolic disturbances of secondary injections (MR), (5) some are photocatalyzed."[6] Benzer an-

swered in turn, on October 27, "I don't think there is any *real* difference between the beliefs 1, 2, 3, 4, 5 which you stated in your letter, and the opinions of Benzer & Jacob." In an effort to keep the discussion going, he asked, "What is your idea of what happens to a U.V'd phage when it infects a bacterium? Have you shown our manuscript to Dulbecco?"[7]

It is not clear why Benzer saw no real differences between Delbrück's view and the views that he and Jacob were espousing, although there may have been nothing overtly contradictory between them. The exchange illustrates the ongoing preoccupation of the phage group with the events of the latent period in spite of their inability, after more than a decade of effort, to find out what these events were. The stakes, however, were very different for the two men. Delbrück, who had abandoned his effort in the early 1940s to analyze intrabacterial phage growth biochemically, was now treating it as a series of matings that he, in collaboration with Niccolo Visconti, analyzed mathematically as a problem in population genetics. He had no need to act on his "beliefs" concerning the nature of these "reactions."[8] Benzer, on the other hand, was now not only thinking about the problem as a series of biochemical "reactions" but was committed to a research program whose principal objective remained "eventually to analyze the different reactions that intervene in the reproduction of phage." By the end of October Benzer had found another way to compensate for the absence of other phage workers at Purdue with whom to engage in daily discussions. "After visiting Luria recently," he wrote Delbrück, "my lonely feeling is cured, since it is clear now that I can get a good argument at any time only 2 hours drive away—not only with Lou and his crowd, but with Sol Spiegelman & his."[9]

He availed himself of that opportunity often in the following years. Benzer's need for such regular contacts highlights the deeply social nature of scientific investigation. A man who enjoyed spending much of his working time in solitude, who was not given to small talk, and who protected his time carefully, he nevertheless believed he could not function fully as an investigator without frequently exchanging views with others similarly occupied. Even as he sought to mark out an investigative pathway that distinguished him from others in his field, he remained, like most effective scientific investigators, an intense collaborator in a collective enterprise.

"After considerable plumbing, dickering, and dishwashing," Benzer had now got his laboratory sufficiently ready to begin experimenting,

although he still lacked a full-time person to maintain it.[10] The project he took up built on some earlier observations of the effects of irradiating phage with ultraviolet light before they were used to infect bacteria. The survival curves of such phage were linear on semi-log plots, suggesting a "single-hit" inactivation phenomenon. In their joint paper Benzer and Jacob had suggested that "irradiation of phage before development could block their future development at a particular stage." In an appendix they had described a method of blocking the development of phage by means of cyanide, then releasing them to grow by diluting the cyanide. Combining these themes, he now worked "on the intracellular 'development' of T2 inactivated *before* infection, doing tricks," as he reported to Delbrück in January 1953, "with ph[oto] R[eactivation] in the presence of cyanide."[11] This was, however, only a temporary plan. By late January he had written and sent to *Biochimica et Biophysica Acta* a paper titled "Induced Synthesis of Enzymes in Bacteria Analyzed at the Cellular Level" and decided to pursue further work on adaptive enzymes. He was, he added in his letter to Delbrück, "waiting for crucial chemicals to arrive from Paris, so that I can take up the problem of the number of enzyme-synthesizing centers per cell for galactosidase (i.e. are the genes the thing?)."[12] Rather than returning to the pathway that he had followed before going to Paris, as he had planned to do in his original proposal for work at Purdue, he was now seeking to integrate what he had done and learned in Paris with his earlier work.

By that time Benzer had found still another way to increase his contacts with fellow workers in the field: by inviting the "local phagophiles" to a get-together at Purdue. Near the end of January Luria and his crew came from Urbana, Cyrus Levinthal and Edwin Lennox came from the University of Michigan, and George Bowen came from Oak Ridge.[13]

In order to prepare for a seminar that he intended to give concerning the size of the gene in February 1953, Benzer read three important recent papers. The first, titled "Genetic Formulation of Gene Structure and Gene Action," was written by Guido Pontecorvo of Glasgow, Scotland. A former student of Hermann Muller after Muller went to Scotland in 1937, Pontecorvo was reviewing fundamental questions about the size, nature, and definition of the gene in which his mentor had played so prominent a role before the war but which, according to Elof Carlson, received less attention in the years afterward.[14]

"There are various ways in which a gene can be defined," Pontecorvo wrote; "they are consistent with one another at certain levels of genetic analysis, but not at others. It is precisely at the levels at which inconsistencies arise that the interest of biochemistry in genetics and of genetics in biochemistry becomes greater." Genes can be defined, in the first place, as the "ultimate parts of chromosomes which are still 'self reproducing,' i.e., if missing they are not replaced in successive nuclear cycles." At the present time, however, there was no way to identify this ultimate unit.[15] In the second definition the gene was the "ultimate part of a chromosome which when changed is reproduced in the changed form in successive nuclear cycles." Such a change was commonly called a "mutation." According to the third definition, the gene was the "ultimate unit of physiological activity." This definition implied a one-to-one correspondence between genes and properties and seemed to "hold better the deeper the analysis of the effects of genes goes. The reason, of course, is that the more easily detected effects of a gene are physiologically several orders of integration removed from its primary activity." Reflecting implicitly the influence of the work of Beadle and Tatum during the previous decade (see Chapter 2), Pontecorvo treated the activity of an enzyme as that most likely to correspond to a primary gene action. The fourth definition of a gene, which, he noted, was "historically the first definition," stated that it was "the ultimate unit in hereditary transmission or more precisely unit of recombination."[16]

Because of the doubtful value of the first definition, Pontecorvo included only the other three in his summary of the definitions. In routine work, he stressed, two genes are clearly distinguishable and the three definitions are "practically consistent," expressing "different properties of one and the same thing. . . . Inconsistencies, however, arise when we test the three definitions in extreme cases, particularly in cases of very close linkage."[17]

If we compare Pontecorvo's definitions with those Muller had given a decade earlier (see Chapter 1), we can see that, although formulated somewhat differently, they were nearly equivalent. As the continued development of the field made it possible to focus increasingly on "cases of very close linkage," however, the problem of the inconsistencies that arose at this level assumed greater urgency.

Pontecorvo described two fundamentally different ways to estimate the size of a gene. "The most reliable method of resolving the chromosome into its linear array of genes," he wrote, "is that of crossing over."

The resolving power is limited only by the number of the products of meiosis that can be counted, and therefore it sets only a maximum size. The lowest measurable recombination frequencies measured up to that point, in microorganisms, were on the order of 10^{-4}. Assuming that the length of the giant salivary chromosome was the same as that of the same chromosome in somatic mitotic divisions or the early stages of meiosis, and that the frequency of crossing over was proportional to the length even at very short distances—an assumption he admitted to be doubtful—he calculated that the technical limits of analysis led to a length of 100 Å. He started with data collected by J. Alan Roper concerning crossing over among three closely linked genes in *Aspergillus nidulans.* From the difference between the total of the recombination distances between the middle and the outer two sites of mutation and the distance between the outer two, and assuming that the length of the chromosome corresponded to that in *Drosophila,* he estimated a length for the middle site of 4,500 Å, which he compared to the value of 1,250 Å. This was the value that Muller and Prokofyeva had obtained for the mean value of four genes in a small region of the giant salivary gland (see Chapter 1). All of these estimates were such, Pontecorvo concluded, that the "value of picturing the gene as of megamolecular size is disputable."[18]

The method of estimating the size of a gene developed by Douglas E. Lea in particular, another adaptation of target theory, used the dependence of the frequency of mutations on the dose of an ionizing radiation given to an organism. "Since irradiation of the order 10^7 to 10^8 r is required for an average of one mutation per gene, and one ionization or a small cluster within a reactive volume is assumed to produce mutation, the 'molecular weight' of this volume can be calculated at 10,000 to 100,000, equivalent to a diameter, if assumed to be spherical, of 20–60 Å." A related method, comparing the efficiencies of different densities of ionization, gave a diameter of 40–90 Å. Although the assumption of a spherical target was probably in error, these estimates were so much smaller than those based on genetic analysis that he could conclude tentatively that the "gene as a unit of physiological activity is based on a chromosome segment longer, in certain cases at least, than any part of a chromosome where mutation may occur." There may, therefore, be several sites of mutation within genes defined as units of physiological activity, and crossing over may take place within the gene between the various mutable sites.[19]

Drawing on questions Muller had raised in 1940 about whether

there were invariable, well-defined lines of demarcation between genes (see Chapter 1, p. 25) and on the more radical views of Richard Goldschmidt, Pontecorvo asked whether the "picture of a gene as a sharply delimited portion of a chromosome—the 'corpuscular' gene—still had the heuristic value which it unquestionably had in the early days of genetics." The units of crossing over and mutation may be integrated in more than one way to make up genes. They may be shared by more than one gene, and the integration of mutational units may differ in different stages of cellular development. "The genes, as units of physiological action, emerging from the preceding considerations," he declared, "are processes, or functions, not atomic edifices: and in first approximation their structural basis lies in a more or less clearly delimited chromosome segment of a length tentatively estimated of the order of 1000 Å, i.e. too long to be usefully considered as a mega-molecule."[20]

The second paper Benzer read, published in 1949 by Hershey and his research assistant, Raquel Rotman, was "Genetic Recombination Between Host-Range and Plaque-Type Mutants of Bacteriophage in Single Bacterial Cells." Continuing the program he had begun in 1946 to investigate the genetics of phage by mixed infections of mutant forms, Hershey had by this time isolated nine independent r mutants causing rapid lysis, one "h," or "host-range," mutant, unable to infect some strains of bacteria that the wild type could, and one "m," or "minute," mutant that produced a "very small halo less plaque."

"In principle," Hershey and Rotman wrote,

> the experimental technique we have to describe is very similar to that of genetic crossing, and will be referred to in this paper in genetic terms. One starts with a pair of mutants, each corresponding to a mutant haploid germ cell differing from the wild type by a different unit change. Bacterial cells are infected with both members of the pair, and during viral growth the pair interact to produce viral progeny corresponding to the germ cells of a new generation, but now including some individuals differing from wild type by both unit changes, and other individuals differing from wild type not at all.[21]

In their paper they illustrated with a photograph the visible differences in plaques by which they identified these forms (fig. 5.1).[22] By plating samples of the total yield of the progeny virus they could make differential counts of the various types of progeny yielded by crosses

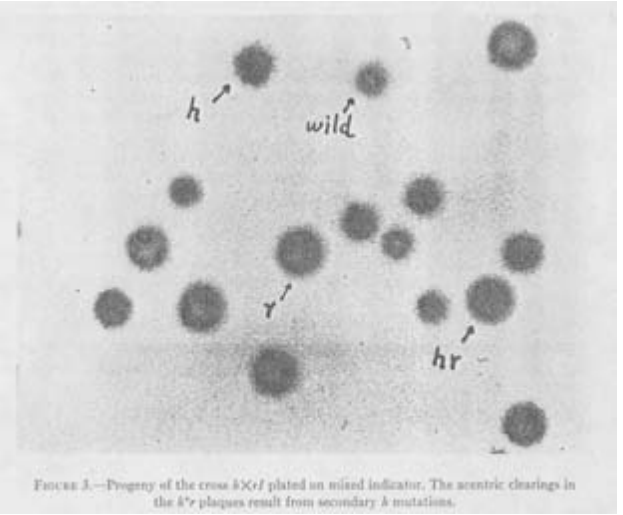


Figure 5.1. Photograph of plaques made by wild-type and host-range mutants of bacteriophage T2. (Reproduced by permission from A. D. Hershey and Raquel Rotman, "Genetic Recombination Between Host-Range and Plaque-Type Mutants of Bacteriophage in Single Bacterial Cells," *Genetics* 34 [1949]: 44–45, fig. 3.)

between each of the pairs of mutants. From these results they constructed a diagram of the "linkage system" that resembled a classical genetic map (fig. 5.2).[23] Accepting a view recently put forth by Luria that the multiplying units were not whole phage particles but "simpler structures" into which the phages became divided, Hershey and Rotman interpreted the system as representing "genetic action between two sets of independently multiplying chromosome-like structures." Although "the linkage data support fairly well the idea of linear structure, . . . independent evidence for crossing over is meagre." The reason for their caution was that the correlation between the numbers of reciprocal recombinants that one would expect to find from crossing over was not evident in their results.

Finally, Benzer read a paper already well known to the phage group, that by Hershey and Chase in which the central experiment using the Waring blender had shown, as they expressed it in their discussion, that "when a particle of bacteriophage T2 attaches to a bacterial cell, most of the phage DNA enters the cell, and a residue containing at least 80 per cent of the sulfur-containing protein of the phage remains on the cell surface. This residue consists of the material forming the protective membrane of the resting phage particle, and it

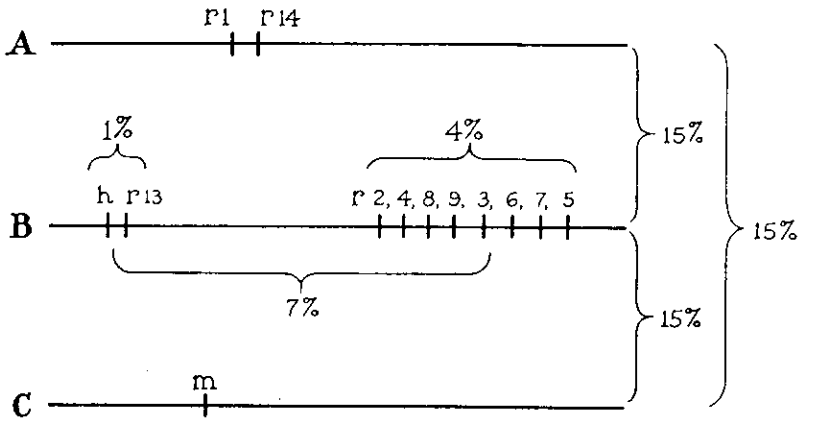


FIGURE 4.—Linkage relations among mutants of *T2H*. The percentages indicate yields of wild type in two factor crosses.

Figure 5.2. Linkage system for host-range mutations in bacteriophage T2. (Reproduced by permission from A. D. Hershey and Raquel Rotman, "Genetic Recombination Between Host-Range and Plaque-Type Mutants of Bacteriophage in Single Bacterial Cells," *Genetics* 34 [1949]: 44–45, fig. 4.)

plays no further role in infection after the attachment of the phage to bacterium." They assured themselves that the 20 percent of sulfur-containing protein not accounted for in that experiment did not play a role by showing that "little or none of it" is incorporated into the progeny, in contrast to the phosphorus and adenine of the progeny, which Jim Watson and Ole Maaløe had shown to be derived from that of infecting phage. Hershey and Chase inferred that "protein has no function in phage multiplication, and that DNA has some function."[24] As mentioned in Chapter 4, Watson and other phage biologists had quickly decided that the function to which Hershey and Chase alluded must be that it serves as the genetic material.

Benzer gave his seminar on the size of the gene at Purdue on February 20. According to his notes he proposed three answers to the question, what is the nature of the gene? The first was "a mark in a notebook," the light-hearted nature of which he indicated by adding "(not quite)." The second answer was "unit of genetic thinking," a more serious way to indicate its abstract nature in ordinary genetics. The third, a "unit of *hereditary transmission. self-reproduction. mutation. and physiological activity,*" he took from Pontecorvo's paper.[25]

Mentioning the "gene on string model of [the] chromosome," Benzer

added that the "gene alone [is] old fashioned—[and the] chromosome may be [the] unit." He seemed to reflect the position that Pontecorvo took in his article, but it is not evident whether Benzer accepted or merely mentioned these views. Noting that genes are composed of protein and DNA, he pointed out that the protein was "formerly considered crucial" but since the Hershey-Chase experiment, DNA appeared to be more important. Discussing that experiment, Benzer described the structure of phage and the one-step growth experiment. He also mentioned the "donut" experiment of Gus Doermann, who had shown that when bacteria are lysed prematurely by a chemical agent they release empty protein shells. He summarized the results of both in a circular diagram, which, he noted, is "not really *self*-reproduction."[26] (See fig 5.3.)

Turning to his central theme, "How long [is the gene]?" Benzer noted first that "it may vary in size" and that its "shape [is] unknown." Pointing out that some methods of measurement gave a length and others a volume, he compared the difficulty of answering the question with that of how many angels can dance on the head of a pin. The first way to find out, he said, might be to look in a microscope at giant salivary chromosomes in *Drosophila*. If the bands visible on these chromosomes were "correlated with genes," an assumption supported by the fact that genetic deficiencies matched absences of bands, then from the width of a band, which was estimated to be 20 mμ, and a molecular weight of 50×10^8, he could calculate a length of 200 mμ but stressed that this was only a "maximum number." The source of these numbers is not evident, but the next estimate that he gave, based on the study of four closely linked genes by Muller and Prokofyeva, evidently came from Pontecorvo's summary of their result.[27]

Citing Pontecorvo's use of Roper's data to calculate for *Aspergillus* a length of 450 nanometers (which Pontecorvo had expressed as 4,500 Å) for the length of the "middle" of three "mutation sites," Benzer commented that this represented the length of the "space plus gene" and that the method was no good if "only spaces between the genes count."[28]

Taking up the subject of "phage particles," Benzer summarized the experiments of Hershey and Rotman, copying out their linkage diagram, which he labeled "T2 map." Estimating that there were "about 20 known genes," he made a calculation of their length based on the dimensions of the T2 capsule (derived, presumably, from electron microscopy). The diameter of the capsule being 100 mμ (nanometers), he

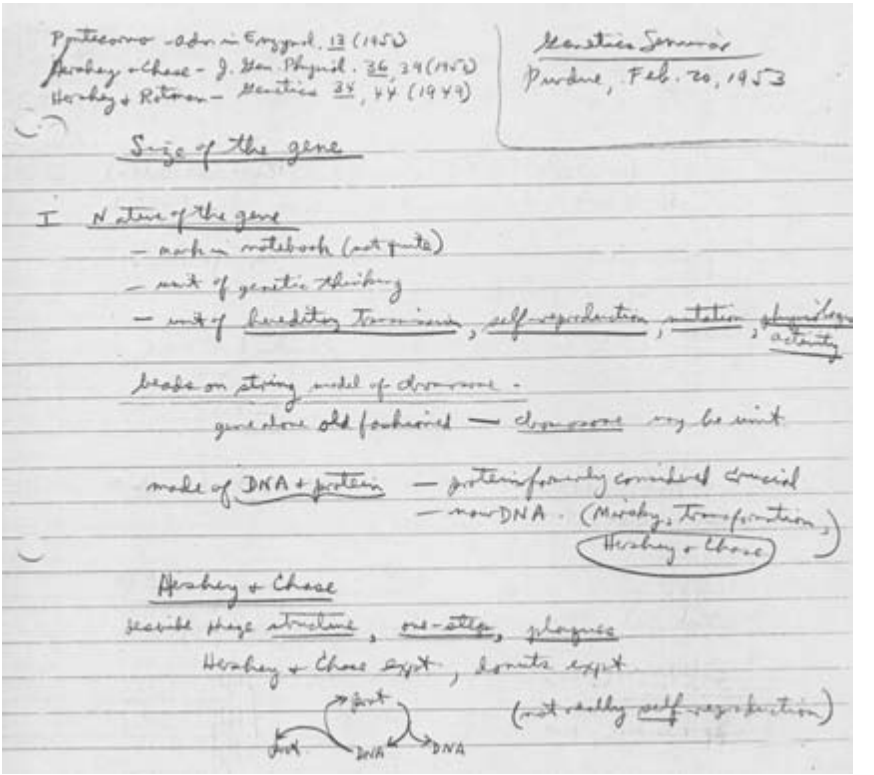


Figure 5.3. Benzer's summary of Hershey-Chase experiment and Doermann's phage growth model from notes for a seminar on the size of the gene presented at Purdue University, February 20, 1953. (BPP.)

divided that figure by the cube root of 20 to obtain a figure of 40 mμ for the diameter of one gene. For T3, whose diameter was 50 mμ , and for which 5 genes were known, the equivalent result was 30 mμ, and for the still smaller phage S13, assuming "at least one gene," it was 16 mμ. "Thus," he concluded, "with phage we can do better than with higher organisms." Though he cautioned that "genes may not be inside," any more than electrons are in the nucleus, he undoubtedly thought, in keeping with the Hershey-Chase experiment, that they most likely were within the protein capsule.[29]

Did Benzer think that the smaller dimensions calculable for phage genes challenged Pontecorvo's view that they are far too large to be megamolecules? The notes do not indicate that Benzer took any position regarding this issue. In any case, the lengths that he calculated

were derived from detectable mutations and represented, therefore, not the unit of physiological activity for which Pontecorvo wished to reserve the term *gene* but a "site of mutation," which Pontecorvo acknowledged could be "one or two orders of magnitude shorter" than the unit of physiological activity.[30]

Taking up the two target theory methods of determining the size of the gene that Pontecorvo had discussed, Benzer likened the first to a "blind man shooting bullets" at a circular target. "Every hit inside is registered, everyone outside is not." After describing the application to ionizing radiations, survival curves, and single- and multiple-hit curves, he made some calculations independent of those Pontecorvo had mentioned and came up with a minimum length of 15.8 mμ for S13 phage, assuming that the target was spherical with sharp boundaries and that all mutations were detected. He reached the same result applying the second method, which compared the results for three different types of radiation.[31]

In teaching a seminar on the size of the gene, Benzer was performing his new pedagogical role, a function not directly connected with his research interests, although the enthusiasm he showed for the target theory method derived from his research experience. That he should have chosen this subject for one of the few such seminars he gave in his first year at Purdue suggests, however, a more than passing interest in the question. Reading Pontecorvo in preparation for the seminar made him more aware than he had been of the questionable nature of the gene, and reading Hershey and Rotman probably stimulated his interest in the emergence of genetic mapping as a major subfield of phage biology. It did not immediately alter the direction of the experimentation he was carrying on, but preparing the seminar did lead him to think about problems beyond his longstanding preoccupation with the use of radiation to explore the nature of phage reproduction during the latent period.

In the undergraduate course Biology 156, Benzer delivered two lectures on the subject of structural duplication. On April 10 he gave the general background, oriented around the question, what, if any, structures duplicate? The key question in biology, he began, was reproduction. Summarizing the problem abstractly in the statement, "0 = 0 + 0, every part is duplicated," he asked, "Are these 'self-duplicated'?" For historical background he reached back to the seventeenth-century debate between the epigenetic view presented by William Harvey and preformationist views, the latter split between the homunculists, who

viewed the organism as preformed in the sperm, and the ovists, who believed it to be preformed in the egg. This "bitter war," in which both sides turned out to be wrong, seems silly now, he remarked, but it had parallels in the current division between the nucleic acid and the protein "schools" of gene reproduction, and here, too, "both could be wrong."[32]

Drawing again on Pontecorvo's article, Benzer enumerated the meanings of the gene described there but did not adopt Pontecorvo' s position that the genetic loci cannot be megamolecules. The gene, Benzer stated, "could have many forms." If it was a megamolecule, then it must be a "complex structure" with a "specific arrangement of components" that were "always present and directing activities." The gene could also be thought of, however, as "any member of a cycle" in which one molecule gives rise to another and none of them "self-duplicates." In this case the gene could be thought of as a "habit." Finally, we "can consider the gene as a piece of information 'fed into' a machine with instructions, including the instruction to make 2 copies." Then it "loses its identity."[33]

All of these views were, Benzer stressed, only speculations. "Thus, we are not certain that 1) direct self-reproduction occurs, 2) if it does, we do not know if protein does it or nucleic acid, 3) we are not sure of the structures of prot[ein] or NA. So, we are discussing the problem of structure duplication when we do not know the structure and do not know whether duplication occurs." The whole discussion may be premature, he acknowledged. Nevertheless, posing the possibilities might serve as a stimulus to the further experimentation necessary to resolve such issues.[34]

"Part of the difficulty in experimentation," Benzer went on, "is complication due to the complex cellular activities," which include "not only the reproduction of genes, but synthesis of enzymes, cell walls, flagella, etc. We would like to study pure genes, and the closest we can come to this is viruses," which are on "the borderline between living and dead." They "seem to be concerned only with reproducing themselves, and consist of nucleoprotein." Outlining the structure of bacterial viruses and their reproductive cycle, he again reviewed the Hershey-Chase experiment and its significance. "It seems at present," he concluded, "that DNA is more important than protein." He cautioned, however, that this may be temporary. Whatever the role of these two classes of molecules turned out to be, "somehow the specificity is transmitted," and this specificity is "assumed to be in the number

and order of arrangement of the components." This was, in spite of all the uncertainties, a safe supposition. "What else," he asked, "have they got?" It may be that neither molecule was self-reproducing but that "specific DNA → specific protein which makes more DNA." He closed his lecture by mentioning "other cases of self-duplication" thought to take place in cells, including those of enzymes, plastids, and plasmagenes.[35]

In his second lecture, on April 13, Benzer considered specific replication schemes. First pointing out the opposition among current schemes between the concepts of negative and positive replication, the former assuming that a template molecule gives rise to another whose structure is complementary to it, the latter that the template gives rise to a second molecule identical to it, he used the analogy to a tennis ball to suggest that one can have both. The two halves of a tennis ball are both complementary to each other and identical. In the remainder of his lecture Benzer summarized two recently proposed replication schemes, one representative of the "protein school," the other of the "nucleic acid school."[36]

Felix Haurowitz, a member of the chemistry department at the University of Indiana, had for two decades studied antigen-antibody reactions. In an article titled "Biological Problems and Immunochemistry," published in 1949 in the *Quarterly Review of Biology,* Haurowitz had reported on recent advances in the study of these reactions obtained by using uniform chemical substances as antigens. Such analytical methods had greatly clarified the relation between the "determinant groups" contained in antigens and the antibodies formed. The antibodies synthesized in cells are "complementarily adapted negative replicas" of the antigen proteins that carry the determinant groups. "It seems necessary to assume," he had asserted, "an analogous mechanism for the formation of normal proteins." At first, the view that normal cellular proteins are formed as negative replicas of a positive cellular template seems paradoxical, he had acknowledged, because some proteins, such as those of genes and viruses, "are formed by self-reproduction, i.e., as *positive replicas* of their templates." These ideas could be reconciled, however, by assuming two successive steps in the formation of a protein molecule. For the first step, the "formation of the specific peptide chain containing a definite number of each of the amino acids arranged in a definite order," Haurowitz had proposed a mechanism that drew on ideas previously expressed by, among others, Hermann Muller and Linus Pauling, as well as the model presented

by Max Delbrück at the Cold Spring Harbor Symposium in 1941 (see Chapter 2). Haurowitz supposed that this synthesis took place along a monomolecular layer of the template chain, where the short-range forces that Muller had claimed in 1922 to be responsible for gene reproduction can operate. In an analogy to the deposition of molecules identical to those composing a crystal on its surface, monolayers of the amino acids that will compose the protein replica are deposited along the template chain. "We can imagine," Haurowitz had written, "that a 2–dimensional peptide monolayer covered by a solution containing different amino acids will preferentially adsorb amino acids of the same kind, so that tyrosine, leucine, and alanine will be adsorbed to tyrosyl, leucyl, or alanyl residues of the expanded peptide chain. If these adsorbed amino acids in the presence of a non-specific enzyme undergo condensation, a positive two-dimensional replica of the expanded peptide layer will be formed." To account for the complementary nature of antibodies, he had invoked a second step: the folding of the extended chain into a globular form in which the antigen acting as a template causes a small portion of the antibody molecule to acquire the shape of a negative replica.[37] "This picture would . . . be incomplete," Haurowitz had concluded, "if account were not taken of the important role played by the nucleic acids in protein synthesis." That role, he had surmised, is "most probably, to render insoluble the 2–dimensional peptide layer which forms the template," because the template must be rigid and insoluble in order to perform its function.[38]

After outlining Haurowitz's scheme in his lecture, Benzer raised several arguments implying that it did not fully satisfy him. The first was that it "requires two templates." The second took the form of the question, what stops crystallization? The third and probably strongest objection, to his mind, was that "protein is not the thing, anyway." This response was a reflection of the fact that since the publication of Haurowitz's paper the Hershey-Chase experiment had swung opinion toward the view that DNA was more important than protein in the replication of genes, as Benzer had mentioned in his previous lecture. To explain Haurowitz's preference for a protein template, Benzer remarked that "he feels N[ucleic] A[cids] not specific enough."[39] This was only an assumption on Benzer's part, because Haurowitz had not given a reason in his paper for the nonspecific role he assigned to nucleic acids. That nucleic acids were not complex enough to determine the specificities of genetic transmission was, however, a widespread attitude among biochemists of the time and has been invoked

since then by historians as an explanation for the resistance to the evidence produced earlier by Oswald Avery and others that DNA can function as the template in the reproduction of genes or the synthesis of proteins.[40]

To represent the nucleic acid school, Benzer discussed a paper published in 1952 by Alexander Dounce of the Department of Biochemistry at the University of Rochester. The mechanism of duplication that Dounce proposed was based on his conviction that recent extensions of the work of Avery on transformation were making it "increasingly probable that nucleic acids may be the patterns or templates which must govern gene action and hence protein synthesis, since according to the work of Beadle et al. a gene appears to act by causing the synthesis of a specific enzyme or other protein." Dounce was, therefore, already persuaded of the view to which the Hershey-Chase experiment was soon to attract more followers. The purpose of his paper was to "outline a reasonably plausible chemical mechanism that might account for peptide chain synthesis, in the hope of pointing the way to experimental work that might prove decisive in solving the problem." The chemical scheme he proposed

> was based upon the hypothesis that there are at least as many specific nucleic acids in a cell as there are specific peptide chain arrangements in the proteins of that cell. It is furthermore postulated that the specific arrangement of amino acid residues in a given peptide chain is derived from the specific arrangement of nucleotide residues in a corresponding specific nucleic acid molecule, and that in addition the nucleic acid molecule in question is concerned in transferring energy necessary for peptide bond synthesis. The proposed mechanism shows how these postulates can be expressed in chemical detail by the use of familiar types of biochemical reactions. In addition, an extension of the mechanism accounts for the reduplication of the nucleic acid templates by a path which is independent of peptide chain synthesis but which involves reactions of a type similar to those used for the latter purpose.[41]

The mechanism that Dounce proposed assumed that adenosine triphosphate can interact with the DNA molecule to yield a diphosphonucleic acid, in which, through the action of a nonspecific enzyme, the added phosphate groups are attached sequentially to the phosphates that make up the backbone of the nucleic acid molecule (fig. 5.4). This diphosphonucleic acid ought to be able, from an energetic standpoint, to react with the alpha-amino groups of amino acids to form amino-

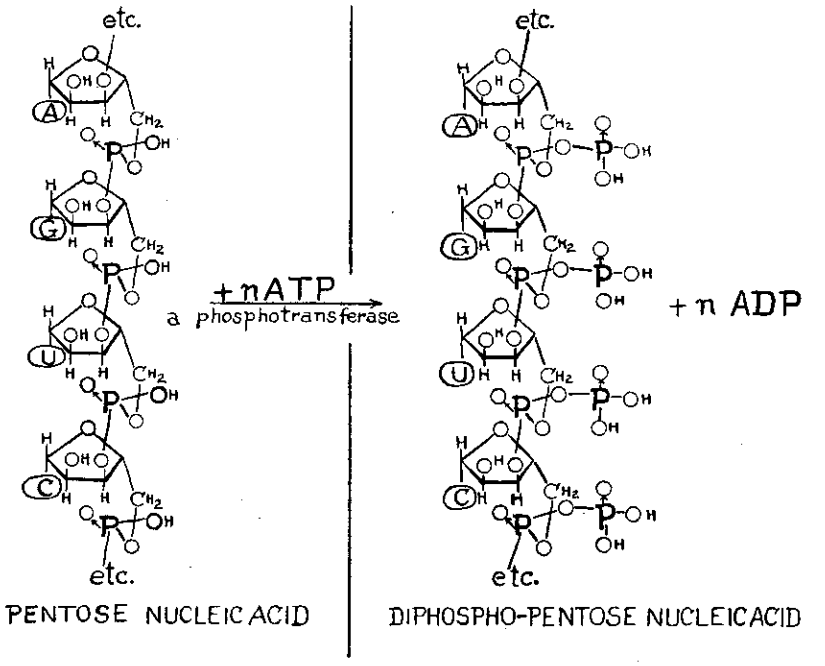


Fig. 1. Formation of diphosphonucleic acid.
A encircled = Adenine
G encircled = Guanine
U encircled = Uracil
C encircled = Cytosine

Figure 5.4. A. L. Dounce's 1952 model for formation of diphosphonucleic acid as the first step in protein synthesis. (Reproduced by permission from A. L. Dounce, "Duplication Mechanism for Peptide Chain and Nucleic Acid Synthesis," *Enzymologia* 15 [1952], fig. 2.)

phosphate compounds, with the loss of inorganic phosphate, thus transferring the energy necessary for the synthesis. The key to Dounce's scheme was the postulate that there existed a class of specific synthesizing enzymes determining which amino acid would be attached at each phosphate site. Each enzyme would be specific not only to the particular purine or pyrimidine at that site in the polynucleotide chain but to its two neighboring bases. Thus, for the case of adenine, there would be ten possible enzymes corresponding to the following possible surrounding bases: A-A-A, G-A-A, C-A-A, U-A-A, G-A-G, C-A-G, U-A-G, U-A-U, C-A-U, and C-A-C. There would, similarly, be ten possible "neighborhoods" for each of the other three bases, cytosine, uracil, and guanine, making forty in all. There were, he pointed

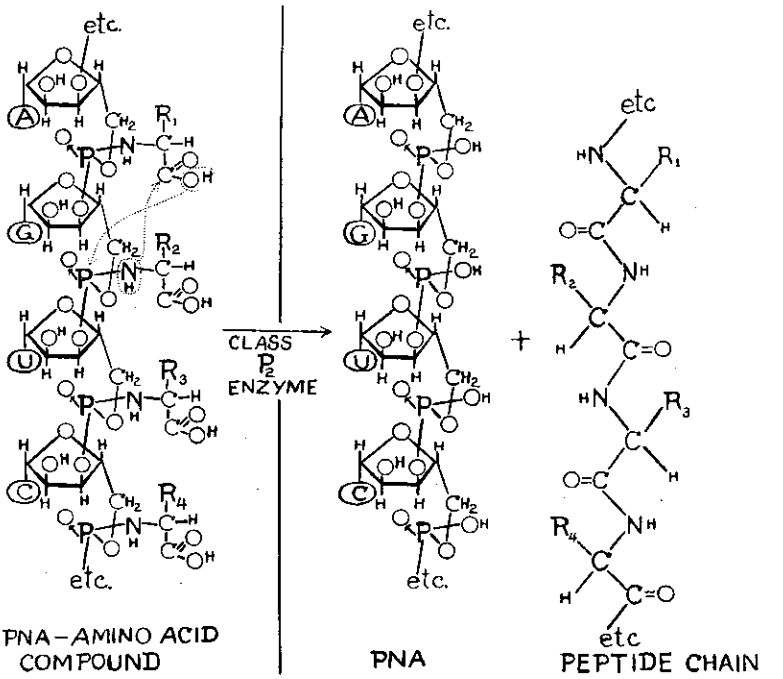


Fig. 3. Formation of peptide chain under influence of a class P_2 enzyme (phospho-carboxy transamidase).

Figure 5.5. A. L. Dounce's 1952 coding scheme for DNA-directed protein synthesis. (Reproduced by permission from A. L. Dounce, "Duplication Mechanism for Peptide Chain and Nucleic Acid Synthesis," *Enzymologia* 15 [1952], fig. 5.)

out, "more than enough configurations to account for all amino acids known to occur in proteins" (fig. 5.5).[42]

A second class of enzymes, in this case nonspecific, would be required to "peel off" the peptide chain by means of a transamidation reaction. From the same diphosphonucleic acid that served as an intermediary in protein synthesis, Dounce showed, it would be possible to form a dinucleotide-nucleic acid using only four enzymes, one specific to each of the four nucleotide bases. A relatively nonspecific enzyme would then suffice to split off the second polynucleotide, resulting in the formation of a new nucleic acid molecule containing the same sequence of bases as the original one. In presenting his scheme, Dounce stressed that other intermediates in place of the diphosphonucleotides could serve as the same basic mechanism.[43]

The system was flexible, Dounce pointed out, in that it should bring

about the synthesis of peptide chains when supplied with ATP and amino acids, nucleic acids if supplied with ATP and mononucleotides, and both if supplied with a mixture of amino acids and mononucleotides, "in ratios varying with the relative concentrations" of these substrates. Although a hypothesis of the type he had presented should "take into account the geometry and special arrangements of the molecules in question," he admitted, he thought that too little was known about the structure of nucleic acids to make such "calculations . . . very profitable at present. Possibly some work with atomic models would be desirable." Meanwhile he was encouraged by the earlier finding of William Astbury that the major spacing found along the fiber axis in x-ray crystallographic analyses of polynucleotides corresponded with the spacing in polypeptides, making it plausible that the amino acids could be aligned along the diphosphonucleotide chain in the manner that his scheme required.[44]

In his lecture Benzer emphasized that Dounce's scheme required "one NA for each peptide arrangement in [the] cell," that the "specific order of amino acids [is] derived from [the] order of nucleotide residues," that nucleic acid is "concerned in [the] transfer of energy for peptide bond synthesis," that the "same template [serves] for both NA and peptide," and that each base condenses "with [a] specific amino acid determined by [the] base and its neighbors." In summary, he said, the scheme "makes NA important," "postulates new intermediates," and "allows variation in rel[ative] amounts of NA and protein formed."[45]

Morange has pointed out that Dounce was the "first person to consider the relation between proteins and nucleic acids to be indirect and thus non-stereospecific and structurally arbitrary." Because template models were at the time thought to require stereospecific interactions with other molecules, as in Pauling's model of antibody formation, the "originality of Dounce's model was not widely recognized."[46] Benzer's response to the model suggests, however, that those who were thinking about the problem of gene duplication and protein synthesis at the time the paper appeared appreciated its significance more than is apparent from the published literature. It was not only plausible and boldly original, but it anticipated brilliantly what later became known as the "sequence hypothesis." Moreover, it solved perceptively the problem of how a sequence of just four bases might specify a larger number of amino acids. That Dounce's mechanism had only a limited, transient influence was due to the unforeseen events of the year after it

was published, which quickly made obsolete his assumption that too little was known about the structure of nucleic acids to take into consideration the geometry and spatial arrangements of the molecules in question.

Two days after giving the second of his lectures on structure duplication, Benzer covered much of the same ground again in a biophysics seminar, where he added a few new points to his previous discussions. Under the heading "Nature of the Gene," for example, he reiterated his views about the gene as a megamolecule and the alternative "cyclic theory," but in dealing with "information theory" he compared the gene to a telegram, which "loses its identity during 'reproduction.' " According to this conception a "mutation = misprint."[47] For these analogies Benzer may have reached back to the reading of Schrödinger's *What Is Life?* that had initiated his shift to biology seven years earlier.

After outlining these positions, Benzer cautioned that "we must bear in mind

a) instantaneous picture does not represent the organism
b) Observation and experiment interfere with events and [there] may be some kind of indeterminacy here—if you determine all the information you kill the cell.[48]

This allusion to the views that he had first encountered in the mimeographed lectures of Max Delbrück that he had read in 1946 (see Chapter 3) confirms that Benzer remained as deeply impressed with these ideas about the biological equivalent to complementarity in physics, long after his initiation into the phage group and the development of his association with Delbrück, as he had been when he first encountered them as a graduate student in physics.

Turning next to the problem of duplication, Benzer again took up the question of positive and negative templates, retold with relish the story of the bitter wars between homunculists and ovists, and outlined the Hershey-Chase experiment, from which he concluded that "DNA [is] most important" and that "NA alone must be sufficient to give duplication plus protein" (although he noted that the "result may be in error" because "a small amount of protein" may enter the bacterium). Under the heading "Now We Are Getting NA Theories," he again summarized the mechanism of Dounce, no longer balancing that theory with a discussion of theories of the protein school.[49]

At the end of his discussion Benzer took into account the newest

development in the nucleic acid field. Pointing out that "duplication theories are handicapped by lack of knowledge about structure," he described the "new theory" of Pauling and Corey that DNA is composed of "3 intertwined chains" with "PO_4 in [the] middle" and "bases sticking out." It is likely that Benzer had only learned about this structure, which had just appeared in the *Proceedings of the National Academy of Sciences,* between the time he prepared his lectures for Biology 156 and the time he gave his biophysics seminar. Using the same methods of model-building and rigorous specification of bond angles and lengths that had enabled them to solve the structure of the protein alpha-helix, Linus Pauling and his associate Robert Corey had formulated a "promising structure for the nucleic acids."[50] They placed the polynucleotide backbones at the core of a three-chain helical molecule. At each level along the fiber axis three phosphate groups were packed around an octahedron, with the four oxygen atoms of the group forming a tetrahedron around the phosphate atoms. The ribose sugars attached to the phosphate groups connected the latter into chains, while the purines and pyrimidines projected toward the outer radius of the helix. While each chain of the molecule formed a left-handed spiral, repeating itself after 3½ turns and eight nucleotide residues, the successive layers of projecting bases made a slow right-handed spiral (fig 5.6).

The atomic parameters that they gave for the molecule, Pauling and Corey wrote, "represent the best solution of this problem that we have found; these parameters, however, probably are capable of further refinement. The structure is an extremely tight one, with little opportunity for change in position of the atoms." In their effort to solve the structure of DNA, Pauling and Corey had worked with limited information. Their attempts to determine the crystal structure of DNA had not yielded accurate results, and they had to rely on the outdated data of William Astbury. Their choice of a triple chain with the phosphate backbone on the inside was influenced by structures that had been suggested earlier without detailed atomic coordinates. Although all of the bond angles and lengths conformed to the accepted limits for each type, some of the dimensions they postulated came very close to these limits. Altogether, their structure only accounted "moderately well for the principal features of the x-ray patterns of sodium thymonucleate and other nucleic acid derivatives." Nevertheless, as the "first precisely described structure for the nucleic acids that has been suggested by any investigator," Pauling and Corey's model seemed at first sight to

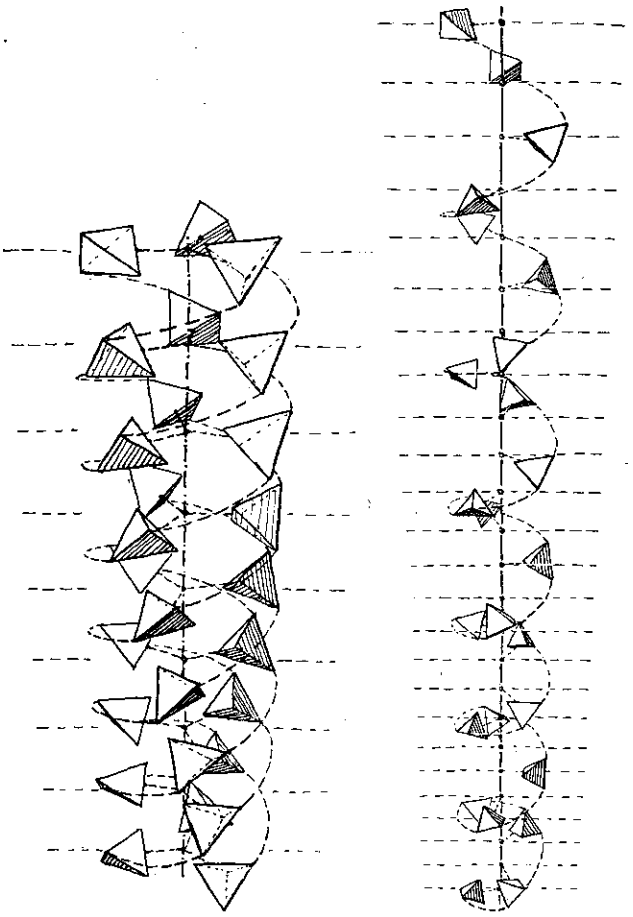


Figure 5.6. The triple-stranded DNA structure proposed by Pauling and Corey in 1953. (Reproduced by permission from L. Pauling and R. B. Corey, "A Proposed Structure for the Nucleic Acids," *Proceedings of the National Academy of Science* 39 [1933]: 84–97.)

fill the strongly felt need for a structure on which to base ideas about duplication and protein synthesis. Although they pointed out that the radially directed hydrogen bonds of the purine and pyrimidine groups on the periphery of the molecule "would permit the nucleic acid molecule to interact vigorously with other molecules," they offered no ideas about how the structure might self-duplicate, and their model, in fact, suggested none.[51] In the brief note that Benzer prepared for his discussion of this new theory in his biophysics seminar, he left no indica-

tion that he saw any way in which their proposal might overcome the "handicap" that the previous absence of knowledge of the structure had posed for solving the problem of structural duplication.

The series of seminars and lectures on the size of the gene and on structure duplication that Benzer gave at Purdue in the spring of 1953 show that during this period these had become topics of intense interest for him, if they had not been earlier. The uncertainties that he expressed and the unsolved problems that he posed for his students are particularly revealing because of their timing, as well as his own position as a well-integrated younger member of the phage group whose central goal was to understand the gene as the basic unit of biological reproduction and mutation. At precisely the same time that Benzer was grappling pedagogically with these issues without knowing the structures of the molecules in question, James Watson and Francis Crick were proposing a radically new structure for DNA that rapidly transformed the scientific landscape within which the issues could be framed. The brief letter to *Nature* in which they announced their helical model appeared on April 25—only ten days after Benzer introduced the "new theory" of Pauling and Corey to his biophysics seminar.

Whereas Pauling and Corey had incorporated three chains of the phosphate-sugar backbones, Watson and Crick chose two chains. Whereas Pauling and Corey had located the chains in the core of the fiber axis with the bases projecting outward, Watson and Crick placed the chains on the outside of the helix with the bases inside. Each base attached to one chain was paired by means of hydrogen bonds to a corresponding base attached to the other chain. The most novel feature of their model was that the regularity of the two backbones required that a purine base on one chain could pair only with a pyrimidine base on the other. Making the additional assumption that the bases exist in the molecule in only one "favored" tautomeric form, they inferred from the resulting locations of the hydrogen bonds that the pyrimidine-base thymine pairs only with the purine-base adenine, and cytosine only with guanine. They illustrated these features in simplified form with a schematic diagram (fig 5.7). Because the bases were stacked horizontally with respect to the vertical fiber axis, there were no restrictions on the order of successive base pairs, and Watson and Crick assumed that any sequence of pairs could occur. "The sequence of bases on a single chain," they wrote, "does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other

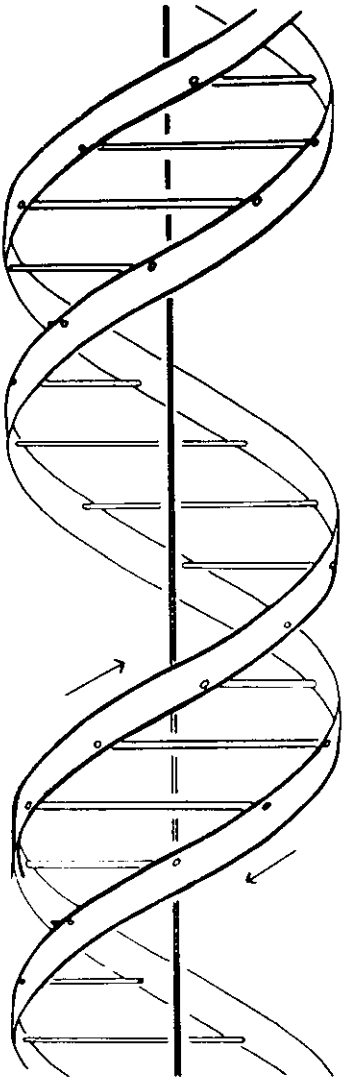


Figure 5.7. The double-stranded DNA structure proposed by Watson and Crick in 1953. (Reproduced by permission from James D. Watson and Francis H. C. Crick, "The Structure of DNA," *Cold Spring Harbor Symposia on Quantitative Biology* 18 [1953], fig. 4, p. 125.)

chain is automatically determined." Each of the two chains was the "complement of the other."[52]

In their succinct discussion, Watson and Crick barely hinted at the consequences of their model for the question of gene replication. "It has not escaped our notice," they wrote, "that the specific pairing mechanism we have postulated suggests a possible copying mechanism for the genetic material." This implication of the model was, in fact, so evident that the authors quickly followed with a longer submission to *Nature* making the mechanism explicit:

> The phosphate-sugar backbone of our model is completely regular, but any sequence of the pairs of bases can fit into the structure. It follows that in a long molecule many different permutations are possible, and it therefore seems likely that the precise sequence of the bases is the code which carries the genetical information. If the actual order of the bases on one of the pair of chains were given, one could write down the exact order of the bases on the other one, because of the specific pairing. . . . It is this feature which suggests how the . . . molecule might duplicate itself. . . . Our model for deoxynucleic acid is, in effect, a *pair* of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken, and the two chains unwind and separate. Each chain then acts as a template for the formation on itself of a companion chain, so that eventually we shall have *two* pairs of chains, where we only had one before. Moreover, the sequence of the pairs of bases will have been duplicated exactly.[53]

In the terminology in which template mechanisms had previously been discussed, the Watson-Crick model combined the merits of a negative and a positive template, fulfilling concretely the conditions Benzer had expressed abstractly with his tennis ball analogy. The single chain on which a complementary chain was supposed to form produced a negative replica in a process understandable in biochemical terms through stereochemical fitting together. The resulting double helix, however, was an exact positive replica of the double helix from which it had arisen, preserving the identity of the genetic material.

The Watson-Crick model immediately drove the Pauling-Corey structure out of contention. Before the publication of the first of the papers to appear in *Nature,* Pauling himself (to whom Delbrück had shown a letter from Watson describing the model) stated at a Solvay Conference on proteins in April that his own proposal for the structure of nucleic acid was "probably wrong" and that "the formulation of their structure by Watson and Crick may turn out to be the greatest

development in the field of molecular genetics in recent years." The primary reason for these judgments was not that the empirical evidence for the double helix was more decisive than that for the Pauling-Corey triple helix. Both were based on similar considerations—model-building, attention to accurate bond angles and lengths, and the compatibility of the model with the existing x-ray diffraction patterns. Although Pauling and Corey had made several assumptions that soon appeared dubious, and although Watson and Crick had benefited by having seen the recent superior x-ray photographs of Rosalind Franklin and found additional support for their base-pairing mechanism in the "Chargaff rules"—that is, the experimental work by Erwin Chargaff showing that in DNA from a variety of sources the ratios of adenine to thymine and of guanine to cytosine always approached very near to 1:1—both sets of authors prudently presented their structures as not yet "proved to be correct." Pauling saw as clearly as did Watson and Crick that the great advantage of their structure was that "in its feature of complementariness of the two chains it suggests a mechanism for the duplication of a chain by a two-step process—a molecular mechanism that may well be the mechanism of hereditary transmission of characters."[54] That this suggested mechanism was inherent in the very structure of the model gave the double helix a double beauty, one of form and of potential function that exerted a powerful attraction on those concerned with this fundamental and long-standing problem.

On June 3 Benzer left Lafayette to travel to Cold Spring Harbor for the eighteenth annual Cold Spring Harbor Symposium on Quantitative Biology. The program, organized by Delbrück, was on the subject of viruses, and he had planned it in order to bring together phage and animal virologists. Building on the "trinity" of virus stages agreed on the previous year at the international bacteriophage congress at Royaumont, France, Delbrück organized the sessions around the infective, or mature virus; the multiplying, or vegetative phase, which, he pointed out, was a term borrowed from its application to sporulating bacteria; and the condition in lysogenic bacteria named provirus by André Lwoff. The prominence given to the last was a mark of the influence of the recent work on lysogeny by Lwoff and his group, most of whom came to the symposium. In his opening remarks Delbrück expressed the hope that the structure of the program would serve both to focus on the "most interesting problems of current research" and "to bridge the gaps between the classes of viruses and between the methods of research." Two hundred and eighty people participated in or attended the meetings, the largest ever.[55]

To judge from Benzer's report on his trip, the program succeeded well in its aim of bringing the two fields closer together:

> Sections of the program were arranged so as focus upon one of these forms [vegetative, infective, or provirus], or the transition between one and another, drawing material from studies of various kinds of diverse viruses. In this way the broader aspects of virus phenomena were stressed. The good effects of this approach could be seen in the dwindling, as the meeting progressed, of the initial good-natured antagonism between animal virologists and phage workers. (Part of this effect could perhaps also be ascribed to the forced contact occasioned by a judicious pairing of roommates.) The paper of Renato Dulbecco on the final day of the symposium, describing the successful application of phage techniques to the study of animal viruses, and pointing out that some phenomena may prove to be more readily studied with animal viruses than with phage, dealt the final blow to any feelings of difference.[56]

Benzer did not give a formal paper, but in one of the discussion sessions he was able to present his "recent observations on the injection step in bacteriophage infection." Reflecting the both the broadly international character of virology and the importance to him of frequent interactions with others in his field, he finished his report with the thought that "the meeting was invaluable for the opportunity it offered to meet virus workers from all over the world."[57]

Delbrück had noted in his introductory remarks that there was a last-minute addition to the list of participants. "The discovery of a structure for DNA proposed by Watson and Crick a few months ago and the obvious suggestions arising from this structure concerning replication seemed of such relevance to many of the questions to be discussed at this meeting that we thought it worth while to circulate copies of three letters to *Nature* concerning this structure among the participants and to ask Dr. Watson to be present at the meeting."[58]

The rather peremptory invitation that Delbrück had issued to Watson to participate had been the outgrowth of a correspondence between them that began when Watson sent a letter to Delbrück in March outlining their model. Delbrück had immediately perceived its genetic implications and potential importance, seeing that if correct it would open a new and turbulent era in molecular biology. He recognized the idea of base pairing as fundamentally important and was ready to bet on its validity. True to his skeptical nature, however, he rejected what was for Watson and Crick the feature that defined the molecule and imposed on it the specificity of its base pairs. Delbrück could not accept

the idea that the two strands of polynucleotides, wound around each other hundreds of times, could be separated from one another without breaking into pieces. Declaring this unwinding or "untwiddling" problem to be "insuperable," he proposed to Watson that the two strands must not be wound around each other in an interlocking, or "plectonemic," form but in a "paranemic" manner, in which two individual coils are placed side by side, then slipped in between each other until their centers roughly coincide. Although he admitted that he and Crick were unhappy about the unwinding problem, Watson objected that two paranemic coils could not fit the crystallographic data to which their model conformed.[59]

Watson presented his paper in the main auditorium of the Cold Spring Harbor laboratory, gesturing frequently with a pointer to a projection of the schematic drawing of the double helix that had accompanied the first of his and Crick's papers in *Nature.* To make matters clearer, he displayed a three-dimensional model that the shop of the University of Cambridge had fabricated for him. Systematically he reviewed the chemical and x-ray evidence for the fibrous nature of the molecule and for the existence of two chemical chains in the molecule, and then he described the proposed helical structure, pointing to the diagrams above his head and to the model on the table before him. He displayed the two base pairs permitted by the spacing between the phosphate ribose backbones and the positions of the hydrogen bonds on the adenine, thymine, guanine, and cytosine bases. After summarizing the evidence for these aspects of the molecule, he stated that "we thus believe that the present experimental evidence justifies the working hypothesis that the essential features of our model are correct and allows us to consider its genetic possibilities."[60]

"A genetic material must in some way fulfill two functions," he began. "It must duplicate itself, and it must exert a highly specific influence on the cell. Our model for DNA suggests a simple mechanism for the first process, but at the moment we cannot see how it carries out the second one." That is, they had no ideas about how the sequence of DNA bases determines the synthesis of proteins. The self-duplication relied on the union of each part with an opposite or complementary part, avoiding the difficulty of imagining how "like attracts like" and also making unnecessary the idea that proteins and nucleic acids are complementary to each other and that self-replication involves the alternate formation of one and then the other. After proposing that the two chains unwind and that each chain then forms a mold

on which free nucleotides can attach themselves to form a new chain, he acknowledged that it was not obvious to them "whether a special enzyme would be required to carry out the polymerization or whether the existing single helical chain could act effectively as an enzyme."[61]

Watson now confronted the difficulties in the replicating scheme. Several of them he dismissed easily, but he admitted the problem of unwinding raised by Delbrück to be a "very fundamental difficulty." He mentioned the solution that Delbrück had proposed based on paranemic coiling but replied that "it is impossible to have paranemic coiling with two regular simple helices going around the same axis. This point can only be clearly grasped by studying models"—perhaps one of Watson's motivations for bringing a model was to allow his listeners to see for themselves why the paranemic scheme would not work.[62]

Estimating that for an organism such as phage the number of DNA turns might be twenty thousand and in higher organisms the upper limit might be a thousand times higher, he described the problem of finding out how the chains could untwist without tangling as a "formidable one." Watson favored the possibility that the untwisting process would be less complicated if the replication began at the ends as soon as the chains began to separate. The danger of tangling would be reduced because a two-stranded structure is much stiffer than a single strand. Such tentative suggestions left the difficulty largely unsolved, but Watson and Crick seemed content to leave to the future the discovery of the "precise mechanism of replication."[63]

According to some of those who were present at this session, Watson's presentation left them spellbound. There was a period of silence, then a few questions were asked, but no objections were raised. François Jacob had the impression that everybody felt that the molecule and its genetic implications were so beautiful that they could not be wrong.[64]

Benzer, who had not read the DNA papers in *Nature* prior to the meeting, took succinct notes on Watson's lecture, taking as its main points the following:

Watson—sugar-phosphate backbone
basic structure seems indep[endent] of source of the DNA
Give crystalline pattern, in spite of irregular sequence of bases
x-ray evidence suggests 2–strand structure
adenine thymine pairs can form
guanine cytosine hydrogen bonds

hydrogen bonds relatively weak compared to ordinary chemical bonds
leads to suggestion as to replication

In an informal "phage jam session" Delbrück again brought up the question of plectonemic and paranemic coiling. He thought that there might be a two-stranded plectonemic helix, the threads of which are interlocked and cannot be separated, but that paranemic supercoiling might alter their relations so that the threads could slip apart. According to Benzer's notes, "Max thinks that changes in radiation & suicide sensitivity due to separation of the threads."[65] Apparently, however, Delbrück eventually accepted Watson's arguments against paranemic coiling. Still not satisfied that the plectonemic coils could unwind, he pondered other solutions during the following months.

Neither his brief notes nor the statement in his trip report that "new developments reported included the promising and suggestive model of Watson and Crick for the structure of DNA"[66] reveals any unusual excitement in Benzer's response to the presentation of the double helix. Given the intensity of his concern with the problem of gene self-duplication during the weeks just before he heard about it, and the potential of the new model to solve the very problems with which he had been grappling indecisively, however, we would expect this news to have made at least as deep an impact on him as we know it to have made on others present at the symposium. In an interview, however, Benzer recalled a detailed discussion on this subject with Sydney Brenner, whom he met for the first time on this occasion.

In a paper about ultraviolet radiation phenomena given at the symposium by George Bowen, a postdoctoral fellow in Hollaender's Biology Division at Oak Ridge, Benzer may have had the satisfaction of being treated as a leader in bacteriophage research in the use of ultraviolet radiation to understand the replication of the genetic material. After describing the general effects of ultraviolet light on bacteria, bacteriophage, nucleic acids, and proteins, Bowen discussed at length the Luria-Latarjet and Benzer experiments. Luria and Latarjet had hoped to determine from the shapes of ultraviolet survival curves of intracellular phage something of the time-course and mode of phage reproduction. "As often happens," Bowen related, "the experimental results eluded expectations and brought to light a new phenomenon." The more precise results that Benzer was able to achieve with his improved techniques showed that within a few minutes there is a remarkable

decrease in the sensitivity of the phage; it drops to about one-twentieth of its initial value halfway through the latent period. Thereafter the curves begin to develop a multiple-target character and to increase in sensitivity. These results supported some of the general schedule of events already observed, as well as the idea that there are profound changes during the vegetative stage, but they "cannot be interpreted in the simple manner that was at first expected." In his further experiments Benzer contributed a "greatly improved technique, a new idea for the use of the phenomenon as a tool in other work, and the extension of the experiment to phage types other than T2." Benzer had also "advanced an hypothesis which at least formally accounts for the results." This hypothesis, which states that the phage passes through a series of ultraviolet-sensitive steps so that the overall sensitivity of the phage decreases as the number of sensitive steps still to be covered diminishes, as well as the similar hypothesis he devised to account for his experiments with Jacob on lysogenic bacteria, have already been discussed (see Chapters 3 and 4). Commenting on Benzer's interpretations, Bowen wrote:

> This picture does account for the observations in a very general way, and in particular it supplies the rationale for the use of the effect as an index to the development of T2. It must be admitted, however, that the hypothesis has so far failed to suggest further experiments which would increase our understanding of the effect. Present ignorance of events occurring during the early part of the latent period makes it difficult to frame any such hypothesis in very precise terms. Nevertheless the Luria-Latarjet effect is potentially such a valuable clue to the processes of vegetative growth that a more specific picture should be sought and tested.[67]

This judgment that the Luria-Latarjet experiment as refined by Benzer had given disappointing results but was still in principle so promising that it ought to be pursued further was probably not far from Benzer's own assessment. In a general lecture on the effects of radiation in biology that he gave on April 23 for Koffler's course on bacteriology, Benzer had concluded by stating, "Radiation experiments still have a lot to offer in solving biological problems. Actually, [they] may be only beginning to pay off—now that the confusions are becoming better understood."[68]

Benzer did continue to search for payoffs in the further application of his refinement of the Luria-Latarjet experimental system, but it did

not lead him to significant advances. In a letter of recommendation written for him later, Delbrück commented that during his years at Caltech Benzer "concentrated on radiological work. The intent of this work was to learn something about the replication of the phages inside their host cells by studying the changes in radiation sensitivity of an infected bacterium during the course of the infection. This work, though of very high quality, did not yield the desired information on the mode of replication, due to basic aspects of replication which we still do not understand."[69] We might make the same assessment of the extension of that work to lysogenic bacteria in collaboration with Jacob and to its further continuation at Purdue.

It is often said that in highly competitive sciences someone who does not make a major discovery within the first few years after attaining a doctoral degree has missed the chance to reach the forefront of the field. Benzer had been awarded his doctorate six years earlier and had yet to do so. Nevertheless, among his seniors and peers alike, he was admired and thought to have great promise. The key to his reputation lies in Delbrück's phrase noting the "very high quality" of his work. When prominent senior scientists recognize that a younger scientist has the talent, skill, and persistence to do sustained high-quality work, they often make the judgment that eventually she or he will do something outstanding. Alfred Hershey implicitly made the same judgment when he wrote Benzer in October, 1953, "I have just read your paper on lactose adaptation, really for the first time. What conception, what execution, what exposition (this should be in French)! Salut! Al."[70]

Benzer was not only admired by influential senior members of the phage group, but he was also well connected with each of the most important centers of phage work. He had spent two years in Delbrück's group at Caltech and one year in Lwoff's group in Paris, and he had met regularly with Luria's group in Urbana. He had become friends with promising younger workers in each of these places and had impressed them with the meticulous, imaginative way he went about his work. At Purdue he had a relatively secure position with the responsibility of leading a research program oriented around his own interests. After one year there, he was promoted to associate professor. In such supportive circumstances he need not have been anxious to prove himself by pushing too hard for a spectacular result.

CHAPTER SIX

Entering the rII Region

In 1965 Benzer gave a compelling account of the chance conjunction of events that had led him nine years earlier to take up his project to map the genome of the bacteriophage T2. The seminar on the size of the gene for which he had read Pontecorvo's paper (see Chapter 5) had already made him aware of the need for high-resolution mapping to distinguish among the several definitions of the gene. Then, as he related the story:

> I had started out to attempt the Hershey-Chase experiment with genetic markers, to show sequential injection of the various parts of the phage genome, as Jacob and Wollman had done with bacterial conjugation. For that experiment, a stock of an r mutant of phage T2 was needed. Now stocks of r mutants grown on strain B of *E. coli* usually have titers much lower than the wild type r^+ stocks, since r^+ phages induce lysis inhibition and hold the cells together for a longer period of intracellular multiplication. But I had just read in George Streisinger's thesis that on certain strains of *E. coli* other than strain B, r mutants of T2 do yield titers as high as r^+. Could that mean that the r mutant can produce lysis inhibition on those strains? To test this possibility, I plated out some T2r and T2r^+ on the strains I had in hand in my laboratory. If r produced lysis inhibition, it should make small, fuzzy-edged plaques similar to r^+, rather than the large, sharp-edged r-type plaques seen on strain B. On that day, I happened to be preparing an experiment on lysogeny for my phage class and was growing cultures of K12(λ) and its non-lysogenic derivative K12S (obtained via Luria from Esther Lederberg). Plating T2r and T2r^+ on those strains certainly gave different results from plating them on strain B. On K12S, r and r^+ both gave small, fuzzy plaques. On K12(λ), r^+ made small, fuzzy plaques, but the plate to which r was supposed to have been added had no plaques at all. I

> was sure that in the rush to prepare for class, I had neglected to add phage to that plate. But repetition confirmed the result.
>
> To me, the significance of the result was now obvious at once; here was a system with the features necessary for high genetic resolution. Mutants could be detected by the plaque morphology using strain B. Good high-titer stocks of the r mutants could be grown using strain K12S. Strain K12(λ) could be the selective host for detecting r^+ recombinants arising in crosses between r mutants. A quick computation showed that if the phage genome were assumed to be one long thread of DNA with uniform probability of recombination per unit length, the resolving power would be sufficient to resolve mutations even if they were located at adjacent nucleotide sites. In other words, here was a system in which one could, as Delbrück later put it, "run the genetic map into the ground." I dropped everything else and embarked on this project.[1]

If we assume that this is a literal and accurate recollection of the earlier events, then Benzer had experienced an extraordinarily rich flash of insight. His recognition of the rare opportunity he had at hand to pursue the problem of the definition of the gene by means of high-resolution mapping appears to be the outcome of an accidental concurrence in time of at least four events causally unrelated to one another: the idea of carrying out a Hershey-Chase experiment, for which he required r mutants, but from which he was then diverted; the reading of a thesis (the reason for which reason he read Streisinger's thesis is not specified); the presence in his laboratory of a particular pair of lysogenic and nonlysogenic bacterial strains obtained through a personal network of colleagues (again for a purpose not specified); and preparations for a class demonstration independent of his immediate research.

In Paris Benzer had found once before that an r mutant of T2 did not plate on a lysogenic strain of *E. coli* K12 when he picked this phage and this bacterium to begin his study of adaptive enzymes. Rather than pause to find out why, he had shifted to *E. coli* strain B, on which the phage did reproduce normally (see Chapter 4). After observing the same behavior for the second time, Benzer regarded his first encounter with the "rII phenomenon" as a discovery that he had failed to recognize then because, "as Pasteur would say, 'my mind was not prepared.' "[2] Benzer credited Pontecorvo's paper with preparing his mind to recognize the phenomenon the second time. As nice an illustration as this juxtaposition of events appears to be for Pasteur's famous saying that "chance favors the prepared mind," it suggests more realistically

the elusiveness of Pasteur's claim. The rII phenomenon was not a preexisting entity waiting to be discovered but rather a product of the entire cluster of events that suggested the mapping method to Benzer. When he had encountered the inability of the mutant to plate on a lysogenic bacterium in Paris, it was just what he had taken it to be then—an obstacle to his research project; his decision to switch to another strain rather than to explore the reason for this behavior was the most effective way to succeed at what he had begun.

How long did it take for the cluster of events culminating in Benzer's insight to take place? His account suggests a sequence compact enough in time to constitute a single encounter with the phenomenon. With the exception of the reading of Streisinger's thesis, which seems to have preceded by a relatively short interval the rest of the story, the steps described above seem to have occurred on the day that he prepared for the class demonstration of lysogeny, although the repetition of the experiment that persuaded Benzer that his first result was not due to a mistake may well have come one or two days later. Similarly, his statement that the significance of the result was obvious at once, the reference to the quick calculation that suggested that the resolving power of the method would reach down to individual nucleotides, and his use of a statement actually made by Delbrück only much later to describe what was obvious at once imply that Benzer had seen the full significance of these events in a unitary "Eureka" experience.

Because of their ephemeral nature, such moments of sudden illumination seldom leave direct documentary traces, and we are left most often with only the testimony of the memory of those who have experienced them. Memory is, however, inevitably in part their construction of past events in the light of subsequent ones. In this case the transfer of a comment made by Delbrück at an advanced stage in Benzer's project to describe Benzer's initial view of it is an explicit clue to the reconstructed nature of his account. In this case also, the record of his investigative activity is dense enough so that we can expect to uncover further aspects of reconstruction. That does not mean that Benzer's recollection of a powerful moment of insight is false; it means only that the passing of time may have expanded the meaning of what he remembered as the significance he had initially recognized in his encounter.

Most, but not all, of the elements making up the event Benzer remembered can be confirmed using surviving documents. The most important of them is the record of the ongoing experiments with r mutants.

They occupy a series of looseleaf notebooks designated "r mutants," in which the earliest experiment was dated January 9, 1954, a time coinciding with Benzer's recollection of approximately when he took up the new project. He recalled in addition that Dotty and the children were in New Jersey at the time visiting her seriously ill father and that he had a full week of "unrestricted time" to begin work.[3]

The first experiment Benzer recorded was the "induction of K12(λ)." After subjecting the bacteria to different doses of ultraviolet light, he determined the number of plaques formed as a function of the dose, as well as the number of bacteria "surviving as colony formers," and plotted the resulting curves on a graph. This was the basic induction experiment established by Lwoff in 1949 and applied the following year to K12 by Joshua Lederberg, as well as by Jean Weigle and Delbrück. Benzer did not state what the objective of his experiment was, but it could have been to prepare for the classroom demonstration of lysogeny mentioned in his account.[4]

On January 11 Benzer recorded the "plating of T2r and T2r$^+$ on K12S and K12(λ)." He arranged the results for eight plates in the form of a table:

	On K12S	On K12(λ)
T2r$^+$	325, 347	388, 358
T2r	386, 435	0, 0

Below the table he wrote:

> —All plaques (both T2r and T2r$^+$) are of the r$^+$ type [that is, small and fuzzy]
> —K12(λ) is resistant to T2r22 but not to T2r$^+$!!

It is not difficult to identify this experiment with the one Benzer later described as intended to test whether r mutants plated on strains other than B yield titers as high as do the wild type. The fact that the r mutant produced r$^+$-type plaques answered positively on the first try his question whether on a strain other than B it might produce lysis inhibition and, therefore, large titers. The two exclamation points suggest that he was surprised by the second result, although not necessarily as disbelieving as he pictured himself in his later account. In any case, the following day he plated the same two strains again with the T2r mutant. For comparison, he also plated the phage on strain B and on mixed cultures of B with K12S and with K12(λ). His reason for doing the latter two tests was to be completely certain that the phage were

preset and that the system was working although they did not plate on K12(λ). He charted the results as follows:

B	379, 365	(large, clear r plaques)
K12S	80, 43	(small)
K12(λ)	0, 0	
On B + K12S	145	(turbid) i.e. mottled character
On B + K12(λ)	281	(turbid) but not mottled—rather large r plaques with many small colonies inside

He commented that the plaques appearing on K12S were probably due to K12(λ) and that he should do a control for this. He drew the conclusions that "T2r is adsorbed on K12(λ)" but that it "does not kill" and "does not induce l (at least not to any great extent)." Finally, he asked, "Do the bacteria become lysogenic for T2r?"[5]

At this point Benzer had obtained all the results that he remembered as having led him to see "at once" that he had a system suitable for high-resolution mapping. If so, it is rather surprising that he recorded nothing to that effect, asking instead a different question about lysogeny. Moreover, his comment that the small plaques formed on K12S may have come from K12(λ) would seem to indicate that he did not yet interpret these results in a way that would have made the system for genetic mapping visible to him. The absence of a written trace, however, does not rule out the possibility that the insight occurred to him at that point. Laboratory records seldom reveal all of the significant thoughts that accompany or immediately follow a particular experiment—the flash might easily have taken place after he had put his records away. A stronger test would be whether the steps he took soon afterward are consistent with his recollection that he "dropped everything else and embarked" on a mapping project. [Editor's comment: These experiments, however, do not allow the quick calculation of the high resolving power of such a mapping method; because Benzer assayed two 0.1 ml aliquots of a 10^{-5} dilution of phage stocks of a nominal titer of about 10^9 phage/ml, the discrimination of the system between mutant and wild type was approximately 1/500 but possibly much better. Later he carried out experiments he described as "transmission" studies in which he plated low dilutions, that is, high numbers, of mutant phage on K12(λ) to estimate the "breakthrough" of the mutant phenotype, a number essential to the calculation of the resolving power of the method.]

Because he did not know on what host the initial T2r$^+$ stock that he had obtained from Luria had been grown, on January 14 Benzer plated the same three bacterial strains with T2t grown on strain B, the same host strain that had served for the T2r he had used. With B itself the phage formed regular r$^+$ plaques with halos. On K12(λ) and on K12S it produced fuzzy plaques with a halo, "small and variable in size." He concluded that "the sens[itivity] of K12(λ) to T2r$^+$ (as contrasted with T2r)" that he had observed in the experiment of January 11 "is *not* due to the fact that it had been grown in any different host."[6] By eliminating this possibility, the experiment assured him that the contrast between the two when plated on the lysogenic bacteria was due to the difference between the mutant and the wild type.[7]

In order to explore further the nature and range of the phenomenon he had discovered, the following day Benzer tested "T2r and r$^+$ and T4r and r$^+$ on various bacteria." In addition to the three strains of bacteria previously used, he plated them on strain ML and strain N1, the latter being the same one on which George Streisinger had shown that r mutants could be grown to high titers. Benzer must have written Streisinger for this strain after reading his thesis, and Streisinger sent it shortly after moving from Urbana to Caltech. In contrast to T2r, T4r plated on K12(λ). With ML and N1 both T2 phages behaved as they had with K12S, whereas T4r formed very faint plaques with ML and T4r$^+$ none at all. Benzer concluded that "K12(λ) distinguishes between T2r and T2r$^+$, but not between T4r and T4r$^+$" and that "ML might possibly be sens[itive] to T4r but not T4r$^+$."[8] These results suggested, therefore, only a doubtful extension to T4 phage of a means of discrimination between the mutant and the wild type that he had already found for T2 and no extension to other hosts of the means he had discovered for T2.

Thus far Benzer had used only a single T2 mutant stock, r22, originally isolated by Hershey, that he had had on hand for other purposes. It was already known, however, that the r mutants that rose independently from T2 stocks were not identical, in spite of the fact that they were identified by the same plaque characteristics. The recombination experiments of Hershey had shown that they were located in different places along the genetic map. The natural next step for Benzer to take in exploring the generality of his phenomenon was to test whether it applied to other r mutants. On January 16 he isolated four r mutants from a T2r$^+$ stock and tested each of them the following day on the bacterial strains B, K12S, and K12(λ). Three of them behaved just as his original r mutant had, but the fourth produced plaques with slightly

hazy edges on B and produced r^+-type plaques (as opposed to none for the other three) on K12(λ). This was the first indication that the phenomenon he was examining might not apply to all r mutants. Benzer noted, however, that because the wild-type culture from which he had derived these mutants might not have come from a single plaque, he should seek a "new batch."[9] On the same day he did isolate more mutants from a single T2r^+ plaque. He also isolated a group of T4r mutants from a T4r^+ plaque. Although he did not say why he returned to T4r, it is possible that the evidence of a single T2r mutant not behaving as had the other four that he had tried suggested that the T4r mutant that had plated on K12(λ) was also not characteristic of all T4r mutants.

At the same time Benzer decided to test whether the r mutants behaved with other lysogenic bacteria as they had with the K12(λ) that he had been using until then. In order to do so he exposed stocks of K12S bacterial cultures to phage λ (at this point the designation K12S was shortened to S). Selecting ten isolated colonies that had been so exposed, he induced them with ultraviolet light following the method of Lwoff (a procedure for activating the endogenous λ prophage that he and Delbrück referred to informally as "Lwoffing" the bacteria). Four of them produced many plaques, two of them produced an "intermediate number," and four produced few to no plaques. The first four he considered "probably lysogenic." Choosing one of these, which he called "S(λ presumed)IV," he made comparative spot tests of each of the ten T4r mutants and the 13 T2r mutants he had by then isolated on it and on the K12(λ) stock he had used until then. On the latter, four of the T4 and two of the T2 mutants plated, whereas the rest did not. On the newly lysogenic strain all of the mutants plated. He commented, "Thus, S2λIV [S2 denotes a single-colony isolate of K12S from Luria] does not have the same selective character for r mutants as does K12(λ). Therefore, the selective character of K12(λ) is probably not due to the presence of λ, [provided that this S(λ) is actually lysogenic]. Will test the other S(λ) isolates."[10]

The experiments that Benzer had carried out during the ten days since he had discovered the inability of a T2r mutant to plate on K12(λ) might be seen either as preliminary tests of the generality of a system he had already planned to use as a tool for mapping or as explorations of the nature and extent of the phenomenon itself. Mapping would only be feasible if an extensive group of mutants displayed the same character that the one on which he had made the initial observation did. The testing of these same mutants on another lysogenic strain

might indicate either that he simply wanted to know whether he was limited to using the bacterial strain on which he had made the initial observation or had wider latitude. The testing of a second lysogenic strain could be seen, on the other hand, as an effort to determine whether it was the presence of the prophage or some other peculiarity of the first strain that was responsible for the effect, and the fact that the one he tried appeared not to have the same character might lead him in the direction of seeking another explanation for that effect.

On January 20, on bacterial strain B, Benzer crossed two of his T4r mutants, numbers II and V, that had been "inactive on K12(λ)." First infecting bacteria separately with the two phages, he established the number of the phage contained in the resulting bursts that had resulted from the lysis of infected bacteria by comparing the burst size with that of cells killed with chloroform after infection. The latter number, representing the number of plaques that had been produced by "free," unadsorbed phage, he subtracted from the number representing "total infective centers." From these data he was able to calculate that in the mixed infection "16% of the bacteria should have been infected." By similarly comparing the plaques *formed* before burst from the total infective centers with those formed by free phage in strain B killed and lysed with chloroform, he found that 269 bacteria had been infected. When plated on K12(λ), 53 r^+-type plaques formed. From the ratio between these two numbers he inferred that "20% of the infected bacteria yield recombinants" and commented that "this must represent virtually *all* of the mixedly infected bacteria." The conclusion he drew from the experiment as a whole was that "T4rII and V are not identical and give high frequency of recombinants." Some time later he crossed out the words "high frequency of."[11]

Was the main purpose of this experiment simply to determine whether the two mutants were or were not identical? We may note, in the first place, that Benzer identified as wild-type recombinants the products of the mixed infections that plated on K12(λ). By this time, therefore, if not earlier, he had had the basic insight that his initial observation of the failure of an r mutant to plate on this lysogenic bacterial strain afforded a new means to recognize recombinants and to determine the frequency of recombination. It would have been hard to have such an insight without seeing also that this system provided another way to map mutants which shared that characteristic. There is nothing to indicate, however, that he must also have realized by then that the new method contained the potential to carry mapping to a

much finer degree of resolution than other methods and that he ought to drop everything else and embark on such a project.

On the same day, Benzer returned to the problem of whether the bacterial S strains he had treated with phage λ on January 16 had actually become lysogenic and whether they were able, as Esther Lederberg's K12(λ) was, to distinguish r mutants from the wild type. Expanding the inquiry, he now tested all ten of the bacterial strains originally isolated with each of the T2r and T4r mutants he had isolated, representing the results for T2 on a matrix of vertical and horizontal rows in which "+" indicated "complete clearing (except for resistant colonies)" and "0" indicated an inability of the phage to plate on the bacteria. From the resulting patterns he judged that "only [S(λ?)] numbers I, IV, and IX show any discrimination between r stocks, and these three show *identical* responses." That is, in each of these three cases ten of the twelve T2r mutants were unable to plate, whereas the two phage mutants he had numbered V and X caused clearing. Four of the mutants produced some mottled plaques, due, he presumed, to reversions of some mutants to wild type. For six of the mutants there seemed to be "an inhibitory (colicine-like) effect, with an occasional fuzzy plaque appearing." Colicines are molecules that kill *E. coli* by perforating their membranes, degrading ribosomes, or using other lethal mechanisms, so what Benzer meant was that these mutants appeared to kill their host bacteria but were unable to reproduce in them.[12]

The results with the T4r mutants were very similar, except that one of them did not appear to kill the bacterial cells. The general conclusion Benzer drew was that "those [bacterial strains] which have become lysogenic can distinguish the r mutants." Later he added, "(both T2's and T4's)." But, he went on, "S(λ)IV does not seem to be lysogenic and yet it too can distinguish. This strain appeared to be lysogenic in a previous test." Later he added, "But [it] has been purified by restreaking since then." The strain S(λ)IV was the one he had first chosen, from the ten he had isolated, to test with the mutants. Whereas it had then appeared lysogenic but unable to discriminate, now it appeared nonlysogenic but able to discriminate.[13] The situation remained confusing, therefore, with regard to this particular case, but the fact that two other strains were both lysogenic and discriminating was implicitly inducing Benzer to give up the opinion he had expressed a few days earlier that the selective character of K12(λ) was probably not due to the presence of the prophage in it. He may by this time have begun to think about the question of how interactions between the prophage

and the mutant phage during the latent period might inhibit the growth of those of the latter that could not plate on lysogenic bacteria.

Benzer was still concerned, however, about the paradoxical case of S(λ?)IV (which, now that he was satisfied that it was nonlysogenic, he wrote without the symbol "λ") On January 22 he asked himself, "Is S( )IV resistant to λ even though it is not lysogenic?" Exposing it, as well as S and K12(λ), to phage λ obtained by Lwoffing K12(λ), he found that only S formed plaques. "Thus," he concluded, "S( )IV has the characteristics of K12(λ) with respect to resistance to λ as well as discrimination among r mutants. Is this a case of transduction? If so, it should be interesting to study if it is so frequent."[14] *Transduction* was the term used to describe the importation of characters from one bacterial strain to another by infecting the second with a phage grown on the first. He could interpret the situation as a possible example because he had derived the phage λ to which he exposed S( )IV from the same bacterial strain with which the exposed strain now exhibited a common characteristic. His interest in the phenomenon suggests that Benzer was being drawn further into the question of what properties of a host bacterium might account for the failure of most of his r mutants to reproduce in them.

Among the results of the experiment of January 17 comparing the effects of T2r and T4r mutants on K12(λ) and S(λ?)IV and of the similar but more extensive experiments of January 20, on which Benzer did not comment, was the accumulating evidence that with lysogenic bacteria T4 did not behave differently than T2, as his initial experiment with them had led him to believe. In both cases a similar proportion of the mutants formed no plaques. Therefore, it would seem equally promising to use mutants of either phage type. In his subsequent experiments, however, Benzer seemed to have settled on T4 as his main experimental material, switching only occasionally back to T2 or trying one of the other T-series phages. Whether he had a strong reason for this preference or merely decided that it would be best to focus on a single type and happened to go with T4 is not clear from the record (or from Benzer's recollections). There seemed to be equally viable precedents for either choice. Hershey had produced the initial genetic maps with T2r mutants, whereas Doermann had mapped T4 mutants. In general, those who had worked with Delbrück favored T4 for most purposes, but there is no reason to believe that Benzer would have been unduly swayed by the local ethos in which he had spent two

years. Nor is it evident that the course of his investigation would have differed significantly had he made the other choice. Some contingent choices between alternate potential experimental materials have had major consequences for further developments, but this choice seems to have been less critical to the outcome.

On January 22 Benzer extended the crosses between T4r mutants to include all combinations of the nine stocks he had isolated. This time he detected recombinations semiquantitatively by plating them on strain B rather than on K12(λ) and estimating the proportion of the plaques that were mottled. In his research notebook he divided the results into four groups:

-	= less than 1 % noticeably mottled plaques (on B)
+	= several %
++	= ~10%
+++	= 20% or more

Again he drew a matrix of vertical and horizontal rows to represent the results, from which he drew the following verbal generalizations:

> *II, V, VI, VIII, IX, X* recombine very little with each other, and have similar "action spectra" with respect to rate of recomb[ination] with the other mutants. This is also the group which gave the colicine-like effect (Jan 20) on S(λ)—except for II, which gave a different reaction. Thus, V, VI, VIII, IX and X might be identical or they might be closely linked (II and V have been shown to be different).

Benzer had established that II and V were different mutants when he crossed them on January 20, using the criterion of plating on K12(λ) to infer that they recombine. He borrowed the term *action spectra* from radiation physics, in which the term referred to the efficiency of killing by ultraviolet light as a function of wavelength.[15]

> V and VII form a group (these both gave complete clearing in spot test). These recombine fairly readily with first group, but not with each other.
>
> III stands by itself (gives complete clearing for spot test) & recombines readily with everybody else.

When he reexamined the plates the following day, he found some additional mottling and constructed a second matrix to represent it. "All crosses show some evidence of recombination in the form of peripheral mottling of the plaques," he now wrote, "so −, +, ++, and +++ are

used to indicate roughly the frequency of recombination as judged by the percentage of really patchwork plaques." He did not restate the situation with regard to the three groups, but his revision implies that he now regarded the members of the first group as closely linked rather than identical.[16]

Benzer also examined the plates for what he called a "dominant" reaction, that is, the "interference of one plaque with development of another when they overlap." Again he used a matrix to represent the crosses, with "−" denoting no apparent interference, "+" denoting a weak effect, and "++" denoting a pronounced effect. "When dominant reaction appears," he commented, "the smaller (more r^+-like) plaque is dominant. However, dominant reaction does not appear in every case when the two phages have different sized plaques." The test would be more efficiently carried out, he added, with free phage rather than bacteria infected in various ways, and he wondered whether a general order of domination could be established. "IV seems to be the most 'domineering.' "[17]

During this experiment Benzer had utilized a technique taught to him by François Jacob in Paris to prevent the bacteria in the adsorption tubes from lysing by putting potassium cyanide (0.01 M) in the solution. To test whether the cyanide inhibition would last overnight, on January 23 he repeated two of the crosses: IV × IX and V × VI. Plating the results of the first cross on strain B, he observed, as before, many mottled plaques. The crossing of V × VI, however, gave "very few mottled plaques," a result he would have expected, because these two were in the group that had behaved similarly in the previous experiment. But when he plated this same cross on S(λ)1, he obtained 93 r^+-type plaques. He concluded both that cyanide had kept the bacteria from lysing and that "V and VI recombine to give (wild?) type active on S(λ)1, and are therefore not identical."[18]

Because the complexes had proved stable in cyanide, Benzer was now able to use them again. He decided on the same day to plate on S(λ) the crosses between r mutants that "separately, do not form plaques" on it, that is, numbers II, V, VI, VII, IX, and X. "If recombination takes place," he wrote, "plaques on K [K12S] should be obtained. This has already been shown (today) for the V × VI cross, and the II × V cross." The result was that "all crosses show plaques on S(λ)." He constructed another matrix, in which he represented each cross by the number of plaques formed:

	II	V	VI	VIII	IX	X
II			20	35	30	45
V			93	123	108	101
VI				50	30	55
VIII					52	43

When he treated II × VI with chloroform and plated the result on strain B, he obtained about four hundred plaques, but there were only six on S(λ). "Thus," he wrote, "some of the cells have lysed (not very many) but most of the plaques on S(λ) still come from unlysed complexes." Below the matrix he wrote afterward, "These numbers are not very meaningful."[19]

In his focus on these crosses we might well see Benzer moving purposefully toward the mapping project as he later defined it. He had found that plating on the lysogenic strain under some circumstances provided a more sensitive indicator of recombination between the r mutants than the formation of mottled plaques on strain B. He had moved from the simple observation of recombination in single crosses to a semiquantitative measure of frequencies of recombination in multiple crosses. On this basis he had differentiated three groups of r mutants: those within each group apparently closely linked with others in that group but recombining freely with members of the other groups. If he had been familiar at this point with the papers in which Hershey and his co-workers had mapped mutants of T2, he might well have wondered whether the three groups he had just distinguished corresponded with the three linkage groups Hershey had earlier established.[20] Nothing that he wrote down at this time demonstrates, however, that Benzer yet envisioned such a project in broad terms. The steps he took during the following weeks suggest instead that he was moving in other directions, perhaps exploring further the general nature of the r mutant phenomenon without knowing yet where his probes might lead him.

On the same day (January 23) that he completed the multiple cross experiments, Benzer performed one titled "Fate of T4r mutants on S(λ)I compared to B." He adsorbed four of his r mutants, II, IV, VIII, and IX (of which the latter two were within the closely linked group), as well as the combination VIII + IX, on both strain B and the S(λ) strain, then plated them all on B. This protocol tested the intracellular growth of the phage in the strain to which the phage was adsorbed, but the

resultant progeny were assayed on the B strain, either as free phage or as infective centers. For each case he calculated the fraction of infected bacteria that yielded plaques on B. All of them when adsorbed on B gave large fractions of plaques when plated on B. When adsorbed on S(λ), on the other hand, very small fractions of II and IX gave plaques afterward, whereas substantial fractions of the other two, alone and in combination, did. Benzer concluded that

> II & IX are lost when adsorbed on S(λ)—i.e. yield no plaques on B.
> IV acts the same on S(λ) as on B
> VIII gives an intermediate result (is the stock possibly impure? Is there a multiplicity effect?)
> All the phages adsorb to and kill S(λ), (rate of adsorption seems 1/2 that on B.)

These results seem not to have told Benzer anything new. On January 24 he isolated new T4r mutant stocks, four of which he used the next day, along with mutants VII, IX, and X of the older stock, for a "titration of T4r mutant stocks on $S(\lambda)_I$ and B." For each of them he calculated the ratio between the titers on S(λ) and on B. Two of the new mutants gave ratios of 0.1 and 1.3, respectively, whereas the rest gave ratios of less than 10^{-5}. He commented, "The 'stray' plaques may represent either (or both) reversions to wild type or 'host range' mutations (these might be the large & small plaque types, respectively)." There followed two "mixed infections" of T4r mutants on the same lysogenic bacterial strain, and he used a Klett turbidometer to follow by optical means the changes in the numbers of bacteria over the course of two hours after adding one of the mutants, compared to a control. On January 27 he tested a new T4rV stock on *E. coli* B as the indicator strain, and he adsorbed T4rVIII on S(λ)I at three different multiplicities of infection. The resulting plaques were very variable in size. The results of this experiment were charted as follows:

Input multiplicity	Fraction of infected bacteria yielding plaques on B
5.8	0.34
2.9	0.35
0.29	~0.2

He commented, "This does not seem to be an effect of cooperation of several phage particles, but a fraction of 'agreeable' bacteria." On the

same day he performed another mixed-infection experiment on S(λ)I with three T4 mutants alone and in three combinations, plating them afterward on B as the indicator strain, recording both the fractions of infected bacteria that yielded plaques and those that yielded strongly mottled plaques. Two of the mutants and the three combinations yielded plaques in fractions ranging from 0.14 to 0.54, whereas IX alone yielded only 0.04. None of the mutants alone yielded mottled plaques, whereas two of the combinations yielded fractions of 0.40 and 0.49, respectively, of mottled plaques. In a lengthy comment on the results, he wrote:

> In case of III + IX, 24% of the (mixedly) infected bacteria give yield (and half of these give mottled plaques). Therefore, in the case, > 12% of the bacteria yield III whereas only 4% do when IX is not present. [From the data presented it appears that Benzer meant to write ". . . yield IX when III is not present."] It would be nice to have wider margins between the "yield factor" of the strain, if a clear result is to be obtained in the experiment. Why does III give a "yield factor" of only 0.54 when it plates so effectively on S(λ)?[21]

It is difficult to reconstruct the precise aims that Benzer had in performing this series of experiments or what he was learning from them. When reviewing these pages with the author in May 2001, he was unable to account in detail for what he was doing then. At one point he remarked with a chuckle, "There's a lot of wheel spinning here."[22] What seems clear, however, was that the questions he was asking during that period were not related to mapping. He called the experiments in which he infected the bacteria with two mutants not "crosses" but "mixed infections." He did not attribute the results of adsorption on the lysogenic bacteria, and the absence or presence of small or larger numbers of plaques in the subsequent platings, to recombination, but he hinted at mechanisms such as "cooperation between phage particles" or "agreeable bacteria." He retrospectively identified the curves of bacterial survival after infection with a mutant, plotted using the data from he Klett instrument, as a study of the "dynamics of the process." Taken together, these clues suggest that he was looking at the "r mutant phenomenon" from a different point of view than he had been only a few days earlier: that he was not at this point developing a tool but searching for a mechanism to explain why some of the r mutants could not reproduce in lysogenic bacteria although others could.

A clue that appears particularly cogent in light of the developments of the following weeks was Benzer's reference to the possibility that the "stray" plaques he observed from some of the mutants that had been adsorbed onto S(λ) might be due to "host range" mutations. As he proceeded along similar lines he came increasingly to regard himself as pursuing a prime example of the host range problem: that phage mutations are often expressed as changes in the strains of bacteria in which they are able (or unable) to reproduce.

On January 30 Benzer tested his three standard reference bacterial strains, S, S(λ)I, and B, with wild-type phage T5. Plaques formed on B but not on the lysogenic or the nonlysogenic S strain. Apparently this was not his first attempt to expand the scope of his system to T5, for he remarked that "S(λ) did not acquire the T5 sensitivity of K12(λ), at least not in this particular strain of S(λ)."[23] He did not pursue the question of T5 further, continuing instead to fix on the behavior of T4. On the same day he prepared a second S(λ) stock, which he called λ2, and again plated on B a T4 mutant (V) adsorbed first on S(λ)I. Again he obtained fractions of less than 1 percent that yielded plaques. On the last day of January he treated "S2 with λ from S(λ)I" at a multiplicity of infection of five. Half of the bacteria lysed, forming plaques, and the other half survived to form colonies. The original S and the survivor plates each contained colonies of two morphologies, rough and smooth, from which he chose a number of each type and cultivated them overnight. On February 2 he seeded both types, as well as strain B, with three different phage—λ, T4r$^+$, and T4r. The latter two he had chosen for their property of producing, respectively, "typical r$^+$ and r plaques." In this case, however, he found "identical plaque types for the r$^+$ and r phages. Same number of plaques in all cases." This result led him to comment, "Thus, the smooth and rough variants seem to have no difference in respect to the characters we are interested in. (Searched in vain for plaques showing r character on S. The plaques of r$^+$ on B, however, show plenty of cases of sectored plaques.)" (Sectored plaques were normally taken as an indication that new mutants had arisen during the formation of a wild-type plaque.)[24]

These remarks suggest that a phenomenon that must have been in the back of his mind ever since the beginning of his experiments with r mutants was now moving to the forefront of Benzer's attention. That is, the only characteristic property by which r mutants were identified, the formation of large, sharp-edged plaques easily distinguished from the small, rough-edged plaques of the wild type, was not a constant

property but one dependent on the host in which it reproduced. In the very first plating experiments with the T2r strain he had had on hand on January 11, he had noted that on the nonlysogenic strain S its plaques were of the wild type. The first T4r mutant, on the other hand, produced r-type plaques on S but r^+-type on the strain N1. Within the bounds of distinctly r or r^+ types, moreover, the sizes of the plaques varied extensively from host to host. In the February 3 experiment, his "vain" search for r-type plaques formed on S by a T4r mutant appears to mark the beginning of a new plan to examine systematically the dependence of the phenotypic expression of r mutants on the host bacterium.

Before pursuing this plan, however, Benzer turned to another unanswered question: why some of the T4r mutants were able to "produce a yield from a fraction of the cells of S(λ). The question arises whether this culture contains a mixture of sensitive and insensitive cells." In order to test the homogeneity of one of his S(λ) cultures he chose eighty colonies from it, incubated them, and spread each on a plate. On each plate he then placed a spot of each of five phages. The result was that "all plates show identical response." Lambda had no visible effect on them, and the wild-type T4 gave complete clearing. Of the three T4 mutants he tried, V had "no visible effect," X produced a "barely detectable 'inhibition,' " and VIII gave a "definite general colicine-like inhibition, with occasional tiny plaque-like spots." He concluded that the "culture is *not* a mixture of genetically sensitive and resistant cells."[25] If the cause of these fractional yields of plaques was not in homogeneity in the lysogenic bacteria, Benzer must have wondered whether they were due to homogeneity in the mutant infecting phage. He had already entertained the idea that reversions or host-range mutations might account for the formation of "stray" plaques. For now, however, he left the question unanswered while he turned to another question that had come up several times before: whether the capacity of the lysogenic bacterial hosts to distinguish between T4 mutants, a property he designated "D," was separable from or inherent in their lysogenicity.

"Since, by exposing S to λ, one obtained strains which had the D character (i.e., discriminating between r mutants)," he wrote on February 3, "it is necessary to know whether some of the original bacteria already had the D character or whether the property had been 'transduced.' " From a culture of S2 that he had spread on plates the day before, he chose sixty colonies, incubated them for several hours, and

then plated λ, T4rV, and T4r⁺ on each of them. In all sixty plates each of the phage produced complete clearing. "Thus," he concluded, "the S culture contains none (out of 60) cells showing (genetically) either resistance to λ or discrimination between r mutants.[26]

For this special case, therefore, D seemed to be a property imparted to the S strain together with λ. To give more generality to the question whether lysogenicity and D were separable, Benzer next took each of the twenty-four colonies he had used for the "rough-smooth" experiment, chose ten colonies from each of these clones, and tested 200 of the resulting clones "for lysogenicity, sensitivity to T4r⁺, sensitivity to T4rV, sensitivity to λ, and to T4rVIII." Exposing each of the clones to λ and testing the surviving bacteria with each of these phages, he found only two mixed types in the 200 clones; that is, in 198 of the cases the bacteria were lysogenic and discriminated between the wild and mutant T4 or else were nonlysogenic and did not discriminate. The other two were "not l[ysogenic] but D." Despite the overwhelming preponderance of cases in which the two properties occurred together, Benzer concluded that "one cannot with assurance rule [out] the possibility that the original population contained these. Also, it is not ruled out [that] the 'lysogenic' but not 'D' is possible."[27]

Leaving this small possibility open for the time being, Benzer returned on February 6 to search for "T4r mutants which are r-type on S." He plated T4r⁺ on the bacteria and on the following day chose ten colonies that were "sectored-looking," an indication that mutants had arisen among them. He plated these again on the S strain. The plates were "too heavy, but most show some r plaques." Choosing ten more colonies from these plaques, he found when he plated them on S and B that three of them were not r-type on either S or B, but six of them and one-third of the seventh "are *r type on S* and *also + type on B* with the same efficiency of plating on both types of bacteria. The plaques are somewhat more fuzzy than those on B, but the distinction from r⁺ type is unmistakable." Also, "there is no host-induced difficulty between S and B, since for all cases tested so far, phage arising from either one will plate on the other with 100% efficiency. (This must be tested for S(λ) also.)" Quickly carrying out this last intention, he plated each of the phage that had yielded r-type plaques and one of those that had yielded r⁺ plaques on B, S, and S(λ). The former group formed no plaques on the lysogenic bacterial hosts, but the latter formed wild-type plaques on all three bacterial strains.[28]Although he did not comment on these results, they must have seemed to suggest that the group

of mutants forming r plaques on S might fit within the group previously found to plate on the lysogenic bacteria and that those which formed wild-type plaques on S might be added to the group known to be unable to plate on "D" bacteria.

As he continued his search for more mutants newly arisen from T4r$^+$ that would give r-type plaques on S, it became clear that Benzer was interested not only in finding such mutants but in finding out what factors determined this aspect of their phenotypic expression. In particular, he pursued the possibility that differences in the host on which they had been grown influenced whether they yielded r- or r$^+$-type plaques on S. On February 9 he chose from a "generalized clearing due to r$^+$," produced by T4 wild-type phage grown on S(λ), some "r-like spots" that he then plated on B, S, and S(λ). The plaques on all of the plates were of wild-type character and appeared in equal numbers. "This latter fact shows," he commented, "that phage grown on an S(λ) plates on B and S with the same efficiency as its original host. However, this did not give us a mutant which is r type on S(λ)." Attempting to produce such a mutant, he chose more sectors that appeared to be of the r type from these plaques and plated them, but the "plates on S(λ) were ruined due to overheating of the water bath."[29]

Intensifying his focus on "host-induced influence on T4r mutant[s]," on February 9 Benzer had also begun a test in which he chose plaques from one of his previously isolated mutants (T4rBXI) and plated them on B, S, and S(λ). The results were as follows:

On B	188 r type plaques
On S	174 r$^+$ type
On S(λ)	0

Choosing plaques from the B plates and from the S plates, he plated each of them again on all three strains and obtained the same results in each case that he had in the previous plating. He concluded, "Thus, there is *no* host induced influence on plating efficiency or phenotype."[30] That is, a prior history of having reproduced in a different type of bacterium did not alter the type of plaque, the relative numbers, or the types of bacteria on which the mutant could or could not reproduce again. Luria and Human's host-induced variation was not a confounding phenomenon in Benzer's experiments on various host strains of *E. coli.*

Returning for the first time since his earliest experiments to the

bacterial strain N1 sent to him by Streisinger, on February 13 he plated wild-type T4 on a purified stock. As before, he obtained only some "faint plaques," an indication that heterogeneity in the original stock had not been the cause of the faintness. On these plates four "clear type" plaques appeared. "This is presumably," he commented, "a host range mutant. It is perhaps best not to use this, since it would mean introducing another genetic change. However, mutants which are r type on N, may be sought for T2."[31]

On the same day Benzer resumed the search interrupted two days earlier for "mutants of T4r$^+$ which are r type on S(λ)." By successively choosing plates in the same manner that he had for those plating on other strains, by February 14 he obtained three mutants that were r type on S(λ) alone; two that appeared to be wild type and that he, therefore, discarded; four that were r type on both S(λ) and B; one that was r type on S(λ), B, and S; and one that produced tiny clear r plaques on S(λ).[32] He appeared to be well launched into an inquiry into the complexities of the phenotypic expression of r mutants. Not only was the nature of the plaques dependent on the host on which they were plated, but the mutants appeared to be divisible into several distinct types with respect to the type of hosts that caused these variations.

Simultaneously, during the second week of February Benzer pursued further experiments intended to clarify whether the troublesome bacterial strain S( )IV was an ordinary lysogenic strain. On February 10 he made an "attempt at induction of lysis in S( )IV by UV." Subjecting S, S( )IV, and S(λ) to ultraviolet light for eighty seconds each, he found that "S( ) lyses when induced in the same way as S(λ) does." After testing the phage yielded by the lysis of these two strains, however, he concluded that the titers of the lysates were very different. The one for the problematic strain was 10^{-5} as large as for the other, corresponding to "yields of 25 per induced bacterium [for S(λ)] and 2×10^{-4} per induced bacterium" for S( ). On February 18 he tested his S( )IV stock for free λ, found that there was none present, and concluded that the λ appearing when it was induced was "probably liberated on lysis."[33]

On February 15 Benzer followed up the suggestion he had made to himself the previous day to seek T2 mutants that were r type on the bacterial strain N. As he had done with T4, he plated a T2r$^+$ on the bacterial culture, then chose sectored-looking plaques (which were "pretty hard to find") to replate on N. From them he received very heterogeneous plaques from which he picked an "r looking" one to

replate. This time all of the plates gave the "same weird type of blotchy, clear-center, irregular plaques." From an r-type plaque found on one of the plates on February 17, however, he was able to obtain in the next plating six r-type and fifteen wild-type plaques. On the following day he plated a mutant (T2rB5) from his old stocks on N, as well as on the usual other three strains. On B and N he obtained plaques turbid enough to make him wonder whether this was a tu (turbid) mutant, whereas on S and S(λ) the plaques were tiny. Noting that the plaques on B and N "look the same," he remarked, "However, the r-ness of B is not very marked, so this is not a good mutant for comparison."[34] Another T2r stock yielded only a few wild-type plaques on N and S and r type on B. Thus, although it appeared that he could obtain T2 mutants yielding r type on N, his results up to this point were indecisive. Benzer interrupted his research to attend one of the frequent phage conferences held by Luria at Urbana.

The conference lasted from February 20 to February 23. Jacques Monod and several others talked about adaptive and constitutive enzymes and the latest results of work on the galactosidase system. Maurice Sussman of Northwestern University described mutants of slime molds that could be plated just like phages. Ed Lennox, from Cy Levinthal' s group in Michigan, discussed his efforts to increase the frequency of transduction of *Salmonella* by phages, showing that a combination of two transducing phages was more effective than a transducing and a nontransducing phage together. Joe Bertani discussed experiments involving the linkage between P2 and λ in infections of *Shigella* bacteria. There was much in the papers and discussions that was closely enough related to Benzer's current work to be useful and stimulating to him. He and Ed Lennox bet a bottle of wine each with Joe Bertani on the question, if a phage were "decapitated" when it had been adsorbed onto a bacterium but not yet injected, "does the DNA run out spontaneously?"[35]

During the meeting Benzer summarized his own recent research, beginning with the observation by Streisinger that had caught his attention—that r mutants give high titers on bacterial strain N1—and his own testing of r mutants on S and K12(λ). Presenting a table of his early results with T2 and T4 mutants, he noted that there was a "reduced host range associated with r mutants." When crossed with each other the r mutants that did not plate on K12(λ) showed recombination, from which he inferred that "many different negative mutants may arise."[36]

Next Benzer discussed the question, is K character due to λ? ("K" was the designation he now gave to bacterial strains that discriminate between r mutants. In his notebooks, when he had been uncertain of the answer, he had called this the "D" character.) He was persuaded that all lysogenic bacteria have the K character; some, however, are poorly lysogenic because of resistance to λ but nevertheless yield 10^{-4} λ per induced bacterium. He also discussed his experiments on the "dominance effect," the "fate of T4r neg[ative] on S(λ)," the absence of "host-induced effects," the search for T4 mutants yielding r-type plaques on S, and mixed infections of S(λ).[37]

Sometime later Frank Lanni, a phage worker who had formerly been at Urbana, wrote Benzer: "When we visited Urbana a few weeks ago, we heard in a rather vague way about some interesting experiments you have been doing with T2r$^+$ and T2r in relation to host range. Nobody seemed to know the details in reliable fashion."[38] This report suggests that Benzer may have represented his current research as oriented generally around host-range problems, although it is possible that he had only mentioned that certain of his experiments raised host-range–related questions. As we shall see, evidence from the statements he had made two weeks later supports the likelihood that at Urbana he would have presented much of what he had been doing recently as being aimed at that general problem.

Benzer brought several new bacterial strains back from Urbana, including a C and a C(λ) given to him by Joe Bertani, and set to work with them the following day. During the last week of February he tested two of his T4 and T2 mutants and the λ phage derived from his S(λ) with these strains. With respect to the C(λ) they behaved the same as they had with S(λ). He also tested a T6 phage probably given to him at the meeting.[39]

On March 2 Benzer wrote four letters, each describing his current research projects. From Demerec at Cold Spring Harbor he asked for information about apartments he might rent there. "I am working on a problem of bacteriophage genetics," he explained, "(phenotypic expression of the r mutation) and would like to pass the summer at Cold Spring Harbor, especially in order to be near Hershey."[40]

Benzer wrote O. M. Ray of the National Research Council to request permission to use $250 from his research grant to visit colleagues in Paris, Copenhagen, and London following an international photobiology congress in Amsterdam that he planned to attend in August. In justifying his request, he wrote:

> Given the opportunity of being in Europe, it would be of the greatest value in the execution of my research project to be able to visit certain colleagues for information and advice. I am working on a problem of interference between carried and infective bacterial viruses. While wild type (T2 and T4) can multiply in a lysogenic or a non-lysogenic host, certain mutants can multiply only in the non-lysogenic form. These mutants will adsorb to and kill the lysogenic cell, but, due to the presence of the carried virus, the process of development is blocked at some unknown stage. Dr. F. Jacob (Paris), one of the foremost workers on lysogenic bacteria, has been working on closely related problems and a consultation with him would be invaluable. It should be to good advantage also to visit Dr. O. Maaløe (Copenhagen) for the purpose of learning his new technique of sectioning bacteria for electron microscope observation, which may be useful in tracing the fate of the mutants I am studying when they infect lysogenic cells. With Dr. M. Pollock (London) I wish to discuss his experiments on the inhibition of adaptive enzyme formation by ultraviolet light, which has direct bearing upon the inhibition of virus multiplication by UV.[41]

From these two letters it might appear that Benzer was pursuing two separate projects at once. However, in a letter he wrote to Hershey on the same day requesting some mutants, he described them as connected:

> Dear Hershey,
>
> A: Facts, B: favors
>
> A) Perhaps you know all this already. If not, you may be interested.
>
> The expression of the r phenotype of a (T2, T4) mutant depends upon the host. Also, some r mutations carry an associate loss of host range (in lysogenic bacteria). Thus:

Host	B	K12S	K12S(λ)	
T4r$^+$	r$^+$	r$^+$	r$^+$	plaque type
T4r type I	r	r$^+$	r$^+$	——
Type II	r	r$^+$	no plaques	
Type III	r	r	r	

> r mutants isolated on B are usually types I and II, while r mutants isolated on K12S or K12S(λ) (much more rare than on B since types I and II do not show as r) are type III. Type II adsorbs on & kills

K12(λ) but only a small fraction of the cells yield. The ability of the host to discriminate between types I and II goes with the presence of carried λ. All type II mutants so far tested recombine with each other [he crossed out the words here underlined] are genetically distinct and I think this system may lead to a sort of "physiological" genetics of phage.

I am working on this problem. Looking for "heterozygosis" in mixed infections with two different type II on K12(λ) Rather than work out a whole new map I wonder whether you would let me have . . . [He replaced the passage here underlined with the following:]

B) 1: I would like to know whether these classes are associated with different locations on the chromosome map. Rather than map all these mutants, I would be most grateful for samples of your already-mapped T2 mutants and double mutants (or as many as you are willing to give)

2: Can I persuade you to let me work on this problem in your lab this summer?

See you at Oak Ridge in April
Best Regards,
Seymour[42]

Benzer wrote a similar letter to Gus Doermann requesting his T4 mutants.

From these letters taken together, we can see that Benzer seemed to be working on three problems that were, on one hand, distinct enough to be described by different names—"host range," "phenotypic expression," and "interference between carried and infective bacterial viruses"—but that were, on the other hand, sufficiently related to one another, if not overlapping, to constitute for him a coherent research project. Viewing them together, one might see problems of host range—that is, differences in the hosts in which particular mutants would or would not reproduce—as potentially manifestations of the same factors that might also play a part in determining what form of plaque the mutants were able to form on the hosts in which they did reproduce. In the case of lysogenic bacteria, interference between carried and infective viruses could be viewed as the putative explanation for the host-range limitations that prevented some mutants from reproducing on them. The extent to which these were really manifestations of one problem rather than separate problems could perhaps not be settled until solutions to the problems were reached. Such overlaps and indis-

tinct boundaries between research problems are typical of the intimate level of daily scientific investigation. It is often only after one or more such problems have been solved that they can be presented in publications as well demarcated and separable from one another.

His letter to the National Research Council shows that Benzer was not merely directing the system of phages and bacterial strains he had put together toward the problem of interference between carried and infective viruses but was entertaining ambitious plans to attack this problem with a variety of other techniques that he expected to learn from colleagues in Europe and elsewhere. That he should do so is not surprising, because it is clear that the observations that had prompted him to take this new turn in his research pathway also incorporated major elements of his earlier interests. The whole thrust of his application of the Luria-Latarjet experiment, beginning five years earlier in Oak Ridge, had been to find ways to elucidate the stages of development of phage during the latent period. In Paris, he and Jacob had begun together a project to apply the same methods and objectives to the case of lysogenic bacteria. There, too, he had absorbed some of the great current interest among Lwoff's group in the nature of the prophage and its relation to infective phage. His discovery of the inability of a group of r mutants to reproduce in S(λ) thus provided him with an unexpected but very favorable opportunity to extend these prior interests. At the same time, his plans to learn about such new techniques as those Maaløe was developing was a recognition of the limitations he and others had long experienced in penetrating by irradiation and the other methods commonly applied in the phage group to the events occurring within the bacteria during the phage growth cycle.

Finally, Benzer's letters to Hershey and Doermann reveal that, far from intending to use his experimental system to map the r mutants of T4 or T2, Benzer was, nearly two months after making the observations on which the system was based, seeking to avoid the necessity of mapping, hoping instead to obtain the mutants that had already been mapped from these pioneers of bacteriophage mapping. Even if, at some earlier point, he had seen the potential of his system for higher resolution mapping, and even if the series of crosses he had carried out in January represented a start in that direction, his current priorities seem completely to have overshadowed whatever plans he might have had to devote himself to that project. More probably he had recognized in his system only one more method of mapping, not one sufficiently

superior to those that Hershey and Doermann had used to justify a major effort to surpass them.

Hershey responded on March 5, "I will send you a set of r's shortly." He imagined that they would plate typically on bacterial strain B, although he had always plated them on S. He would also send a phage stock "marked 'u' (for unstable). This stock forms plaques sectored with r's, all of which prove to be linked to r1, which is itself a rare mutant in the wild stock. If your results show any connection between physiology and map position, as I think they will, I would appreciate hearing about the derivatives of u." In a handwritten postscript he wrote that they would be "delighted to have you here this summer." There would be plenty of room, because he would be away for most of the time Benzer would be at Cold Spring Harbor.[43]

While waiting for Hershey's stocks to arrive, Benzer busied himself further with the behavior of the still-troublesome bacterial strain S( )IV, which he had characterized at Urbana as "poorly lysogenic." On March 5 he examined the induction of a purified culture of the strain and carried out a one-step growth experiment on an induced culture. On the same day he began a "Luria-Delbrück" experiment on the inducible fraction of S( ). In 1943 Luria and Delbrück had shown from a study of the fluctuations in the numbers of colonies of bacteria that were resistant to lysis that the resistant bacteria had arisen through mutations (see Chapter 2). When Benzer carried out a similar experiment on S( ) induced by ultraviolet radiation, however, the fraction of yielders was "exceedingly constant, ruling out the idea that the yielders represent *spontaneous* mutations from S( ) → S(λ)."[44]

Four days later Benzer compared the "fate" of another T4r mutant in S(λ) exposed to ultraviolet light with its fate in the same strain not exposed. In both cases about 1 percent of the infected bacteria yielded plaques, from which he concluded that "UV has no effect." From a one-step growth experiment carried out at the same time he inferred that "less than 1% of this 1% give normal yield."[45] These experiments seem to have been carried out in pursuit of his effort to understand the nature of the interference between carried phage and the growth of infective phage. The regular use of the term *fate* with regard to the mutants for such experiments suggests that he meant not only knowledge of the conditions that determined whether phage reproduced normally but eventually knowledge of the stage at which they were blocked when they did not.

Not long afterward Benzer received from Hershey five T2r mutants, two double-r mutants, and the unstable uhr16. These he plated on his standard three bacterial strains, B, S, and S(λ). The results were that "r2, r4, r7, and those double mutants involving them (r1, r4, and r4, r7) are 'negative' type [that is, they gave no plaques on S(λ)]. These are all in the same cluster on the B 'chromosome.' On the other hand, r1 and r13 (and r1r13) are 'positive' type. These are outside the cluster (r13 is also on B, r1 is on A 'chromosome')." He was able to establish these locations by reference to Hershey's genetic map, as had been his purpose in requesting the stocks.[46] To find that the r mutants from Hershey that did not plate on the lysogenic bacteria fell, like his own, into a cluster located apart from those that did plate must have been satisfying to Benzer, and it must have stimulated him to wonder whether his set of "type II" mutants would fall within Hershey's cluster. For the moment, however, perhaps because of Hershey's request, he concentrated on the uhr16 mutant, which was also negative on S(λ).

On March 22 Benzer found that the u mutant yielded "400 plaques of very variable type" on B, 2 faint plaques on S, and 3 faint ones on S(λ). The following day he chose derivatives of the u stock, selecting three from plaques that appeared to be r^+ plaques on B and two each from sectorial plaques on B and from plaques on S. Some of the plaques resulting from further platings on B and on S were r, and some were r^+; most of them were also turbid. On the March 27 he repeated the platings of three of the stocks. All of the plaques, he observed, "are r type, but vary very much in size, so that tiny ones cannot really be distinguished from r^+. However, the plaques on S and S(λ) include lots of r type ones, so these plaques are not due to an r^+ impurity."[47]

Puzzled by these last results, Benzer wrote Hershey on the same day that " 'u' has me a bit perplexed. Would you mind telling me its history? It was marked 'uhr16' and gave such an array of plaques that I don't know which one the stock designation represents." At the same time he reported on the mutants and double mutants that "plate on the non-lysogenic bug but not on the lysogenic." The " 'defect' seems to be associated with the cluster of r mutants in linkage group B. Just to be sure, and if you don't mind the effort, could you let me have stocks of r14, 8, 9, 3, 6, 5, h^c, h^t5, m and any doubles that might be available?" Benzer was asking for the rest of the mutants that Hershey had located in the same cluster, presumably to find out whether the defect was common to all the mutants located there. "From the rough crosses I have made among my T4 derivatives," he went on,

> I think the same conclusion will hold for T4. Out of 9 mutants tested, the 6 defectives form a cluster, 2 non-defectives form a second, and one non-defective forms a third. Presumably the same "region" is involved in both T2 and T4 (T6 also shows this phenomenon, by the way) but this remains to be seen. How much is really known about matching the maps of T2, T4, (and T6)? I have not had any response from Gus [Doermann].[48]

The tentative references to T6 reflect an experiment that Benzer was carrying out at the time he wrote Hershey that involved plating on S(λ) a series of T6 mutant that he had isolated from a single plaque on March 24. Two days later he had preliminary results showing that three of the ten he had chosen formed "smallish r type" plaques on the lysogenic bacterium, the rest forming no plaques.[49] The experiment was one of several explorations of the extent to which his r mutant phenomenon was applicable to other classes of phage, but the availability of Hershey's and Doermann's genetic maps clearly induced him at this stage to fix most of his attention on T2 or T4.

Benzer's letter to Hershey suggests that, although he had sought to avoid extensive mapping, the fit between Hershey's mapped mutants and the results of his own rough crosses was drawing Benzer further into the question of the relation between the "defect" shown by his r mutants and their location on the genetic map. For the first time, he wondered whether a subgroup of r mutants sharing this property might occupy a distinct "region" within a chromosome. There is no indication here or in the next group of experiments he performed, however, that he was contemplating an entry into the field of mapping; rather, he would continue to rely on the work of Hershey and Doermann while he pursued the questions about host range, phenotypic expression, and interference that he had already taken up.

Meanwhile, on March 25 Benzer turned to the study of "mixed infections" of S(λ) with his own r mutants that he had begun during the last week of January. Now, however, instead of using two mutants, he compared the number of r, r^+, and mottled plaques formed by plating the lysogenic bacteria with the wild type, $T4r^+$ and a mutant, T4rBIV, with those produced in a mixed infection with the two. Calculating the percentage of the total number of infected bacteria giving rise to each type of plaque for each of the three cases, he observed,

> Thus, even the absolute # of bacteria yielding mottled plaques is greater [in the mixed infection] than those yielding plaques when r

> alone is used. If it is assumed that killing particles act indiscriminately, the % of mixedly infected (yielder) bacteria giving r or mottled plaques is enormous [20%] compared with only 3% giving a yield when infected with r alone.
>
> Conclusion: r^+ enables r to develop (as well as r^+) in a mixedly infected bacterium, whereas r only would not.[50]

His conclusion rested on the fact that, although with r^+ alone 49 percent of the infected bacteria produced plaques, these were all of the r^+ type. With the mutant alone the bacteria produced only r-type plaques. With the combination, 8 percent of the infected bacteria produced wild-type plaques, 12 percent produced mutant-type plaques, and 8 percent produced mottled plaques. The r^+ phage and r phage together must, therefore, have been able to produce the mottled plaques that neither could produce alone. His reasoning and his focus on this aspect of the results indicate clearly that Benzer's research was still aimed primarily at understanding the mechanisms of phage reproduction in lysogenic bacteria, in particular on interactions between the infective phage (and also the carried phage), rather than on their recombination.

On April 3 Gus Doermann finally responded to Benzer's request in a letter that began with apologies for the delay, which had been caused by his mother's illness and his own strep throat. "I will be glad to send you stocks for your most interesting problem," Doermann went on, cautioning, "You may find some of them difficult to use." He referred Benzer to technical directions contained in his publications. "The markers that I have mapped," he went on, "are all given on the map in the C[old] S[pring] H[arbor] symp[osium] paper. Sending you my stocks, however, has one condition. This arises from the fact that everyone wants to use genetically known material, but no one is willing to do the more or less thankless and dull job of mapping the markers. Therefore the condition is that you must promise to locate on the T4 map at least two of your independently arising mutants."[51] Benzer marked this paragraph and underlined the last sentence, signifying that he took seriously the obligation under which the receipt of Doermann' s stocks would place him. Doermann must have sent the stocks at nearly the same time as the letter, because five days later Benzer was able to carry out "tests of stocks of T4 mutants received from Doermann." There were six stocks in all, including the wild type, two single mutants, and three "rtu" double mutants (r that was tu, meaning "turbid"). Doermann had indicated in his letter that he would leave it to

Benzer to separate the markers in these three. Plating each of the phage in the usual way on bacterial strains B, S (which he again began to call "K12S"), and S(λ)—again called K12S(λ)—he found that two of the mutants gave no plaques on the lysogenic phage, whereas the other five gave r-type plaques. "Thus," he concluded, "r47tu41 and r51 are 'defective' while r48 (and its two tu mutants) are r type on all three bacterial strains. The tu character does not appear to affect the 'defectiveness.' "[52]

Fourteen additional phage stocks arrived from Hershey at about the same time, and Benzer tested them in the same manner on April 9. Three of these, including the wild type, proved "non-defective." Five plated on B but gave no plaques on λ lysogens, and he added them to his list of "defective" T2r mutants. In the remaining five cases the titers were too low to judge. He commented that "all plaques are quite poor, possibly due to the fact that the incubator did not work properly (temp. was only 33C.) All plaques on S and S(λ) are r^+." Despite this flawed result, by checking Hershey's map he was able to affirm that "the generalization still holds that only mutants in the cluster on the B linkage group are defective." On April 14 he repeated the experiment on the mutants whose titers had previously been too low and was able to add one more of them to the list of defective ones.[53]

Benzer left no comment about the location of the T4r mutants that had proved defective, but r47 and r51 were close enough together on Doermann's genetic map to be compatible with the generalization Benzer made about Hershey's mutants. These further encounters with previously mapped markers must, therefore, have reinforced Benzer's surmise that the defective r mutants occupied a distinct region in both T2 and T4. Nevertheless, these results did not change the dominant direction of his investigation.

Among the experiments Benzer carried out during the first half of April that reflected his previous interests were two on the dominance effect, which he regarded as a "real test of r and r^+ phenotypes when phages are plated on K12S or K12S(λ)," and another "mixed infection of S(λ) with two 'defective' r's" that he had earlier isolated.[54]

On April 17 Benzer left Purdue to take part in a symposium on genetic recombination at Oak Ridge. He arrived the following evening, "in good time to participate in the pre-symposium mingling." Many of the prominent leaders in the emerging field of molecular genetics were there. During the next three days formal symposia were held concern-

ing recent developments in the study of fundamental genetic phenomena, including discoveries about "DNA-mediated transformation and phage-mediated transduction of genes from one bacterium to another, and with the structure of the DNA molecule which appears to be a major determinant of genetic specificity." When Benzer suggested to Francis Crick that since he and Watson had established the beautiful DNA structure they could now study the effect of x-rays on many further problems related to the structure, Crick responded that he would prefer to leave that work to others. Afterward Benzer, Ed Lennox, Gunther Stent, Sol Spiegelman, Jim Watson, Otto Landman, and Gus Doermann drove to Gatlinburg, in the Smoky Mountains, where they spent a day hiking and engaging in "very profitable informal discussions of our various private and joint researches." In the evening all seven shared one room, an arrangement that made Benzer feel somewhat claustrophobic.[55] No explicit traces remain of suggestions that Benzer may have received for his ongoing work from these intense discussions, but he so regularly benefited from such interactions that it is plausible that the first experiments he took up when he returned may have reflected such influences.

On April 27 Benzer tested the "UV sensitivity of T4r$^+$ when plated on B or on K12(λ)." Exposing one sample of the wild-type phage to ultraviolet radiation and using another sample as a control, he plated both on each of the two bacteria. The idea behind the experiment was probably that, if the radiation knocked out the r$^+$ gene, then the phage might survive better on B, which did not require the wild type, than on K12(λ), which did. There was, however, "little if any difference in sensitivity," and he did not pursue the question further.[56] At the same time he prepared six new stocks of T4r mutants grown on K12S for use in a study of what he called in his laboratory notes the "transmission factor." Having noticed already that not all of the defective mutants were totally unable to reproduce on his lysogenic bacterial strains but that the degree of the disability varied greatly from one mutant to another, he now turned to investigate these differences more systematically.[57]

Plating his six new mutants together with three of Doermann's on B and on K12S(λ) at several dilutions, he found that "none of the S(λ) plates show any plaques at the undil[uted] and 10x dil[uted] platings." At greater dilutions progressively fewer of the mutants were unable to form plaques. He calculated the titers of each mutant on B and on

K12(λ), and in a third column he calculated their ratios. The latter, representing his transmission factors, ranged in his own mutants and in one of Doermann's from 2×10^{-7} to 5×10^{-4}. Two other mutants, obviously nondefective, gave ratios near unity. He called the r mutants that plated at greater dilutions "minority phages," four of which he tested further at two dilutions on April 29. He was struck by a qualitative rather than a quantitative distinction: "X, IX, 51 [roman numerals signified his own mutants; arabic numerals were those of Doermann's mapped markers] plate as r^+ on B and are therefore merely reversions. However VIII is still r type and perhaps represents a kind of 'host range' mutation enabling it to grow on K12S(λ)." It is a mark of his continued attachment to the host-range problem that Benzer turned next to the "isolation of 'host range variant' of T4rBVIII." Plating derivatives of the mutant, the wild-type T4, and mixtures of the two on bacterial strains B, K12S(λ), and mixtures of these two cultures, he recorded the results on April 30 without comment. Perhaps the fact that, unlike the wild type, the mutant derivative now produced no plaques discouraged him from pursuing the lead further at this time. (Looking back at these experiments in May 2001, Benzer characterized the event as a "false alarm.")[58]

After this brief digression Benzer returned on May 4 to measurements of transmission coefficients, this time comparing the fraction of total infective centers yielding plaques with those of infected bacteria, by using chloroform lysis in control tests. He obtained similarly very low fractions for the defective mutant phage. Did these measurements represent another means to pursue the problems of host range, phenotypic expression, and interference with which he had already been engaged, an exploration of the scope and limits of his experimental system, or the potential emergence of a new research problem? Possibly at this incipient stage Benzer need not have distinguished among such alternatives. Means and ends shift rapidly and subtly during the course of daily laboratory life. In any case, at this point he turned aside once again, to prepare to fulfill his obligation to locate two of his own T4 mutants on Doermann's map. A cross between T4 wild type and Doermann's double mutant, T4r47tu41, also carried out on May 4, was intended to separate the two markers on the latter. Doermann had written Benzer that since he did not "know in what combinations they will be useful, I will let you take them apart and put them into the desired combinations." Using the ratio between the number of r and of turbid-

type plaques formed and the total plaques, including combinations, as a measure of the recombination frequency, Benzer came up with 31 percent, compared to Doermann's value of 25 percent for the same markers.[59]

For the first cross of T4r mutants, performed on May 10, Benzer chose from the latest group of mutants that he had isolated the only one that had not plated on K12S(λ) at any dilution, as well as the two from Doermann's stocks that also had not plated on K12(λ). One of them, r47, he had probably obtained six days earlier by separating the two markers of the double mutant Doermann had sent. Doermann had listed the other, r51, as a single mutant, but during the course of the experiment Benzer came to suspect that it was actually an rtu double mutant. In three separate crosses between the three mutants taken two at a time, which he then plated on both B and K12(λ), he calculated the frequency of recombination as the ratio of two times the number of wild types formed to the total number of plaques. Although his laboratory notes are not entirely clear on the matter, it seems that he recognized wild types as those able to plate on B and on K12(λ); he was, in other words, relying on his own system rather than on the methods used by Hershey or Doermann to map markers. The results were as follows:

T4rBV × r47	=	7.7% recombination
V × r51	=	0.6% recombination
r47 × r51	=	7.8% recombination

At the top of the page on which he summarized the results of this experiment he represented the three markers as a genetic map like those of Doermann and Hershey (fig. 6.1). He placed his mutant (number V) 0.6% to the right and to the left of r51 because without further crosses between V and other markers it was not possible to decide which was the correct location.

This simple diagram, appearing almost as an afterthought on the borders of the page, can be seen as a historical landmark, the earliest progenitor of those that Benzer eventually constructed. Was it also the decisive turning point along Benzer's investigative pathway? The circumstances of the experiment strongly suggest that he had undertaken it not as a change in the direction of his research project but to comply with Doermann's stern request that he map at least two of his mutants.

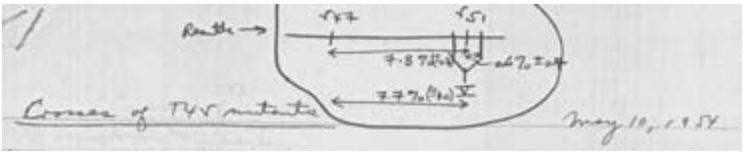


Figure 6.1. Benzer's first map of r mutants. (RN no. 3, May 10, 1954.)

The fact that his own most defective mutant fell within the boundaries of the two r mutants of Doermann that shared this property, whereas those of Doermann that lay outside these boundaries were able to plate on K12S(λ), must have further strengthened Benzer's conviction that the r mutants that were unable to reproduce in lysogenic bacteria constituted in T4, as in T2, a cluster within a specific region of the chromosome. His next moves show that, although for the moment he might still have regarded the venture as a diversion from his prior interests, the promise he now saw in further mapping rapidly came to overshadow those interests.

On May 10 and 11 Benzer mapped T4 wild type and three mutants on a strain of presumably lysogenic bacteria (BB) that Gunther Stent had sent him from Berkeley, comparing the types of plaques formed with those formed by his own strain B. He noted that "these r and r^+ characterizations are entirely *un*ambiguous. This strain discriminates among the r's." This experiment can be regarded as a continuation of his occupation with host-range and phenotypic expression problems, but it may simply have been a favor he did for Stent, who needed to know about these properties of the strain for his own investigative purposes.[60]

On May 13 Benzer examined the transmission factor of one of his T4 mutants "vs. age of culture" and obtained curves showing a marked effect. This was obviously a continuation of the project he had taken up shortly before mapping his mutant. On the same day, however, he crossed five more of his mutants with Doermann's T4r47, and two days later he crossed the same strains with r51(tu). Then he became completely preoccupied with further crossing experiments and their analysis. By May 21 he had produced and revised a genetic map that represented twenty-four crosses and the linear relationships between five of his mutants and Doermann's r47 and r51. All of his mutants lay between Doermann's on the map (fig. 6.2).

On May 28, on three sheets stapled together in his laboratory notebook, Benzer wrote a long meditation under the title "thoughts on the

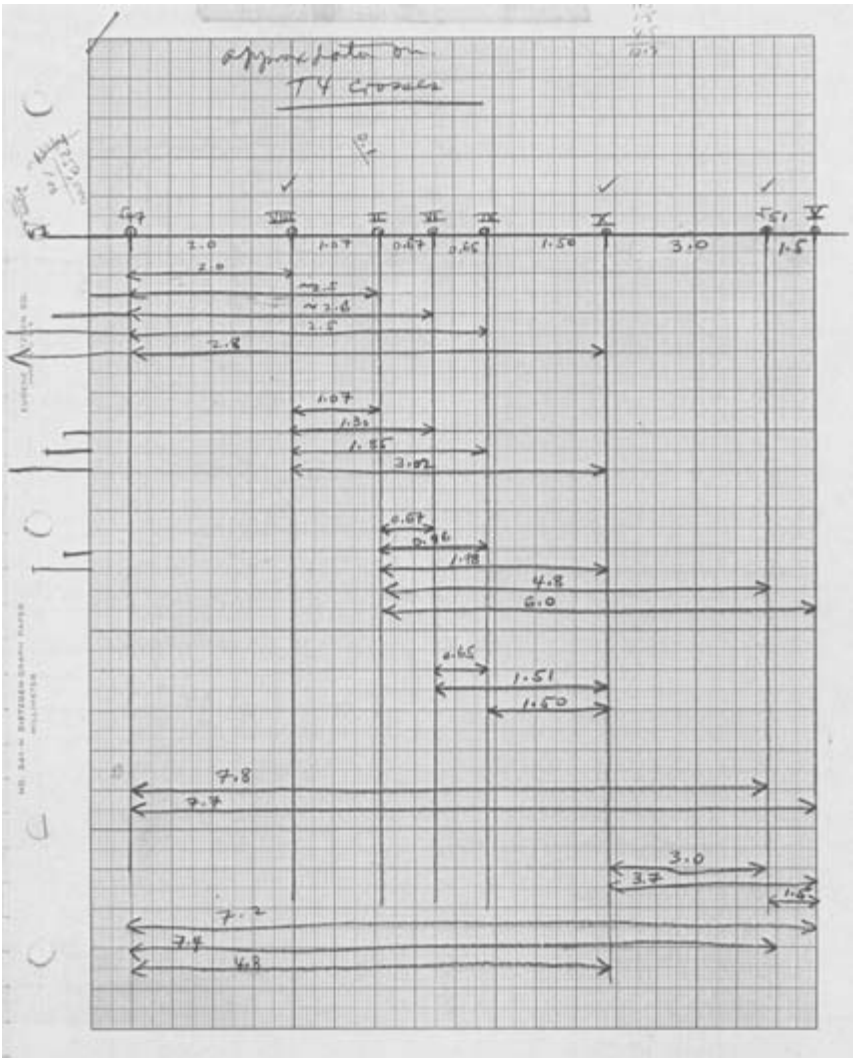


Figure 6.2. Summary in the form of a genetic map of bacteriophage T4r crosses, drawn between May 15 and May 21, 1954. (RN no. 3.)

gene." The character and significance of these passages require that they be quoted in full:

> It occurs to me that the "r" region under study can be interpreted as a *single gene.* The length of the region corresponds to the order of 10,000 nucleotides per strand. If one considers that each nucleotide pair determines [a] particular amino acid, then this region could

correspond to the synthesis of a polypeptide chain 10,000 amino acids long. This would give a molecular weight of the order of one million. A defect anywhere along the DNA chain could lead to a crucial defect in the protein, although the seriousness of the defect could depend upon the particular nucleotide affected or its position in the chain.

The frequency of reversion could be expected to vary with the particular nucleotide responsible for the defect. A reverse mutation could be due to an accidental slipping in of the "correct" partner to an "incorrect" defective nucleotide.

This case gives a clear distinction between the gene as a unit of recombination or mutation and as a unit of physiological action. The nucleotide (or, a very small number of them) is probably the unit of recombination or mutation, while the whole string of nucleotides (10,000 of them) is the unit of physiological action.

An upper limit on the number of nucleotides involved per unit of recombination can be obtained by isolating many more r mutants and seeing at what point "allelic" ones start to occur. (Thus far, no iso-local ones have occurred.) Also, more loci would enable one to set the outside limits of the region.

It would be nice to be able to isolate the specific protein involved and see if its properties are different for r mutants & and wild type.

The other r regions (r1 and r14; r13) (in T2) presumably correspond to different "genes." Although they lead to the same phenotypic effect on B, their effect is not the same as for the "r negative" gene, as can be shown by the behavior on BB and K12S and on K12S(λ).[61]

On the third page Benzer diagrammed his conception of the physiological gene (fig. 6.3):

This passage displays several strong characteristics of a spontaneous expression of a flash of insight. Most obvious is the title and the explicit opening phrase, "It occurs to me." The placement of these thoughts in a notebook normally used to record immediate plans and procedures for and data, calculations, results, and conclusions drawn from daily experimental operations also suggests that he might have been putting down in a place near at hand something that seemed new and important to him—something that perhaps came to mind while he was transferring data from the rough penciled sheets on which he customarily put down notes in the laboratory to the neater daily records in which he summarized results and reduced his data. The phrase "If

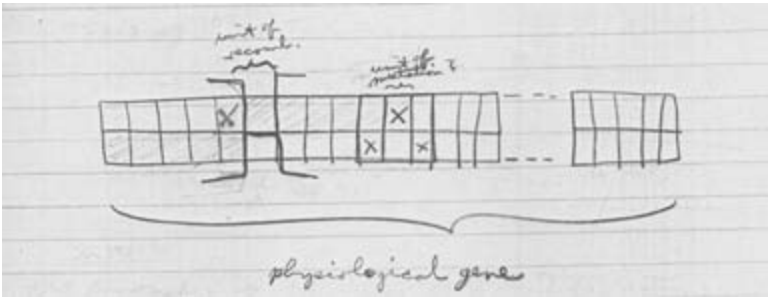


Figure 6.3. Benzer's schema for the physiological gene. (RN no. 4, May 28, 1954.)

one considers that each nucleotide pair determines a particular amino acid" reinforces the view that these were incipient ideas that he had not thought over for very long before writing them down. Although the coding problem was just beginning to be discussed and the relation between nucleotide sequences and amino acids was not yet established, it would have taken only a little reflection to notice that a single nucleotide could not determine each amino acid. When reading over this statement with the author in 2001, Benzer could only comment, "That's pretty stupid: knowing that there are more than twenty amino acids [and only four bases]. So that's dumb."[62] As a well-formulated conjecture this idea would have been as far off base as he retrospectively took it to be, but a more likely interpretation is that he put down this idea rather hastily, with his attention focused mainly on other aspects of his thoughts; consequently he had not thought out what was coming into his mind in the rush of a new insight.

Was this the original occurrence of the insight that Benzer recalled in much-altered form a decade later as the starting point for his mapping project? Memory frequently transfers events into time sequences that differ from reality or projects details belonging to one event onto another one instead. In the case at hand, however, the necessary alterations would have been so pervasive as to constitute essentially a substitution rather than a modification. What the two versions have explicitly in common is only that they assume the unit of mutation to be a nucleotide and provide alternative accounts of what may have prompted Benzer to drop whatever else he had been doing in order to concentrate all his effort on mapping the r region of the T4 "chromosome."

Implicitly, however, there is sufficient overlap so that a transfer of the time at which he remembered the event to have occurred from late

May to early January may account for most of the differences between what is explicitly mentioned. In the original written expression there was no need to include the origin of his experimental system because he had already been using it for three months. By remembering a single momentous flash of insight as the starting point for both the experimental system and the mapping project, Benzer would have been constrained also, without noticing it, to reconstruct the event as including the factors giving rise to both.

If we choose to rely more on the contemporary record than on Benzer's recollection, we see not only a different sequence of events but a diffuse pattern of gradual development in place of an abrupt rupture between what he had been doing up to that point and what he did thereafter. Soon after observing the r mutant defect, he saw that he had at hand an experimental system that could be used for gene mapping and actually tried a few crosses, but he has left no indications that he had by then recognized in it a system capable of much higher resolution than methods that others were already using, and he did not give that application of the system special priority. He turned instead to other problems for which the system seemed to offer considerable promise and which were more closely connected with his prior interests. Some of these were explorations of the scope and nature of the r mutant phenomenon itself. Under the rubrics of the host-range problem, phenotypic expression, and interference between carried and infective phage, he explored, sometimes in sequence, sometimes in parallel, three questions overlapping sufficiently to make it doubtful whether these were separate projects. Several times along the way he performed further crosses intended to locate his r mutants, but these could not yet be said to constitute a coherent mapping project. When he did begin to map a number of his mutants systematically, his motivation at first seemed to have been more to reciprocate the favor that Doermann had done for him by sending him previously mapped mutants than to embark on his own mapping project. What, then, was the decisive factor that finally led him over the course of a few days in early May—that is, about four months after the events that had led him to devise his r mutant system—to devote that system entirely to the construction of a genetic map?

If we associate proximity in time with causal immediacy, then the experimentation that Benzer performed in order to fulfill Doermann's request appears to be the strongest candidate. Most likely, however,

none of these encounters with crossing, some of which he described as "rough" mapping experiments, was, by itself, decisive enough to cause him to put aside everything else in favor of the new project. Rather, they cumulatively raised his interest in the possibilities offered for mapping until a threshold was crossed at which he decided, temporarily at least, to give it priority. It is not necessary to assume that when he dropped his other projects he intended to do so permanently. Moreover, when he had embarked on the project and come to the insight quoted above, he may not yet have foreseen the degree to which his system would be capable of raising the resolution of mapping beyond that of the current methods. It is notable that of the three definitions of the gene mentioned in the passage (which were nearly identical to those he had presented in his seminar on the gene a year earlier, before he had known about the double helix and nucleotide sequences), Benzer gave the most attention to the unit of physiological action, that is, a segment of DNA that he estimated to be about ten thousand nucleotides long. The advantage of his experimental system that seems to have captured his attention most strongly at this point seems, therefore, to have been that his r mutants exhibited a common physiological action and were also grouped together in a particular region of the chromosome.

His emphasis on the differences and similarities between the phenotypic expressions of the three classes of r mutants he had identified suggest, moreover, that in taking up the mapping project Benzer was at first not obviously dropping his previous interests but extending them. Mapping the r region, in other words, can be seen as another approach to the problem of phenotypic expression that he had described at the beginning of March to Hershey and others.

If high resolution was not the most prominent objective that Benzer foresaw when he wrote out these thoughts, however, neither was it far beneath the surface. The most operational statement embedded in what were otherwise mainly "thoughts" about the gene was that "an upper limit on the number of nucleotides involved per unit of recombination can be obtained by isolating more and more r mutants and seeing at what point 'allelic' ones start to occur." The necessity of mapping more and more r mutants was clearly the force that now began to push aside his other projects and to fix his attention on this one.

To the three sheets on which he had written these thoughts Benzer stapled two more on which he added further quantitative calculations and arguments:

$$2 \times 10^{-11} \text{ mg P per T2 particle}$$
$$\text{atom. wt.} = 31 \text{ g}$$
$$6 \times 10^{23} = 31 \text{ g}$$
$$2 \times 10^{-11} = \frac{2 \times 10^{-17}}{31} \times 6 \times 10^{23} = \frac{12}{31} \times 10^{6} = 400{,}000 \text{ nucleotides}$$

Benzer probably carried out this calculation of the number of nucleotides in a T2 particle for that phage type rather than for T4, whose map he had constructed, because he had crucial information, perhaps the amount of phosphorus per particle, that was not yet available for T4. For the rest of his considerations and calculations he turned to T4:

> Total known markers → let us say 50 recombination units (per mating)
> Assume this 50% represents 400,000/2 nucleotides per strand.
> Then 1 % represents 4,000 nucleotides
> 0.01% represents 40 nucleotides
> To measure 0.01% recomb., need to detect fraction $10^{-4}/2$ of K plates over B plates
> Fraction 10^{-6} recombination measures distance between two neighboring nucleotides
> At this level, recomb. rates should [he replaced "should" with "might] become multiples of a fundamental unit.

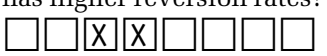
In this calculation of the degree of resolution that he would require in order to detect markers one nucleotide apart, Benzer assumed that a T4 particle contained the same number of nucleotides as a T2 particle and that the particle was a Watson-Crick two-stranded chain of nucleotides. After drawing a line across the page he went on:

> 0.5% recomb. for 5 rounds of mating represents only 0.2% per mating.
> 0.2% per mating corresponds to 800 nucleotides.
> This is an upper limit, which must be reduced if—the linkage groups are found to be longer and if only a fraction of the DNA of the phage is needed

What was the unit to which his calculations led him to assign an upper limit of eight hundred nucleotides? These pages do not say. From a similar calculation that he summarized a few weeks later in the first seminar he presented on the subject, however, we may surmise that he had in mind the region between Doermann's r47 and r51 onto which he

had just mapped his own type II r mutants. On the second page of his calculations and queries, Benzer wrote:

Is a single nucleotide diff[erence] associated with gene mutation?
Stable r mutants
Are these double mutants?
Can they be separated into two singles, each of which has higher reversion rates?



Stable double mutants → 10^{-8} for reversion freq.
If single nucleotides separate.
xx X ++ → 10^{-6} fraction of singles which should revert with 10^{-4} frequency.
But: How to detect 10^{-6} fraction of singles?[63]

I have not identified the stable mutants to which Benzer referred in these statement and therefore am unable to interpret the meaning of these last calculations. It is, however, significant that the 10^{-6} fraction, which he did not yet know how to detect, was the same as the distance between neighboring nucleotides, which he had computed on the previous page.

The fact that these two pages are written in pencil, whereas the previous three are in ink, strongly suggests that he did not write them at the same time. That he stapled them together suggests, on the other hand, that he afterward considered them to be closely related. The most plausible interpretation is that he wrote down these considerations shortly after writing out his thoughts on the gene; consequently, it was the qualitative association of the units of recombination and mutation with one or a few nucleotides, and of the gene as a unit of physiological action with a much longer chain of nucleotides, that induced him to try to determine these distances.

There are no records of earlier computations such as this, and its character also strongly suggests a first effort. These pages may well represent the "quick" calculation that Benzer later recollected having carried out as soon as he realized that he had a system of very high resolving power. If so, however, he did not make it immediately after his realization but four months later, when he had begun mapping.

Equally significant is that these first computations did not show that his system was capable of resolution down to the individual nucleotide but left him wondering whether it was possible to reach low

enough recombination frequencies to detect the "fundamental unit" of mutation and recombination. Sometime within the next month Benzer did make a calculation that answered his question positively. When he did so, he had completed the last step in what he later remembered as a unified insight. In other words, this insight was not one that came in anything resembling a flash or a single "Eureka" experience but one that unfolded gradually during the course of the investigations that he carried out with his experimental system. During this period there were at least several smaller "Eureka" experiences interspersed along his research pathway.[64]

When examination of the laboratory records in May 2001 persuaded Benzer that he had spent nearly four months on host-range and other problems instead of starting at once on the mapping project, he became impatient with his former self. He described himself as wasting time on "side issues" or "fiddling around with the host-range problems." He compared himself to his own recent graduate students, who preferred to apply methods they had already mastered to subsidiary problems, oblivious to central questions staring them in the face that might require new approaches.[65] Why did the actual course of events that can be recovered from the record baffle him when he confronted them half a century later?

Compression, simplification, and reordering of events experienced are general and mostly functional attributes of memory. Whenever we recollect parts of a past too complex to hold them fully in mind, we simultaneously reconstruct them in ways that are adaptive to our current circumstances. For scientists, a particular form of reconstruction is intrinsic to the process of transforming an ongoing investigative stream into arguments supporting a conclusion eventually reached. Most often such reconstructions omit steps not seen afterward as essential to the outcome and rearrange them to make the progression appear more logical than the actual course of events had been. To do otherwise would be to clutter the presentation with details no longer regarded as relevant to the scientific community. Having made such transitions, the authors themselves are most likely, at least in part, mentally to substitute the presented version of events for the research trails they have actually followed.

Benzer's account does not fit entirely into this pattern, for he presented it not in a research report but in an informal essay intended to reveal behind-the-scenes aspects of his earlier work. Moreover, the

story he told was not a logical succession of events but a fortuitous concurrence of logically unrelated events leading to an unexpected insight. The characteristic elements of compression and simplification are, on the other hand, prominent. Why did his memory of the events omit the period in which he had focused on host-range problems so completely that it was a total surprise to him fifty years later to find in his laboratory records the evidence that he had spent nearly three months in this way? When he left his studies of host range, phenotypic expression, and interference behind for the more promising prospects offered by the mapping project, the former were still in an incipient phase and had not yielded any significant conclusions. They would later have seemed unnecessary to the highly successful mapping project. Some of the elements that had belonged to these earlier problems became embedded in the mapping project, where they appeared thereafter as subsidiary problems or research tools, rather than as independent projects. These were subtle, complex transformations, easily left out and eventually forgotten as memories continually reconstruct themselves.

Chapter Seven

Crossing into the Fine Structure

Mapping the r mutants onto the region occupied between Doermann's r47 and r51 was not a one-step conversion of recombination frequencies into distances along a linear diagram but a process of successive approximations. Before constructing a complete diagram, Benzer sketched a partial one when he had done only the crosses of r47 and of his II with each of the other seven. The diagram showed the distances of II between r47, and r51 and the distance between II and V. The latter, which he had placed to the left and to the right of r51 in his first map of the three mutants, now turned out to be to the right. Below this he mapped the distances of VIII, IX, XI, and X from r47, and in a third diagram he mapped the distances of the same four from II. These latter two diagrams, however, did not give the same ordering, and he could not yet tell in which direction these four lay in relation to II (fig. 7.1).[1]

When he had completed all of the twenty-two crosses between all of the mutants, Benzer was able to establish an unambiguous order for the eight mutants on the map, but some of the distances between more distant markers as measured by sums of the distances between neighboring markers differed greatly from the distances directly measured between the two more distant ones (see fig. 6.2). In an effort to refine the map, he replated each of the crosses and in most cases obtained recombination frequencies sufficiently different from the original ones to alter the distances. Discrepancies still remained, however, between the distances as measured by summing neighboring distances and those measured directly between the more distant ones. Clearly the map was still a work in progress.

Rather than working further with his map of this region, Benzer turned to mapping the r mutants outside of the region, that is, those that "do plate on K," K being his abbreviated designation for K12S(λ).

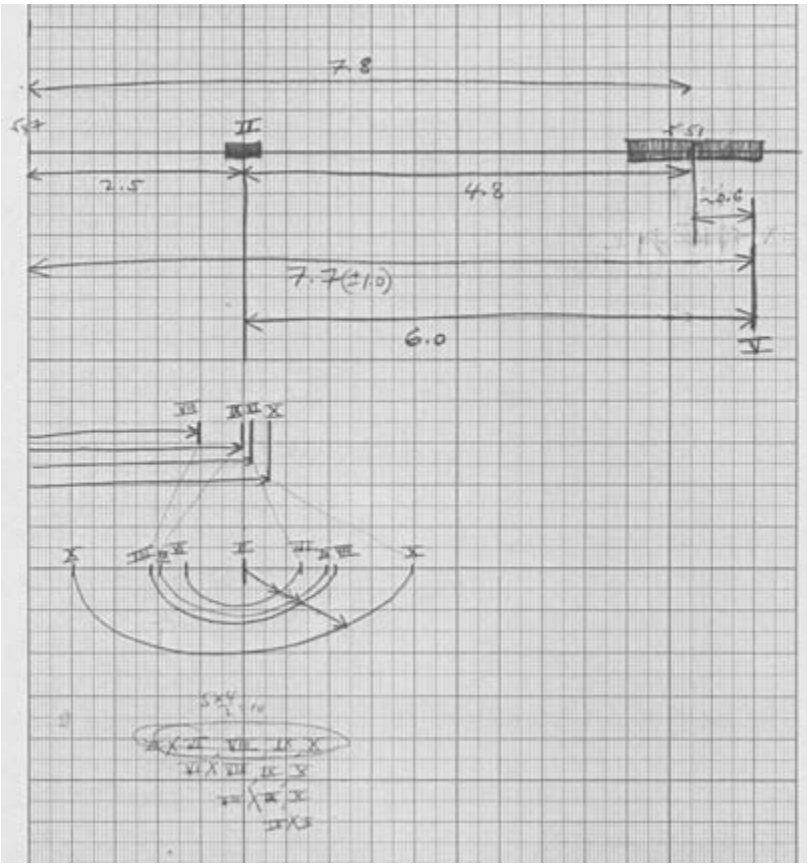


Figure 7.1. Diagram showing a map of four T4 r mutants drawn in both directions. (RN no. 2, May 17, 1954.)

In order to do so he used some previously isolated stocks but gathered new ones as well. Doermann's r48 served as a reference point for the other markers. Benzer drew some rough maps of these mutants but did not develop them further. Most of the markers were relatively far apart, compared to those within the region that did not plate on K.[2]

On June 1 Benzer began "picking and testing 'reversions.'" Several times during the spring he had encountered the phenomenon that among the r mutants that did not normally plate on K a small fraction did and that nonplating mutants gave rise to ones that did. After he began mapping, these events posed an obstacle, because if the plaques they formed were not distinguished from those formed by wild-type recombinants, they would introduce inaccuracies into the counts on

which he based the recombination frequencies. To differentiate reversions from mutants of which a small proportion were able to plate on K, on June 3 he plated the plaques formed there afterward on bacterial strain B. Those that formed wild-type plaques were reversions. For those that formed r-type on B, "the plaques forming on K were not reversions, but probably cases of 'fortuitous leaking.' " Soon afterward he named such mutants "leakers."[3]

Replating the following day on B and on K the six mutants that appeared to be leakers, Benzer found that three of them formed wild-type plaques on both strains. They seemed, therefore, to be "true reversions to the wild type." The other three formed plaques smaller than the wild type, some of them "tiny," on K in numbers ranging from half to the same number as formed on B. "In these cases," he commented, "it appears that these are leakers of much higher efficiency than the original parent stocks." Later he added, "These may be suppressors" (a second mutation at a different locus that in some way suppressed the phenotype of the original r mutant).[4]

In order to broaden his inquiry, on May 29 Benzer had already begun to isolate new r mutants, thirteen of which he tested in the same way on June 3. Most of them turned out to yield reversions. On June 2 he had begun measuring the reversion rates of the older mutants, and he extended the measurements to the new one on June 5. He defined reversion frequencies as the ratio of plaques formed on K to those formed on B. After checking the "authenticity" of his revertants on June 7 by replating and again receiving r^+ plaques on B, he summarized these ratios ($\times 10^{-6}$) for twelve of them on June 8. They were as follows:

T4r47	0.00
T4rBV	0.00
13	0.00
23	0.01
14	0.13
17	0.56
IX	1.8
II	4.5
r51	172
11	179
24	208
(12	<0.2)[5]

Benzer bracketed the first four and last four of these ratios, perhaps because he saw that the reversion rates might fall into groups of differing orders of magnitude. He saw that such a pattern would raise interesting theoretical questions, but he left no trace of his thoughts on this subject. On June 9 he performed one-step growth experiments on three mutants, one of which he had identified as a leaker, and graphed the curves representing the formation of plaques over time. Perhaps he was attempting to quantify his earlier remark that leaking leads to a plaque "eventually." The unusual curve he obtained for the leaker led him to write on his graph, "What is going on here?"[6]

In an attempt to answer this question, Benzer tried two methods to disrupt bacteria infected with a leaker mutant. In the first, he placed them in a Waring blender containing glass beads. The second method was to decompress them. "The decompression would appear more effective than glass beads for disrupting the bacteria," he commented, but "in no case is there any indication of the liberation of additional free phage."[7] Pursuing the question no further, he returned to mapping more mutants outside the region in which his r mutants did not plate on lysogenic bacteria.

Between June 10 and he 13 Benzer carried out several crosses of this type and ran into complexities that led him to suspect that Doermann's r47 was a double mutant containing, in addition to the r marker, one for turbid plaques. To straighten the situation out he crossed Doermann's wild type with his own. He also isolated a mutant containing a marker for minute plaques. No further experiments are recorded until June 22, when he replated some of his earlier crosses between r mutants to "see how many true recombinants are present" and also constructed another one-step growth curve for a leaker. Simultaneously he returned to the problem of reversions. This time he tried the "Lwoff experiment," using a method devised by Jacob to induce reversions. He found, however, that "there is little (if any at all) induction of reversion under these conditions." On June 25 he carried out several mixed-infection experiments and drew up a list of mutants that he classified according to whether they were "strong leakers."[8] Although he may still have been thinking about how to examine further the nature of the leaking process, his most immediate concern was that strong leakers would disturb the accuracy of his recombination experiments and should, perhaps, be avoided.

The experiments that Benzer carried out in June can be seen retrospectively to have been dealing with side issues, which, like host range

and related problems, only delayed him from getting on with mapping the many r mutants that would be needed to reach the degree of resolution he had contemplated during his meditations at the end of May. But again it is difficult to tell whether he was being diverted from his main course by other interesting questions, such as reversions, that came up along his path or was examining factors that he had to make sure would not interfere with the mapping project once he got it under way.

Benzer left Lafayette by air coach on June 29, 1954, to spend the month of July at Cold Spring Harbor. Hershey placed his laboratory, as well as his house, at Benzer's disposal. There is no record of Benzer's performing experiments there, however, and he does not recall having done so. Instead he wrote a paper about the mapping project and its prospects, attended the seminars that took place there frequently during the summer, and engaged in intense discussions of his "current work on the fine structure of genes and mutations in bacteriophage and its relation to their experiences with the genetics of higher organisms."[9] Among the "most fruitful" of these conversations were those with Sydney Brenner, a South African biologist working in England, who had instantly become an avid convert to the double helix when he had seen the model at Cambridge three weeks before the publication of Watson and Crick's first paper in *Nature* in 1953.[10] Brenner quickly became an enthusiastic supporter of Benzer's project. Other significant talks took place between Benzer and Ernest Caspari, Norton Zinder, and several others from the Rockefeller Institute, as well as Milislav Demerec, the laboratory's director.[11]

That Benzer reported discussing his work with others who had experience in the genetics of higher organisms, rather than exclusively with other phage workers, is significant. He later recalled that when he began he knew little about classical genetics and improvised his own method of mapping as he went along. Although his memory probably exaggerated his ignorance of the older genetics, reflecting the general self-image of phage biologists with backgrounds in physics as having made a fresh start on the fundamental problems of life, it is true, as we have seen, that he had come into the mapping field by chance and that his principal models were his fellow phage workers, Hershey and Doermann. We have also seen that Benzer relied heavily on conversations with colleagues to open new vistas: for example, during his year in Paris he absorbed many of the methods and viewpoints of his laboratory partners to complement those which he had gained at Caltech

from Delbrück and his group. His direct contacts at Cold Spring Harbor with geneticists who had worked in the older tradition may have been begun to lead Benzer to view his new project within a broader and deeper context.

At a seminar at Cold Spring Harbor Benzer made the first public presentation of his new work. He began with a general description of the r mutants, comparing their properties with those of the wild type. He enumerated their respective plaque types, their effects on lysis inhibition, the dominance effect, the titers of their lysates, the "selection" effect, and the reversion rates of mutants to wild type. Next he displayed a little chart showing the "phenotypic expression of r mutants" on bacterial strains B and BB, the latter being the same as for K12(λ). The mutants whose expression he included were his types I and II, together with the wild type. Except for the omission of type III mutants, the chart was similar to the one he had put in his March 2 letter to Hershey (see Chapter 6). As he had done earlier, he called the property of discrimination in the bacteria on which type II mutants did not plate "D." The D character, he said, "goes with (λ)" and with defective mutants.[12]

Turning to the "mapping of r mutants," Benzer first mentioned the Hershey and Doermann maps, the fact that type I and type II mutants fell into separate groups, and the properties of double mutants. "The K/B method of mapping," he pointed out, offered "great sensitivity." He then presented a rough diagram of the map of the II region between r47 and r51. Now, however, he identified four of the markers as leakers. One of them was a high-frequency leaker, "but all have same phenotypic effect."[13]

Taking up the "fate of rII in K12(λ)," he stated that they kill the bacteria but yield no phage and no lysis and that ultraviolet induction "does not help (even if T4 [is] added after 40 minutes" and described leakers. "Some can get through," he said, with a long latent period and low yield. The degree of leakage depended on the age of the bacteria and on temperature. Implicitly he was associating his earlier examination of "transmission coefficients" with the properties of leakers. Finally, he described the reversion rates of rII mutants.[14]

Having completed his summary of the experimental work, Benzer turned, in the usual manner of a scientific paper, to a discussion of the results. The first topic he brought up was the nature of the "rII effect." He thought it "may be a metabolic effect of the prophage." He ruled out the idea of an "immunity" effect of the prophage on the ground that

such an effect should not be different for the wild and the mutant phage. He mentioned the pre-induction Lwoff experiments, but his notes do not say how he thought they were related to the question of the rII effect. Finally, he stated that "leakers are very interesting. They can be swayed one way or the other, and might reveal the key to the effect."[15]

More important than the mechanism of the effect, he went on, is "the interpretation of the 'region.'" The many markers with similar qualitative phenotypic effects were distributed randomly within a limited region. Their quantitative effects differed mainly because of differences in their reversion rates, but these differences were not correlated with their positions in the region. From a calculation similar to the one that he had made in his laboratory notes at the end of May, he concluded that the upper limit of the region was 10^4 nucleotides. The frequency of recombination between neighboring nucleotides he now computed to be 5 percent/10^4, or 5×10^{-6}. This was, he stressed, "easily detectable" with his method. He did not, at least in his notes, state what the limit of resolution of his system was.[16]

A striking feature of Benzer's presentation up to this point was the continuity it expressed between his most recent work on mapping and that which had come before he had fixed on that project. In his description of the properties of the two types of r mutants and the wild type, he used the same term, "phenotypic expression," that he had when he had described himself as working on that problem. In his discussion, he first brought up the question of the nature of the rII effect. Searching for an answer to that question had been one of the goals of his study of the host-range problem before he took up mapping. These did not yet look to him like topics that he had dropped in order to pursue his mapping project. On the other hand, his judgment that the interpretation of the region was more important suggests that he was already distancing himself from these earlier concerns.

Benzer began the last part of his lecture with the statement, "I wish to propose that this region constitutes a single "'gene'" and asked, "What is a gene?" He cited the same three definitions he had long ago adopted from Pontecorvo:

Unit of physiological activity (one gene–one enzyme)
[Unit of] Recombination
[Unit of] Mutation,

He asserted that arguments about which is the correct definition of the gene were "academic unless one has sufficient resolution to get down within the gene."[17]

Under the heading "New ideas" Benzer described DNA as the "sole carrier of specificity." The structure of DNA in a series of base pairs provides the specificity in the sequence that also determines the sequence of amino acids. He then indicated how the insertion of an incorrect base in a base pair can cause spontaneous mutations.

Benzer finished with a set of "predictions:"

> A mutation can occur randomly anywhere in the chain (same bases used over and over).
> Any one causes similar phenotypic effect
> Can be fatal or crippling
> Reversion frequency depends upon individual "incorrect" base
> Should [, therefore,] have a few groups of frequencies
>
> All these are shown by our system
> Furthermore, [we] can *map* down to individual nucleotides.[18]

These were remarkably bold predictions. Up to that point Benzer had mapped only eight mutants within the region of his rII mutants. They were closer together than those of Hershey and Doermann, but not by the orders of magnitude necessary to reach the distance between neighboring nucleotides. That his system could show all these things was, therefore, based on his calculation of the limits of resolution that were theoretically possible, and there might well be practical obstacles in the way of reaching that goal.

On July 17 Benzer wrote to Gunther Stent,

> I have gotten into an offshoot of the r problem. The applications of the effect are more interesting than the effect itself. It is technically possible to study, by genetic means, the structure of the r "gene" down to the individual nucleotides, since I can detect recombination frequencies that small. So I am going to be isolating and mapping hundreds of r mutants this year.[19]

The statement that he had "gotten into an offshoot of the r problem" supports the interpretation that Benzer did not at first see the r mutant effect he had observed in January as an opportunity to map but that his primary interest had been in the effect itself until the events described in Chapter 6 gradually drew him into the mapping project. That he

expected to map hundreds of mutants reveals how much work lay ahead of him in fulfilling the predictions he made for his system in his seminar.

Benzer spent much of his time at Cold Spring Harbor preparing a manuscript about his mapping project for publication.[20] He sent a copy to Delbrück, who was at the University of Göttingen in July, and expressed the hope that they could meet somewhere during Benzer's trip to Europe. Concerning Benzer's manuscript, Delbrück wrote on July 27,

> Dear Seymour, I think your paper is tremendously courageous. In fact, I think you must have had a triple highball before writing it. I object to publishing it in the present form because two thirds of it may be proved to be wrong by your own experiments (or may be vindicated) even before the paper is out. In this situation, if you don't want to be patient enough to do the experiments which will vindicate or disprove the two basic ideas, viz.
>
> (a) that c[rossing] o[ver] can occur between any pair of nucleotides
> (b) that mutation is a substitution of a single false base
>
> I would suggest that you write a paper of half the length in which you say
>
> (1) "I have a dandy system for studying a cluster of closely linked loci, namely so-and-so."
> (2) "this cluster has the earmarks of a system of pseudoalleles. It appears to be subdivisible by mutation and c[rossing] o[ver]."
> (3) "A rough estimate gives 16000 nucleotide pairs for this 'gene.' It seems practicable with this material to attempt a direct test of the ideas (a) and (b) above. About this we hope to report in the near future."[21]

Although Delbrück was famous within the phage group for expressing initial skepticism about any new idea presented to him, especially by those for whom he had high regard, his objection seems well-placed. I have not seen a copy of Benzer's original manuscript (Sidney Brenner told me he has preserved a copy) but to judge from the presentation at Cold Spring Harbor, he must have included among his predictions ideas that could only be demonstrated by carrying the resolution of his system down to the dimension of distances between nucleotides. Delbrück was urging him to carry out his plan to do so before publishing a paper that made assertions involving the level of the nucleotide. Part of his skepticism, however, went behind Benzer's assertions to the DNA

structure of Watson and Crick on which they were based and in particular to their hypothesis that a mutation is caused by a tautomeric shift in a single base, resulting in the insertion, at the next replication, of an incorrect base in the nucleotide.[22]

On July 26 Benzer left Cold Spring Harbor for Flushing, New York, where he stayed with family members until his departure for Europe on August 6. In Paris from the eleventh to the thirteenth, he consulted with his old bench partner at the Cold Spring Harbor phage course, Peggy Lieb, about "the kinetics of lysogeneity when bacteria are injected with a temperate phage." The following day he was in Berlin, discussing with Karlfried Gawehn of the Max-Planck Institut für Zellphysiologie the "quantum yield in photobiological effects." Crossing into East Berlin, he felt as though he were "going through the looking glass." Moving on to Copenhagen, he spent August 17–22 at the State Serum Institute talking with Gordon Lark, Ole Maaløe, and Niels Jerne about "the synchronization of bacterial divisions, the serum inactivation of viruses, and the photoreactivation of viruses." There he also took part in a charming birthday dinner in Maaløe's home at which each person present had to make a short speech.[23]

From August 23 to August 28 Benzer attended the Congress on Photobiology in Amsterdam, the purpose for which he had originally planned his trip. There he participated in a symposium on the "effect of non-ionizing radiation on the genetic elements of cells." He had "valuable discussions" with Raymond Latarjet and several others but also managed to offend Latarjet following a talk that the latter gave on radiating phage and bacteria. Having obtained a result opposite to Latarjet's in his own experiments, Benzer "contradicted" the co-developer of the method during the discussion period. Later Peggy Lieb told him that Latarjet had walked down the aisle muttering, "I wash my hands of the Delbrück group." Benzer managed to meet with Delbrück in Amsterdam and consult with him further on "my work on gene fine structure."[24] Benzer did not report on whether these discussions modified either Delbrück's views or his own about his manuscript on the subject. Taking some time out from professional concerns, he enjoyed the famous boat rides through the picturesque canals of Amsterdam.[25]

August 29–31 found Benzer at the University of Geneva, where he talked with Jean Weigle and other members of the Department of Biophysics about "lysogenic forms of bacteria, [the] induction of mutations in viruses, [and] the effects of ultraviolet light on lysogenic

bacteria." He attended the Congress of Cell Biology in Leiden from September 1 to September 5. In addition to attending sessions, he had "especially fruitful discussions" there with Pontecorvo, Mogens Westegaard, and others "about gene structure and function." Back in Paris from September 6 to September 11 with his old associates at the Institut Pasteur, he engaged in wide-ranging conversations with Monod, Jacob, Wollman, Georges Cohen, and Lane Barksdale about enzyme synthesis, phage genetics, adaptation, and antigenic conversion of bacteria. He had time there to carry out several experiments in which he tested his r mutants on some special forms of defective lysogenic bacteria that Elie Wollman had isolated. His results showed that "cells containing prophage in any one of a series of defective forms had the property of being unable to support the reproduction of my mutants." These experiments thus significantly supported and generalized the conclusion he had reached in the spring: that it was the prophage, not some other intrinsic property of K12(λ) and the other lysogenic bacteria he had used then, that discriminated between the rII-type mutants and other r mutants of T4.[26]

Crossing the Channel, Benzer arrived on September 12 at the Postgraduate Medical School of London, where he discussed bacterial genetics, enzymatic adaptation, and photoreactivation with William Hayes, Martin Pollock, and Neville Symonds. Hurrying onward, he traveled to the Genetics Institute of the University of Glasgow the following day, talking with J. A. Roper and Guido Pontecorvo about pseudoallelism in the plant mold *Aspergillus*.[27] *Pseudoallelism* was a term introduced in 1951 by Edward B. Lewis to refer to a series of genetic markers in *Drosophila* that appeared to be multiple alleles: that is, they affected the same phenotypic character of the organism but in different ways. These markers turned out, however, to be separable by crossing over. Although they were very close together, they did not occupy the same position on the chromosome. Unlike Pontecorvo, who treated Lewis's pseudoalleles as subunits of one gene, Lewis considered pseudoalleles to be component genes, "closely linked in function, and adjacent to one another on the chromosome." Pseudoalleles displayed a *position effect* (a term that Lewis borrowed from Sturtevant but used in a somewhat different sense). When two pseudoallelic genes were located on one of a pair of chromosomes, the other chromosome being wild-type, the phenotype of the heterozygous fly was wild-type. When the pseudoalleles were on opposite chromosomes, the heterozygote was mutant. To explain the difference between the

two situations, Lewis postulated a scheme in which the two pseudoalleles controlled separate but closely related metabolic reactions.[28] In discussing analogous situations in *Aspergillus* with Roper and Pontecorvo, Benzer must have recognized the similarity between such pseudoalleles and his r mutants. All of the mutants affected the same phenotypic phenomenon, namely, a more rapid lysis of the host bacterium. Those within the region in which his rII mutants mapped, however, shared an additional property: that of not plating on a lysogenic bacterium. Delbrück had already described this "region" as having the "earmarks of a pseudoallelic system" in his critique of Benzer's manuscript and may have discussed the meaning of that identification further in Amsterdam. These conversations must not only have broadened Benzer's appreciation for the analogies between what he had just begun to explore in phage genetics and what classical geneticists were already doing in the genetics of higher organisms but also suggested that the proposal he had made in his seminar in July—that the region of his rII mutants constituted a single gene—was problematic. Not only were there three different definitions of the gene, but its definition as a unit of physiological action was in doubt. What was a unit of physiological action, and were the genetic units that controlled closely linked functions subdivisible single genes or components of separate genes? Classical geneticists themselves seemed not to be in agreement on such questions.

The indefatigable traveler was back in Lafayette on September 15. He concluded his report on his whirlwind trip by writing, "In all of these places I discussed my current work with the persons listed, obtaining valuable information and suggestions. In several cases I was called upon to give seminars on my work."[29] Although it is hard to identify the specific suggestions and information that he applied to his work, when he resumed it in September, some of the new elements he incorporated must have emerged from these many discussions with leading scientists in his own field and in neighboring ones. It is also notable that these conversations took place in five European countries. The field in which he practiced was truly international.

While Benzer was in Europe, Sydney Brenner, to whom he had also given a copy of his manuscript, read it at the annual phage meeting at Cold Spring Harbor, held from August 25 to August 27. In September Brenner wrote Benzer, "I presented it on your behalf at the phage meeting, and must say it was very favorably received. There were basically no arguments against it. There are a couple of difficulties on back

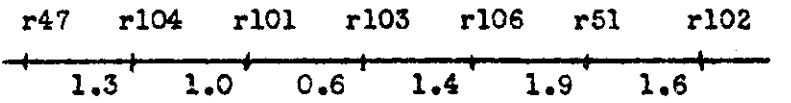


Figure 7.2. Benzer's early map of the T4 rII region. (*Phage Information Service Bulletin* 8 (Fall 1954.)

mutation, but we can discuss these when I come to Purdue." At the insistence of Al Hershey, Brenner then prepared a summary of the paper to include in the next issue of Delbrück's *Phage Information Service* bulletin. Because he had not consulted with Benzer beforehand, Brenner added, "I hope you don't mind my doing that."[30]

Brenner's abstract was titled "The Fine Structure of a Gene in Bacteriophage." It began:

> One class of r mutants (rII) in T2, T4, and T6 is characterized by a failure of the phages to form plaques on *E. coli,* K12, and other strains of the prophage lambda. This property permits the detection of very low frequencies of recombination in crosses between two rII mutants since selection can be exerted for wild type. Eight T4 r mutants of independent origin (including the r47 and r51 mutants of Doermann) have been isolated. All yield wild type recombinants and hence are genetically distinct. The linkage relations are shown below, together with the percentages of recombination in crosses with nearest neighbors.[31]

This diagram (fig. 7.2) was similar to the one Benzer had shown in his Cold Spring Harbor seminar but included two changes. First, whereas he had earlier numbered his mutants using roman numerals, he now numbered them beginning with 101, in the order not of their placement on the map but of their isolation. This change allowed him to express the fact that his mutants were part of the same series as Doermann's without confusing Doermann's mutants with his. Second, Benzer had left out one of the markers between r101 and r106 that had been incorporated into the earlier one. It is not clear why he had done so. Perhaps he regarded the intermediate distances as too uncertain, in part because the marker was a leaker. In any case, the changes indicate that his genetic map of the rII mutants had not finished evolving. The second paragraph of the abstract continued the discussion of Benzer's immediate results:

> All of these mutations have the same phenotypic effect of interfering with the ability of the phage to plate on K12(λ). On some mutants the loss is absolute; in others, however, the defect is not total and such "leakers" can form a small number of tiny plaques on K12. These "leakers" show temperature dependence. It is considered that these mutations are located at different points in a length of DNA constituting a single gene and that each causes some "error" to be made in the structure of a protein or enzyme synthesized under the control of the gene. The quantitative differences between the mutants would depend on whether the "error" was inactivating or only partially inactivating.[32]

Most of the elements of the position expressed in the abstract had been present in Benzer's lecture at Cold Spring Harbor but mingled with earlier ideas that he retained from the period in which he had not yet taken up the mapping project in earnest. Now, whether it was Brenner's work in abstracting or whether the original manuscript had the same character, his focus was narrower, fixed on the linkage relations among a class of mutants, the effects of leakers, and theoretical implications for the nature of mutations. He thus achieved a unity of presentation expressed in the title "The Fine Structure of a Gene." The abstract went on to mention quantitative considerations also similar in objective to those he had previously discussed but considerably revised in detail:

> From the experimental data it is possible to estimate the length of DNA in the rII gene. Given that (i) the DNA of the phage has the structure described by Watson and Crick and that the genetic information is represented in one double-stranded helix; (ii) the number of nucleotide pairs is 2×10^5 (Hershey, Dixon, and Chase, 1953); (iii) the total linkage distance between known markers in T4 is 100 units (Doermann and Hill, 1953); (iv) recombination frequencies per unit length are constant over the entire strand; then the rII gene which extends over 8 units corresponds to about 1.6×10^4 nucleotide units. This is clearly an upper limit, since the linkage distance is probably an underestimate and it is not at all certain that all of the DNA of the phage is genetically active in determining phage phenotype. A lower estimate of the size of the gene can be made from the finding that the eight mutations occur at different sites. If there are N possible sites with equal probability of mutation then the chance of obtaining a duplication in n tries would be given by $\frac{1}{2}(n^2/N)$. Since no duplication has been found in 8 tries, N is probably greater than 32. This rather broad range for the size of the rII gene, 32 to 10^4 nucleotide pairs, will narrow rapidly as more mutations are found.[33]

To a colleague who asked him where the formula ½ (n^2/N) had come from Benzer later explained, "Assuming N sites with a 'multiplicity of infection,' so to speak, of n/N, the probability that a site will be singly infected is $(n/N)\, e^{-(n/N)}$, doubly infected ½ $(n/N)^2 e^{-(n/N)}$, etc. The ratio of doubly infected to singly infected sites will be ½ (n/N). (We assume small values of n/N so that almost all cases of duplication are doubles.) If ½ n/N is the (small) probability that a given case is a double, then the chance of getting one double out of n tries is ½ n^2/N." By "duplications" and "doubles" Benzer meant "independently arising indistinguishable mutants," none of which had occurred among those he or Doermann had isolated and mapped.[34] The strategy of performing experiments with multiplicities of infection low enough to be able to assume that only single or double mutants could arise was one that Benzer had first developed while performing Luria-Latarjet experiments in his last year at Caltech (see Chapter 3). This analysis was, of course, based on the application of the Poisson distribution, the stock-in-trade of radiobiologists and bacterial geneticists. That he could now reach back to apply that method in a wholly different context is one example of the benefits that enable an experienced investigator to deploy a broader range of approaches to a problem than would have been possible in an apprenticeship.

In this paragraph either Benzer or Brenner made a small change that may appear merely stylistic but that probably represents a solidifying of concepts. Hitherto Benzer had proposed that the "region" in which his type II r mutants clustered was a single gene. In this paragraph, for the first time it was named the "rII gene." That identification afterward remained firmly in place. "It is also possible," the abstract continued,

> to estimate the fundamental recombination frequency, i.e., the frequency of recombination between markers separated by a distance of one nucleotide pair. This is not less than 5×10^{-6} since the maximum size of the rII gene is 1.6×10^4 nucleotide pairs, which corresponds to 8 percent recombination. Since the present procedure allows the detection of wild type recombinants in proportions as low as 10^{-8}, it is feasible to establish this fundamental frequency experimentally. Thus extremely fine mapping of a single gene is possible and uncertainty of positioning would only arise with markers with high reversion rates.[35]

The figure of 8 percent recombination was the total distance across the genetic map that Benzer had constructed between May 17 and May 21

(which differed from the sum of the distances between neighboring markers). This paragraph estimated for the first time what Benzer considered the limits of resolution of his system.

All of the major ideas expressed in these four paragraphs were present in nascent form in the thoughts about the gene that Benzer had written down on May 28 or in the calculations on the two additional pages he had attached to them. In the period of three weeks at Cold Spring Harbor during which he prepared his manuscript, he had refined and clarified these ideas and arranged them into a coherent picture of the rII gene. Much of his argument was, however, still theoretical, and on his return he faced the task of mapping many more mutants in order to provide the experimental foundations his picture required.

The last paragraph of the abstract, dealing with reversion rates, stood somewhat apart from the rest. The rates of reversion he had measured fell into four classes: 10^{-8}, 10^{-8}, 10^{-6}, and 10^{-4}. Forward mutation rates (wild type to r) were all approximately 10^{-4}, and since there were at least thirty-two mutation sites, the rate at each site could not be greater than 3×10^{-6} (10^{-4} divided by 32). These results did not conform to the predictions of the Watson-Crick tautomeric model of mutation, according to which the forward and backward rates should be the same, and according to which there should be only two classes of back-mutation—one due to the guanine-cytosine pair, the other to the adenine-thymine pair. Benzer thought that these anomalies might be due to second-site suppressor mutations or to the possibility that the rates of mutation can be influenced by neighboring bases. It was undoubtedly this discussion that raised, as Brenner reported to him, a "couple of difficulties at the Cold Spring Harbor meeting."[36]

Benzer was not the only biologist seeking to map bacteriophage to higher degrees of resolution in 1954. That a key to the question of the nature of the gene lay in the examination of very closely linked mutations was evident among the workers in classical and phage genetics. Delbrück had tried to arrange a meeting between Benzer and Carston Bresch at Göttingen during Benzer's summer travels. "Bresch would very much like to see you," Delbrück wrote in July, "particularly to discuss locus–fine structure problems, since he has been working on fine structure in T7."[37] The meeting did not take place because Benzer could not go to Göttingen before Bresch left for vacation. From Doermann's lab in Rochester, David Krieg corresponded with Benzer in the

fall about problems he was encountering in the study of close linkages among the r mutants.[38] No one else, however, appears to have devised a system with which he expected to reach all the way down to the distance between neighboring nucleotides.

As he returned to the bench in mid-September, Benzer faced a daunting task if he was to realize that aim. Even if his system was theoretically capable of detecting two mutants so close together, if it was a matter of isolating and crossing more and more mutants in the rII region until he found two of them that happened to be that close, it might be a very long time before he succeeded. He needed a shortcut.

Benzer must have brought back such a strategy for moving quickly to closely linked markers with him from Europe, because he applied it almost immediately after he resumed his experiments. After checking the titers of several of his mutant stocks and finding that they had not changed during his absence, he carried out a series of "spot tests for mapping rII mutants" on September 18. His idea was to test a given mutant by plating it on K12(λ), then "spotting" the plate with drops of several other mutants whose positions in the rII region were known. Within each spot a few plaques would be formed when wild-type recombinants arose between the two mutants plated there. If the known mutants were selected from different groups located along the r region, then he could quickly establish the group to which the new marker belonged. For reasons that I was not able to ascertain, Benzer thought when he began these tests that the maximum number of plaques formed by markers that were far apart from each other would be smaller by two orders of magnitude than the number formed by those at close distances. In any case, if successful, this procedure would enable him more quickly to establish roughly mapped subgroups within the overall rII cluster. He could then refine the maps of these subgroups by making crosses between the mutants closely linked there. The procedure as Benzer described it in his experiment of September 18 was as follows:

> Seed plate with 10^9 bacteria (K12 (λ)).
>
> 10^7 particles of test phage
>
> Then spot with drops of known phages at 5×10^6 particles/ml, using drops of ≈ 0.02 ml.
>
> This should give a maximum of 10^3 plaques per spot for large map distances, and a number of plaques corresponding to $\approx 10^5$ progeny for close distances.

After listing the six known mutants used in the experiment with their titers, he gave the result:

> rb13 is closely linked to rbVI [the test mutant]. The spot test procedure works very well, except that II and X should not be used because of strong background (on the II plate there were around 200 large plaques and on the X plate around 500 tiny ones). With the others, there is no background, and the plating bacteria grew quite heavily, so that possibly even higher phage titers could be used to increase the sensitivity.[39]

The background to which Benzer referred was caused by plaques formed by mutants that were leakers or had high rates of reversion to wild types. To test whether it was possible to work with leakers at lower temperatures, he compared the plaques formed on K12(λ) by a leaker with those formed by the wild type at 37°C and at 23°C. At the lower temperature the two formed the same number of plaques, but "the wild type does not do very well either. Perhaps an intermediate temperature (≈30°) would be better," he commented. "Otherwise leakers must be studied in one-step experiments with plating on B." He did not pursue either of these possibilities, probably because he thought that he would be better off working with markers that were not leaky.[40]

On September 20 Benzer carried out spot tests with seven T4r mutants. For each mutant he made a plate on K12(λ), then spotted each plate with the other six. They fell into a group of three and a group of four, each forming only a few plaques with each other but "many" with members of the other group. He placed the spot tests in a matrix showing this result, then drew rough maps of the two groups (fig. 7.3).[41] He expressed the result as follows:

> r47, r203, r209 very closely linked to each other
> r202, 210, 213, 212 very close to each other
> but these groups are very far apart
> Based on the extremely rough counts of plaques within the spots:
> [He repeated the maps shown in fig. 7.3, again renumbering his own mutants as r numbers above 100.]
> VI (r203) appears to be much closer to r47 [shown as mutants 1 and 2 in fig. 7.3] than hitherto believed.[42]

Although the mutants he used in this case may not have been new ones, the numbers he assigned to them show that they were more recent than

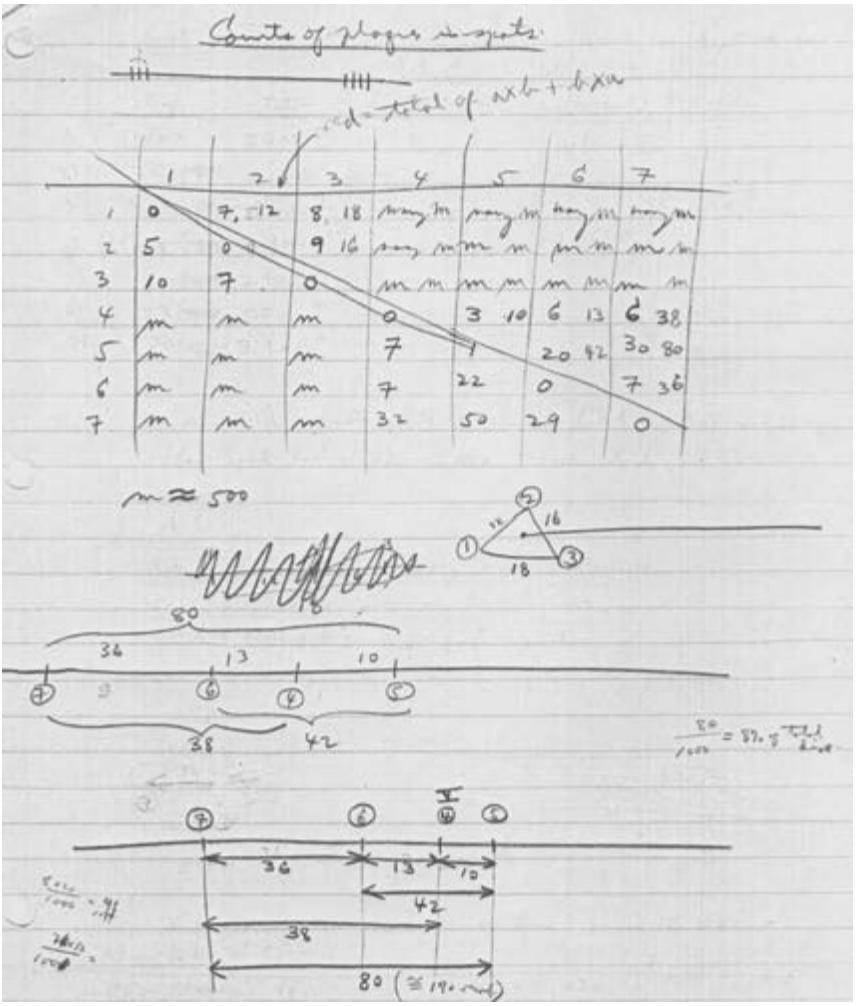


Figure 7.3. Spot-test results and rough map of seven T4 r mutants. Page drawn in pencil following the spot test of September 20, 1954. (RN no. 4.)

those he had used in June to produce his map of the entire rII region. Because his aim was now to fill in further markers within each of his subgroups, he began to isolate new sets of T4r mutants in order to test them. On September 18 he isolated twenty, which he tested on the twenty-first by plating on strains B and K. Three of these he marked as "usable for crossing" because they showed "no leaking." After repeating the tests with the remaining seventeen mutants, he was able to pick out six more that either did not leak or leaked little enough that they

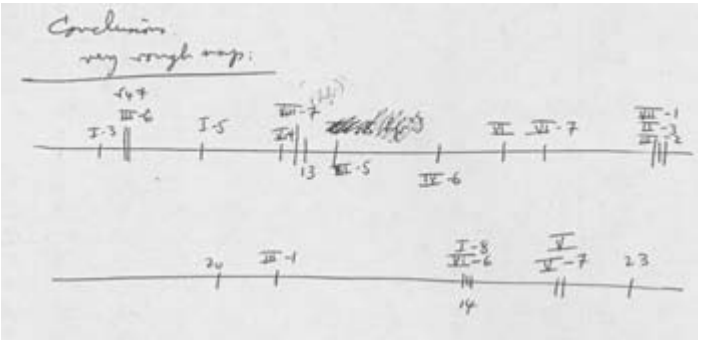


Figure 7.4. "Very rough map" of closely linked T4 r mutants drawn on October 6, 1954. (RN no. 4.)

might be used. On the twenty-sixth and the twenty-seventh he tested two further series of ten newly isolated mutants. In the first case none showed a leaky background; in the second case one appeared to be slightly leaky and a second was "rather leaky." On October 1 he tested three additional series of new mutants on K12(λ). Most of them formed many large plaques, which meant that they were probably rI mutants. From the three series of mutants he chose a total of seven that formed few or no plaques and marked them as "good for crossing of close markers." Between October 2 and October 5 he was able to obtain four more such markers from three further series of new mutants.[43]

Between the first and the fourth Benzer also tested for a "possible suppressor reversion." I have not analyzed these experiments.

On October 5 Benzer spot-tested on r47 and on rBV each of the fifteen T4r "low reversion rate" mutants that he had selected from the seven series of new mutants as suitable for crossing. Almost all turned out to be close to one and distant from the other or the converse.

He put his conclusion in the form of a "very rough map" of each of the two subgroups of closely linked markers (fig. 7.4). Below this he commented:

> These two sets are effectively distant from each other
> IV–3 appears to be a double mutant with mutations located at ≈ I–3 and around VI–7.
> Is VIII a hfr [high-frequency reverter] type?[44]

The first set included, in fact, three subgroups of very closely linked markers. As "tests for close linkage," on October 12 he crossed each of the three mutants found within each of the three subgroups in one

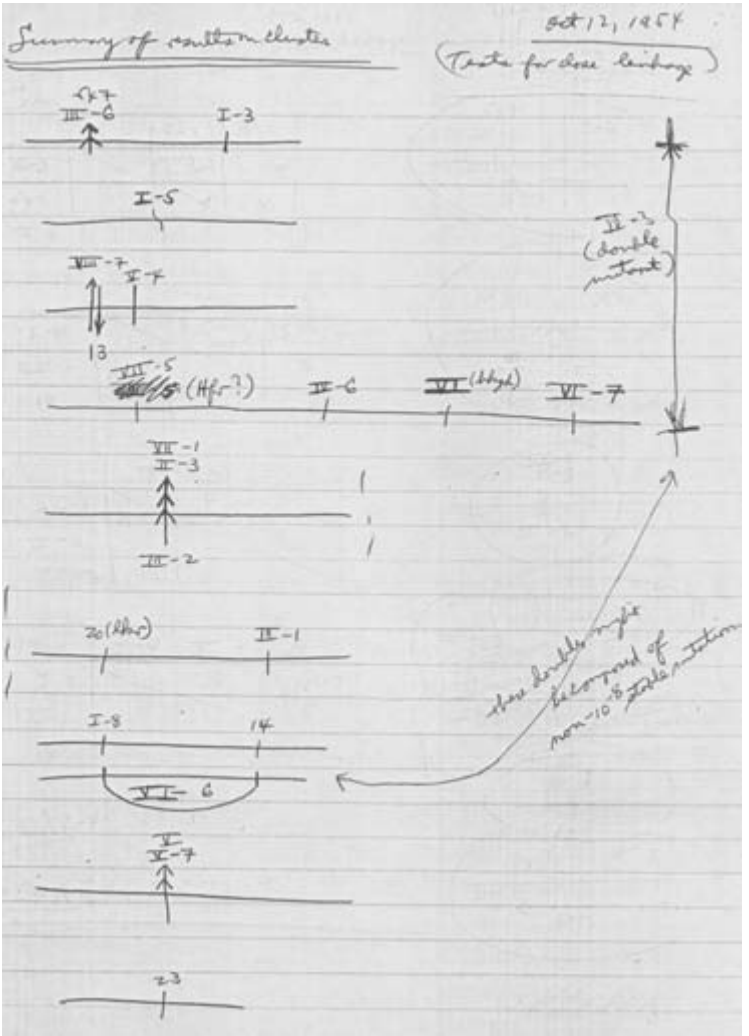


Figure 7.5. Four cluster maps of T4 r mutants drawn on October 12, 1954. (RN no. 4.)

set and those within the single subgroup of the other set. The numbers of plaques formed on K12(λ) were in most cases very low, and he was able to draw maps of the locations of the subunits. Two of them gave no plaques, leading to him wonder whether these mutants were "doubles," that is, identical mutations arising independently. He drew rough maps of the four clusters, each beside the results of the crosses on which it was based (fig. 7.5).[45] To test the likelihood that the mu-

tants which mapped in the same place were duplicates or triplicates, he calculated the number that should be so according to the Poisson distribution. "Given N sites of equal probability," he began, "and assuming that n independently arising mutants are collected,"

$$\frac{P(2)}{P(1)} = \frac{\text{prob. Doubles}}{\text{prob. Singles}} = \frac{x^2/2e^{-x}}{xe^{-x}} = \frac{x}{2} \text{ where } x = \frac{n}{N}$$

The numbers of his mutants that had mapped in the same place gave the following as the "observed" frequencies:

	Calculated:
No. of quadruples = 0	0
No. of triplets = 1	0.4
No. of doubles = 3	3
No. of singles = 14.	14

Inserting the observed numbers of singles and doubles into the formula he had derived for the ratio between numbers of mutants collected (14 as singles, 6 as doubles, and 3 as triples) and numbers of sites (x/2), he obtained the result x = 6/14 = 0.43. Since n, the number of mutants collected, = 23, N = 23/0.43 = 54 sites. "The relative no. of 1, 2, and 3's," he noted at the bottom of the page, "is compatible with a Poisson distribution with 54 total sites (the VI-G and IV–3 'double mutants' alter those data somewhat)."[46]

The initial purpose of Benzer's calculation was, apparently, to help him decide whether the mutants that showed no recombination were actually independently arising identical ones or separate ones too close together for the distances between them to be detected. The intermediate result, that there were fifty-four mutation sites, also had further implications, however. On the next page Benzer explored some of these:

> 54 sites giving rise to stable mutants
>
> Out of 23 mutants studied, at least two are double mutants
>
> Since these may be composed of 10^{-4} or 10^{-6} or 10^{-8} singles, the apparent frequency of doubles is exaggerated by a factor of 3.
>
> Thus we have $x/3 \times 23 = x/69 = 0.03$
>
> 0.03 is ratio of double mutants/single mutants.
>
> If all mutations are independent this ($0.03 \times 2 = 0.06 = 6 \times 10^{-2}$) should be the average frequency of occurrence of a stable mutation. Actually, however, the frequency of observation is something like $10^{-4} \times 0.2 = 2 \times 10^{-5}$

He drew no further conclusion from this large discrepancy between the calculated and the observed average frequency of mutation. The fifty-four sites, however, had another meaning:

> 54 sites = minimum number of crossover units
> 10,000 nucleotides = maximum [number] of nucleotide pairs
> 10,000 max./54 min. = 200 max./1 min. = 200 max. N.P. per crossover unit
> 54 amino acids = MW 10,000[47]

These last calculations were similar to those Benzer had presented at Cold Spring Harbor (via Brenner). At that time, however, he had collected only eight mutants and found no duplicates. The number of sites, N, he had found at that time to be "greater than 32" and predicted that the range between the minimum and the maximum possible sizes of the rII gene would be reduced as new mutants were found. Now he had twenty-three mutants, which included two or three duplicates and raised the minimum number of sites to fifty-four. The maximum size of the gene remained the same for the time being. His calculation of the molecular weight of a protein (which, however, is off by a factor of about two, given that the average molecular weight of an amino acid is about 105 daltons) corresponding to the maximum size of the rII gene indicates his continuing interest in the gene as a unit of physiological action.

Benzer needed more and more mutants. Between October 9 and October 14 he prepared titers of four more. On the nineteenth he titrated "new hi titer stocks" of seven additional mutants. Then he turned to several other tests that might prove useful for future experiments. One was to measure the rate of inactivation of several of his r mutants by the anti-T4 serum of the Danish immunologist Niels Jerne, whom he had visited during his European trip. A second test was of "mixed indicators for detecting rII in presence of rI." For this purpose he seeded plates with mixtures of bacterial strains B and K12(λ) in five different proportions. As "input" phage he used an rII mutant, an rI mutant, and a wild type. In two cases he preadsorbed the phage on B before adding the drops of K. From the results he concluded that when "preadsorbed on B, add equal amount of K for best results." On the nineteenth and the twentieth he crossed one of the new mutants whose reversion rate was high with a wild-type T4 to test for the presence of a "suppressor" mutation. The three thousand plaques that resulted when he replated on his mixed indicator showed that they were proba-

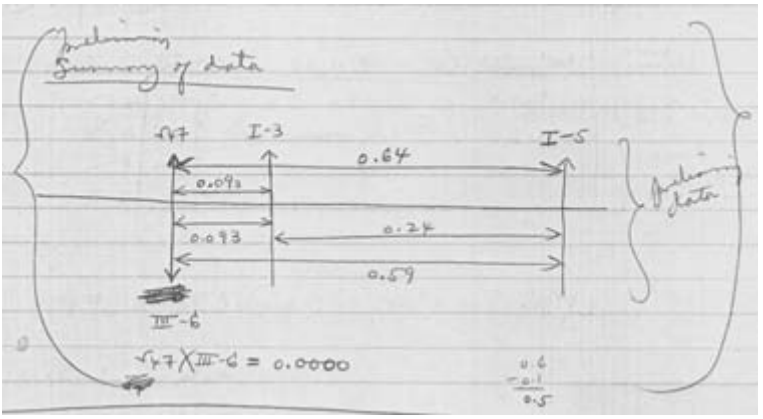


Figure 7.6. Preliminary map drawn on October 23, 1954, showing additive distances between closely linked T4 r mutants. (RN no. 4.)

bly all "small r type," a result that tended to rule out the suppressor hypothesis in favor of a true reversion because suppressors often gave variable and incomplete restoration of the wild-type phenotype.[48]

On October 21 Benzer tested freezing and thawing on bacterial cultures. He froze one sample from an overnight culture of K12S , then plated it along side an unfrozen sample. On the twenty-sixth he thawed part of the frozen sample to room temperature and again plated it. Only two colonies formed on each plate, and Benzer merely concluded that "this procedure is n.g." There is no indication of what he would have done with the procedure if it had worked. The brief episode is representative, however, of the way in which experimentalists often interrupt a line of research at a very promising stage in order to try out a new idea, then return quickly if the first step is not encouraging.

On October 23 and 24 Benzer returned to the map of one of the subgroups of the rII gene, which he now named "microclusters." Plating and replating the mutants that his previous experiments had shown to cluster around Doermann's r47, he refined step by step the distances between the markers composing this cluster. His preliminary summary of data is shown in fig. 7.6. His results are shown in fig. 7.7.

The following day Benzer crossed eight of the markers previously found in the microclusters that gave no plaques on K, and which he therefore considered perhaps to be identical mutants, and two that gave the very low frequency of 0.15 percent recombination. He summarized these results also in graphical form (fig. 7.8).

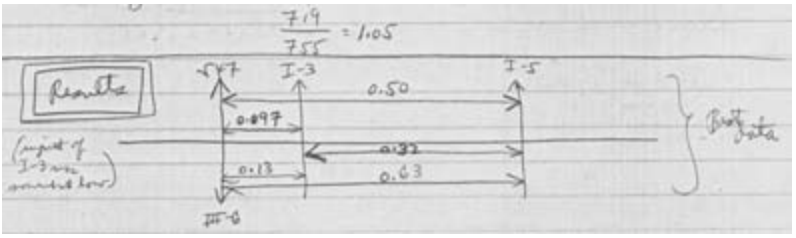


Figure 7.7. Map drawn on October 23, 1954, using best data. (RN no. 4.)

He also summarized the recombination frequencies in other representations comparing the observed frequencies with the expected Poisson distribution for doubles and singles.

On October 26 Benzer wrote down some further thoughts in which he attempted to calculate rates of forward mutation and reversion. He began with an "index" frequency of forward mutation of 2×10^{-4}. This was, apparently, his estimate of the overall rate of mutation for the rII gene. "Suppose," he wrote, a "0.13% fundamental frequency" (by which he apparently meant the frequency of recombination between adjacent mutable sites as estimated from his lowest nonzero recombination frequency). "Then the gene contains 8%/0.13% = 62 elements." (By *elements* he seems to have meant the same thing as sites, N.) He again compared the numbers of doubles, triples, and quadruples expected from a Poisson distribution, this time for twenty-one mutants collected from the sixty-two sites, with an observed distribution. (The latter were different from those he had previously used, and I was not able to identify the experiments from which they came.) Assuming, as before, the equal mutability of the sites, he set the limit of forward mutation (that is, $r^+ \rightarrow r$) frequency per site at $2 - 10^{-4}/62 = 3 \times 10^{-6}$. If each of these elements can mutate with "equal probability to a 10^{-8}, 10^{-6}, or 10^{-4} like state [that is, to an r phenotype with one of these discrete levels of reversion], then the forward frequency per site [$= 1 \times 10^{-6}$]." Realizing, perhaps, that this calculation was not valid, he replaced the numbers shown here in brackets with question marks. He went on, "If [the] gene is composed of equal numbers of 10^{-6} and 10^{-8} type elements[, t]hen we would pick up only (99 out of 100) the 10^{-6} type, which should have a 10^{-8} reversion frequency."[49]

These calculations were also versions of ones he had already presented in the Cold Spring Harbor paper, revised to take account of the larger numbers of mutants, including doubles, that he had since col-

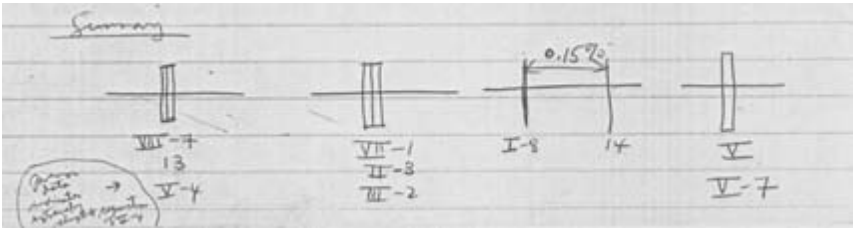


Figure 7.8. Drawing made on October 25, 1954, of map positions of microclusters of T4 r mutants showing relationships between identical or inseparable mutations. (RN no. 4.)

lected and the resulting increase in the maximum number of mutable sites on the rII gene. Whether his interest in calculating forward mutation and reversion rates was still based on the fact that they brought the Watson-Crick tautomeric model of mutation into question is not clear, but whereas Benzer had previously thought about mutations only as the insertion of incorrect bases in individual nucleotides, he began, on the next page, to consider another type: mutations due to inversions of a section of a chromosome:[50]

> spont[aneous] break and inversion—N bonds
> assume prob[ability] of break at any point = α
> prob[ability] of having one break = αN (assume $\alpha N \ll 1$)
> prob[ability] of having two breaks = $(\alpha N)^2$
> assume that healing can occur with either polarity with equal prob[ability],
> then prob[ability] of an inversion occurring = $(\alpha N)^2/2$
>
> To get a reversion must have breaks occurring at exactly the same points, followed by a "correct" inversion.
> Prob[ability] of break at bond a = α
> Prob[ability] of break at bond b = α
> Prob[ability] of getting breaks at both = α^2
> Prob[ability] of getting an exact reversion = $\alpha^2/2$
> So, ratio of forward frequency/reversion frequency = N^2
> For N = 62, $N^2 = \approx 4000$
> For forward frequency 2×10^{-4}, reversion frequency would be $2 \times 10^{-4}/4 \times 10^3 = 5 \times 10^{-8}$

Why did Benzer begin at this point to consider the probabilities of an inversion occurring? Perhaps it was his growing awareness of the parallels between mutations of phage and those of higher organisms, where, as we saw in Chapter 1, the distinction between "point" mutations and those involving larger chromosomal rearrangements played

a major part in the thinking of classical geneticists. A stimulus closer at hand might have been his recent crosses of mutants within the microclusters. Those that gave no detectable recombination might be identical mutations arising independently or might be separate mutations so close together that he had not yet been able to resolve them. A third possibility is that one or both of such mutations were inversions of such a length that recombination between them would not produce a wild type. Benzer did not, however, write down what his reasons may have been.

He left Lafayette by train on October 28 to attend a meeting at Urbana that brought together workers from Midwestern universities to discuss their latest results with microbial genetics, enzymatic adaptation, and bacterial viruses. On the second day of the meeting he described his work on "the fine structure of a bacteriophage gene, for which it has been possible to show that while crossing over within the gene is readily possible, there is a limit at which one runs into atomicity, i.e. there are subunits within which crossing over can not occur. The size of the subunit can be estimated in molecular terms and amounts to no more than a few hundred nucleotides (the molecules of which genes are made)."[51]

To judge from this brief abstract of his talk, Benzer did not regard the cases in which he could detect no recombination as beyond the limits of resolution of his method, which he believed to be less than the distance between neighboring nucleotides, but thought them to be limited by the inherent structure of the gene. Its "atoms" were some kind of subunits a few hundred nucleotides long. Perhaps that is what he had in mind when he began to consider that these units of mutation might be inversions rather than substitutions of single bases.

Shortly after returning from this meeting Benzer gave a physics seminar on November 4 concerning the fine structure of the gene. He placed his own work for the first time within the broad context of the development of classical genetics. He introduced his topic to his audience of physicists by comparing the "troubles of biology and physics," juxtaposing the "elementary particles" of the latter with genes in the former. He showed how the idea of "genes as fundamental particles of heredity" had emerged from crossing experiments done with plants and from Mendel's particulate theory. Quickly running through the ideas of linkage (as in the case of red flowers and long stems and their explanation by crossing over), the seriation of genes, and the identification of linkage groups, he showed how the concept that crossing over

took place between but not within genes had arisen, so that the genes became also the units of crossing over and of mutation. All of this had been carried out with terrific success and had been confirmed microscopically by the identification of chromosomes with linkage groups and of bands on the chromosomes with genes. There was, however the "difficulty of separating close markers by crossing over," so that one could never say that two mutants are different. What was needed was a "good selective system" for separating them. Benzer ended this portion of his lecture by reiterating the three definitions of the gene as the unit of "function, crossing over, and mutation."[52]

Moving on to gene function, Benzer covered "chemical genetics," focusing on the one-gene-one-enzyme concept of Beadle and Tatum. A "mutant makes a defective enzyme," he noted. The enzyme is a protein with a determined sequence of amino acids, and a mutation "may cause an error in assembly." Reviewing the composition of the gene, he summarized the way in which the bacterial transformation experiments and the Hershey-Chase experiments had shifted opinion about the chemical nature of the gene from the protein to the view that "the gene seems to be DNA." He illustrated the situation in phage, where protein forms the coat surrounding the DNA. Summarizing the structure of the double helix, he referred his listeners to a paper by Francis Crick that had just appeared in *Scientific American* for the "fascinating story" of its discovery.[53]

The double helix functioned, according to Benzer, like a tape recorder whose information was contained in a four-letter alphabet. In this connection he mentioned the model recently proposed by George Gamow in which the sixty-four possible triplet combinations of base pairs defined differently shaped grooves in the DNA model into which amino acids could be inserted. Benzer was obviously following closely the emergence of the "coding-problem" at this time, and he viewed the relation between genes and proteins, in the manner of the new molecular genetics, as the transfer of information.[54]

"Now turn to phage (king of organisms)," Benzer announced with what must have been a flourish. Quickly running through the basics of phage genetics, he diagrammed the appearances of r and r^+ plaques and explained the definition of crossing in the haploid phage as a mixed infection. After showing a diagram of the three linkage groups of Hershey and Doermann, he narrowed his focus to his own work on the r mutants. He described the selective feature of his system provided by the inability of rII mutants to plate on K and said that his procedure

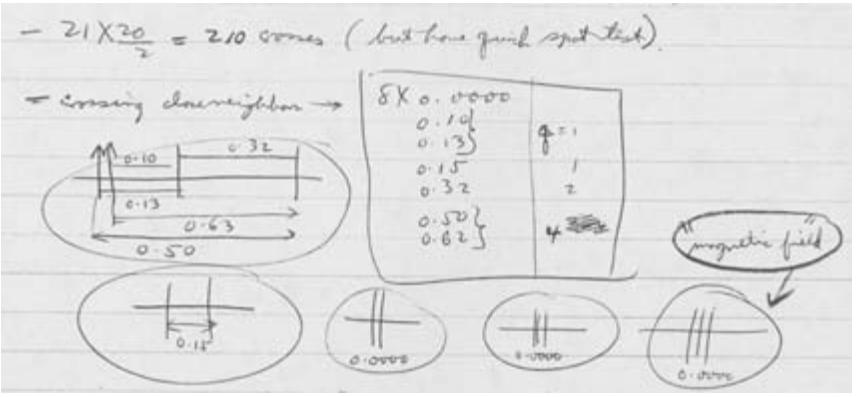


Figure 7.9. Diagram of the crossing of close neighbors, November 4, 1954. (BPP.)

was to "isolate a lot of rII mutants and then map them by crossing them with each other."

Fixing on the group of eight rII mutants that he had mapped during the summer, Benzer summarized the calculations that had led to an estimate of the crossover frequency per nucleotide pair of 5×10^{-4} percent, or 0.0005 percent. "So we can extend down to [this] limit," he stressed, "and then expect 'quantization.' " That is, just as in physics a quantum of light cannot be further subdivided, one could expect that recombination frequencies could not become smaller than those representing the distance between neighboring nucleotides. He cautioned, however, that "we do not know that the n[ucleotide] p[air] is the unit of all DNA genetics."

Bringing his audience to the latest stages of his ongoing research, Benzer described the twenty-one mutants he had mapped in four microclusters of the rII gene. This required 210 crosses, but he reassured them that we "have [a] quick spot test" to make things easier. Showing them diagrams of some of these crosses and a table of the recombination frequencies (fig. 7.9), Benzer concluded, "So, our gene consists of 'fundamental particles' of 0.13% length." That is, he took the lowest recombination frequency above zero to represent the length of this fundamental particle. He went on to consider forward mutation and reversion frequency rates and again pointed out that the fact that the forward rates could range anywhere up to 2×10^{-4} while the reversion rates fell into three groups (10^{-4}, 10^{-6}, and 10^{-8}) was contrary to the implication of the Watson-Crick model of mutation, according to which there should be only two frequencies, similar in the forward and in the backward direction.

"Thus," he stated, "this becomes a solid state problem." Mentioning three other types of possible mutation—addition, deletion, and inversion—he pointed out that the ratio of forward to reversion frequencies for the latter would be N^2. The very brief notes for this part of his lecture do not indicate whether he favored any of these alternatives, but they do reinforce the indications that he was entertaining possibilities other than base pair substitutions.

The conclusions Benzer drew were remarkably open-ended:

> [The] gene as a unit of function is not a "fundamental particle."
> [The] units of c[rossing] o[ver] and mutation are smaller.
> However, may have smaller unit of "fundamental particle" and so on.

Benzer left the following as "future questions":

> Total linkage length
> Is all DNA genetic? (injection experiments)
> Can one have more than one type of mutation at a site?
> Find the enzyme.[55]

To those attending the seminar who were introduced to the problems of biological genetics and who were able to follow Benzer's rapid, soft-spoken tour through the field this must have been a breathtaking lecture. In one hour he had led them from the beginnings of classical genetics to his research of the previous few weeks. At the end, however, they must have been left uncertain where these problems stood. The gene as traditionally understood was no longer the fundamental particle of genetics, but neither were the units of mutation or recombination identified as fundamental particles. Perhaps it was something larger than a nucleotide pair, but Benzer could not yet tell. Perhaps he was closer than anyone else in the field to answering that question, but he was also entirely forthright in acknowledging that he was not yet there.

The first experiment Benzer tried after returning from Urbana was to test an idea that could, if the experiment were to work, illuminate the underlying mechanism of the rII effect on which his experimental system rested. "The fact that rII mutants plate as r^+ on K12S might suggest," he thought, "that K12S supplies ('constitutively') an enzyme corresponding to the rII gene. If so, plating K12(λ) infected with rII on K12S might produce plaques." *Constitutively* was the term originated by Monod to designate the process whereby certain mutant bacteria

produce continuously an enzyme normally requiring the presence of its substrate to induce its production. Benzer was suggesting, therefore, that K12S analogously supplies constitutively an enzyme whose production rII cannot induce and which requires the presence of r^+ to induce in other bacterial strains such as B.[56]

The result was ambiguous. The K12(λ) infected with one of his rII mutants and then plated on K12S produced about one thousand r^+ plaques plus about two thousand tiny (centered) ones. Benzer commented that the latter "are probably due to λ which, of course, obscures the experiment. There does not seem to be any great number of small size r^+ type plaques." Although this result might not in other circumstances rule out further pursuit of the question, the question itself would have been a side issue compared to getting on with the extension of mapping to its limit. Benzer dropped the matter.

Benzer made several unsuccessful attempts between November 2 and November 7 to isolate a T6 lysate that might help T4 r mutants to grow on K12(λ), but the main line he followed was to isolate and then begin making spot tests of the " '10^{-6} class' of mutants," that is, of the group of rII mutants that reverted to wild type at a frequency of about 10^{-6}. We have seen that since the summer he had been impressed by the fact that the reversion rates varied over a large range but seemed to cluster around three widely separated frequencies. In his physics seminar he had hinted that these three groups might be associated with different types of mutation mechanisms. Now he intended to give more definite shape to that surmise by studying the three "classes" of mutants individually.

On November 2 Benzer titrated twenty-three different stocks of "10^{-6} class" mutants selected from the several series of mutants he had isolated during the previous month. On the ninth he plated each of them on K12(λ), using two plates for each mutant, and placed spots of fifteen stable mutants on each pair of plates, drawing a diagram to keep track of the order of spots (fig. 7.10). He summarized the results in the form of a small, rough map of the order of the markers along the rII gene. The following day he carried out tests for "allelism" of the mutants, crossing eight of them that had been very close together according to the spot tests with each of two other mutants, I–8 and 14, and plating them on K12(λ). Two of the eight, I–9 and VIII–9, formed, respectively, 1 and 0 plaques with 14, and the other combinations each produced several hundred plaques. He concluded that "I–9 and VIII–9 are allelic to 14 (and same distance as 14 from I–8)." The others "are

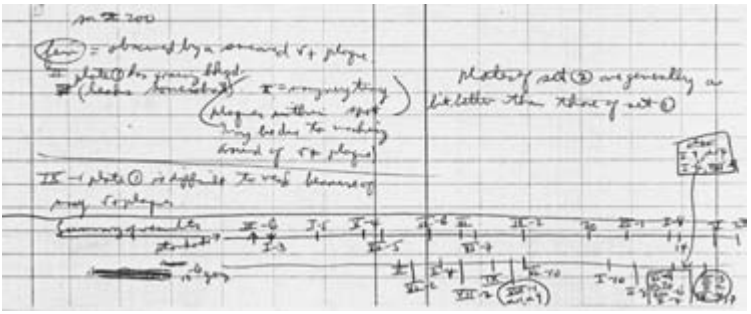


Figure 7.10. Summary mapping by spot-test crosses of T4 r-mutants with reversion frequencies of about 10^{-6}, November 9, 1954. (RN no. 4, bottom of page.)

not allelic to either I–8 or 14, nor are they *between* them. (Cannot say whether they are all allelic to each other.)" After two experiments that tested whether a T6r lysate could affect the transmission coefficient of a T4rII mutant but that revealed no such effects, Benzer turned on November 10 to preparations for spot tests of the "10^{-4} reversion class" of T4rII mutants. He chose eleven mutants from his earlier stocks and isolated five new ones that showed high K/B ratios, spotting each of them with five stable rII mutants. In a table he indicated the spots with plaques by "+," those without as "0." These results, however, were "not very clear, since all plates have quite a bit of background, and it is difficult to be sure that small plaques are not due to 'washing around' of background plaques."[57]

During the rest of November and December, Benzer performed only a few experimental operations. One was the preparation of stocks of T1, T3, and T7 phage for a purpose that is not recorded. On December 17 he asked himself to "suppose mutation is a small inversion of length *l.* If centers fall at random throughout region of length a," and drew a diagram of the possible result (fig. 7.11).

He continued, adding another diagram (fig. 7.12):

> Position of center = x, then centers fall on graph.
>
> If wild recombination frequency depends on distance between nearest *ends* of inversions, then those with centers less than or equal to a distance *l* apart give zero crossover frequency.
>
> This represents a fraction of approximately $2l/a$
>
> For group of 21 mutants studied,
>
> 8 pairs give zero out of [a] total of $21 \times 20/2 = 210$ pairs.
>
> $p(0) = 8/210 = 2l/a$
>
> $l = (8/210 \times 2) \times 8\% = 64/420 = 0.15\% =$ length of the inversions

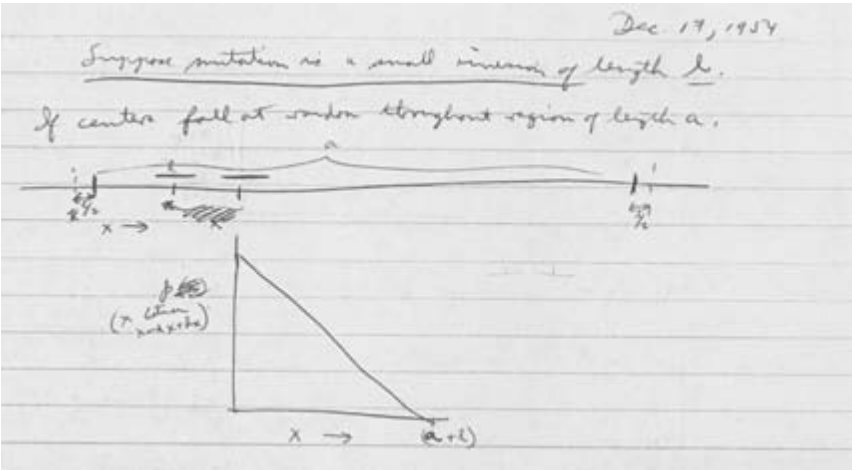


Figure 7.11. Model of inversion mutations, December 17, 1954. (RN no. 4, top of page.)

In considering that mutations might be inversions, Benzer was pursuing both the possibility that his groups of reversion frequencies were incompatible with the Watson-Crick mutation model and an explanation for the number of zero recombination frequencies he had observed that did not require the mutants involved to be independently arising identical ones. Perhaps he was also seeking an alternative for the view he had expressed in his physics seminar that there might be a fundamental genetic particle considerably longer than one pair of nucleotides.

In October Frank Lanni had written Benzer saying, among other things, "Your use of the term 'gene' [in the abstract that appeared in the *Phage Information Service*] to describe the rII complex also puzzles me, but this may reflect only my ignorance, since Demerec uses a similar concept in his note in the same PIS. Do you have the same justification as he?"[58]

When Benzer answered Lanni, belatedly, in early December, he wrote, "The terms 'gene,' 'complex,' 'locus,' etc. are used by different people to mean different things. My gene, which should have been in quotation marks in the abstract, is the physiological unit and is larger than the units of mutation and recombination."[59]

A more strongly worded criticism from Hershey on December 22 moved Benzer beyond the response he had made to Lanni. Hershey had not seen Benzer's full draft, but Brenner's abstract led him to make "one minor suggestion":

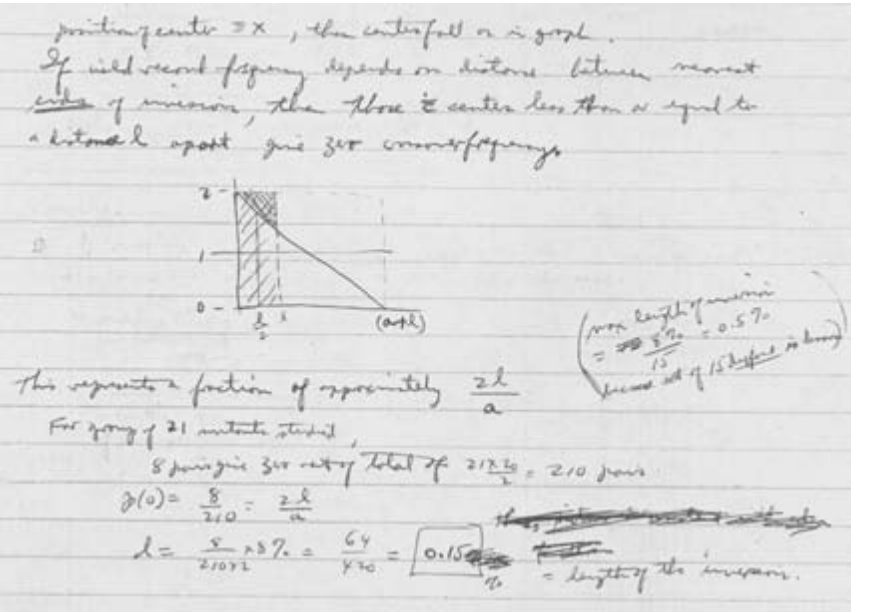


Figure 7.12. Model of inversion mutations, December 17, 1954. (RN no. 4, bottom of page.)

> The word gene is full of confused notions among geneticists. For a sad example see Stadler's posthumous paper in Science (Nov. 19). You are introducing a new (to phage) and satisfactory definition: that region of the phage in which mutations produce a given class of effects. This, however, disagrees in this instance with all previous definitions (see concluding remarks in my paper in Advances in Genetics). In that paper I proposed that when such disagreements arose, the word gene should be abandoned for the descriptive equivalent—in your case some sort of "action unit." I still think this is a good idea. At the same time we should stop using the word gene in any of its other senses. Stadler's paper convinced me that this is more important than I had thought before, because Stadler was not a fool.[60]

What Hershey had written in *Advances* was that there was only one known instance of alternative mutations at the same locus in T2, producing two different h alleles:

> In this instance the alternative mutations occur at a single mutative and combinative locus. Conversely, independent mutations always occur at different combinative loci. For as long as genetic tests

> continue to yield consistent identifications of mutative loci, combinative loci, and map positions, there can be no ambiguity in referring to the loci as gene. When inconsistencies arise, the more explicit terms will have to be used. In the meantime they are useful when it is desired to make clear what method of identification of the locus has been used.[61]

The article by the eminent maize geneticist Lewis J. Stadler (see Chapter 1) that caused Hershey to decide that the word *gene* should be used in fewer senses was submitted to *Science* at the time of Stadler's death in May 1954 and published in November. In it Stadler reviewed the working definition of the gene as a unit of mutation that had been developed since the beginning of the century, and especially Muller's role in defining the problem of the nature of the gene, discussed the question of whether mutations result from structural changes within a gene or rearrangements external to it, and summarized Delbrück's atomic model of the gene, popularized by Schrödinger's book. He made no mention of DNA. The hypothetical model of the gene could not be verified, according to Stadler. The operational gene was real, for it implied no unproved properties, but at that point geneticists lacked criteria for formulating an operational definition.[62]

Benzer replied to Hershey's letter on December 30:

> I agree with you about "genes." In fact, I plan to use that dirty word only once—in order to state that I am not going to use it. At the time of the first draft, I did not realize how bad the situation was, but since then I have talked to geneticists and no two seem to agree. How do you like "functionally related region of a linkage group"? This is a jaw-breaker, but almost always can be abbreviated by "region." I like region better than locus, because the geneticists are just as confused about locus as about gene. Pontecorvo, with whom I have discussed this question, likes region also. However, there remains the difficulty of defining "functionally related" without specifying the function. The specification of a particular amino acid is a function, as is the specification of an entire enzyme.[63]

It is hard not to conclude that it was Hershey's critique that moved Benzer to the decision to do without the word *gene*. We have seen that, from the time of his Cold Spring Harbor seminar in July, he had proposed that the rII region constituted a gene, and he continued to use that word until his physics seminar of November 4 without raising questions about it. To be sure, his discussions with geneticists during his European trip must have reinforced doubts originally stimulated by reading Pontecorvo's paper more than a year earlier, but as recently

as the time he wrote to Lanni he had maintained only that *he* defined the gene as the unit of physiological action, not of mutation or recombination. There was one additional recent observation, however, that concurred with Hershey's view to push him past that position. As he wrote, "Incidentally, I have a sneaking suspicion that the 'rII region' can be divided into two parts. This needs elaboration which I would rather defer until after you have seen the paper, but it worries me more than ever about definitions."[64] What Benzer had observed to arouse this suspicion was that in his spot tests of close markers, not only did those beyond a certain distance away produce more plaques than those that were closest but there was a jump from relatively few to many. This result made him think that perhaps only very close markers required wild-type recombination to produce wild-type plaques and that the others plated because the markers controlled different functions, and, therefore, in a mixed infection, each mutant could supply what the other lacked. Was each of the two parts of the rII region, therefore, a gene, or would it be better not to apply the word *gene* to either the whole or its parts? Together with Hershey's remarks, this new development made Benzer lean toward the second alternative.

Meanwhile the word about Benzer's venture into the rII region was spreading in the *Phage Information Service* bulletin, as well as by word of mouth, throughout the phage community and attracting admiring attention. On December 5, George Streisinger wrote from Caltech: "We have been overwhelmed with your experiments—the idea is really beautiful. What is the latest poop? How close can you get two markers? Inspired by you I have been thinking of starting some experiments along the same lines with the h locus—the whole thing will depend on whether the locus is complex. The beauty here would be that the protein is accessible."[65] That others were beginning to follow his example might not have been entirely welcome news to Benzer, who had yet to reach the closeness of markers of which he thought his system capable. As Streisinger's remarks suggest, it was not so much the achievements so far reported but those promised by the idea behind the experiments and the anticipated results that most interested his colleagues. Nevertheless, what had already been done was enough to lift Benzer in the eyes of his fellow phage workers from a well-regarded investigator to a rising young star.

In the work of Benzer, as well as in that of other scientists that I have reconstructed in fine detail, an interruption in research is often followed by a noticeable shift in emphasis or the introduction of new directions

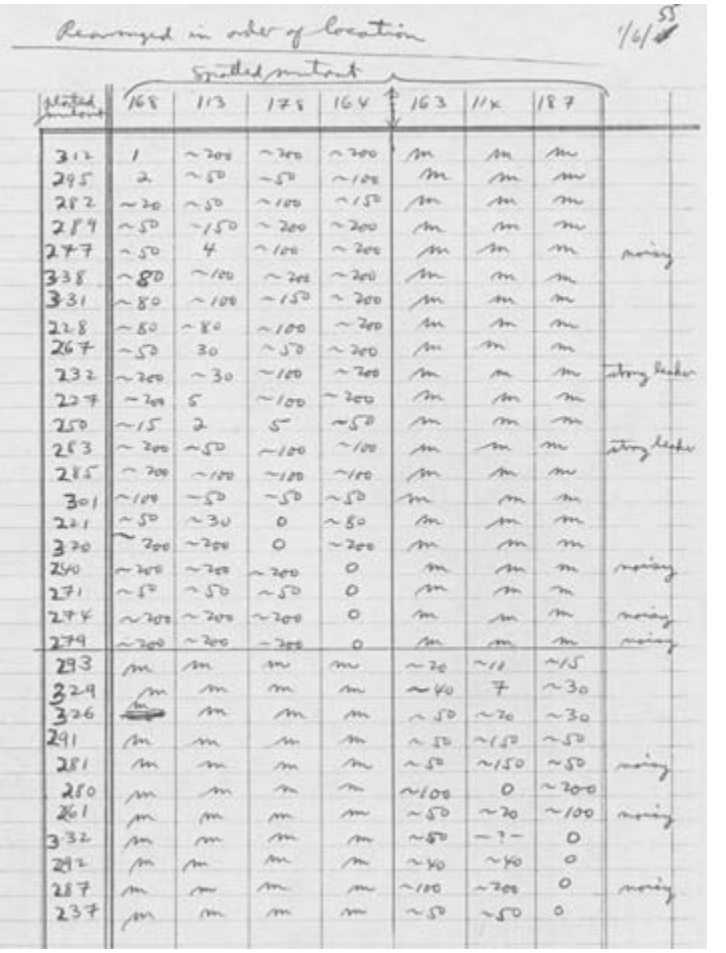
in the investigative pathway. We have seen this in the beginning of the spot-test shortcut discovered on Benzer's return from Europe. Such a change took place also when he resumed his experiments in January 1955, after the Christmas holidays. In some cases the changes can be attributed to the stimulation of meetings and conversations with others, but they can also result simply from the opportunity to distance oneself temporarily from the work while pondering priorities and the state of the broader problem.

The most evident new factor in the experiments that began in January was that Benzer had come to realize that in order to achieve his goal of carrying the resolution down to nucleotide levels he would have to map many more mutants than he had done until then. Thus, when he next isolated a new series of T4r mutants, he spot tested eighty of them on January 4. (He numbered them 251–336.) Twenty-five turned out to be stable.[66]

After tabulating the tests in the usual way according to the order in which they were numbered, on January 6 Benzer rearranged them in the order of location. This ordering made immediately evident the sharp breaks between mutants that produced relatively few plaques and those that produced many. He drew vertical and horizontal lines across his table at these boundaries (fig. 7.13).[67] Although he made no verbal comment on the page, these lines seem to be visual representations of the sneaking suspicion he had expressed to Hershey a few days earlier that the rII region is divided into two parts.

The following day Benzer did a spot test to locate T4rBX, one of his older stable mutants, with respect to two other mutants, III–2 and 20. The result was "no clearing on III–2 spot" and "confluent clearing on 20 spot." He concluded that T4BX "belongs in the left hand (III–2) part of the region."[68] This comment confirms that Benzer now recognized that the rII region was divided into two parts. The procedure followed here became his standard method for quickly determining in which of the two parts a newly isolated rII mutant was located.

During the following weeks Benzer drew up on a double sheet a sum of the plaques produced in spot tests of fourteen different mutants crossed in every possible combination and determined the transmission coefficients of several mutants. Between the middle of January and the middle of February, however, he performed relatively few experiments because he was preoccupied with revising the manuscript he had written the previous summer and with the preparation of an application for a National Science Foundation (NSF) research grant.


Rearranged in order of location 1/6/55

Spotted mutant

plated mutant	168	113	178	164	163	11x	187	
312	1	~200	~200	~200	m	m	m	
295	2	~50	~50	~100	m	m	m	
282	~20	~50	~100	~150	m	m	m	
289	~50	~150	~200	~200	m	m	m	
277	~50	4	~100	~200	m	m	m	mixing
338	~80	~100	~200	~200	m	m	m	
331	~80	~100	~150	~200	m	m	m	
228	~80	~80	~100	~200	m	m	m	
267	~50	30	~50	~200	m	m	m	
232	~200	~30	~100	~200	m	m	m	strong leaker
227	~200	5	~100	~200	m	m	m	
250	~15	2	5	~50	m	m	m	
283	~200	~50	~100	~100	m	m	m	strong leaker
285	~200	~100	~100	~100	m	m	m	
301	~100	~50	~50	~50	m	m	m	
221	~50	~30	0	~50	m	m	m	
320	~200	~200	0	~200	m	m	m	
240	~200	~200	~200	0	m	m	m	mixing
271	~50	~50	~50	0	m	m	m	
274	~200	~200	~200	0	m	m	m	mixing
279	~200	~200	~200	0	m	m	m	mixing
293	m	m	m	m	~20	~11	~15	
329	m	m	m	m	~40	7	~30	
326	m	m	m	m	~50	~70	~30	
291	m	m	m	m	~50	~150	~50	
281	m	m	m	m	~50	~150	~50	mixing
280	m	m	m	m	~100	0	~200	
261	m	m	m	m	~50	~70	~100	mixing
332	m	m	m	m	~50	~?	0	
292	m	m	m	m	~40	~40	0	
287	m	m	m	m	~100	~200	0	mixing
237	m	m	m	m	~50	~50	0	

Figure 7.13. Arrangement of T4 r mutation data into two groups, January 6, 1955. (RN no. 4.)

The NSF proposal was submitted on February 14, 1955. Because the process of approval at Purdue would have taken some time, Benzer must have composed the text of the proposal during the previous weeks—whether concurrently with or before completing the revisions to his paper is not easy to determine by internal evidence.

The abstract of his application began boldly: "This proposal is an attempt to bridge the gap in our knowledge between the genetic properties of hereditary material and its molecular structure." Outlining the limitations of previous methods for studying crossovers of very closely

linked markers, the abstract claimed that a "system has been discovered in bacteriophage that has uniquely favorable properties for the detection of recombinants; this extends the attainable limits of resolution of crossing over experiments down to the molecular limits of the hereditary material." With this system it appeared possible to determine "(1) the molecular sizes of the structures involved in genetic function, crossover, and mutation, (2) the nature of the molecular changes involved in mutation."[69]

Under the heading "Research Plan" Benzer gave a straightforward description of the "biological system" constituted by the rII mutants and how their properties made it possible to detect rare wild-type recombinants. "The rII region," he wrote, "appears to be a unit of physiological function: a mutation arising anywhere within the region affects the same character, whereas this effect is not shown by any mutations outside the region" (1). Although he clung to the idea that the entire rII region is a single unit of function despite his new evidence that it has two parts, nowhere in the proposal did Benzer refer to the region as a gene. He seems to have been truer than his word to Hershey, not even mentioning the word to say that he would not use it.

His estimate of the upper limit on the length of the rII region (sixteen thousand nucleotides) was based on the same considerations as his earlier discussions of the topic, but for the grant he proposed to obtain a better estimate by determining more accurately the total length of the linkage map. He would do this by finding new mutants outside the rII region that might be located in regions not previously represented on the map (2).

Benzer next explained how the "size of the molecular unit which is indivisible by crossing over is to be determined by isolation and crossing of a large number of rII mutants" (2). Here, too, much of the analysis by which one can compute the percentage of recombination to be expected if the unit of mutation is a single nucleotide pair (0.0005 percent) was a repeat of Benzer's earlier formulation. Because his selective technique was capable of detecting much lower values, down to 10^{-7}, it was

> possible to identify as "absolute" alleles two mutants which fail to give even the minimum value of 0.0005%. Several such pairs of allelic mutants have already been obtained. This demonstration of absolute allelism has not before been possible in any other organism. The molecular size of the elements involved in crossing over can be determined by finding the smallest non-zero distance by

> which two mutants can be separated. The present data place an upper limit on this distance of some 260 nucleotide pairs.
>
> In order to find the lower limit of this value, it will be necessary to isolate and cross a very large number of rII mutants, searching for extremely closely linked "clusters." (2–3)

We may note that it was not the extreme sensitivity of his method alone that permitted Benzer to claim the first demonstration of "absolute allelism" but also his confident acceptance of the corollary of the Watson-Crick structural model of DNA as the carrier of hereditary material. That no lower limit could hitherto be set on the distance between nonallelic markers had been in part due to the limits of resolution of earlier methods, but in addition there had been no conclusive demonstration of the molecular dimensions of the "gene."

Comparing Benzer's proposal with his discussion of the same questions in his November 1954 physics seminar, we see that in the proposal he made a distinction between the minimum length between mutations (which, he now stated, was one nucleotide pair) and the "molecular size of the elements involved in crossing over" (at that time, somewhere between 1 and 260 nucleotide pairs). But he did not speculate about a possible "fundamental particle" of genetics that might be much longer than, but conceivably also shorter than, a base pair. Most likely the difference was due to the different audiences—a group of physics students would be impressed by such analogies to the fundamental particles of physics, whereas an NSF panel would not. But it is also plausible that he had clarified the situation further in his own mind between November and January.

The most evident difference, however, between this and earlier statements was Benzer's emphasis on the need to map very large numbers of rII mutants. This was, I have suggested, a realization that somehow became clear to him during the Christmas holidays. It was, moreover, the rationale for the substantial sum he requested of the National Science Foundation: $29,000 over three years: "It is estimated that several hundred mutants will be needed. To cross 100 mutants with one another in all possible pairs requires almost 5000 individual crosses. Although some short-cut methods are possible for preliminary tests, this will certainly be a large undertaking. It is for this reason that a rather large personnel is required for the project" (3). The budget request included the salary for a full-time postdoctoral research associate and a graduate student assistant, as well as funds for permanent

equipment, supplies, and travel. The person he proposed for the research associate was his colleague Ed Lennox, presently a research fellow with Luria.

In the section titled "Significance of the Problem," Benzer wrote: "The system under discussion is a special one, chosen for its unique technical advantages. However, we believe that the basic mechanism for genetic structural changes may apply as well to all organisms. The possibility of extending the resolving power of classical methods of genetics into the realm of structural chemistry, thus joining two disciplines, is more than sufficient inducement to justify the effort required" (4).

Benzer has maintained that he had initially studied little genetics and that he "sort of made it up as he went along."[70] As we have seen, he did, in fact, gradually enter his mapping project on rather narrow grounds, within the boundaries of recent phage genetics. Over the course of the following months, however, from his reading and conversations, he steadily widened his perspective. At this point he was justifying his project on the broadest possible grounds: those of the merger of classical genetics with structural chemistry. Although the section about the significance of a grant proposal is often the occasion for grandiose boilerplate, in Benzer's case the claim made was a prescient prediction of the wide-ranging impact that his research plan could have on the disciplines at whose intersection he was now standing.

Benzer wrote Delbrück on February 3:

> Here is a more subdued second draft of the rII story. Actually, I am so grateful for your advice against publishing the first one that I will be almost delighted if you find fault with this. Renato [Dulbecco] has already seen it during his visit and his suggestions have been incorporated. (Ink drawings will follow.) It suffers from not quite answering any of the $64 questions, but may nevertheless be acceptable as a preliminary report. I believe I can get support for the extra page costs required for PNAS.[71]

Delbrück's advice had been not to make the changes he had proposed in July but to postpone publishing until Benzer had done more experiments. The new draft incorporated much that Benzer had not yet known when he wrote the first draft.

In his revision Benzer acknowledged, among others, "Max Delbrück for his invaluable moderating influence." When he received the manuscript, Delbrück wrote back at once, "Could you come here for a week, as soon as possible, to submit yourself to some more influence, moderat-

ing or otherwise? I have $200.00 travel allowance. Say when." Not content to wait for Benzer to come or even to reply, Delbrück "jotted down some thoughts" about the manuscript and sent them to Benzer the next day. "Your present discussion section seems to me to stand too much under the influence of the pre-conceived notion of an ideal WC chromosome. Since the principal results of your experiments show up a gross incompatibility between this ideal and the facts of life" it would be better to focus the discussion on the latter and point out only marginally that the ideal is a "far cry from your findings."[72] As in his NSF application, Benzer avoided the word *gene* in his paper, titled "Fine Structure of a Genetic Region in Bacteriophage," substituting throughout phrases similar to those he had proposed in his letter to Hershey. He began: "This paper describes a functionally related region in the genetic material of a bacteriophage that is finely subdivisible by mutation and by genetic recombination. The group of mutants resembles similar cases which have been observed in many organisms, usually designated as 'pseudo-alleles.' (See reviews by Lewis and Pontecorvo.) Such cases are of special interest for their bearing on the structure and function of genetic determinants."[73] After summarizing the general phenomenon of genetic recombination and the selective feature of his rII mutants that allowed him to map the genetic material of phage to a resolution smaller than the distance between nucleotides, Benzer stated, "Some preliminary results are here presented of a program designed to extend genetic studies to the molecular (nucleotide) level."[74]

Describing first the r mutants in general, then the particular features of the rII mutants, Benzer pointed out that, while all of the latter showed the same "phenotypic effect of poor multiplication on K12(λ), they differ in the degree of this effect." A certain proportion of the bacteria infected with rII liberate some progeny. This yield of rII phage was measured as the "transmission coefficient," which can be used as an index of the "leakiness" of the mutant. Similarly, the rates of reversion indices for rII mutants covered a wide range. He next described genetic mapping of the rII region, presenting the same "large-scale" map of six of his own rII mutants together with Doermann's r47 and r51 that he had constructed during the previous June (See Chapter 6).[75]

Turning next to what he now called "tests for pseudo-allelism," Benzer pointed out that when two closely linked mutations cause similar defects, their functional relationship can be tested by constructing diploid heterozygotes in two configurations. If both mutations are on the same chromosome, the *cis* form, the heterozygote behaves as a wild

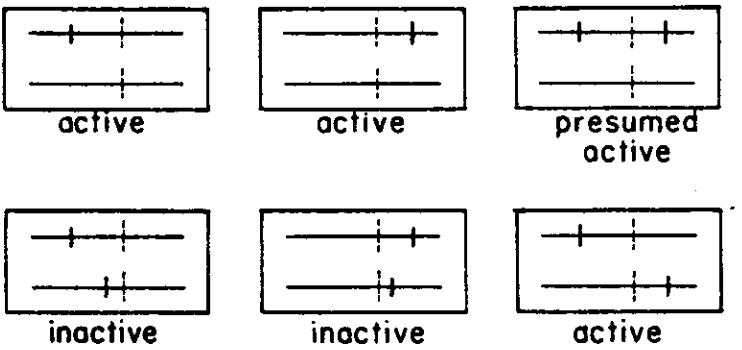


Fig. 3.—Summary of tests for "position-effect pseudo-allelism" of rII mutants. Each diagram represents a diploid heterozygote as simulated by mixed infection of a bacterium (K) with two types of phage containing the indicated mutations. *Active* means extensive lysis of the mixedly infected cells; *inactive* means very little lysis. The dotted line represents a dividing point in the rII region, the position of which is defined by these results.

Figure 7.14. Model of pseudoallelism for division of the rII region into two parts. (Reproduced by permission from S. Benzer, "Fine Structure of a Genetic Region in Bacteriophage," *Proceedings of the National Academy of Sciences* 41 [1955], fig. 3, p. 350.)

type would because the second chromosome is intact. The second configuration, the *trans* form, may or may not act as a wild type would, depending on whether the two mutations are located on the same or on different functional units. In the case of the haploid phage, this test can be "simulated" by mixed infection of the bacteria with the two mutants. Up to that point he had tested only the trans form, which enabled him to divide the rII region into two segments. "On the basis of this test," he concluded, "the two segments of the rII region correspond to independent functional units." He summarized the pseudoallelism test with a set of diagrams (fig. 7.14).[76]

As we have seen, Benzer seems to have discovered this division of the rII region in late December, not long before he began revising his manuscript. Perhaps it was during the writing that he noticed the similarity between what he had found and the cis-trans test devised by E. B. Lewis, which was accepted in classical genetics as the means to determine whether two very closely linked markers belonged to a single gene or to neighboring ones. His paper made reference to the papers of Lewis and of Pontecorvo for the test.

The "rough mapping by spot test" now became a test for pseudoallelism as well. Inspection of a plate on which K12(λ) has been seeded

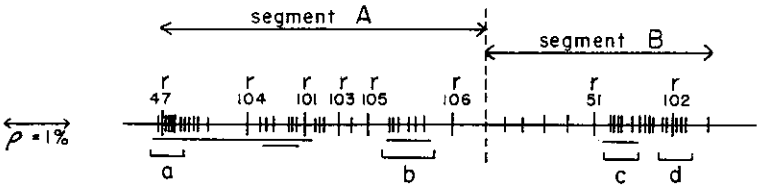


FIG. 4.—Preliminary locations of various rII mutants, based upon spot tests

Figure 7.15. First published fine-structure map of the rII region of the T4 phage showing its division into A and B segments. (Reproduced by permission from S. Benzer, "Fine Structure of a Genetic Region in Bacteriophage," *Proceedings of the National Academy of Sciences* 41 [1955], fig. 4, p. 351.)

with an unknown mutant and then spotted with another mutant "immediately places the unknown mutant in the proper segment, since spotting any mutant of segment A against any mutant of segment B gives a very clear plaque, due to the extensive lysis of mixedly infected bacteria. However, for a pair of mutants belonging to the same segment, plaques are produced only by the relatively few mixedly infected bacteria which give rise to wild recombinants." If a group of mutants has previously been "seriated" along this segment, then the new mutant can be easily located within that group. The method worked best, he pointed out, with mutants of low reversion that are nonleaky.[77]

From the application of this test, Benzer went on, a large group of stable, nonleaky mutants were approximately located, as he showed in a figure (fig. 7.15). Some of the mutants could only be located within a certain span, indicated by a bar in the figure, because they gave very little recombination within that distance but acted normally beyond it. The spot test enabled him to select several "microclusters" (a, b, c, and d) for further study. He presented diagrams of four of these clusters, indicated by brackets.

In his discussion section Benzer emphasized that the bounded set of rII mutants defined a functionally connected region within which all mutations affected the same phenotype. The failure of rII mutants to mature in K12(λ) could be understood if the function of this region in wild-type phage was to control the production of one or more substances required for the reproduction of the phage. The division of the region into two functionally distinguishable segments could be imagined to affect two sequential events or a single substance both parts of which were necessary for it to be active. For example, each segment might control the formation of a polypeptide chain, both chains being

necessary to produce an enzyme. If one is to characterize a unit of function, one must define what function is meant. One can say that the entire rII region is unitary in the function of producing the rII phenotype, but the region is also divisible into two functionally separable segments, and the specification of each individual amino acid can also be considered a unitary function.

These considerations, which dominated his discussion, are clearly further developments of the proposal Benzer had made in his summer seminar that the region in which rII mutants were located constituted a gene. That proposal had been made more subtle as well as more solid by the further evidence he had gathered since then and by his abandonment of the word *gene*. He discussed more briefly the long-term goal of his research:

> By extension of these experiments to still more closely linked mutations, one may hope to characterize, in molecular terms, the sizes of the ultimate units of genetic recombination, mutation, and "function." Our preliminary results suggest that the chromosomal elements separable by recombination are not larger than the order of . . . [260] . . . nucleotide pairs (as calculated from the smallest non-zero recombination value) and that mutations involve variable lengths which may extend over hundreds of nucleotide pairs.[78]

In the outline for a revised discussion and summary that he sent to Benzer on February 8, Delbrück essentially rewrote and combined Benzer's discussion and summary. Because Benzer's draft has not survived, it is not possible to analyze fully the changes Delbrück proposed. Much of the language is similar to that in Benzer's final version, but that could be either because Delbrück retained those parts of the draft or because Benzer accepted some of his suggestions. Benzer did not, however, adopt the most interesting part of Delbrück's version:

> Our principal interest attaches to a group of questions which could be given the heading "the topological structure of the map in relation to the topological structure of DNA."
>
> The WC model and the EM pictures do not suggest the existence of any structural units in the DNA larger than those of the single nucleotide link. Or perhaps that of a turn of the helix. The resolving power of our method would be ample to detect recombinations between mutants which are only one nucleotide link apart, if map distance is simply proportional to DNA distance and if the whole map (abut 100 units) corresponds to the whole DNA (about 200,000 links). It is obvious at a glance that our random sample of mutants

does not correspond to a random sample from an ideal map, the ideal map being defined as one in which

a) every mutation is a change in a single nucleotide pair,
b) every nucleotide pair mutates with equal probability,
c) recombination can occur at every nucleotide pair with equal probability.

Indeed, the real sample differs vastly from an ideal sample, and most probably in the following features:

a) we find double and even triple repeats in a relatively small sample from a region of map length 8%. Corresponding to 16,000 links,
b) we find more than two classes of reversion rates,
c) we find a smallest non-zero map distance of 0.1%.

These deviations from the ideal sample may have one or more of three obvious causes:

a) recombination cannot occur at every link, but only in selected points which lie about 200 DNA links apart,
b) the mutations are not point mutations, i.e. alterations affecting a single nucleotide pair only, but they affect a stretch of about 200 links,
c) mutation rates at different points in the region differ enormously and what we pick out are the few points of relatively enormously great mutability.

It seems to me that the strongest method for exploring these possibilities would be a precision study of the mapping problem. Possibility (b) should give measurable deviations from the additivity law.[79]

Delbrück was not merely moderating Benzer's conclusions but recasting his results to transform them into an attack on the genetic implications of the "ideal" Watson-Crick DNA molecule. What motivated the person who had served as a kind of mentor to Watson, who had received from Watson the first news of the double helix by letter, and who had called it a "discovery that might rival that of Rutherford in 1911" to do so?

It is easy in retrospect to overestimate the solidity of the support for the double helix in the first years after Watson and Crick proposed it. From the beginning Delbrück had had both praise for and deep doubts about some aspects of Watson and Crick's formulation. He thought

base-pairing extremely important, but he did not see how the double helix could replicate without its two strands breaking. At about the time Benzer sent him his manuscript, Delbrück was pondering an alternative mode of replication in which the single strands did break into pieces at every turn. His doubts had sufficient impact that even Watson was moved to try to construct a ribbonlike model of the DNA molecule to avoid the untwiddling problem, as Delbrück had called it. Early in 1955, in fact, Watson wrote Benzer depicting his new model. "The replicating form of DNA," he stated, "is a two shouldered ribbon, not a helix." He added, "For me, pairing is impossible to imagine as long as we think of DNA replicating in the helical configuration."[80] With such doubts about the validity of the original Watson-Crick replication model afflicting one of its authors, it is not surprising that Delbrück saw in Benzer's experiments further evidence against the reality of that molecule.

Although Benzer questioned the mutation model that Watson and Crick had derived from their structure, he seemed to harbor no doubts about the validity of the double helix itself. He seems to have been among those, including Sydney Brenner, who were so deeply impressed by the beauty and promise of the structure as to be confident that any apparent anomalies would be worked out without discarding its essential features. Benzer rejected Delbrück's attempt to enlist his work against it.

Chapter Eight

Is *Gene* a Dirty Word?

While discarding the rewritten discussion with which Delbrück supplied him, Benzer replied tactfully, "Your comments and suggestions on the manuscript are apt and much appreciated, and I am working on the revisions." Moreover, he warmly welcomed the idea of coming to Caltech to discuss the paper further. "I am sorely tempted by your invitation to (literally) 'fly off' to Pasadena, but cannot get away until after March 25. Will the invitation hold until then? I propose March 28 and would like to stay for a longish week, if that is agreeable to you."[1] Delbrück accommodatingly arranged matters to fit Benzer's schedule.

Meanwhile, Benzer was also receiving comments from other colleagues, including especially those at the two laboratories that had preceded him into genetic mapping of phage. Hershey suggested, "You could change the title of this [paper] to something like 'Regional Differentiation in the Genetic Material of T4,' substitute a more physiological discussion, and then write a discussion paper on 'fine structure' for the Brookhaven symposium. The advantage would be economy, and perhaps a chance to do more justice to the beautiful new facts and techniques in this paper." Benzer had been invited to deliver a paper at the Brookhaven National Laboratory the following summer. Hershey's comment was so subtle that Benzer may have missed an implicit point: the manuscript was essentially two papers in one. It described a differentiation within the rII region that Benzer had already observed. Juxtaposed with this was work still in progress toward the eventual limits of resolution, which he had not yet reached. By summer he might have more impressive results to report than he had now. Benzer did not take the hint. Overall, Hershey thought, "Your paper is very pretty, and I hope you will feel it puts you under moral obligation to continue the work. Unfortunately, this seems to call for an Institute."[2]

As we saw from his NSF application, Benzer, too, was aware of the magnitude of the task that lay still ahead.

Doermann wrote back two days later, "I believe everyone in our group has read [the manuscript] and, of course, has found it very interesting. You have certainly developed a powerful attack on the problem of understanding the genetic material, and we are unanimous that this constitutes a most important contribution." Most of Doermann's comments consisted of requests for more detailed information about certain points and an objection to Benzer's use of the term *cross reactivation*. In addition, he noted, "We feel that the order on the microclusters (Fig. 4) is slightly ambiguous. In those cases where recombination is 10^{-6} or less, it may be misleading to assign two loci. In (1), if there is any order within the microclusters, the data that are given suggest the order 168–47–145, rather than 47–168–145."[3]

Without the original diagram it is difficult to assess this comment in detail, but the general point—that Benzer was assigning the order of mutants down to a level at which it was questionable—seems clear. In fact, Doermann's letter may have helped prompt Benzer, as he continued his research, to reconsider how to interpret both the mutants that showed very small nonzero recombination frequencies and those that showed no recombination. Throughout the second half of February he concentrated on the further study of his microclusters.

On the seventeenth Benzer crossed r168 with six other rII mutants from his 100, 200, and 300 series, all grown on K12(λ)—to which, following his example, I shall henceforth mainly refer as "K"—and with r145 grown on B. The crossing of 168 × 312 gave no recombination; nor did 164 × 163. The latter he considered to have been an incorrectly labeled cross of the former two. (In the absence of the manuscript, it cannot be discerned whether these crosses were the same as those in one of the microclusters presented in the paper. They are mainly different from those presented in the final paper.) He drew a map showing the distances of five of the mutants from 168, and the latter separated from 312 by 0.000 percent. Presumably he considered 312 and 168 at this time as "allelic."[4] He also noted that Doermann's old r47 (not represented on this map) was allelic to r168 when he sent these two mutants the following day to Frank Stahl at Rochester.[5]

Benzer gave microbiology seminars on genetic fine structure at Purdue on February 22 and March 1. By now he had finely honed the story and was able to move through classical and phage genetics to his own work with great economy. Little was changed from his previous talks

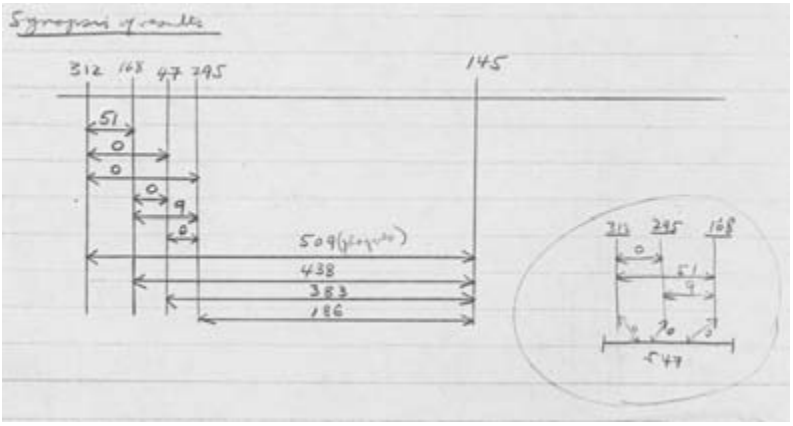


Figure 8.1. Synopsis of allelism tests, showing initial maps, February 24, 1955. (RN no. 4.)

on the subject. As in his NSF application of a month earlier, he estimated that the upper limit of the unit of recombination was "260 N[ucleotide] P[airs] (so far)."[6] Evidently none of his more recent crosses had enabled him to revise that figure downward.

In a series of thirteen more crosses carried out on February 23, Benzer noted an anomalous result involving two double crosses, including (47, 168) × (295, 312), which formed very few plaques on K. "In cross # (13)," he noted, "obviously the four mutants are not all alleles. The number of plaques corresponds to a rather small interval, which would appear to be much less than the unit interval." This result induced him to perform allelism tests the following day by crossing each of these four with the others. The outcome was that 47 formed no plaques with any of the other three, and 312 formed none with 295. Benzer mapped these mutants showing "0" for the distances between those that formed no plaques, although these were in conflict with intervals shown between each of them and the others. These three showed substantially different distances from the widely separated 145. Similarly, 312, 295, and 168 showed no recombination with r47, yet 295 and 168 were separated from one another and from 312. He drew two maps representing these situations (fig. 8.1).[7] By that time he must have begun to suspect that certain mutants behaved quite differently from the rest of them. He then pasted a small piece of paper over the righthand map and represented the four mutants depicted on it as horizontal rather than vertical lines.[8] This critical move appears to

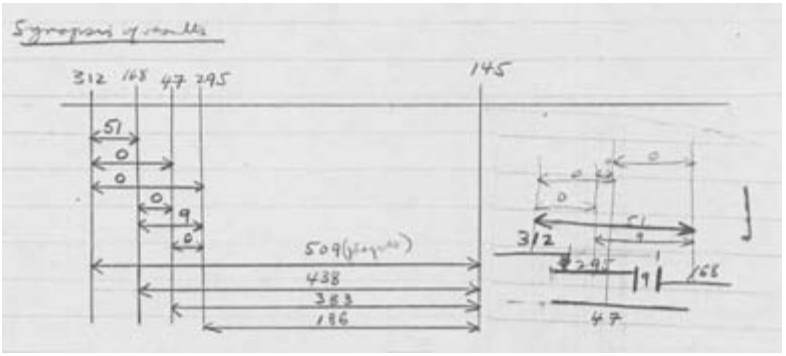


Figure 8.2. Synopsis of allelism tests showing the revised map on a slip of paper covering the initial maps, February 24, 1955. (RN no. 4.)

mark the point at which Benzer recognized that these four mutants could not be point mutations but must be alterations extending along a portion of the chromosome. That explained why they did not recombine with other mutants overlapping with them, which did recombine with each other (fig. 8.2). After recrossing ten of these mutants, on the twenty-sixth he drew a fuller map of the region to 145, noting, however, that one of the distances "does not fit this scheme." He commented also that the maximum distance between the ends of 245 and 168, both extending over an interval, was "25 nucleotide pairs."[9]

Seeking more such anomalous instances, Benzer mapped ten different mutants (from one of the other microclusters he had identified in his draft?) on March 2. Four of them showed no recombination with each other, but only one of the ten—196—also gave no recombinants with the more distant 150. Consequently he mapped this group with the four very close together and 196 extending across the map to 150 (fig. 8.3).[10]

Taking time out from his main concerns of the moment, on March 4 Benzer tested the method of counting plaques on K against the "standard" direct count method at a level of the latter set at 0.01 percent recombination. For the latter he determined the "count of r^+ particles on plates on B containing also many r." That is, choosing two r mutants whose recombination frequency was about what he wanted, he counted the number of wild type formed by recombination on B against the number of mutants that also plate on B. This he compared to the ratio of plaques formed on K to plaques formed on B, *his* usual method. The result was that 0.101 percent by direct count corresponds to 0.88 percent by the K/B count. Later he must have revised this calculation,

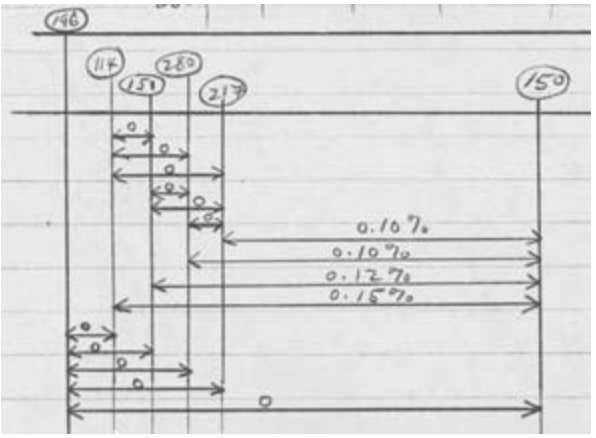


Figure 8.3. Anomalous mapping results from March 2, 1955. (RN no. 4.)

for he reported in his final paper that "for two mutants separated by 1 percent linkage difference (measured by a standard cross on B) the proportion of mixedly infected K yielding any wild type particles is about 0.2 percent."[11]

Responding to a question raised by Hershey, Benzer tested whether the progeny of leakers plated on K and found that, as Hershey expected, their "efficiency of plating" on the lysogenic bacteria was low. (Like their progenitors, they formed no plaques.)[12]

Returning to his microclusters, Benzer crossed six of them in pairs on March 7. Only r164 failed to recombine with any of the others. Accordingly, he mapped this cluster with 164 shown as a line all the way across the top. The others that formed no plaques with near neighbors he showed as 0 distance apart. He found no need to correct for reversions, which would have accounted for only four plaques per plate (fig. 8.4).[13] Answering Hershey's question—In estimating fraction of total linkage distance in rII group, do you use the Visconti-Delbrück correction, or doesn't this make too much difference?—Benzer made the calculation and found about an 8 percent correction for nonadditivity.[14]

Taking up one more microcluster of six mutants on March 13, Benzer found two of them to be anomalous. Number 102 recombined only with two mutants, 187 with none. The other four formed no plaques with their nearest neighbors but did so with more distant ones. He mapped them accordingly (fig. 8.5).

Benzer traveled to Baltimore on March 16 to give an invited paper at an American Physical Society symposium on nucleic acids

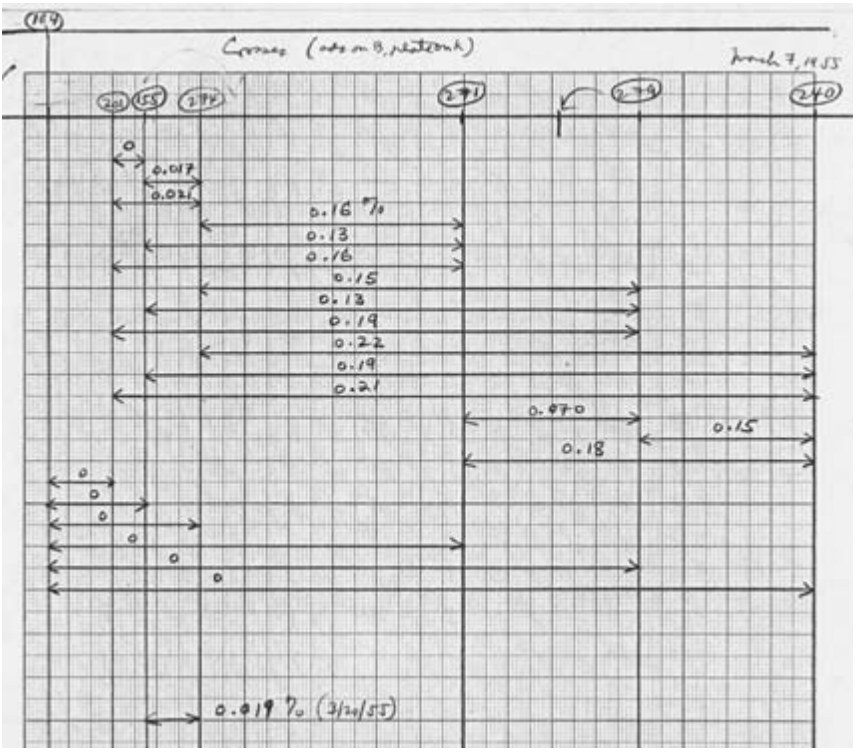


Figure 8.4. Maps of microclusters from crosses performed on March 7, 1955. (RN no. 4.)

sponsored by the High Polymer Physics Division and held on the next day. He covered the usual ground of definitions of the gene and of phage as a very good tool for genetic mapping, concentrating on his own work. As he often did when addressing physicists, he likened genetic events to those in physics. The transitions brought about by mutations, for example, he called a kind of "biological nuclear spectroscopy." A noteworthy change from earlier presentations was that he described reversion rates as covering a very wide range but no longer as falling into three clusters of values. (Had his later results blurred this pattern?) Reflecting his most recent experiments on the microclusters, he pointed out a "complementarity" in the three meanings of *gene:*

1. for "gene"—definite length of chromosome
2. c[ross] o[ver] element much smaller (at least down to 20 nucleotides)
3. some mutations involve "large" pieces, anywhere within the length

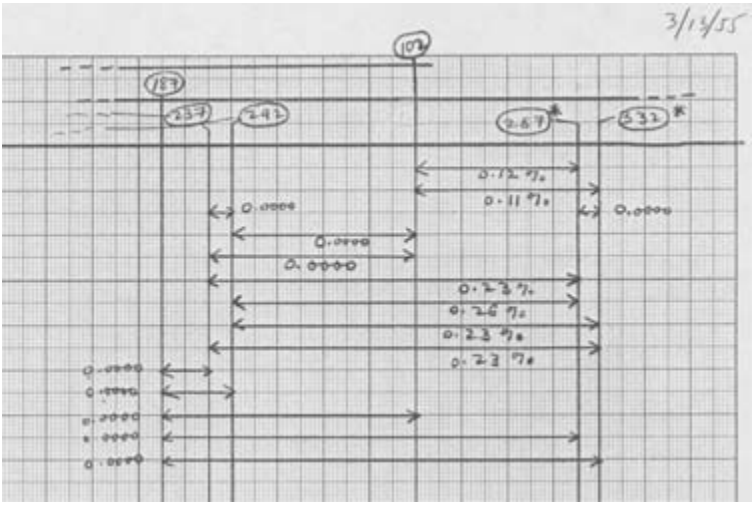


Figure 8.5. Maps of microclusters from crosses performed on March 13, 1955. (RN no. 4.)

This cryptic outline of what he actually said suggests that Benzer had quickly assimilated to his framework his discovery of the anomalous mutants stretching across all or large portions of a microcluster. Less easy to explain is what observation had induced him to reduce the upper limit of a crossover unit from 260 to 20 nucleotides.[15]

Satisfied that mutants extending over a portion of the chromosome occurred often enough to be significant, Benzer turned to other last-minute problems related to his manuscript. Among the unresolved questions was whether the "apparent reversions" of r mutants to wild type were true returns to the wild type or instead constituted recombinations with a mutant containing a suppressor of the rII phenotype. To test this possibility, on March 20 Benzer carried out back-crosses between mutants suspected to contain a suppressor and wild-type T4. If the suppressor was recombining with the wild type in large numbers, one ought to observe on B both r and r^+ plaques. Spot-testing several suspected suppressors with the wild type, he found that "all yield clear spots on B & K. Thus, 96 have been tested and no (ordinary) r recombinants found, thus if there is a 'suppressor' mutation present in the r_{198}, it is linked within 0.5% of the r198 site. This restricts it to the A segment of the rII region."[16]

David Krieg sent Benzer word that he, too, had found very few r-type recombinants in similar back-crosses. These results did not prove that the reversions were all genuine returns to the wild type, but

they did restrict the range of possible suppressors to closely linked mutants in the rII region.[17]

Benzer and Dottie left on the 11:32 A.M. train on March 22, 1955, for St. Louis, where he presented a seminar to an "active" group of microbiologists. They stayed for two days while he engaged in his customary intensive discussions—with Mel Cohn, an old confrere from the Institut Pasteur, Robert De Mars, and Arthur Kornberg. Departing by air for Pasadena, they were whisked immediately upon their arrival to a desert camping trip in Joshua Tree National Monument. The camping was delightful except for a mishap that befell Niels Jerne. After indulging in too much wine, Jerne decided to jump over the campfire but landed in a pile of stones and sprained his ankle. The next morning he crawled out of his sleeping bag with blood on his face, having attempted to go rock climbing. No permanent harm was done, however.[18]

Back in Pasadena the Benzers were invited to a number of parties.[19] The main event, however, was a three-day symposium that Delbrück had set up. On March 27 Naomi Franklin discussed the fine-structure mapping of the h (host-range) mutants of T2 that she had been carrying out with George Streisinger. Starting with an h mutant, she had isolated a group of mutants to wild type, which she had plated onto bacterial strains B and B/2 (that is, a strain of B on which T2 does not reproduce). Some of the mutants had turned out to be unstable, forming turbid plaques. She had spot-tested the h^+ mutants with one another. If recombination were to take place, the plaques would clear. The new h^+ mutants that had been spotted on themselves or with the original h^+ mutants had caused no clearing, but the turbid ones had given "lots." Crossing the h^+ mutants, adsorbing them on B, and plating them on B/2, she had found, after counting the plaques and subtracting the "background," that they formed a cluster separated by intervals varying from 1 to 2 percent crossover frequency. One of the intervals, however, was much smaller—0.004 percent. The figure was "uncertain" because of the background but nevertheless so small as to impress Benzer strongly. These two mutants were closer together than any he had yet observed in T4.[20]

Benzer was appreciative of the potential importance of Streisinger and Franklin's finding. He took careful notes of the talk and later wrote to Brenner and others that they had found a cluster that "so far looks very similar to rII." Their work also provided a good analogy to rII, "since one starts with an 'active' segment and isolates defec-

tive mutants, whose mutations can be located anywhere within the segment."[21]

He was impressed, but not entirely pleased. Streisinger had begun this work under the inspiration of Benzer's preliminary report on the rII region, and he was, in a sense, copying him. In short, Benzer was now "feeling the hot breath of competition."[22]

The following day Benzer gave his own seminar, focusing on his work from the time he had taken up the r mutants one year earlier. After thanking the "Dottie Foundation" for supplying cookies for the symposium (Delbrück had informed him that speakers at symposia at Caltech were responsible for providing them), Benzer related that the origin of his research on the rII region had been an "accidental discovery" prompted by a "parenthetical remark" in Streisinger's dissertation that high-titer r mutants can be prepared with some bacterial strains.[23]

Although he had avoided the word *gene* in his manuscript on the fine structure of a genetic region and in his NSF proposal, he had, as we have seen, continued to deal with the several meanings of the word in his seminar discussions. At Caltech he again brought up genes, but with a strong hint of irony. The experimental system that he had discovered, he pointed out, "turns out to be of interest in understanding of 'genes.' " He added, "We all know *genes*." They are a "Black Box." He mentioned four properties associated with the word: a "function (enzyme); duplication; mutation; and crossing over." In his discussion he probably stated the reasons that he had mentioned in his letter to Hershey in December for doubting the usefulness of the gene.[24]

After leading his audience through the properties of the rII mutants and the prediction that his system had the potential to reach the dimensions of neighboring nucleotides, he concluded that the unit of crossing over was "no larger than around a dozen nucleotide pairs" or maybe as few as three or four pairs according to the results that Franklin had presented the previous day. One of his conclusions was that the gene equals a "length of DNA."[25]

Dottie left for home after Benzer's seminar, while he "buckled down" to writing the final draft of his paper, which he gave to Delbrück to submit to *PNAS* just before leaving Caltech. "It was wonderful," he afterward wrote," to be in Pasadena after four years' absence. Perfect weather, hard discussions and paper-writing during the day, and chamber music [with Renato Dulbecco] every other evening."[26]

The extent to which the "hard discussions" with Delbrück and

others at Caltech further modified Benzer's manuscript cannot be fully ascertained. At any rate, the most significant changes were probably due to the further experiments he had carried out since sending his draft to others for comment. The three microclusters he had earlier included he replaced with the four he had constructed during March in which he represented the anomalous mutants as horizontal lines. These mutants he now described as "violently anomalous." The result can be understood, he commented, "if it is assumed that each [anomalous] mutation extends over a certain length of the chromosome, and production of the wild type requires recombination *between* those lengths."[27]

This situation, he went on, "raises the question of whether there exist true 'point' mutations (i.e., involving an alteration of only one nucleotide pair) or whether all mutations involve more or less long pieces of chromosome." This terminology suggests that he was aware of similar uncertainties discussed by Muller and others in the 1920s and 1930s (see Chapter 1). The issue was now replaying itself in the era of the double helix, an era in which theory supported the point mutation theory but practice seemed to indicate that, at most, the majority were of that type. Benzer was also aware, however, that the procedure he had followed—selecting only stable mutants in order to minimize inaccuracies—may have favored mutants containing gross chromosome alterations. In order to avoid such anomalies in future experiments, he thought it advisable to use only mutants that showed some reversion.

Like most modern scientific papers, Benzer's "Fine Structure of a Genetic Region in Bacteriophage" is a logical reconstruction of the experiments, observations, and arguments supporting his conclusions. It has little narrative structure and does not purport to follow the investigative pathway from which it came. Hidden within it, however, is a chronological structure based on the breaks in time over which he wrote its various sections. We cannot make a precise demarcation of these boundaries, because his later revisions altered earlier portions and because of the absence of the first and second drafts. Nevertheless, it appears that the early sections describing the rII region, the operation of his system, and the construction of the "larger-scale map" of eight mutants (which he had completed by early summer) must have been included in the first draft that he sent to Delbrück, Brenner, and others in July. The next section, however, the one about pseudoallelism and the cis-trans test, could only have been written in its final form after

Benzer had come to understand the significance of that test—that is, after the beginning of January 1955. "Rough-Mapping by the Spot-test" could have been written during the fall of 1954, probably in the early stages of his revision of the first draft. "Microclusters" was written as part of the second draft circulated in January but must have been largely rewritten at Caltech at the end of March. The paper thus contains various strata tacitly representing successive stages in the development of Benzer's ideas. A further differentiation was obscured by Benzer's decision to eliminate references to the gene. The paper bears only subtle traces of his earlier belief that the rII region constituted a functional gene. As a literary production it is less than unified. As a preliminary report on a method for fine-structure mapping, however, it was powerful, and it was seen that way by contemporaries.

From Pasadena Benzer flew to Berkeley, where he gave seminar at Wendell Stanley's Virus Laboratory. There he was also received with general enthusiasm. Although he was slightly annoyed, as he teasingly wrote to Sydney Brenner a few weeks later, to be told "Frankly, Sydney tells [the rII story] better than you do," overall Benzer judged his seminars to have been "pretty successful. In each case, at least one person whom I regard highly seemed excited about it. ([Martin] Kamen [in St. Louis], [Richard] Feynman [at Caltech], and [Roger] Stanier [at Berkeley])."[28]

When he returned home, Benzer was, nevertheless, "very anxious to start the mutants going again." Perhaps his anxiety was focused on the fact that he had still to fulfill his now long-held objective to reveal recombination rates of the order of a single nucleotide; it was perhaps also in part because of the competition posed by work such as that of Streisinger and Franklin.

Benzer had more than increasing numbers of mutants in mind as he resumed his work. On April 14 he wrote in his notebook a scheme of "pathways to explain the r story" (fig. 8.6).

> This scheme accounts for the behavior of rI, rII, and rIII mutants. A wild type phage can use the central pathway, leading to lysis inhibition and growth regardless of the blocks present in strain B or in strain K12S(λ). RIII or rI mutants block L[ysis] I[nhibition] but not growth (in all hosts), and rIIA or rIIB mutants fail to produce L.I. in B or growth in K. . . . Strain BB or K12S have intact upper and lower pathways so that the phage genotype is immaterial, except for rI mutants.[29]

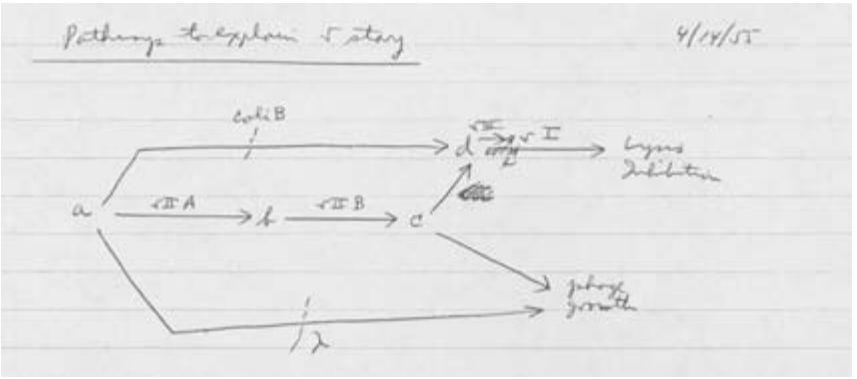


Figure 8.6. Schema of April 14, 1955, "Pathways to explain r story." (RN no. 5.)

What kind of pathways were these? Was Benzer thinking about bacterial metabolic pathways, and consequently the possession of enzymes by the respective mutants, enabling them to induce metabolic sequences in the respective strains with which they were matched? Although there is no evidence that he sought to test this overall scheme experimentally, Benzer began to "think through polypeptide-glasses" after his return from Caltech.[30] That is, he began to search for an "rII substance"—an enzyme that might correspond with the mutant genetic region.

On April 18 Benzer tested for the rII substance in T6r$^+$ (on B) lysate. Plating each of five rII mutants on bacterial strain K/6 without and with the lysate, he obtained with four of them only slight effects in comparing the first and second results. With high-titer r104, however, there was a "convincing effect of T6r$^+$ lysate on development of rII in K/6." When he tested the action of yeast extract on this effect the following day, he found none.[31]

Seeking now to locate the rII substance in the T6 lysate, Benzer plated r104 on the lysate itself, on a pellet formed by centrifuging the lysate, on the supernatant, and on broth. The latter two gave no effect. With the lysate there was "quite pronounced clearing + a few feeble plaques." With the pellet there was "stronger clearing . . . with many feeble plaques." Responding with enthusiasm to this lead, he wrote, "Conclusion: We have something here! It is sedimentable, similar to phage."[32]

Fractionating the lysate further on the twenty-fifth, he found some stimulation of growth in one plate representing pellet IB. On the next

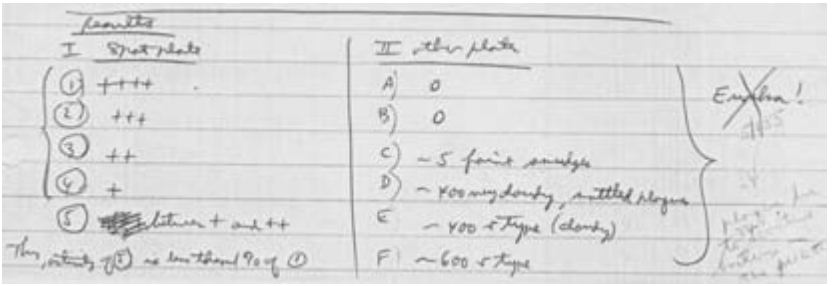


Figure 8.7. Results of spot tests performed with T4 rII mutant in the presence of lysate components on T6–infected cells. The initial results of April 29, 1955, induced the "Eureka!" reaction, later crossed out. (RN no. 5.)

day he tested "fractions of T6r+ lysate for stimulation of r104 growth on K/6." Comparing the decanted lysate, a high-speed pellet, a medium-speed pellet, and the supernatants, he concluded that "once the first heavy sediment (low speed) is removed the activity sticks with the phage. However, there would appear to be lots of activity lost in the low speed pellet (This activity could be spurious—due to presence of some B/6 in the lysate which can be utilized by T4r104 for growth.—Must test *filtered* lysate. Overall recovery of phage seems very good."[33]

On the twenty-ninth Benzer spotted a pattern in the sequence of spot tests of T4r104 with the successive supernatants and lysates of T6. With the lysate, the rI supernatant, rI pellet, rII lysate, rII supernatant, and rII pellet, respectively, there was a progressive decrease in activity. He was prompted to write "Eureka!" beside the result (fig. 8.7).[34] Repeating the test on the same day, however, he got a different result and had to conclude that "something must have been wrong with the previous test plate." He concluded from the new result that "there seems to remain some activity in the hi speed supernatant. This fraction might be easier to purify than the sediment. Also, it could be that the sediment contains rII enzyme (stuck to cell walls) while the supernate contains its product. Either of these could have X activity."[35]

Thus, although he had to cross out his Eureka moment, Benzer still believed at the end of April that he might "have something here." Might the rII enzyme actually be within reach? He wrote to a colleague on May 8:

> Since returning home I have been searching for the hypothetical "substance" produced by the rII "gene." So far, I have a few encouraging but not conclusive spots on a plate. The long range story will

> be the problem of deducing the relation between the specific structure of the genetic elements and the resultant structure of the protein it manufactures—i.e. the "code."[36]

Never a member of the small circle of insiders who had joined with George Gamow to solve the coding problem by the methods of statistical analysis and cryptanalysis, Benzer was nevertheless influenced by these efforts to produce such a code. By the end of 1954 Brenner had shown that no overlapping code could satisfy the known order of amino acids in such proteins as insulin, recently sequenced by Frederick Sanger.[37] Brenner was Benzer's closest contact among the members of this group. Benzer's approach to the problem was experimental. If he could identify an enzyme produced by a mutant whose base composition was known and correlate that with a change in composition of the amino acid, he could begin to correlate the two sequences. As his letter indicates, that would be the end of a "long story," but this intense if narrowly shared contemporary interest in the coding possibilities undoubtedly stimulated him to think he could take an important first step.

In a broader assessment of his position, Benzer wrote to Brenner on May 26:

> There are now so many mutants on the map that we can start looking at the thing through protein colored glasses, but no great truths have yet emerged from this. Twice repeats are commonplace (although some of the first ones were false—-due to overlapping mutations). I have one 16–fold repeat! This is nice, because one can measure forward and reverse (and back again) rates at the same site. But the mutation rates still make no sense in terms of simple-minded ideas (i.e. without recourse to very long range "paragenetic resonance" or "benzerine").[38]

These comments suggest that after sixteen months Benzer's r mutant project remained open-ended, its main goals still fluid. If he portrayed it regularly as intended to pursue mapping to the point at which genetic dimensions merged with molecular ones, he was sometimes more interested in other questions such as the relation between functional genetic sequences and amino acid sequences, the significance of the wide range of mutation and reversion rates, and the meaning of repeats. Moreover, that no great truths had yet emerged seems a confession of doubt about what his work to that point had revealed that was important.

François Jacob has contrasted vividly the triumphant "science of the day," with its secure achievements, to the tentative, stumbling "science of the night," or science still under way. The latter wanders blindly, corrects itself, doubts all. It is a workshop of the possible, where hypotheses remain vague presentiments, cloudy sentiments; where phenomena are solitary ideas unconnected with one another.[39] The contrast is striking but too strongly drawn. Science in process is built at the transition between the achieved and the unknown. Benzer's position in the spring of 1955 is representative. He had already established much on which he could count. Nevertheless, the way ahead remained unclear; it was a continual probing from the light into the darkness still ahead.

Despite his evident excitement about the possible existence of the rII substance, Benzer performed only a few more indecisive experiments in this direction at the beginning of May before returning to the mapping of more mutants. It may have been during his stay at Caltech in March that Delbrück made the remark, later quoted repeatedly by Benzer, that to achieve his objective would require "running the map into the ground."[40] One senses in his approach in May and June that Benzer was tightening his focus around the goal of reaching the limits of resolution of his method. He did not discard his protein-colored glasses, but he deferred the search for the enzyme. The most plausible reason for prioritizing mapping was that he had to prepare to give the first public presentation of his work to a large audience of diverse geneticists.

From May 12 through the first week of June, Benzer crossed rII mutants and mapped almost continuously. He crossed previous stocks of mutants and isolated further mutants. He did ordinary and spot-test crosses. There is no clear indication that he introduced new techniques, except that he utilized two of the long anomalous mutants, 164 (which stretched across segment A of the region) and r 196 (which stretched across segment B), to establish quickly to which segment a new mutant belonged. He was apparently establishing further microclusters similar to the four he had constructed in February.[41] Typical of the maps he drew are those shown in fig. 8.8.

On June 10, 1955, Benzer left by auto for Long Island to take part as an invited speaker in the annual Brookhaven Symposium on Biology; the subject for the year was mutation. The symposium included classical geneticists and phage biologists. According to the program, Benzer

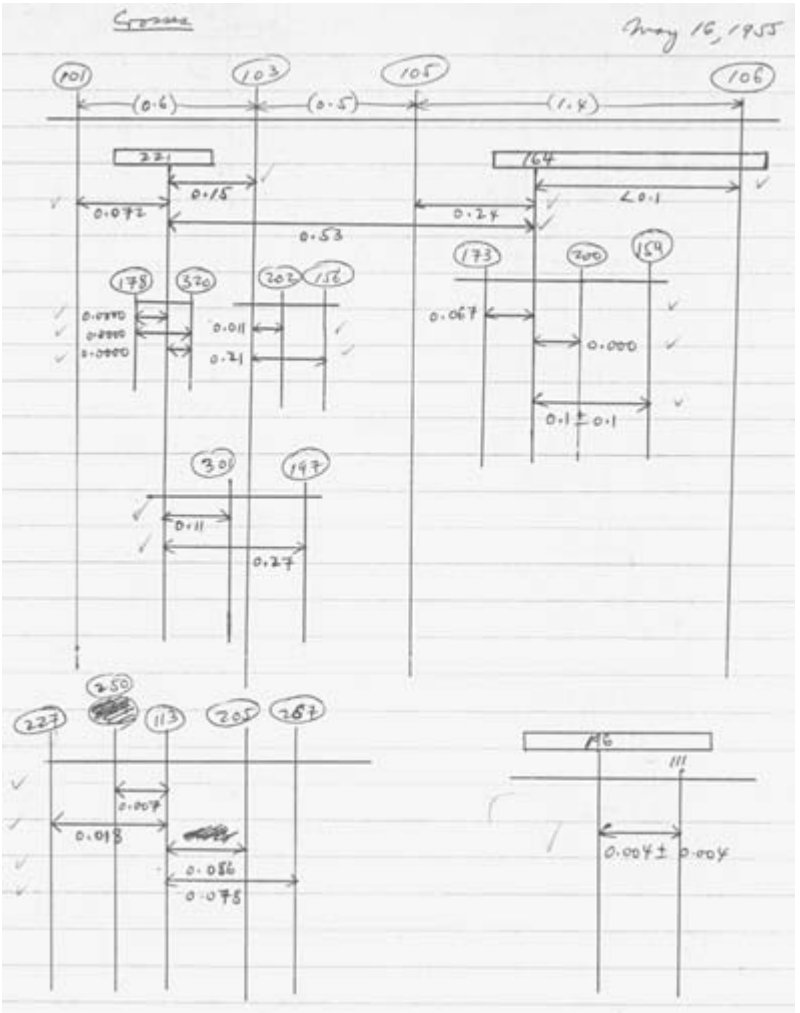


Figure 8.8. Typical map of the rII region drawn on May 16, 1955. (RN no. 5.)

gave the first paper in the opening session, chaired by Kurt Stern and titled "The Chemical and Physical Organization of Genetic Material and Its Bearing on the Mutation Process." Benzer's topic was genetic fine structure and its relation to the DNA molecule.

Beginning with a brief discussion of the concept of genes along chromosomes in *Drosophila* genetics, Benzer pointed out that genes themselves are black boxes and that there is a fine structure within the box. He hoped to establish that this structure, too, is linear. "The word

'gene,'" however, "has become a dirty word" because of the different meanings attributed to it. The difficulties arose particularly in the study of pseudoallelism.[42]

Turning next to phage genetics, Benzer discussed the properties of his rII mutant system that made it so useful for the study of these questions. Repeating his usual rough calculations, he concluded that there was an order of magnitude of 0.001 percent recombination per nucleotide pair. "But," he added, "one can detect 10^{-6}%!" This resolution was equivalent to 0.003 angstrom units, clearly absurd, because it is less than the size of an electron. "But, after all, DNA is made up of atoms," so we "can only resolve to units separable by genetic recombination." It becomes "fascinating to see whether [a] linear relation can be established" down to the finest details of the genetic material.[43]

Describing the procedures with which he had selected "clusters within clusters" for further examination, Benzer presented slide diagrams of the successive degrees of resolution he had reached (fig. 8.9). Summarizing his progress, he said: "From the smallest non-zero distance between mutations the chromosome would seem to be divisible by recombination at least to the extent of pieces ten nucleotides in length. The size of a mutation, i.e., the length of the chromosome in which alteration is apparent, varies from several hundred nucleotides for the 'anomalous' mutants to no more than ten for the others."[44] Slowly, he appeared to be closing in on his goal. Since writing his *PNAS* paper he had reduced his estimate of the lowest recombination number from a "dozen or so" to ten. He no longer seemed to doubt whether there were any point mutations, or if he did, he chose not to mention it.

According to his prepared notes for the talk, Benzer went on to discuss the cis-trans test for functional allelism, reverse mutations and their frequencies, and other related topics. He concluded by mentioning that "to make [an] enzyme, [you] need about 3 Nucleotide Pairs per amino acid, or 4000 nucleotide pairs for a protein." It "remains to be seen whether such a protein can be found and its properties correlated with genetic structure."[45] The published version of his paper, which was taken from a stenographer's transcription of his talk, does not include these topics. Perhaps he did not deliver this part of the talk, or else it was eliminated from the publication because of space considerations. At any rate, the original notes show that the identification of the enzyme and its correlation with the genetic material remained ensconced in his mind as the ultimate goal of the work.

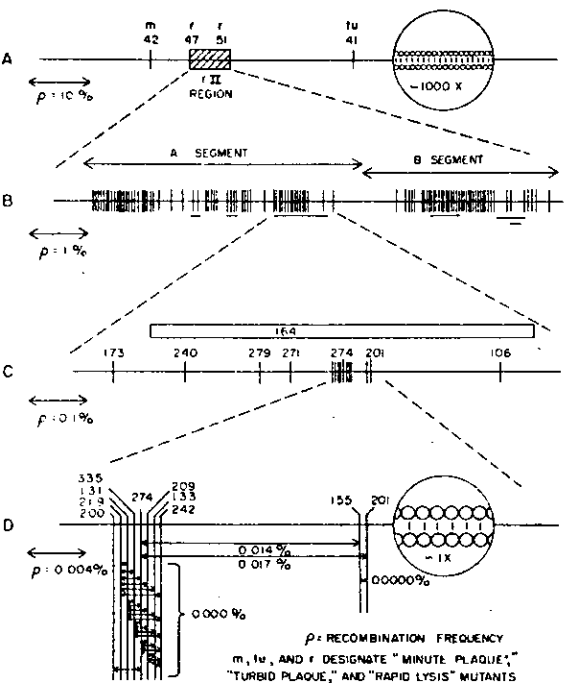


Figure 1. Linkage maps of T4 phage. A) The location of the rII region is shown with respect to mutants (mapped by Doermann and Hill (3)). The inset shows the estimated corresponding spacing of nucleotides (circles) according to the structure of Watson and Crick (4), on the assumption that the phage "chromosome" is such a fiber of DNA. B) The locations are shown of a number of rII mutants within the region. (There is some distortion due to the difficulty of drawing lines sufficiently close to one another.) All mutants within a segment are functionally related, whereas the A and B segments are functionally independent. A horizontal line represents an "anomalous" mutant which produces no wild recombinants in crosses with any mutant covered by its span. C) A selected group is shown on larger scale. Wild recombinants are given by r164 with r173 but not with any of the others. D) Further magnification. The group of eight mutants on the left all have similar reversion rates and give less than 10^{-3} percent recombination with each other in the indicated crosses. They are presumed to be recurrences of identical mutations. Similarly, r155 and r201 are probably recurrences.

Figure 8.9. Linkage map of T4 phage presented at a symposium held at the Brookhaven National Laboratory, in June 1955. (Reproduced by permission from *Brookhaven Symposia in Biology—Number 8: Mutation* [Upton, NY: Brookhaven National Laboratory, 1955], fig. 1, p. 4.)

At the end of the session in which Benzer gave his talk, an older man came up to offer him a piece of cake. The man was Hermann Muller, and the cake was a gesture of congratulation from the geneticist who had so long ago asked the questions about the nature of the gene that the younger man now seemed poised to answer.[46]

Figure 8.10. Photograph of the audience at the Brookhaven symposium, June 1955. Benzer is third from the left in the first row on the center aisle. (Reproduced by permission from *Brookhaven Symposia in Biology—Number 8: Mutation* [Upton, NY: Brookhaven National Laboratory, 1955], frontispiece.)

Until the Brookhaven Symposium, Benzer's work had been admired mainly by the small group of phage workers within which he moved and others who came into personal contact with them. Francis Crick followed the work with interest with Sidney Brenner as intermediary. George Beadle, head of the Biology Division at Caltech, was taken by its elegance. At Brookhaven, for the first time the work came directly to the attention of a broader array of "persons interested in basic genetic mechanisms."[47] As the foreword to the in-house publication of the papers noted, "The remarkable phenomenon of mutation continues to fascinate scientists of all disciplines." In this symposium "an attempt was made to examine the current status of research in . . . this flourishing subject. To accomplish this end, scientists working on diverse species and studying mutation from different perspectives were brought together to report recent findings and their bearing on earlier work." The sixteen invited papers were presented to an audience of more than three hundred and fifty scientists (fig. 8.10). Benzer's contribution to the flourishing field thus began to move beyond his own circles toward a larger position of promise for genetics and biology.

After the Brookhaven meeting Benzer spent one day in New York City, then went to Cold Spring Harbor to join bacteriophage workers there for discussions and seminars on current problems of microbial genetics, including in particular virus reproduction. On the way home he motored for several days through Canada, resuming his work at Purdue on July 7.

During his stay at Cold Spring Harbor, Benzer probably discussed with Alan Garen, who was working there, the possibility of Garen's coming to Purdue as a research associate funded by Benzer's National Science Foundation grant. On August 1 Garen received a formal offer to come for twelve months beginning on September 1, 1955.[48]

Between July 7 and July 20 Benzer carried out various crosses, including three factor crosses. They were not restricted to the r region but included some h and tu markers. It is not clear what the general thrust of his investigation was at this point. He remapped some of the mutants of Doermann, Hershey, Streisinger, and Martha Baylor. On July 25 he summarized the experiments he had done on "Enzyme X" in April and May. The following day he did spot tests with a "fraction of $T6r^+$ lysate grown on B/2,4,7" but found no effect of any of them. On the twenty-seventh he found that "B alone gives a perfectly lovely [positive] spot test!" Perhaps seeking to extract his enzyme X, he tested the effects of $CHCl_3$ on the cells of cultures infected with $T6r^+$. Three hours after infection he found about 2×10^8/ml. of "happy looking cells" under the microscope. After shaking the cultures in $CHCl_3$, he saw only "shriveled walls." Spot testing with the $CHCl_3$ at different concentrations showed "no effect visible in any of the spots." He tested the relation between time and the breakage of cells exposed to the reagent, and on the thirtieth he tested the $CHCl_3$ extract of $T6r^+$ grown on B on the development of plaques by r104 on K/6. "The extract is sterile," he wrote, "but shows no effect whatever on the development."[49] The investigation of the rII substance thus ended indecisively.

During the month of August Benzer did only a few experiments. On the twenty-third he traveled by air back to Cold Spring Harbor for the annual conference of phage workers. More than fifty attended from all over the United States and Canada. Benzer took notes on papers by Mark Adams, Ernest Pollard, Gunther Stent (on his work with ^{32}P irradiated "suicide" phage), Alan Garen (on *Salmonella*), Norton Zinder (on transduction), Seymour Lederberg, Joe Bertani, Doermann and his student Robert Edgar, Hershey, George Streisinger, and others. The main themes of the conference, as he summarized them, were "basic

problems of genetic structure and function, biochemical properties, and the effects of radiation."[50]

Going to a conference on an average of once every two months seemed to satisfy Benzer's need for frequent interaction with his colleagues. He had been cured of the isolationism he had once felt at Purdue when Jim Watson told him that he was leaving Caltech because "there is no one there to talk to."[51]

In September, October, and much of November Benzer crossed and calculated the recombination frequencies of more mutants, occasionally mapping them. By November he had treated more than four hundred mutants in this way. Most of them gave rates between 0.1 percent and 1 percent, with a few an order of magnitude smaller. He was apparently no closer to determining the distance between neighboring nucleotides than he had been the previous summer, and the procedures had become routine. By December he was probably looking for some further trick to shorten the task.[52]

In January the stable mutants showing no reversion had appeared as "violently anomalous" because they did not recombine with any of a number of nearby mutants, and Benzer had thought to avoid them in future experiments. Now, however, these same stable mutants seemed to point a way toward the more rapid location of these and other mutants. On December 2 he plated four stable mutants located on segment B, three of which (r362, r377, and r426) were new and one (r196) of which already known, on the usual lysogenic bacterial strain K. He then spot tested these with four other stable segment B mutants. The result is shown in the following table, with + and ++ representing degrees of recombination and 0 no recombination:

Spots	Plated	102	189	237	292
196	+	+	+	++	
362	0	0	0	0	
377	0	0	0	0	
426		+	+	++	++

He performed similar spot tests with B segment stable mutants on December third, then on the fifth tested which of seventeen "old" B segment mutants were "under 196," that is, whether they recombined with r196, a stable B segment mutant that extended a considerable length along segment B. Seven of them turned out to be under 196, ten not to be.[53]

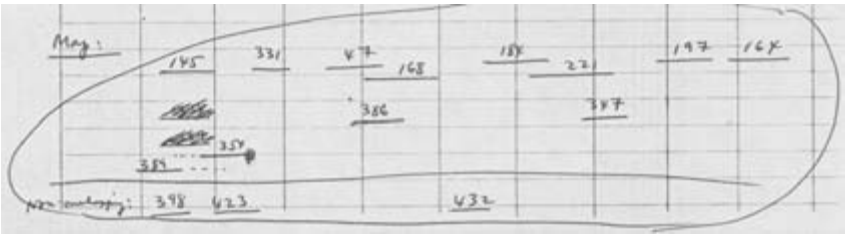


Figure 8.11. Rough map drawn on December 20, 1955, to show the extents of deletion mutations in the rII region of the T4 phage. (RN no. 5.)

By crossing stable mutants, regarded as deletions of a length of chromosomal material, with each other, Benzer was able to determine which of them overlapped with one another along the B segment. Once he had established an order of several of them, he could then order ordinary revertant mutants as well by crossing them with the deletions and finding out which ones they were "under." Thus, by what he later called a "divide and conquer" strategy,[54] he might be able quickly to locate a new mutant along the segment, greatly reducing the number of further crosses necessary to reach the lowest nonzero recombination values.

On December 20 Benzer tested fifteen B segment mutants with the stable mutant 426. All of them formed many plaques on K, and he concluded that "none of these is 'under 426.' " Turning to the A segment, he crossed eight "old" stable mutants with one another and seven new ones with each of the old ones. From the patterns in which they did or did not form plaques, he was able to ascertain which deletions overlapped with one another along the segment. Some of these were surprises: "Discovery!—184 overlaps 221, which was not previously suspected." Moreover, 354 and 389 overlapped 145; 386 overlapped both 47 and 168; 347 overlapped 221 but not 184; and three of the new stable mutants, 398, 423, and 432, "do not overlap any of [the] old stables."

As these tests began to reveal which stable mutants overlapped, they began to build a structure of overlapping and nonoverlapping deletions. Benzer represented them in a rough map (fig. 8.11). The last test Benzer undertook that year was to cross thirteen T4r mutants with r164 to see which ones were under the deletion known to extend along the A segment. Only 192 proved to be "under 164." As can be seen from the map, Benzer was building up a system of stable deletion mutants

that were beginning to cover the territory of the A and B segments of the rII region.[55] With each addition the system became a more powerful tool for locating any new mutant immediately according to the deletions under which it was located. That procedure invited him, in turn, to isolate many more mutants than he had done during the first two years of his project, in effect, to saturate certain portions of his map.

In January, February, and March 1956, Benzer crossed stable mutants with each other, in particular to establish which were under r164 and r196. These two extended a long way through segments A and B, respectively, and could quickly determine to which segment the mutant belonged. In April and May he isolated many more mutants, bringing his total to more one than one thousand. These, too, he located first as under or not under r164 or r196.[56]

As he identified shorter stable mutants in this way, Benzer was able to use them in turn to locate other mutants more closely within the segment. On April 29 he spot-crossed nineteen stable B mutants against one another. Two of them, r196 and r187, together covered about half of the segment (fig. 8.12). From the pattern of crosses that did and did not give recombinants, he was able to conclude that

> 196, 620, 426, 497, 739, and 199R254 were independent from each other and all others (that is, they recombined with all others except for 638).
>
> 102 and 575 were both under 187
>
> 237, 202, 467, 471, 625, 707, 565, 199R2411, 377, 102 were indistinguishable: that is, they gave identical patterns of recombination with the other mutants.
>
> Finally, 638 "is most anomalous!" It recombined with none of the others.

Representing these relations on a map, he drew the anomalous 638 all the way across the segment (fig. 8.13).[57]

Several more such experiments brought him a complete set of overlapping deletions for the B segment, though there remained gaps in the A segment. By spot testing new r mutants first with r164 and r196, then according to the segment in which this test placed them, with less extended deletion sequences from the same segment, Benzer was now in a position to achieve his goal: to saturate the map at a particular location where he could then test the limits of resolution of his system and the length of the unit of recombination. He had become able to do

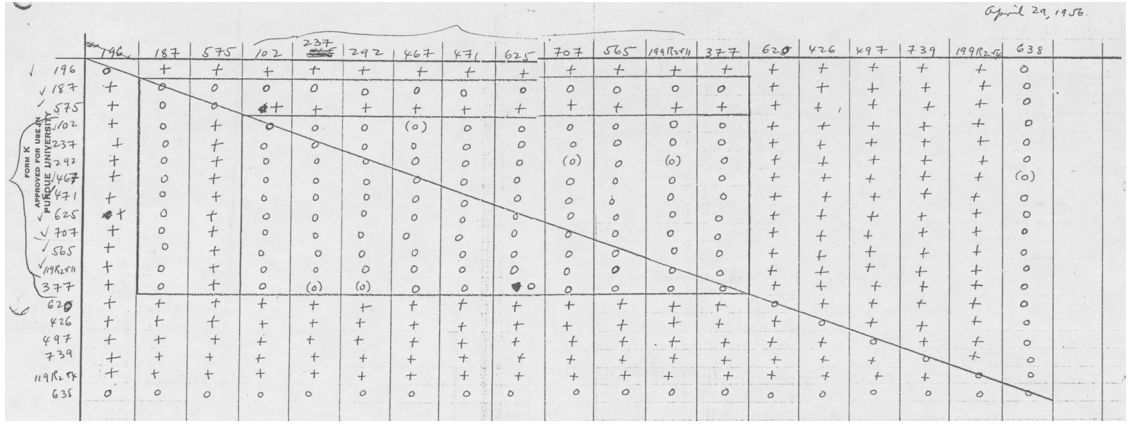


Figure 8.12. Deletion mapping across the rII region, April 27, 1956. (RN no. 6.)

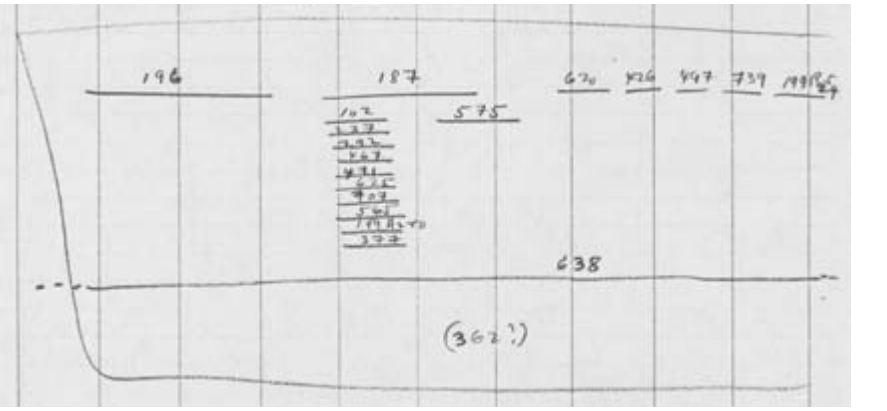


Figure 8.13. Map drawn on April 27, 19567 showing anomalous deletion mapping of T4 rII638. (RN no. 5.)

so not by gathering a large staff to proceed by brute force, as he had thought necessary a year earlier, but by ingenious methods enabling him to arrive there as quickly as possible by himself.

By the beginning of June Benzer was preparing his slides for a talk titled "The Nuclear Unit of Heredity" that he was scheduled to present on the nineteenth at the prestigious McCollum-Pratt Institute Symposium on Chemical Basis of Heredity. All the major figures in the emerging field of molecular biology would be there. For this occasion Benzer chose to break once and for all with the dirty word *gene*. In his introduction he pointed out that it was "significant that the word gene is missing from the title. Beadle had used it to pick out one function, but *one gene one enzyme* was a definition, not a hypothesis. We have even more kinds of alleles than of genes (multiple, pseudo, iso, complex, hetero, and homo). All this difficulty of terminology," he averred, "is a hangover from the classical 'gene,' which was a unit of recombination, of mutation, and of function."[58]

"We should say what we mean." With that he introduced the terms *recon, muton,* and *cistron*. The *recon,* or unit of recombination, "will be defined as the smallest element in the one-dimensional array that is interchangeable (but not divisible) by genetic recombination." The unit of mutation, or *muton,* he defined as the "smallest element that, when altered, can give rise to a mutant form of the organism."[59]

Benzer admitted that the unit of function was more difficult to define, because it depends on what level of function is meant. One may

have in mind a group of enzymatic steps leading to one particular physiological effect or the synthesis of one of the enzymes involved or the specification of a single critical amino acid. To define a functional unit independent of "biochemical information," he advocated the "elegant *cis-trans"* test of E. B. Lewis. If the infection of a bacterium by two viruses, one defective in one respect, the other in another, results in a mutant phenotype, then the two are regarded as defective in the same function, or "non-complementary." A map segment within which such noncomplementary mutants fall is defined as a cistron.[60]

We may note that in defining his unit of function as a cistron, Benzer was retreating from the position he had hoped to attain by identifying the rII substance. His original choice had been to propose the rII region as a unit of physiological function, which he would define biochemically by finding its enzyme. The cistron preserved the sense of an extended length of genetic material further divisible into mutons and recons at the expense of leaving unsaid what function it played.

In the body of his paper, Benzer once more described the features of his rII system that made extraordinarily high resolution possible. For his wide audience he was careful to include photographs illustrating the appearance of r and wild-type plaques (fig. 8.14), as well as the appearance of the spot tests (fig. 8.15).

The smallest recombination frequency found among the crosses would set the upper limit at the size of the recon. Up to that time that had been between r240 and r359 and amounted to around 0.02 percent. The main problem was how to relate distances on the genetic map to molecular distances. To complicate matters, the distances along the map were not strictly additive (large distances tended to be greater than the summation of small distances), that Cyrus Levinthal's recent experiments suggested that only 40 percent of the T4 DNA may carry genetic material, and that a "negative interference" tends to get worse at very small distances. A summation based on the smallest distance between markers would be several times as long as one based on distant markers.[61]

Making reasonable assumptions to get around these uncertainties, Benzer inferred that the ratio of recombination per unit molecular length at small distances would be 800 percent recombination divided by 80,000 nucleotide pairs, or 0.01 percent recombination per nucleotide pair. The smallest observed recombination frequency, 0.02 percent, would, therefore, give a recon "limited to no more that two nucleotide pairs."[62]

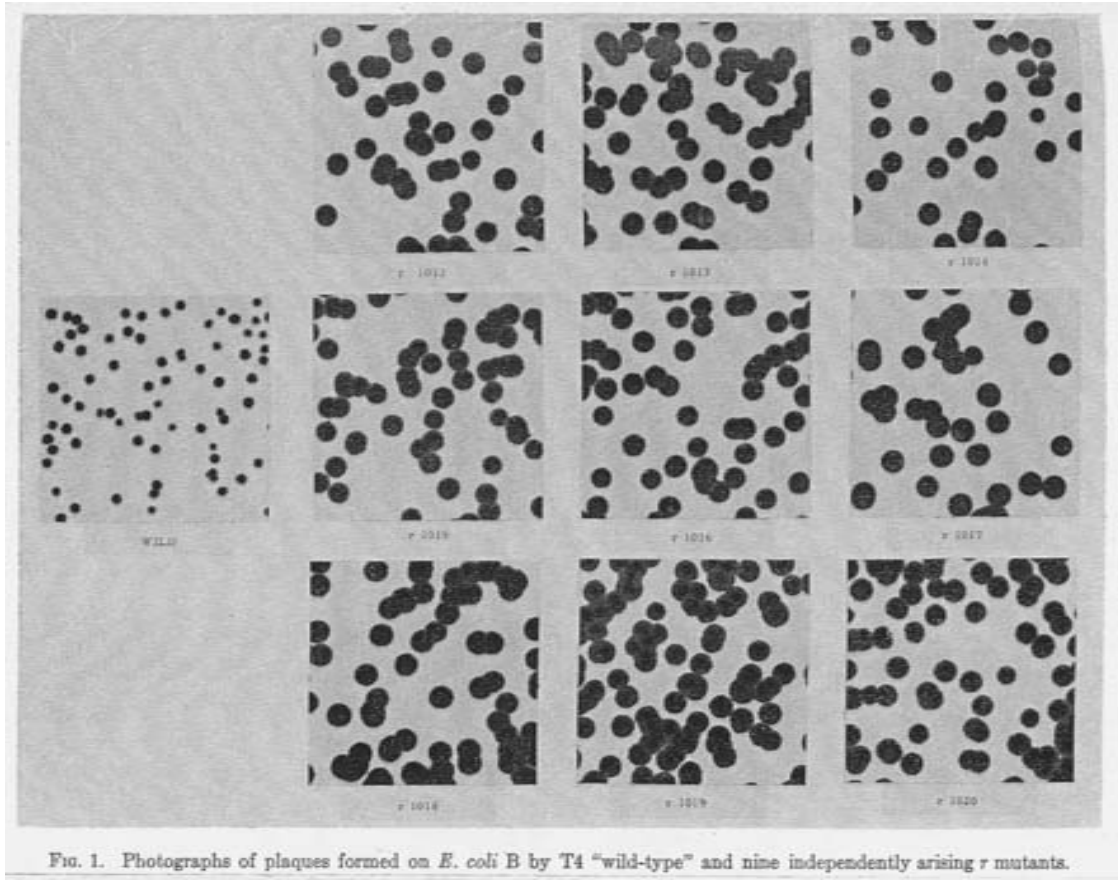


FIG. 1. Photographs of plaques formed on *E. coli* B by T4 "wild-type" and nine independently arising *r* mutants.

Figure 8.14. Photographs of plaques of wild-type and T4 r mutants on the permissive *E. coli* B host. (McElroy, William D., and Bentley H. Glass, eds. *A Symposium on the Chemical Basis of Heredity,* JHU 1957, p. 25, fig. 1. © 1957. Reprinted with permission of the Johns Hopkins University Press.)

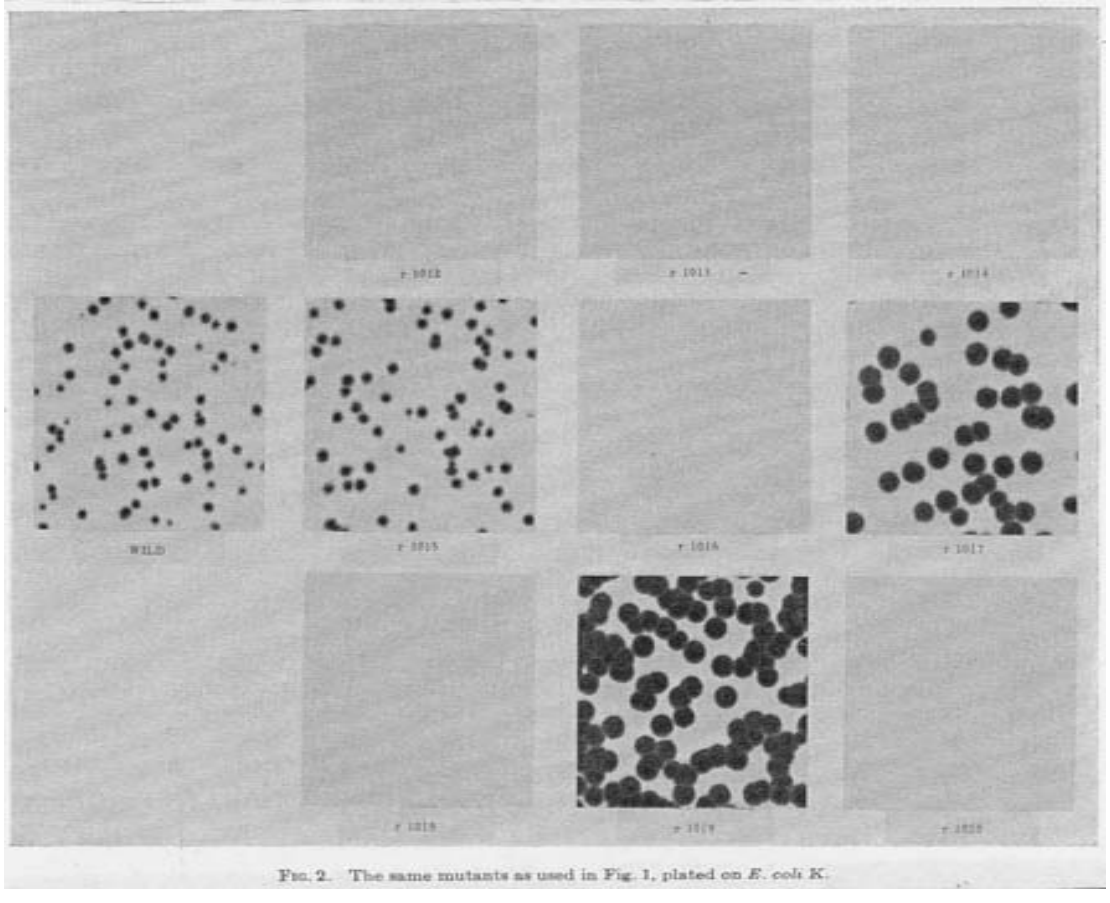


FIG. 2. The same mutants as used in Fig. 1, plated on *E. coli* K.

Figure 8.15. Photographs of plaques of wild-type and T4 r mutants on the nonpermissive *E. coli* K host. (McElroy, William D., and Bentley H. Glass, eds. *A Symposium on the Chemical Basis of Heredity,* JHU 1957, p. 26, fig. 2. © 1957. Reprinted with permission of the Johns Hopkins University Press.)

Stable mutons may involve map segments of various length, but reverting mutants had not been found to give a mutation size exceeding 0.005, an alteration of no more than five nucleotides. A cistron, on the other hand, turned out to be a complicated structure, modifiable by mutation at many locations. It seemed safe to conclude that in the A cistron alone there were more than one hundred locations at which a mutation leads to an observable phenotypic effect.[63]

George Beadle opened the discussion of Benzer's paper: "I would like to say that I don't want to get into any argument with Benzer about terminology. If he wants to call a functional unit a cistron that's all right with me. I would like to say, however, that I agree that his is a beautiful work."[64]

Beadle's judgment was widely shared among those concerned with the relation between DNA and the genetic material. Benzer had fallen just short of his goal. Had he been able to find one or more mutants with half the recombination frequency shown between r240 and r359 and then run into the limit that he had previously called "atomicity," the proof that the elementary units of recombination were base pairs would have been decisive. In view of the strong theoretical predisposition toward such a conclusion, however, he had come close enough to be persuasive, and his work did have the effect of leading many classical geneticists to accept the identification of their gene with a segment of DNA.

Raphael Falk, who was a postdoctoral fellow in the laboratory of Hermann Muller at the time Benzer's work appeared, has recalled that "The feeling of the beauty . . . was very strong at the time, it had a very important effect. Benzer's work was, in many aspects, the bridge that helped the transmission geneticists to adapt to the molecular age. The elegance of the experiments, the methodological reductionism which his physicist's concept brought into the idea was of great value. . . . I have rarely experienced that beauty, and the excitement which this beauty brought about."[65] The adaptation was not entirely painless. At the Brookhaven Symposium, at which Benzer had presented his paper in 1955, the molecular biologists had dominated events with a boisterous style of dress and manner that contrasted with the more sedate style of the classical geneticists. Muller and his students were largely ignored or viewed as figures from the dim past. In April 1957 Muller wrote Benzer:

> I have the greatest admiration for the recent work in microorganisms, most notably by yourself, and the supporting work on other material, as by Lewis and by Green on Drosophila, showing the divisibility of the gene as defined by allelism tests. The carrying of this down to or nearly to the level of the nucleotides is certainly a first-magnitude discovery. I think no one can realize this better than those of us who many years ago had been thinking along such lines but did not have the right methods to go far in that direction. Yet it makes us feel a little more futile than we really should feel when we find our earlier gropings along these lines ignored, just as though we had been too crude to conceive the possibilities.[66]

Feeling ignored by a younger generation is not uncommon among scientists and other scholars. As we have seen, Benzer did reach out to a number of geneticists to discuss his work and profited from their views. Nevertheless, by his own testimony, he never immersed himself thoroughly in the older literature and was undoubtedly unaware of the extent to which Muller had grappled with these issues. More broadly, Benzer was a member of a group known as the phage biologists, who prided themselves on breaking with the style of their predecessors and who thought they could quickly solve long-baffling problems with the tools they brought with them from physics. In doing so they attracted a great deal of admiration but also some ill will.

Chapter Nine

The Survival of the "Gene"

Benzer was not alone in these years in his quest to subdivide the genetic material into its finer subunits. His nearest counterparts, Streisinger and Franklin, did not carry their projects through to publication, but others were working on different organisms to the same general end. In 1955 Milislav Demerec was using autotrophic mutants of the bacterium *Salmonella typhimurium* to examine the "structure and organization of gene loci." Taking advantage of "transduction"—the process in which bacteriophage grown in one bacterial culture can carry a small fragment of the bacterial chromosome into the bacteria they subsequently infect—to create heterozygote regions of bacterial chromosomes, Demerec, too, was able to gather evidence that "a gene can by crossing over be subdivided into small, lineally arranged units" that he called "sites."[1] At Yale University, David Bonner used *Neurospora* to explore the possibility that the "genetic unit as described in physiological terms may well be comprised of specific genetically separable subunits."[2] In Glasgow, Guido Pontecorvo, J. A. Roper, and Robert H. Pritchard were studying the "organization of the chromosome over minute regions" in the plant mold *Aspergillus.* In linkage experiments with this organism, Pontecorvo and Roper were able to achieve recombination fractions of "the order of 10^{-6}."[3]

Into this field of activity Benzer's work was absorbed with alacrity and placed quickly at the forefront. Among the first to respond to it was Pontecorvo, whose thoughts about the different meanings of the word *gene* had been Benzer's initial inspiration to question its size. At the Cold Spring Harbor symposium on genetic mechanisms held in June 1956, Pontecorvo commented that "the remarkable work of Benzer (1955) on bacteriophage and Demerec (this Symposium) on S*almo-*

nella" provided support for the "model of the gene structure" that Pontecorvo had suggested in 1952.[4]

In a paper coauthored with Roper, Pontecorvo focused more particularly on Benzer's work: "We may . . . attempt to give a chemical meaning to what genetic analysis resolves, and perhaps even to what is the basis of the process on which genetic analysis is based, that is, crossing over. In this direction a remarkable paper by Benzer on bacteriophage has taken the lead. In the present paper, Benzer's attempt will be used in a comparative approach." From a comparison of the number of mutational sites identified up to that time with the recombination fractions observed in the experiments of Lewis on *Drosophila,* Benzer on T4, and Roper on *Aspergillus,* Pontecorvo calculated "what part of the genetic map the smallest recombination fractions" in each part of these organisms represented. Then, giving "chemical meaning" to these results by following "Benzer's argument," he calculated that the distances were equivalent to "eight nucleotide pairs for *Aspergillus,* 216 for *Drosophila,* and 12 for phage."[5]

It was not the argument of Benzer alone but the spreading influence of the Watson-Crick structures that had impelled Pontecorvo to entertain this chemical meaning of the results of genetical analysis. But this classical geneticist who had so recently asserted that genes are not atomic edifices at all made his concession guardedly. "All these crude estimates," he wrote, "are amusing exercises in numerology. They may even have some heuristic value if they are taken for nothing more than what they are." The analysis of the genetic map by crossing over possessed an "amazing sensitivity, a sensitivity which biochemistry still lacks."[6]

The historian Elof Carlson has written that it was the theoretical "daring" with which Benzer translated "genetic concepts . . . into molecular terms," rather than his experimental results by themselves, that distinguished his work from that of others such as the more conservative Demerec. Pontecorvo's reaction does support the view that the chemical meaning Benzer gave to genetical analysis forced classical geneticists to confront the "era of DNA."[7] But the elegance of the genetic experiments themselves, as Falk recalled, surely also contributed to the geneticists' acceptance of this conjuncture.

More problematic is the effect the introduction of three new and unfamiliar terms may have had. Benzer recalls no particular inspiration for their choice, except that as a physicist he would have been inclined to name a particle with a word ending in *-on*.

In a letter of recommendation, Delbrück wrote in 1957 that "Benzer picked up a casual observation regarding the behavior of certain mutants of T4 and developed from this a powerful method for the study of genetic fine structure, with sensational results. . . . He worked out the method in minute detail and the results obtained with it then strongly influenced our whole way of thinking (and terminology) concerning the organization and method of functioning of genetic material."[8] Delbrück's assessment was astute as usual. Benzer had not had a single dramatic discovery such as a fixed limit on the resolvability of the genetic material that would have identified the base pair as the fundamental unit. There was still the possibility that future researchers would attain that goal, but it really mattered less than Benzer's interpretation of the results gained up to that point. He was seeking to bring together the language of genetics and of "chemistry," encouraging geneticists and molecular biologists to use words that discarded what he regarded as vestiges of the past. His success at influencing the thinking of those around him was due to several factors: the beauty and elegance of the experiments themselves; their timeliness—others were also attempting what he was doing, but without the advantages of the special properties of his rII system that gave him a competitive edge; and his position within the community of scientists who were avidly interested in the nature of the genetic material.

We have seen that Benzer followed a well-recommended pathway from physics to phage biology. He began with the Cold Spring Harbor phage course, did the nearly mandatory apprenticeship at Caltech, and spent a year in Lwoff's highly regarded laboratory. He became a personal friend of some of the central figures in the group, such as Sydney Brenner and François Jacob, and was acquainted with the leading ones, Crick and Watson. He attended conferences and seminars assiduously, engaging in intensive discussions about his work and the work of others. In short, by virtue of the qualities of his personality as well as his work, he gained privileged access to the views of the group who defined the direction of movement at this formative period.

Paradoxically, Delbrück, at a deeper and more personal level, remained unpersuaded—not only of the meaning that Benzer and others gave to his work but of the basic assumptions about genetic mapping on which it rested. He gave voice to his doubts in a lecture titled "Atomic Physics in 1910 and Molecular Biology in 1957" that he presented at MIT in November 1957. *"If"* the linear genetic map whose fine structure Benzer had been studying "is a direct image of its mate-

rial carrier, the DNA molecule," Delbrück acknowledged, "then it can be estimated from Benzer's studies that a point on the genetic map cannot be larger than a few units of the molecule." The fact that points on the genetic map can be equated with points on the molecule, however, did not convince him that it necessarily followed that they were direct images of one another. Certain odd features, such as the positive correlation of markers that were very close together in three-factor crosses and the small heterozygote regions in phage genomes, had not yet been accounted for in this scheme. Possibly, he inferred, the topology of the material carrier is not really a "simple line" but involves branches or ladders. Or the "non-simplicity results from the fact that we are confronted with a true complementarity" in the sense of Niels Bohr's argument that "experimental situations used to define observations may be mutually exclusive." In this case, "a rational account which includes both sides of the picture, chemical structure *and* genetic map, may turn out to be an abstract one, not a visualizable one."[9]

Notes

Abbreviations

BPP	Benzer's personal papers
CTA	California Institute of Technology Archives
FLHP	Professional correspondence of Frederic Lawrence Holmes, Section of the History of Medicine, Yale University
MD, CTA	Max Delbrück Papers, in the California Institute of Technology Archives
RN	Research Notebooks, numbered in BPP

Chapter One. Classical Mendelian Genetics

1. See, among others, Robert Olby, *Origins of Mendelism,* 2d ed. (Chicago: University of Chicago Press, 1985); Elof Axel Carlson, *The Gene: A Critical History* (Philadelphia: Saunders, 1966); Garland E. Allen, *Thomas Hunt Morgan: The Man and His Science* (Princeton: Princeton University Press, 1978); Robert E. Kohler, *Lords of the Fly:* Drosophila *Genetics and the Experimental Life* (Chicago: University of Chicago Press, 1994); Jonathan Harwood, *Styles of Scientific Thought: The German Genetics Community, 1900–1933* (Chicago: University of Chicago Press, 1993).

2. Gregor Mendel, "Versuche über Pflanzen-Hybriden," *Verhandlungen der Naturforscher Vereins in Brünn* 4 (1865): 24–32.

3. See "The Unit-Character Fallacy," chap. 4 in Carlson, *The Gene,* pp. 23–32.

4. T. H. Morgan, "What Are 'Factors' in Mendelian Explanations?" *Proceedings of the American Breeder's Association* 5 (1909): 365–368 (emphasis in original).

5. Carlson, *The Gene,* pp. 20–22.

6. T. H. Morgan, "Sex Limited Inheritance in *Drosophila,*" *Science* 32 (1910): 120–122. For accounts of the circumstances surrounding this event,

see Allen, *Thomas Hunt Morgan,* pp. 148–153; Kohler, *Lords of the Fly,* pp. 37–43.

7. T. H. Morgan, "The Origin of Nine Wing Mutations in *Drosophila,*" *Science* 33 (1911): 496–499; "The Origin of Five Mutations in Eye Color of *Drosophila* and Their Modes of Inheritance," *Science* 33 (1911): 534–537.

8. A. H. Sturtevant, "The Linear Arrangement of Six Sex-Linked Factors in *Drosophila,* as Shown by Their Mode of Association," *Journal of Experimental Zoology* 14 (1913): 43–44.

9. T. H. Morgan, "Random Segregation Versus Coupling in Mendelian Inheritance," *Science* 34 (1911): 384.

10. Ibid. (emphasis added).

11. Carlson, *The Gene,* p. 43.

12. Ibid., pp. 58–65.

13. T. H. Morgan, "An Attempt to Analyze the Constitution of the Chromosome on the Basis of Sex-Limited Inheritance in *Drosophila,*" *Journal of Experimental Zoology* 11 (1912): 365–411.

14. Ibid., p. 384.

15. Ibid., p. 404.

16. See Elof Axel Carlson, *Genes, Radiation, and Society: The Life and Work of H. J. Muller* (Ithaca: Cornell University Press, 1981), p. 67.

17. Ibid., p. 66.

18. Sturtevant, "Linear Arrangement," pp. 45–52.

19. Ibid., pp. 49, 58.

20. Kohler, *Lords of the Fly,* pp. 56–65.

21. T. H. Morgan, "Factors and Unit Characters in Mendelian Heredity," *American Naturalist* 47 (1913): 5–10.

22. Ibid., pp. 10–16.

23. Ibid., p. 11.

24. Ibid., pp. 11–12.

25. A. H. Sturtevant, "A Third Group of Linked Genes in *Drosophila Ampelophila*," *Science* 37 (1913): 990–992.

26. H. J. Muller, "A Gene for the Fourth Chromosome of *Drosophila,*" *Journal of Experimental Zoology* 17 (1914): 325–334.

27. Ibid., pp. 334–335.

28. C. B. Bridges, "Direct Proof Through Non-Disjunction That the Sex-Linked Genes of *Drosophila* Are Borne by the X-Chromosome," *Science* 40 (1914): 107–109.

29. Carlson, *Genes, Radiation, and Society,* pp. 70–90.

30. Kohler, *Lords of the Fly,* pp. 62–64.

31. T. H. Morgan, A. H. Sturtevant, H. J. Muller, and C. B. Bridges, *The Mechanism of Mendelian Heredity* (New York: Johnson Reprint Corporation, 1972), p. vii.

32. Carlson, *Genes, Radiation, and Society,* p. 89.

33. Morgan et al., *Mechanism,* p. 3.

34. Ibid., pp. 204–205, 213–214.

35. Ibid., pp. 224–225.

36. Ibid., pp. 48–69, 131–135, 149–154. Although the volume briefly mentioned experiments performed by Bridges, Muller, and Sturtevant, it did not associate the theoretical inferences drawn from them with any of the four coauthors. This feature of the book fit with Sturtevant's recollection that ideas were so freely exchanged in the fly room that none of them was concerned about which of them originated them. As Carlson and Allen have pointed out, however, Muller felt that his own contributions to the theoretical underpinnings of the mechanism of Mendelian inheritance was too little recognized in this practice. See Carlson, *Genes, Radiation, and Society,* pp. 77–90; Allen, *Thomas Hunt Morgan,* pp. 203–208.

37. Morgan et al., *Mechanism,* pp. 60, 131–132.

38. Ibid., p. 226.

39. H. J. Muller, "The Mechanism of Crossing-Over," *American Naturalist* 50 (1916): 193–221, 284–305, 350–366, 421–434.

40. T. H. Morgan, "The Theory of the Gene," *American Naturalist* 51 (1917): 513–515.

41. Ibid., pp. 515–544.

42. Ibid., pp. 518–519.

43. Ibid., p. 535 (emphasis in original).

44. T. H. Morgan, *Theory of the Gene* (New Haven: Yale University Press, 1926), p. 25 (emphasis in original); Lindley Darden, *Theory Change in Science: Strategies from Mendelian Genetics* (New York: Oxford University Press, 1991), pp. 3, 168–199.

45. Morgan, *Theory of the Gene,* p. 1.

46. Ibid., p. 25.

47. Ibid., pp. 1, 309–310.

48. H. J. Muller, "Variation Due to Change in the Individual Gene," *American Naturalist* 56 (1922): 32–34.

49. Ibid., pp. 36–42.

50. Ibid., p. 43.

51. Ibid., pp. 44–49.

52. Ibid., p. 49.

53. Carlson, *Genes, Radiation, and Society,* pp. 137–146.

54. H. J. Muller, "Artificial Transmutation of the Gene," *Science* 66 (1927): 84–87.

55. Carlson, *Genes, Radiation, and Society,* p. 150.

56. H. J. Muller, "The Production of Mutations by X-rays," *Proceedings of the National Academy of Sciences* 14 (1928): 714–717.

57. Ibid., pp. 723–724.

58. Ibid., pp. 720–721.

59. H. J. Muller, "The Effects of Roentgen Rays upon the Hereditary Material," in *The Science of Radiology* (Springfield, IL: Charles C. Thomas, 1932), pp. 305–318; Carlson, *The Gene,* pp. 131–133.

60. T. S. Painter, "A New Method for the Study of Chromosome Rearrangements and the Plotting of Chromosomal Maps," *Science* 78 (1933): 585–586.

61. Carlson, *Genes, Radiation, and Society,* p. 195.

62. Ibid., pp. 184–203.

63. H. J. Muller and A. A. Prokofyeva, "The Individual Gene in Relation to the Chromomere and the Chromosome," *Proceedings of the National Academy of Sciences* 21 (1935): 16; H. J. Muller, "On the Dimensions of Chromosomes and Genes in Dipteran Salivary Glands," *American Naturalist* 69 (1935): 405.

64. Muller and Prokofyeva, "Individual Gene," pp. 16–26.

65. Carlson, *The Gene,* pp. 131–139.

66. Muller, "On the Dimensions of Chromosomes," pp. 409–411.

67. Muller, "Effects of Roentgen Rays," p. 305.

68. See a similar comment by Carlson, *The Gene,* p. 128.

69. Daniel Raffel and H. J. Muller, "Position Effect and Gene Divisibility Considered in Connection with Three Strikingly Similar Scute Mutations," *Genetics* 25 (1940): 539–566.

70. Ibid., p. 569.

71. Ibid., p. 570.

72. Ibid., pp. 570–578.

73. Carlson, *The Gene,* pp. 124–126.

74. Raffel and Muller, "Position Effect," p. 576 (emphasis in original).

75. Ibid., p. 563n1; Carlson, *Genes, Radiation, and Society,* pp. 229–249.

76. H. J. Muller, "Discriminatory Effect of Ultraviolet Rays on Mutation in *Drosophila*," *Nature* 143 (1939): 83–84.

77. Kohler, *Lords of the Fly,* pp. 133–293.

78. Carlson, *The Gene,* pp. 156, 253–254.

Chapter Two. Genetics and the Phage Biologists

1. H. J. Muller, "Physics in the Attack on the Fundamental Problems of Genetics," *Scientific Monthly* 44 (1936): 210–214.

2. Elof Axel Carlson, *Genes, Radiation, and Society: The Life and Work of H. J. Muller* (Ithaca: Cornell University Press, 1981), pp. 187–188.

3. K. G. Zimmer, "The Target Theory," in *Phage and the Origins of Molecular Biology,* ed. John Cairns, Gunther S. Stent, and James D. Watson (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory of Quantitative Biology, 1966), pp. 33–36.

4. Carlson, *Radiation, Genes, and Society,* p. 188. One should evaluate this statement in the context in which Muller made it: as part of a toast at Delbrück's wedding in 1941.

5. Zimmer, "Target Theory," pp. 36–37.

6. Seymour Benzer, "Adventures in the rII Region," in *Phage and the Origins of Molecular Biology,* ed. John Cairns, Gunther S. Stent, and James D. Watson (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory of Quantitative Biology, 1966), p. 158.

7. Ernst Peter Fischer and Carol Lipson, *Thinking About Science: Max Delbrück and the Origins of Molecular Biology* (New York: Norton, 1988), pp. 85–86 (emphasis in original). The directly quoted passages are translated from the German by Fischer.

8. Fischer and Lipson, *Thinking,* p. 69.

9. N. W. Timoféeff-Ressovsky, K. G. Zimmer, and M. Delbrück, "Über die Natur der Genmutation und der Genstruktur," *Nachrichten von der Gesellschaft der Wissenschaften zu Göttingen: Nachrichten aus der Biologie* 1 (1934–1935): 189–245. I thank Kathryn Olesko for obtaining a copy of this paper for me from the Göttingen University Library.

10. Ibid., pp. 190–217.

11. Ibid., pp. 217–223.

12. Ibid., pp. 223–225.

13. Ibid., pp. 225–226.

14. Ibid., p. 226 (emphasis in original).

15. Ibid., pp. 227–234.

16. Ibid., p. 226.

17. Fischer and Lipson, *Thinking,* pp. 95–97.

18. See Angela N. H. Creager, *The Life of a Virus: Tobacco Mosaic Virus as an Experimental Model* (Chicago: University of Chicago Press, 2002), pp. 57–78.

19. The text of a later English translation of the essay is published in Fischer and Lipson, *Thinking,* pp. 99–101.

20. Muller, "Physics," pp. 213–214.

21. Ibid., p. 109.

22. William C. Summers, "How Bacteriophage Came to Be Used by the Phage Group," *Journal of the History of Biology* 26 (1993): 261–262.

23. Ibid., pp. 256–267; Fischer and Lipson, *Thinking,* pp. 112–117.

24. Summers, "How Bacteriophage Came to Be Used," p. 258.

25. E. L. Ellis and M. Delbrück, "The Growth of Bacteriophage," *Journal of General Physiology* 22 (1938–1939): 365–384 (quotation is on p. 367).

26. Fischer and Lipson, *Thinking,* p. 114.

27. Ellis and Delbrück, "Growth," pp. 365–384.

28. Summers, "How Bacteriophage Came to Be Used," pp. 258–259, 264.

29. Fischer and Lipson, *Thinking,* pp. 122–124.

30. M. Delbrück, "Adsorption of Bacteriophage Under Various Physiological Conditions of the Host," *Journal of General Physiology* 23 (1939–1940): 631–642; Delbrück, "The Growth of Bacteriophage and Lysis of the Host," *Journal of General Physiology* 23 (1939–1940): 643–660.

31. Summers, "How Bacteriophage Came to Be Used," p. 258.

32. Fischer and Lipson, *Thinking,* p. 124.

33. Ibid., pp. 125–128.

34. M. Delbrück, "Radiation and the Hereditary Mechanism," *American Naturalist* 74 (1940): 350–362.

35. Ibid., p. 361.

36. Ibid.

37. M. Demerec, "Foreword," *Cold Spring Harbor Symposia on Quantitative Biology* 9 (1941): v–vi.

38. M. Delbrück, "A Theory of Autocatalytic Synthesis of Polypeptides and Its Application to the Problem of Chromosome Reproduction," *Cold Spring Harbor Symposia on Quantitative Biology* 9 (1941): 122–124; H. M. Kalckar, "The Nature of Energetic Coupling in Biological Syntheses," *Chemical Reviews* 28 (1941): 76–77, 143–144; K. Linderstrøm-Lang, "Proteolytic Enzymes," *Annual Review of Biochemistry* 8 (1939): 48–50.

39. Delbrück, "Autocatalytic Synthesis," p. 123.

40. Ibid., p. 124.

41. Ibid., pp. 124–126.

42. H. J. Muller, "Résumé and Perspectives of the Symposium on Genes and Chromosomes," *Cold Spring Harbor Symposia on Quantitative Biology* 9 (1941): 305–306.

43. H. M. Kalckar, "High Energy Phosphate Bonds: Optional or Obligatory?" in *Phage and the Origins of Molecular Biology,* ed. John Cairns, Gunther S. Stent, and James D. Watson (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory of Quantitative Biology, 1966), pp. 43–44.

44. Fischer and Lipson, *Thinking,* p. 126.

45. M. Delbrück, "Bacterial Viruses (Bacteriophages)," *Advances in Enzymology* 2 (1942): 1–32 (quotationss are on pp. 3–4).

46. Ibid., p. 30.

47. Ibid., p. 28.

48. S. Luria, *A Slot Machine, a Broken Test Tube: An Autobiography* (New York: Harper and Row, 1984), pp. 11–22.

49. Ibid., pp. 21–24.

50. Ibid., pp. 69–70; E. Wollman, F. Holweck, and S. Luria, "Effect of Radiations on Bacteriophage C_{16}," *Nature* 145 (1940): 935–936.

51. Luria, *Slot Machine,* pp. 30–31, 70.

52. M. Delbrück and S. Luria, "Interference Between Bacterial Viruses: I. Interference Between Two Bacterial Particles Acting Upon the Same Host, and the Mechanism of Virus Growth," *Archives of Biochemistry* 1 (1942): 111–141.

53. Ibid., pp. 111–141.

54. Ibid., pp. 136–137.

55. S. Luria and M. Delbrück, "Interference Between Inactivated Bacterial Virus and Active Virus of the Same Strain and of a Different Strain," *Archives of Biochemistry* 1 (1942): 207–218.

56. Thomas F. Anderson, "Electron Microscopy of Phages," in *Phage and the Origins of Molecular Biology,* ed. John Cairns, Gunther S. Stent, and James D. Watson (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1966), pp. 63–63. See also Nicolas Rasmussen, *Picture Control: The Electron Microscope and the Transformation of Biology in America, 1940–1960* (Stanford: Stanford University Press, 1997).

57. Anderson, "Electron Microscopy," pp. 65–66; S. Luria, M. Delbrück, and T. F. Anderson, "Electron Microscope Studies of Bacterial Viruses," *Journal of Bacteriology* 46 (1943): 57–76 (quotations are on pp. 60, 65).

58. Luria, Delbrück, and Anderson, "Electron Microscope," pp. 63, 64; Anderson, "Electron Microscopy," pp. 66–71.

59. Delbrück and Luria, "Interference Between Two Bacterial Particles," p. 113.

60. Luria, *Slot Machine,* pp. 74–78.

61. Ibid., p. 79.

62. S. Luria and M. Delbrück, "Mutations of Bacteria from Virus Sensitivity to Virus Resistance," *Genetics* 18 (1943): 491–511.

63. Ibid., p. 492.

64. Fischer and Lipson, *Thinking,* p. 142.

65. J. B. S. Haldane, *New Paths in Genetics* (New York: Harper and Brothers, 1942), pp. 22–24, 47–82.

66. G. W. Beadle, "Biochemical Genetics: Some Recollections," in *Phage and the Origins of Molecular Biology,* ed. John Cairns, Gunther S. Stent, and James D. Watson (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1966), pp. 26–27; Robert E. Kohler, *Lords of the Fly:* Drosophila *Genetics and the Experimental Life* (Chicago: University of Chicago Press, 1994), pp. 211–227; G. W. Beadle and Boris Ephrussi, "The Differentiation of Eye Pigments in *Drosophila* as Studied by Transplantation," *Genetics* 21 (1936): 225–247.

67. Beadle and Ephrussi, "Differentiation," pp. 242–244.

68. Kohler, *Lords of the Fly,* pp. 224–226.

69. G. W. Beadle and E. L. Tatum, "Experimental Control of Development and Differentiation," *American Naturalist* 75 (1941): 107–116.

70. Ibid., pp. 109, 115.

71. Beadle, "Biochemical Genetics: Some Recollections," p. 29.

72. Ibid., pp. 24–25.

73. G. W. Beadle and E. L. Tatum, "Genetic Control of Biochemical Reactions in *Neurospora*," *Proceedings of the National Academy of Sciences, U.S.A.* 27 (1941): 409–502.

74. G. W. Beadle, "Genetics and Metabolism in *Neurospora*," *Physiological Reviews* 25 (1945): 646–650.

75. Beadle, "Biochemical Genetics: Some Recollections," p. 29.

76. Beadle and Tatum, "Genetic Control," p. 502.

77. Ibid., p. 504.

78. Beadle, "Biochemical Genetics: Some Recollections," p. 30.

79. G. W. Beadle, "Biochemical Genetics," *Chemical Reviews* 37 (1945): 15–96 (quotation is on p. 19).

80. Elof Axel Carlson, *The Gene: A Critical History* (Philadelphia: Saunders, 1966), pp. 166–173.

81. Beadle and Tatum, "Genetic Control," p. 499.

82. Ibid., p. 500.

83. Beadle, "Biochemical Genetics," p. 16; Carlson, *The Gene,* pp. 172–173.

84. M. Delbrück, "Problems of Modern Biology in Relation to Atomic Physics," 2d printing (distributed in typescript form by the Committee on Growth of the National Research Council, 1946), pp. 1–2.

85. Ibid., pp. 3–4.

86. Ibid., p. 4 (emphasis in original).

87. Ibid., pp. 6–10.

88. Ibid., pp. 11–25.

89. Ibid., pp. 26–28.

90. Ibid., pp. 29–31; Curt Stern, "Genic Action as Studied by Means of the Effects of Different Doses and Combinations of Alleles," *Genetics* 28 (1943): 441–475.

91. Delbrück, *Problems,* p. 31.

92. Ibid., pp. 31–33. The exception was that the original source for Delbrück's views about the resonance structure of the radical had been Linus Pauling's *The Nature of the Chemical Bond* (Ithaca: Cornell University Press, 1939).

93. Delbrück, *Problems,* p. 34.

94. Ibid., pp. 34–37.

95. Ibid., pp. 37–39.

96. Ibid., pp. 40–44.

97. Ibid., frontispiece.

98. Ibid., p. 45.

99. Delbrück, "Bacterial Viruses," pp. 21–22.

100. S. Luria, "Mutations of Bacterial Viruses Affecting Their Host Range," *Genetics* 30 (1945): 84–99.

101. Ibid., p. 97.

102. F. W. Stahl, ed., *We Can Sleep Later: Alfred D. Hershey and the Origins of Molecular Biology* (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2000), pp. 3–4.

103. Luria, *Slot Machine,* pp. 42, 81.

104. A. D. Hershey, "Mutation of Bacteriophage with Respect to Type of Plaque," *Genetics* 31 (1946): 620–640.

105. Ibid., p. 639.

106. M. Delbrück and W. T. Bailey Jr., "Induced Mutations in Bacterial Viruses," *Cold Spring Harbor Symposia on Quantitative Biology* 11 (1946): 33–37.

107. A. D. Hershey, "Spontaneous Mutations in Bacterial Viruses," *Cold Spring Harbor Symposia on Quantitative Biology* 11 (1946): 67–76.

108. Ibid., p. 75.

109. Fischer and Lipson, *Thinking,* pp. 153–154.

110. Hershey, "Mutation of Bacteriophage," pp. 20–21.

111. M. Demerec and U. Fano, "Bactriophage Mutants in *Escherichia Coli,*" *Genetics* 30 (1945): 119–120.

112. Fischer and Lipson, *Thinking,* pp. 159–162; Kalckar, "High Energy Phosphate Bonds," p. 43.

113. Ibid., pp. 176–177.

114. S. S. Cohen, 'The Synthesis of Bacterial Viruses in Infected Cells," *Cold Spring Harbor Symposia on Quantitative Biology* 12 (1947): 35–48; S. S. Cohen to F. L. Holmes, August 28, 2002, FLHP.

115. Fischer and Lipson, *Thinking,* p. 164.

116. Ibid., p. 162. Aaron Novick, on whose description of the course in 1947 Fischer and Lipson drew for this characterization, stated only that they were introduced by Delbrück to a "biology made comfortable for people with backgrounds in the physical sciences." Aaron Novick, "Phenotypic Mixing," in *Phage and the Origins of Molecular Biology,* ed. John Cairns, Gunther S. Stent, and James D. Watson (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1966), p. 134.

117. Erwin Schrödinger, *What Is Life? The Physical Aspect of the Living Cell* (Cambridge: Cambridge University Press, 1946), pp. 1–2.

118. Ibid., pp. 7–17. In the following paragraphs I discuss primarily the chapters of the book treating genetics and related problems. For an analysis of the chapters dealing with the more general problem of biological order and those who responded to these aspects of the book, see E. J. Yoxen, "Where Does Schroedinger's 'What Is Life?' Belong in the History of Molecular Biology?" *History of Science* 17 (1979): 17–52.

119. Schrödinger, *What Is Life?* p. 19.

120. Ibid., pp. 18–45.

121. Ibid., p. 30.

122. Yoxen, "Where Does Schroedinger's 'What Is Life?' Belong?" p. 34.

123. Schrödinger, *What Is Life?* pp. 42–45.

124. Ibid., p. 56.

125. Ibid., pp. 56–67.

126. Ibid., p. 57.

127. Ibid., pp. 60–62.

128. Yoxen suggests that the source for Schrödinger's idea may have been a brief passage about the self-reproduction of the chromosomal gene in the neuro-

physiologist Charles Sherrington's philosophical treatise *Man on His Nature.* See Yoxen, "Where Does Schroedinger's 'What Is Life?' Belong?" pp. 34–35.

129. M. Delbrück, "What Is Life? and What Is Truth?" *Quarterly Review of Biology* 20 (1945): 370–372.

130. Ibid., p. 371.

131. For a recent critique of these retrospective attributions, see Lily Kay, *Who Wrote the Book of Life? A History of the Genetic Code* (Stanford: Stanford University Press, 2000), pp. 59–66. Kay contends that Schrödinger's ideas about "code-scripts" came from a much older discourse comparing memory to an engram and had little in common with views about genetic codes originating after 1953.

132. Robert Olby, *The Path to the Double Helix* (Seattle: University of Washington Press, 1974), p. 246.

Chapter Three. The Physicist Becomes a Phage Biologist

1. Jonathan Weiner, *Time, Love, Memory: A Great Biologist and His Quest for the Origins of Behavior* (New York: Knopf, 1999), p. 35; S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 3.

2. Weiner, *Time,* pp. 36–37; S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 3.

3. S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 3.

4. Ibid.

5. Weiner, *Time,* pp. 40–42.

6. S. Benzer, interview with F. L. Holmes, January 5, 2002, tape 2.

7. Ibid., tape 3.

8. Ibid.

9. Weiner, *Time,* pp. 42–45.

10. Dorothy Klem to S. Benzer, December 4, 1946, BPP.

11. S. Benzer, interview with F. L. Holmes, January 5, 2002, tape 2.

12. John L. Danforth to S. Benzer, November 1, 1945, BPP.

13. P. Edelman to S. Benzer, February 9, 1946; S. Benzer to "Dr. Darrow," May 1, 1946; S. Benzer to J. H. Dellinger, February 12, 1946, BPP.

14. S. Benzer to G. P. Harnwell, November 27, 1946, BPP.

15. S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 3.

16. Weiner, *Time,* p. 45.

17. S. Benzer to K. S. Cole, March 3, 1947, BPP.

18. K. S. Cole to S. Benzer, March 5, 1947, BPP.

19. Frank C. Hockman to W. L. Ayres, April 26, 1947, BPP.

20. John L. Lewine to S. Benzer, July 26, 1947, BPP.

21. M. M. D. Williams to S. Benzer, October 3, 1947; Benzer to M. M. D. Williams, October 10, 1947, BPP.

22. M. M. D. Williams to S. Benzer, December 17, 1947, BPP.

23. S. Benzer to S. Luria, December 1, 1948, BPP.
24. S. Benzer to A. Hollaender, November 13, 1947, BPP.
25. S. Benzer to S. Luria, January 5, 1948, BPP.
26. S. Luria to S. Benzer, January 6, 1948, BPP.
27. S. Benzer, interview with F. L. Holmes, January 5, 2002, tape 2.
28. S. Benzer to G. W. Beadle, January 23, 1948, BPP.
29. S. Luria to S. Benzer, February 12, 1948, March 20, 1948; S. Benzer to S. Luria, March 22, 1948, BPP.
30. K. Lark-Horovitz to Elizabeth Randell, February 27, 1948, BPP.
31. S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 3.
32. S. Benzer, "Notes on Phage Course: June 28–July 17, 1948 at Cold Spring Harbor, L.I.," handwritten notebook, unpaginated, BPP. All course notes in the discussion that follows are from this source.
33. S. Benzer, interview with F. L. Holmes, 4 January, 2002, tape 3.
34. Ibid.
35. Ibid.
36. Ibid.
37. Weiner, *Time,* p. 45; S. Benzer, interview with F. L. Holmes, January 3, 2001, tape 1.
38. Gunther S. Stent, *Nazis, Women and Molecular Biology: Memoirs of a Lucky Self-Hater* (Kensington, CA: Briones, 1998), p. 310.
39. S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 3.
40. Stent, *Nazis,* p. 311.
41. E. Eugene Banker to S. Benzer, April 21, 1948, BPP.
42. S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 3.
43. S. Benzer to V. A. Johnson (draft), October 19, 1948, BPP; S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 2.
44. S. Benzer, interview with F. L. Holmes, January 3, 2001, tape 1.
45. S. Luria and R. Latarjet, "Ultraviolet Irradiation of Bacteriophage During Intracellular Growth," *Journal of Bacteriology* 53 (1947): 149–163.
46. [S. Benzer], "Proposed Plan of Research," TS, copy dated in ink "Feb 1, 1949," BPP.
47. Ibid.
48. Ibid.
49. S. Benzer, interview with F. L. Holmes, January 5, 2002, tape 2.
50. S. Benzer to M. Delbrück, January 14, 1949, BPP.
51. M. Delbrück to S. Benzer, January 18, 1949, BPP.
52. S. Benzer to M. Delbrück, January 28, 1949, BPP.
53. [S. Benzer], "Proposed Plan of Research," TS, copy with handwritten annotations by Delbrück, BPP.
54. S. Benzer to K. Lark-Horowitz, January 24, 1949, BPP.
55. S. Benzer to W. L. Ayres, January 18, 1949; K. Lark-Horovitz to S. Benzer, January 18, 1949; J. S. Karling to S. Benzer, January 24, 1949, BPP.

56. [Benzer], "Proposed Plan of Research."

57. S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 3.

58. S. Benzer to J. S. Karling, March 1, 1949, March 14, 1949, March 29, 1949; S. Benzer to K. Lark-Horovitz, March 1, 1949, March 28, 1949; S. Benzer to W. L. Ayres, March 29, 1949; S. Benzer to S. Luria, March 28, 1949, BPP.

59. S. Benzer to Director, Hopkins Marine Station, April 8, 1949; S. Benzer to C. B. van Niel, April 8, 1949; Francis Stroberugh to S. Benzer, April 26, 1949, BPP.

60. S. Benzer to S. Luria, April 2, 1949; R. G. Gustavson (National Research Council) to S. Benzer, April 8, 1949, BPP.

61. S. Benzer, interview with F. L. Holmes, January 5, 2002, tape 2; *Phage Information Service* no. 4, April 7–9, 1949.

62. S. Benzer to K. Lark-Horovitz, May 25, 1949, BPP; S. Benzer, "Adventures in the rII Region," in *Phage and the Origins of Molecular Biology,* ed. John Cairns, Gunther S. Stent, and James D. Watson (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1966), p. 157; S. Benzer, interview with F. L. Holmes, January 5, 2002, tape 2.

63. [S. Benzer], "Quarterly Report," May 1949, TS, BPP, pp. 2, 4.

64. Ibid., p. 2–3.

65. Ibid., p. 3.

66. Ibid. pp. 3–4.

67. [S. Benzer], "Quarterly Report," August 12, 1949, TS, BPP.

68. S. Benzer to R. Dulbecco, July 19, 1949, BPP.

69. [Benzer], "Quarterly Report," August 12, 1949.

70. Ibid.

71. S. Benzer to R. Dulbecco, July 29, 1949.

72. Ibid.

73. R. Dulbecco to S. Benzer, May 31, 1949 (inserted in S. Benzer, laboratory notebook "Oak Ridge 1948–1949, vol. 1"); S. Benzer to R. Dulbecco, July 29, 1949, BPP.

74. S. Benzer to K. Lark-Horowitz, November 7, 1949, BPP.

75. J. J. Weigle, "Dark Field Microscopy of Phages," in [M. Delbrück], "N.F.I.P. Semiannual Report July 1–December 31, 1949," TS, BPP.

76. [S. Benzer], "Progress Report, Seymour Benzer, 12/26/49," TS, BPP.

77. Ibid.; S. Benzer, "Resistance to Ultraviolet Light as an Index to the Reproduction of Bacteriophage," *Journal of Bacteriology* 63 (1952): 59–72 (quotation is on p. 64).

78. S. Benzer to A. Hollaender, January 2, 1950, BPP.

79. [Benzer], "Progress report, Seymour Benzer, 12/26/49."

80. Ibid.

81. Ibid.

82. [M. Delbrück], "N.F.I.P. Semiannual Report July 1–December 31, 1949."

83. S. Benzer to K. Lark-Horowitz, December 30, 1949, BPP.

84. S. Benzer to K. Lark-Horowitz, November 7, December 30, 1949, BPP.

85. Ibid.; S. Benzer to A. Hollaender, January 2, 1950, BPP.

86. M. Delbrück, ed. *Viruses 1950* (Pasadena: California Institute of Technology, 1950), pp. 3, 100–146.

87. [Delbrück], "N.F.I.P. Semiannual Report, July 1–December 31, 1949."

88. Benzer, "Adventures," p. 158.

89. S. Benzer to Ole Maaløe, May 9, 1950, BPP.

90. Ibid.

91. [S. Benzer], "Progress Report, Seymour Benzer, 6/14/50," TS, BPP.

92. [S. Luria], "A Preliminary Study of the Effect of Temperatures Above Optimum on the Growth of Bacterial Viruses," pp. 3, 6, TS with handwritten note "S. E Luria," BPP.

93. [Benzer], "Progress Report, Seymour Benzer, 6/14/50."

94. Ibid.

95. Ibid.

96. Ibid.

97. "UV Method for Following Early Stages of Phage Development," TS with handwritten note "Revision of syllabus, Jan. 15, 1951," BPP.

98. R. Dulbecco and S. Benzer, "Ultraviolet Experiments on Phages," *Phage Information Service Bulletin* no. 5, pp. 11–13.

99. S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 3.

100. [S. Benzer], "van Niel course 1950" (notation on looseleaf cover containing typewritten notes of lectures), p. 1, BPP.

101. Ibid., pp. 1–9.

102. Ibid., pp. 9–24 (quotation is on p. 11).

103. Ibid., p. 25.

104. S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 3.

105. S. Benzer, laboratory notebook, "Cal Tech II, 1949–1951," BPP.

106. Charles Galperin, "Le bactériophage, la lysogénie et son déterminisme génétique," *History and Philosophy of the Life Sciences* 9 (1987): 175–224.

107. [Delbrück], " N.F.I.P. Semiannual Report, July 1–December 31, 1950," pp. 3–4.

108. Benzer, "Adventures," p. 159; S. Benzer, interview with F. L. Holmes, January 5, 2002, tape 4.

109. A. Lwoff to S. Benzer, September 19, 1950, BPP.

110. S. Benzer to K. Lark-Horovitz, October 5, 1950, BPP.

111. K. Lark-Horovitz to S. Benzer, November 2, 1950, BPP.

112. Benzer, "Adventures," p. 159; M. Delbrück to C. E. Richards, November 3, 1950, MD, CTA, box 2, file 24.

113. M. Delbrück to Richards, November 3, 1950.

114. Robert K. Merton, "The Sociology of Science: An Episodic Memoir," in *The Sociology of Science in Europe,* ed. Robert K. Merton and Jerry Gaston (Carbondale: Southern Illinois University Press, 1977), pp. 89–93.

115. [S. Benzer], "Brief Outline of Proposed Research: Intracellular Development of Bacteriophage in Lysogenic Bacteria," TS with handwritten annotation "Oct. 1950, application for Cancer Fellowship renewal for Paris," BPP.

116. [S. Benzer], "Report of Seymour Benzer," in [Delbrück], "N.F.I.P. Semiannual Report, July 1–December 31, 1950," BPP (emphasis in original).

117. Ibid.

118. [Delbrück], "N.F.I.P. Semiannual Report," p. 2.

119. S. Benzer to S. Luria, February 12, 1951, BPP.

120. Benzer, "Adventures," p. 158.

121. S. Benzer, "Resistance to Ultraviolet Light as an Index to the Reproduction of Bacteriophage," *Journal of Bacteriology* 63 (1952): 59–72 (quotation is on p. 71).

122. Ibid., pp. 59–64.

123. Ibid., pp. 65–69 (emphasis in original).

124. Ibid., p. 70.

125. Ibid., pp. 66–67.

126. S. Benzer to S. Luria, June 23, 1951, BPP.

127. S. Benzer to K. Lark-Horovitz, June 23, 1951, BPP.

Chapter Four. To Paris and Back

1. S. Benzer, interview with F. L. Holmes, January 5, 2002, tape 2.

2. Ibid.; S. Benzer to Delbert Anderson, August 14, 1951, BPP.

3. François Jacob, *La Statue intérieur* (Paris: Seuil, 1987), pp. 235–237, 256.

4. Ibid., pp. 256, 290; S. Benzer, interview with F. L. Holmes, January 4, 2002, tape 1.

5. Michel Morange, *A History of Molecular Biology,* trans. Michael Cobb (Cambridge: Harvard University Press, 1998), pp. 150–163.

6. For a vivid portrait of the personality and scientific achievements of Lwoff, see Jacob, *La Statue,* pp. 244–252.

7. Ibid., pp. 253–255; Morange, *History,* pp. 151–152.

8. Ibid.

9. Charles Galperin, "La Bactériophage, la lysogénie, et son déterminisme génétique," *History and Philosophy of the Life Sciences* 9 (1987): 175–224.

10. Ibid., pp. 177–194.

11. Ibid., pp. 187–210; M. Delbrück, "Bacterial Viruses or Bacteriophages," *Biological Reviews* 21 (1946): 30–40 (quotations are on pp. 30, 33–34).

12. Galperin, "La Bactériophage," pp. 210–213.

13. André Lwoff and Antoinette Gutmann, "Recherches sur un *Bacillus*

megatherium lysogène," *Annales de l'Institut Pasteur* 78 (1950): 711–739 (quotations are on 711–712).

14. André Lwoff and Antoinette Gutmann, "Les Problèmes de la production du bactériophage par les souches lysogènes: La lyse spontanée du Bacillus megatherium," *Comptes rendus de l'Académie des Sciences* 229 (1949): 605–607.

15. Richard Burian, "Symposium: The Mapping Cultures of Twentieth-Century Genetics: From the Fly Group to the Human Genome Project" (Max-Planck Institut für Wissenschaftsgeschichte, 2–4 March 2001).

16. Lwoff and Gutmann, "Les Problèmes," pp. 605–607; André Lwoff and Antoinette Gutmann, "Production discontinue de bactériophages par une souche lysogène de *Bacillus megatherium*," *Comptes rendus de l'Académie des Sciences* 229 (1949): 679–682; Lwoff and Gutmann, "Recherches," pp. 713–733.

17. Lwoff and Gutmann, "Recherches," pp. 733–737.

18. Ibid., p. 713.

19. André Lwoff, Louis Siminovitch, and Niels Kjeldgaard, "Induction de la production de bactériophages chez une bactérie lysogène," *Annales de l'Institut Pasteur* 79 (1950): 817–859.

20. Ibid., pp. 856–857; André Lwoff, Louis Siminovitch, and Niels Kjelgaard, "Induction de la lyse bactériophage d'un population microbienne lysogène," *Comptes rendus de l'Académie des Sciences* 231 (1950): 190–191.

21. Lwoff, Siminovitch, and Kjeldgaard, "Induction," p. 856.

22. Jacob, *La Statue,* p. 260.

23. Ibid., pp. 260–262; François Jacob, "Induction de la lyse et de la production de bactériophages chez un *Pseudomonas pyocanea* lysogène," *Comptes rendus de l'Académie des Sciences* 231 (1950): 1585–1587.

24. Jacob, *La Statue,* p. 262.

25. François Jacob and Elie L. Wollman, "Induction of Phage Development in Lysogenic Bacteria," *Cold Spring Harbor Symposia on Quantitative Biology* 18 (1953): 101–122.

26. Jacques Monod and Elie Wollman, "L'inhibition de la croissance et de l'adaptation enzymatique chez les bactérie infectées par le bactériophage," *Annales de l'Institut Pasteur* 73 (1947): 937–956 (quotations are on pp. 938, 941–942).

27. Ibid., pp. 944, 953–954.

28. Ibid., pp. 953–955.

29. Jacques Monod and Melvin Cohn, "La Biosynthèse induite des enzymes (adaptation enzymatique)," *Advances in Enzymology* 13 (1952): 67–119 (quotation is on p. 68).

30. François Jacob, "Influence du régime carbone sur le développement des bactériophages chez un *pseudomonas pyocyanea,*" *Annales de l'Institut Pasteur* 82 (1952): 578–603.

31. Monod and Cohn, "La Biosynthèse induite," pp. 88–90 (emphasis in original).

32. Roger Stanier, "Enzymatic Adaptation in Bacteria," *Annual Review of Microbiology* 5 (1951): 35–56 (quotation is on p. 38); Seymour Benzer, "Adventures in the rII Region," in *Phage and the Origins of Molecular Biology,* ed John. J. Cairns, Gunther. S. Stent, and James. D. Watson (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1966), pp. 159–160.

33. Benzer, "Adventures," pp. 159–160.

34. See Chapter 3, [p. 102].

35. Benzer, "Adventures," p. 160.

36. S. Benzer, "Induced Synthesis of Enzymes in Bacteria Analyzed at the Cellular Level," *Biochimica et Biophysica Acta* 11 (1953): 383–395.

37. Benzer, "Adventures," p. 160; Benzer, "Induced Synthesis," p. 384; Melvin Cohn and Jacques Monod, "Purification et Propriétés de la β-Galactosidase (Lactase) d'*Escherichia coli,*" *Biochimica et Biophysica Acta* 7 (1951): 153–174 (quotations are on pp. 153–155).

38. It was later realized that this phage was nearly identical to T7 phage in Delbrück's T series. See R. W. Hyman, I. Brunovskis, and W. C. Summers, "DNA Bbase Sequence Homology Between Coliphages T7 and ϕII and Between T3 and ϕII as Determined by Heterodupex Mapping in the Electron Microscope," *Journal of Molecular Biology* 77 (1973): 189–196; R. W. Hyman, I. Brunovskis, and W. C. Summers, "A Biochemical Comparison of the Related Bacteriophages T7, ϕI, ϕII, W31, H, and T3," *Virology* 57 (1974): 189–206.—WCS.

39. Cohn and Monod, "Purification et Propriétés"; Benzer, "Induced Synthesis," p. 384.

40. Benzer, "Induced Synthesis," p. 385. The reconstruction of Benzer's investigation in this and the following paragraphs is based on Benzer's later publication and does not necessarily recover the actual chronological order in which he performed the experiments.

41. Ibid., pp. 385–386.

42. Ibid., p. 388.

43. Ibid., pp. 388–389.

44. Ibid., pp. 389–391.

45. J. Monod, A. M. Pappenheimer, and G. Cohen-Bazire, "La Cinétique de la biosynthèse de la β-galactosidase chez *E. coli* considérée comme fonction de la croissance," *Biochimica et Biophysica Acta* 9 (1952): 648–660.

46. Ibid., p. 659.

47. Benzer, "Induced Synthesis," p. 392.

48. S. Benzer, interview with F. L. Holmes, January 5, 2002, tape 2.

49. Jacob, *La Statue,* p. 291.

50. S. Benzer to K. Lark-Horovitz, March 7, 1952, BPP.

51. Jacob, *La Statue,* pp. 290–291.

52. S. Benzer to K. Lark-Horovitz, March 7, 1952, BPP.

53. K. Lark-Horovitz to S. Benzer, February 26, 1952, BPP.

54. Undated TS accompanying S. Benzer to K. Lark-Horovitz, March 7, 1952, BPP.

55. Monod, Pappenheimer, and Cohen-Bazire, "La Cinétique," pp. 650–651.

56. S. Benzer to K. Lark-Horovitz, May 14, 1952, BPP.

57. Jacob, *La Statue,* pp. 292–294; Robert Olby, *The Path to the Double Helix* (Seattle: University of Washington Press, 1974), p. 310; Horace Freeland Judson, *The Eighth Day of Creation* (New York: Simon and Schuster, 1979), p. 130; James D. Watson, *The Double Helix* (New York: Atheneum, 1968), p. 119; S. Luria, "An Analysis of Bacteriophage Multiplication," in *The Nature of Virus Reproduction: Second Symposium of the Society for General Microbiology,* ed. Paul Fildes and W. E. van Heyningen (Cambridge: Cambridge University Press, 1953), pp. 99–116. The discussion, including Watson's comment, is on pp. 113–116.

58. Benzer to Lark-Horovitz, May 14, 1952, BPP.

59. Ibid.

60. Undated TS accompanying S. Benzer to K. Lark-Horovitz, March 7, 1952, BPP; Jacob, *La Statue,* p. 291.

61. S. Benzer and F. Jacob, "Etude du développement du bactériophage au moyen d'irradiations par la lumière ultra-violette," *Annales de l'Institut Pasteur* 84 (1953): 193–197; F. Jacob, laboratory notebook, JAC/B2,2, Archives of the Institut Pasteur. I assume that the experiments reported in their joint paper as having been done by Jacob alone are the ones referred to by Benzer before they began the experiments performed together.

62. Benzer and Jacob, "Etude du développement du bactériophage," pp. 192–193.

63. S. Benzer, interview with F. L. Holmes, 2002; Benzer and Jacob, "Etude du développement du bactériophage," pp. 197–198.

64. Jacob, *La Statue,* p. 291.

65. Ibid., pp. 295–297; M. Delbrück, "Introductory Remarks About the Program," *Cold Spring Harbor Symposia on Quantitative Biology* 18 (1953): 1; M. Delbrück to S. Benzer, September 30 1952, CTA.

66. S. Benzer to K. Lark-Horovitz, March 7, 1952, BPP.

67. Ibid.

Chapter Five. Teaching and Research at Purdue

1. S. Benzer, "Biophysics Program," TS, BPP.
2. M. Delbrück to Bernard Davis, August 12, 1957, MD, CTA.
3. S. Benzer to K. Lark-Horovitz, March 7, 1952, BPP.
4. S. Benzer to M. Delbrück, September 26, 1952, MD, CTA.

5. S. Benzer and F. Jacob, "Etude du developpement du bactériophage au moyen d'irradiations par la lumière ultra-violette," *Annales de l'Institut Pasteur* 84 (1953): 198–201.

6. M. Delbrück to S. Benzer, September 30, 1952, MD, CTA.

7. S. Benzer to M. Delbrück, October 27, 1952, MD, CTA.

8. N. Visconti and M. Delbrück, "The Mechanism of Genetic Recombination in Phage," *Genetics* 38 (1953): 5–33.

9. S. Benzer to M. Delbrück, October 27, 1952, MD, CTA.

10. Ibid.

11. S. Benzer to M. Delbrück, January 23, 1953, MD, CTA.

12. Ibid.

13. Ibid.

14. Elof Axel Carlson, *The Gene: A Critical History* (Philadelphia: Saunders, 1966), pp. 188–195.

15. G. Pontecorvo, "Genetic Formulation of Gene Structure and Gene Action," *Advances in Enzymology* 13 (1952): 121–149 (quotation is on pp. 121–123).

16. Ibid., pp. 124–125.

17. Ibid., p. 129.

18. Ibid., pp. 129–131.

19. Ibid., pp. 133–134.

20. Ibid., p. 134.

21. A. D. Hershey and Raquel Rotman, "Genetic Recombination Between Host-Range and Plaque-Type Mutants of Bacteriophage in Single Bacterial Cells," *Genetics* 34 (1949): 44–45.

22. Ibid., p. 45.

23. Ibid., p. 49.

24. A. D. Hershey and M. Chase, "Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage," *Journal of General Physiology* 36 (1952–1953): 39–56 (quotations are on p. 54).

25. S. Benzer, "Genetics Seminar, Purdue, February 20, 1953," MS, BPP (emphasis in original).

26. Ibid.

27. Ibid.

28. Ibid.

29. Ibid.

30. Pontecorvo, "Genetic Formulation," p. 133.

31. Benzer, "Genetics Seminar."

32. S. Benzer, "Purdue: Bio 156 Lecture—April 10, 1953," MS, BPP.

33. Ibid.

34. Ibid.

35. Ibid.

36. S. Benzer, "Structure Duplication, Second Lecture, Bio 156, April 13, 1953," MS, BPP.

37. F. Haurowitz, "Biological Problems and Immunochemistry," *Quarterly Review of Biology* 24 (1949): 93–101.

38. Ibid., p. 99.

39. Benzer, "Structure Duplication, Second Lecture."

40. Robert Olby, *The Path to the Double Helix* (Seattle: University of Washington Press, 1974), pp. 73–121; Michel Morange, *A History of Molecular Biology,* trans. Matthew Cobb (Cambridge: Harvard University Press, 1998), p. 34.

41. A. L. Dounce, "Duplicating Mechanism for Peptide Chain and Nucleic Acid Synthesis," *Enzymologia* 15 (1952): 251–258 (quotation is on p. 251).

42. Ibid., pp. 251–253.

43. Ibid., pp. 253–256.

44. Ibid., pp. 256–257.

45. Benzer, "Structure Duplication, Second Lecture."

46. Morange, *History of Molecular Biology,* pp. 126–127.

47. Benzer, "Biophysics Seminar, Purdue, April 15, 1953," MS, BPP.

48. Ibid.

49. Ibid.

50. Ibid.; Linus Pauling and Robert B. Corey, "A Proposed Structure for the Nucleic Acids," *Proceedings of the National Academy of Sciences* 39 (1953): 84–97 (quotation is on p. 84).

51. Pauling and Corey, "Proposed Structure,"

52. James D. Watson and Francis H. C. Crick, "A Structure for Deoxyribose Nucleic Acid," *Nature* 171 (1953): 737–738.

53. James D. Watson and Francis H. C. Crick, "Genetical Implications of the Structure of Deoxyribonucleic Acid," *Nature* 171 (1953): 965–966.

54. Pauling is quoted in Olby, *Path,* p. 422.

55. "List of Those Attending or Participating in the Symposium," *Cold Spring Harbor Symposia on Quantitative Biology* 18 (1953): ix–xiv; M. Delbrück, "Introductory Remarks About the Program," ibid., pp. 1–2.

56. S. Benzer, "Report of Trip to Virus Symposium," TS, n.d, BPP.

57. Ibid.

58. Delbrück, "Introductory Remarks," p. 2.

59. For a detailed account of these exchanges between Delbrück and Watson, see Frederic L. Holmes, *Meselson, Stahl, and the Replication of DNA: A History of the "Most Beautiful Experiment in Biology"* (New Haven: Yale University Press, 2001), pp. 1–48.

60. James D. Watson and Francis H. C. Crick, "The Structure of DNA," *Cold Spring Harbor Symposia on Quantitative Biology* 18 (1953): 123–131.

61. Ibid., pp. 127–128.

62. Ibid., p. 128.

63. Ibid., pp. 128–129.

64. F. Jacob, *La Statue intérieur* (Paris: Seuil, 1987).

65. S. Benzer, "Notes on Cold Spring Harbor Symposium on Viruses," BPP.

66. S. Benzer, "Report of Trip to Virus Conference," TS, BPP.

67. G. H. Bowen, "Studies of Ultraviolet Irradiation Phenomena—An Approach to the Problems of Bacteriophage Reproduction," *Cold Spring Harbor Symposia on Quantitative Biology* 18 (1953): 245–253 (quotation is on p. 248).

68. S. Benzer, " 'Biological Effects of Radiation,' lecture for Koffler course on Bacteriology, Purdue, April 23, 1953," BPP.

69. M. Delbrück to B. D. Davis, August 12, 1957, MD, CTA.

70. A. D. Hershey to S. Benzer, October 15, 1953, BPP.

Chapter Six. Entering the rII Region

1. S. Benzer, "Adventures in the rII Region," in *Phage and the Origins of Molecular Biology,* ed. John Cairns, Gunther S. Stent, and James. D. Watson (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1965), p. 161.

2. Ibid., p. 159.

3. S. Benzer to F. L. Holmes, September 14, 1999, FLHP; S. Benzer, "Research Notebook r mutants no. 1," BPP (hereafter RN no. 1, etc.).

4. J. J. Weigle and M. Delbrück, "Lysogenesis of Strain K12 of *E. coli*," in "N.F.I.P. Semiannual Report, July–December 31, 1950," BPP; RN no. 1, January 9, 1954. All tables are drawn from the notebooks.

5. RN no. 1, January 11, 12, 1954; S. Benzer, interview with F. L. Holmes, May 23, 2001, tape 1.

6. RN no. 1, January 14, 1954.

7. Benzer's concern with host effects was based on the recent findings of Luria and Mary L. Human (S. Luria and M. L. Human, "A Non-Hereditary Host-Induced Variation in Bacterial Viruses," *Journal of Bacteriology* 64 [1952]: 557–569) that growth of phages on some bacterial strains was affected by the particular strain of bacteria in which the last cycle of growth had taken place. This was the phenomenon called "host-induced modification," and it was puzzling because it seemed to represent a case of transient heredity. Later this phenomenon was found to be the result of the host-strain–specific modification and degradation of the phage DNA by so-called restriction and modification enzymes.—WCS.

8. RN no. 1, January 15, 1954; G. Streisinger to S. Benzer, January 8, 1954, BPP; [S. Benzer], "Trip to Urbana, Feb. 20–23, 1954," BPP.

9. RN no. 1, January 17, 1954.

10. Ibid., January 17–19, 1954.

11. Ibid., January 20, 1954.

12. Ibid.; 1954; S. Benzer, interview with F. L. Holmes, May 23, 2001, tape 1.

13. RN no. 1, January 20–21, 1954.

14. Ibid., January 22, 1954.
15. S. Benzer, interview with F. L. Holmes, May 23, 2001, tape 1.
16. RN no. 1, January 22–23, 1954.
17. Ibid.
18. Ibid.; S. Benzer, interview with F. L. Holmes, May 23, 2001, tape 2.
19. RN no. 1, January 23, 1954.
20. See A. D. Hershey and Raquel Rotman, "Genetic Recombination Between Host-Range and Plaque Type Mutants of Bacteriophage in Single Bacterial Cells," *Genetics* 34 (1949): 44–71 (quotation is on p. 49).
21. RN no. 1, January 23–27, 1954.
22. S. Benzer, interview with F. L. Holmes, May 23, 2001, tape 2.
23. RN no. 1, January 30, 1954.
24. Ibid., January 30–February 2, 1954.
25. Ibid., February 3, 1954.
26. Ibid.
27. Ibid., February 3–6, 1954.
28. Ibid., February 6–9, 1954 (emphasis in original).
29. Ibid., February 9, 1954.
30. Ibid., February 10, 1954.
31. Ibid., February 13, 1954.
32. Ibid., February 13–14, 1954.
33. Ibid., February 10–13, 18, 1954.
34. Ibid., February 18, 1954.
35. Benzer, "Trip to Urbana."
36. Ibid.
37. Ibid.
38. F. Lanni to S. Benzer, March 31, 1954, BPP.
39. RN no. 1, February 24, 26, 1954.
40. S. Benzer to M. Demerec, March 2, 1954, BPP.
41. S. Benzer to O. M. Ray, March 2, 1954, BPP.
42. S. Benzer to A. D. Hershey, March 2, 1954, draft letter, BPP. I have not seen the final copy of the letter. I have not included all of the phrases eliminated or the changes in order that Benzer made in correcting the draft.
43. A. D. Hershey to S. Benzer, March 5, 1954, BPP.
44. RN n0. 1, March 2–5, 1954.
45. Ibid., March 9, 1954.
46. Ibid., March 19, 1954.
47. Ibid., March 22–27, 1954.
48. S. Benzer to A. D. Hershey, March 27, 1954, BPP. This is a rough draft of the letter. I have arranged the quoted passages according to the corrections Benzer made on the draft.
49. RN no. 1, March 24–29, 1954.
50. Ibid., March 25, 1954.

51. A. H. Doermann to S. Benzer, April 3, 1954, BPP.
52. RN no. 1, April 8, 1954.
53. Ibid., April 9–15, 1954.
54. Ibid.
55. S. Benzer, "Report on Trip to Oak Ridge by S. Benzer," TS, n.d., BPP.
56. RN no. 2, April 27, 1954; S. Benzer, interview with F. L. Holmes, May 23, 2001, tape 3.
57. The determination of the transmission factor, or the "leakage" of the mutant phenotype when plated on K12(λ), was crucial to estimate the limits to the resolution of mapping the r mutants. This experiment may have been motivated by this realization.—WCS.
58. RN no. 2, April 27–30, 1954; S. Benzer, interview with F. L. Holmes, May 23, 2001, tape 3.
59. RN no. 2, May 4, 1954; A. H. Doermann to S. Benzer, April 3, 1954, BPP.
60. RN no. 2, May 10–11, 1954; G. Stent to S. Benzer, June 16, 1954, BPP.
61. RN no. 2, May 28, 1954.
62. S. Benzer, interview with F. L. Holmes, May 23, 2001, tape 4.
63. RN no. 2, undated, between May 28 and 29, 1954, BPP.
64. For a discussion of these aspects of "Eureka experiences," see Howard Gruber, *Darwin on Man: A Psychological Study of Scientific Creativity,* 2d ed. (Chicago: University of Chicago Press, 1981), pp. 1–15.
65. S. Benzer, interview with F. L. Holmes, May 23, 2001, tape 4.

Chapter Seven. Crossing into the Fine Structure

1. RN no. 2, May 13–21, 1954.
2. Ibid., May 29–June 1, 1954.
3. Ibid., June 1–3, 1954.
4. Ibid., June 5, 1954.
5. Ibid., May 29–June 8, 1954. All tables are drawn from the notebooks.
6. Ibid., June 9, 1954.
7. Ibid.
8. Ibid., June 10–13, 22–25, 1954.
9. S. Benzer, "Trip to Cold Spring Harbor, L.I.," TS, BPP.
10. Victor M. McElheny, *Watson and DNA: Making a Scientific Revolution* (Cambridge, MA: Perseus, 2003), p. 68.
11. Benzer, "Trip to Cold Spring Harbor."
12. S. Benzer, "Seminar—Cold Spring Harbor, July 1954," handwritten notes, BPP.
13. Ibid.
14. Ibid.
15. Ibid.

16. Ibid.
17. Ibid.
18. Ibid.
19. S. Benzer to G. Stent, July 17, 1954, Gunther Stent Papers, Bancroft Library, University of California, Berkeley. I thank Bill Summers for drawing this letter to my attention.
20. Benzer, "Trip to Cold Spring Harbor."
21. M. Delbrück to S. Benzer, July 27, 1958, BPP.
22. James D. Watson and Francis H. C. Crick, "The Structure of DNA," *Cold Spring Harbor Symposia on Quantitative Biology* 18 (1953): 123–131.
23. S. Benzer, "Report on European Trip," handwritten draft, BPP; S. Benzer to F. L. Holmes, May 23, 2001, FLHP.
24. Benzer, "Report on European Trip."
25. Ibid.; S. Benzer to F. L. Holmes, May 23, 2001, FLHP.
26. Benzer, "Report on European Trip."
27. Ibid.
28. E. B. Lewis, "Pseudoallelism and Gene Evolution," *Cold Spring Harbor Symposia on Quantitative Biology* 16 (1951): 159–174.
29. Benzer, "Report on European Trip."
30. S. Brenner to S. Benzer, September 22, 1954, BPP. Some accounts of this meeting state, obviously incorrectly, that Brenner delivered Benzer's paper for him when Benzer was suddenly called away from the meeting.
31. S. Brenner, abstract for S. Benzer, "The Fine Structure of a Gene in Bacteriophage," *Phage Information Service* no. 8 (August 1954).
32. Ibid.
33. Ibid.
34. F. Lanni to Benzer, October 21, 1954; Benzer to Lanni, December 7, 1954, both in BPP.
35. Brenner, abstract for Benzer, "Fine Structure."
36. Ibid.; S. Brenner to A. Benzer, September 22, 1954, BPP.
37. M. Delbrück to A. Benzer, July 17, 1954, BPP.
38. D. Krieg to S. Benzer, undated; S. Benzer to D. Krieg, October 21, 1954, both in BPP.
39. RN no. 4, September 18, 1954.
40. Ibid.
41. RN no. 4, October 12, 1954.
42. Ibid., September 20, 1954.
43. Ibid., September 18–October 6, 1954.
44. Ibid., October 6, 1954.
45. Ibid., October 12, 1954.
46. Ibid.
47. Ibid., October 13, 1954.

48. Ibid., October 19–20, 1954; S. Benzer, "Fine Structure of a Genetic Region in Bacteriophage," *Proceedings of the National Academy of Sciences* 41 (1955): 344–354 (quotation is on p. 348).

49. RN no. 4, October 26, 1954.

50. Ibid.

51. S. Benzer, "Research Conference at University of Illinois," TS, BPP.

52. S. Benzer, "Physics Seminar, November 4, 1954," MS, BPP. All quotations in the following discussion refer to this MS.

53. Ibid.

54. Ibid.

55. Ibid.

56. RN no. 4, November 3, 1954.

57. Ibid., November 8–14, 1954.

58. F. Lanni to S. Benzer, October 21, 1954, BPP. I have not been able to find a note by Demerec in the same issue of *Phage Information Service.*

59. S. Benzer to F. Lanni, December 7, 1954, BPP.

60. A. D. Hershey to S. Benzer, December 22, 1954, BPP.

61. A. D. Hershey, "Inheritance in Bacteriophage," *Advances in Genetics* 5 (1953): 89–106 (quotation is on pp. 103–104).

62. L. J. Stadler, "The Gene," *Science* 120 (1954): 811–819.

63. S. Benzer to A. D. Hershey, December 30, 1954, BPP.

64. Ibid.

65. G. Streisinger to S. Benzer, December 5, 1954, BPP.

66. RN no. 4, January 4, 1955.

67. Ibid., January 6, 1955.

68. Ibid., January 7, 1955.

69. S. Benzer, "Research Proposal for Application for Grant from National Science Foundation," February 14, 1955, p. 1, BPP. Further page references to the proposal appear in the text.

70. S. Benzer, interview with F. L. Holmes, January 3, 2001, tape 1.

71. S. Benzer to M. Delbrück, February 3, 1955, MD, CTA.

72. S. Benzer, "Fine Structure," p. 354; M. Delbrück to S. Benzer, February 8, 1955, BPP.

73. Benzer, "Fine Structure," pp. 344–345.

74. Ibid., p. 345.

75. Ibid., pp. 345–349.

76. Ibid., pp. 349–350.

77. Ibid., pp. 50–51.

78. I have not seen the draft manuscript that Benzer sent to Delbrück and others at this time. No copy survives in his own papers. The number given in this passage in the final version is "a dozen." There is reason, however, to infer that he revised the number in a later revision (see Chapter 8). I have taken the number 260 from his NSF report, composed about the same time as the draft.

79. M. Delbrück to S. Benzer, February 8, 1955, containing "Outline for a Revised Discussion and Summary."

80. J. D. Watson to S. Benzer, April 20, 1955, BPP. For a full account of the replication problem and its aftermath, see Frederic L. Holmes, *Meselson, Stahl, and the Replication of DNA: A History of "the Most Beautiful Experiment in Biology"* (New Haven: Yale University Press, 2001).

Chapter Eight. Is *Gene* a Dirty Word?

1. S. Benzer to M. Delbrück, February 1955, BPP.
2. A. D. Hershey to S. Benzer, Feburary 10, 1955, BPP.
3. A. H. Doermann to S. Benzer, February 12, 1955, BPP.
4. "Research Notebook r mutants No. 4," February 17, 1955, BPP (hereafter RN no. 4, etc.).
5. S. Benzer to F. Stahl, February 18, 1955, BPP.
6. S. Benzer, "Microbiology Seminar, February 22 [to] March 2, 1955," MS, BPP.
7. RN no. 4, February 23–25, 1955.
8. Ibid., February 24, 1955.
9. Ibid., February 26, 1955.
10. Ibid., March 2, 1955.
11. Ibid., March 4, 1955; S. Benzer, "Fine Structure of a Genetic Region in Bacteriophage," *Proceedings of the National Academy of Sciences* 41 (1955): 344–354 (quotation is on p. 350).
12. A. D. Hershey to S. Benzer, February 10, 1955, BPP; RN no. 4, March 6, 1955.
13. RN no. 4, March 7, 1955.
14. A. D. Hershey to S. Benzer, February 10, 1955, BPP; RN no. 4, March 11, 1954.
15. S. Benzer, "Short Outline, Baltimore, March, 1955"; "APS Symp on nucleic acids, March 17, 1955"; "Trip to Baltimore, March 16–18, 1955," all in BPP.
16. Benzer, "Fine Structure," p. 348; RN no. 4, March 20, 1955.
17. RN no. 4, March 20, 1955.
18. S. Benzer to K. Lark-Horovitz, May 8, 1955, BPP.
19. Ibid.
20. S. Benzer, "Cal Tech March 27 1955," MS, BPP.
21. S. Benzer to S. Brenner, May 26, 1955; S. Benzer to Martha Baylor, May 10, 1955, both in BPP.
22. S. Benzer to F. L. Holmes, May 23, 2001, FLHP.
23. Benzer, "Cal Tech Seminar, 3/28/55"; "Fine Structure in the Genetic Map of Phage," BPP.
24. Ibid.

25. Ibid.
26. S. Benzer to K. Lark-Horovitz, May 8, 1955, BPP.
27. Benzer, "Fine Structure," pp. 352–353.
28. S. Benzer to K. Lark-Horovitz, May 8, 1955; S. Benzer to S. Brenner, March 24, 1955, both in BPP.
29. RN no. 5, April 14, 1955.
30. S. Benzer, "Genetic Fine Structure and Its Relation to the DNA Molecule," in *Brookhaven Symposia in Biology—Number 8: Mutation* (Upton, NY: Brookhaven National Laboratory, 1955), p. 5.
31. RN no. 5, April 18–19, 1955.
32. Ibid., April 22, 1955.
33. Ibid. April 25–26, 1955.
34. Ibid., April 29, 1955.
35. Ibid., April 29, 1955.
36. S. Benzer to K. Lark-Horovitz, May 8, 1955, BPP.
37. For a detailed account of these efforts, see Lily Kay, *Who Wrote the Book of Life? A History of the Genetic Code* (Stanford: Stanford University Press, 2000), pp. 136–155.
38. S. Benzer to S. Brenner, May 26, 1955, BPP.
39. F. Jacob, *La Statue intérieur* (Paris: Seuil, 1987), p. 330.
40. S. Benzer, "The Elementary Units of Heredity," in *A Symposium on the Chemical Basis of Heredity,* ed. William D. McElroy and Bentley Glass (Baltimore: Johns Hopkins University Press, 1957), p. 74.
41. RN no. 5, May 12–22, 1955; RN no. 6, May 23–June 7, 1955.
42. S. Benzer, "Brookhaven Lecture, 6/17/1955: Genetic Fine Structure in Its Relation to DNA," MS, BPP.
43. Ibid.; Benzer, "Genetic Fine Structure," *Brookhaven Symposia,* p. 3.
44. Benzer, "Genetic Fine Structure," p. 5.
45. Benzer, "Brookhaven Lecture."
46. S. Benzer to F. L. Holmes, undated, FLHP; S. Benzer, interview with W. Summers, June 6, 2003.
47. Benzer, "Trip Report, June 20–July 2, 1955," BPP.
48. R. M. Whaley to A. Garen, August 1, 1955, BPP.
49. RN no. 5, July 25–30, 1955.
50. S. Benzer, "Trip to Cold Spring Harbor, Aug. 23–28, 1955," TS; "Phage Meeting, Cold Spring Harbor, Aug. 25–27, [1955]," MS, BPP.
51. S. Benzer to S. Brenner, May, 26, 1955, BPP.
52. RN no. 5, September 6–November 25, 1955.
53. Ibid., December 2–5, 1955.
54. Benzer, "Elementary Units," p. 77.
55. RN no. 5, December 20, 1955.
56. RN no. 6, January–March, 1956.
57. Ibid., April 29, 1956.

58. S. Benzer, "Baltimore Lecture June 19, 1956: The Sizes of the Nuclear Units of Heredity," handwritten notes, BPP.

59. Ibid.; Benzer, "Elementary Units," pp. 70–71.

60. Benzer, "Elementary Units," p. 71.

61. Ibid., pp. 90–91.

62. Ibid., p. 92.

63. Ibid., pp. 92–93.

64. Ibid., p. 129.

65. Raphael Falk, tape recording of discussions at workshop "Gene Concepts in Development and Evolution," Max-Planck-Institut für Wissenschaftgeschichte, Berlin, 1996.

66. Quoted in Elof Axel Carlson, *Genes, Radiation, and Society: The Life and Work of H. J. Muller* (Ithaca: Cornell University Press, 1981), p. 391.

Chapter Nine. The Survival of the "Gene"

1. Milislav Demerec, "A Comparative Study of Certain Gene Loci in *Salmonella,*" *Cold Spring Harbor Symposia on Quantitative Biology* 21 (1956): 113–121; Elof Axel Carlson, *The Gene: A Critical History* (Philadelphia: Saunders, 1966), pp. 200–206.

2. D. M. Bonner, "The Genetic Unit," *Cold Spring Harbor Symposia on Quantitative Biology* 21 (1956): 163–170.

3. G. Pontecorvo and J. A. Roper, "Resolving Power of Genetic Analysis," *Nature* 178 (1956): 83–84.

4. G. Pontecorvo, "Allelism," *Cold Spring Harbor Symposia on Quantitative Biology* 21 (1956): 172–174.

5. Pontecorvo and Roper, "Resolving Power," pp. 83–84.

6. Ibid.

7. Carlson, *The Gene,* pp. 202–203.

8. M. Delbrück to B. Davis, April 12, 1957, MD, CTA.

9. M. Delbrück, "Atomic Physics in 1910 and Molecular Biology in 1957," MD, CTA.

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