Molecular Biology Through Discovery
Companion to Benzer (1959) *On the Topology of the Genetic Fine Structure*
Proc Natl Acad Sci USA (1959) 45:1607-1620

A. What are genes?

In 1934 the first gene-related Nobel Prize was awarded to Thomas Morgan, whose lab had exploited the fruit fly to gain the first understanding of inheritance through the filter of the chromosome. He was less than magnanimous to the gene however, stating in his Nobel address:

> *What are genes?... There is no consensus of opinion amongst geneticists as to what the genes are – whether they are real or purely fictitious – because at the level at which the genetic experiments lie, it does not make the slightest difference…*[1]

Morgan was far from alone in relegating the gene to a useful construct.[2] If there was no concept of what genes were as physical entities, then they had to be defined by what they did. Genes were defined as the unit of physiological function, the unit of inheritance, the unit of mutability, and the unit of recombination – a set that carried no guarantee of self-consistency.[3] Whatever the definition, genes were generally thought to be indivisible, the atom of genetics.[4]

…at least the atom up to the 20th century! Over the course of that century, the atom has been repeatedly divided and subdivided. From the chaos of the early 1900's, an order emerged. Perhaps the new chaos of genetics appealed to the generation of physicists that had tamed the atom. In any event, a significant number of them switched fields and became major players in the birth of molecular biology. The book, *What is Life?*, written by Erwin Schrödinger, one of the founders of quantum mechanics, heightened the interest of many physicists in the molecular basis of genetics.

The most influential renegade from physics was Max Delbrück (**Fig. 1**), who at Cal Tech became unofficial guru of the phage group, a loose confederacy of those who used the new field of bacteriophage biology to study the nature of the gene. Characteristic of the phage group was the uninhibited exchange of views that Delbrück had experienced as a physicist in the lab of Niels Bohr. Jim Watson was a member of the phage group, and his work with Crick represented a wildly successful marriage between the biological sensibilities of his home environment and the structural sensibilities that prevailed at the Cavendish.

Figure 1: Max Delbrück (left) and Salvador Luria. Luria was Watson's mentor. Taken at Cold Spring Harbor, 1953.

The third cathedral of molecular biology was Institut Pasteur in Paris – actually just a short hallway with André Lwoff's lab at on one end and Jacques Monod's on the other. We'll talk extensively about this group later in the semester and its role in understanding gene regulation. It enters our story now because another renegade physicist in the phage group, Seymour Benzer (**Fig. 2**), visited Lwoff's group and combined the lessons of both groups to devise an experiment capable of seeing inside the not so indivisible gene and discerning its internal parts.

Figure 2. Seymour Benzer.

B. How to dissect genes?[5]

The first thing Seymour Benzer did after securing his first faculty position, in physics, at Purdue University was to leave it. He managed to arrange an extended leave of absence that enabled him to learn the ways of the phage, through a tour with Delbrück at Cal Tech and then a year with Lwoff. In 1952, Benzer returned to Purdue to begin his tasks as a faculty member, teaching and doing research as he could find time.

He was also asked to give a seminar to the Biochemistry Department. Benzer had just read an article by Guido Pontecorvo on the nature of genes. The article described efforts at estimating the size of genes by the sensitivity of chromosomes to known intensities of x-rays and made the claim that genes were not indivisible points as most thought but had structure. Pontecorvo predicted that genetic recombination (of the type well studied in flies Morgan's group) could take place not merely between genes but ***within*** a single gene. But such an experiment was impossible. Recombination within so small a portion of the chromosome was just too rare to be detected. Benzer took this opportunity to explore the topic in his seminar, given the provocative title, "The size of the gene". This may not seem provocative now, but suppose I changed the title to "The size of consciousness"? It was not at all obvious that a gene was something that could have a size.

The seminar, coupled with his recent experiences in Paris and Cal Tech, gave him a unique perspective on an accident that has since entered the lore of molecular biology. Benzer had the notion that phage DNA should be injected into a cell in a set gene order, and he tried to figure out an experiment that would test this idea, using Escherichia coli phage T2, one of the standard objects of study in the phage group. This phage (and the phage, T4, that he eventually focused on) infects E. coli, multiplies within its host, and then lyses (breaks open) the cells to release progeny phage, which go on to infect new *E. coli* cells. If the infection takes place on a Petri plate, you can see dense growth of *E. coli* (represented by gray in **Fig. 3A**), except in small regions where a phage and its progeny have lysed the cells in spreading circles of death called "plaques". Wild-type T2 makes small fuzzy plaques. Phages mutant in the rII region ("r" stands for "rapid lysis") make large plaques with sharp boundaries on the phage group's favorite variant of E. coli, strain B (**Fig. 3B**). Unfortunately, this mutation raised a technical problem. The mutant phage lyses the bacteria before all the phage had been made, so the phage yield was pretty poor. Benzer had to grow up large cultures of E.coli to get a sufficient number of phages.

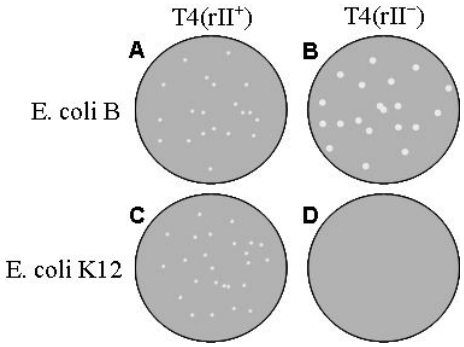

Figure 3. Plaque formation by phage T4 wild-type and rII mutant on two strains of E. coli.

Benzer was teaching a phage course and therefore had growing second strain of E. coli, strain K12, that was used in Paris to study phage regulation. Before class, he decided to try infecting this strain with the mutant T4(rII⁻) phage and the wild-type T4(rII$^+$), hoping (without much basis) to get a better yield of phage. When he returned from class, class he found (**Figs. 3C and D**) that the wild-type strain had lysed the bacteria, as expected, but there was no infection at all with the mutant phage! Naturally, he suspected that he had merely forgotten to add the phage in his rush to class, but a repetition of the experiment squashed that theory. At this point, most would have shelved the experiment as a failure, but Benzer's recent seminar had predisposed him to recognize the import of what he had discovered – the experimental system Pontecorvo longed for – a system Benzer used to break open the gene for the first time.

The 1959 article by Benzer, the culmination of years of work, is unusual in many respects. One is that it reads like a novel rather than a typical research article. Second, it was written for an audience that knew nothing about phage genetics, and Benzer made a concerted effort to explain his system without presuppositions. For these reasons, I'll suggest the unusual practice of reading this article without the skipping around that is generally the route to success. I'll read it along with you.

Introduction

Paragraph 1: The one thing Benzer could count on his audience knowing something about is the triumph of Mendelian genetics and its connection to the chromosome, achieved in large part through the work of Thomas Morgan and his followers on fruit flies. To this audience, a gene was a useful construct, but it was one that would run away if you looked at it too closely. Benzer put the word in quotation marks. The

questions at the end of this paragraph are doubly audacious, first by suggesting that genes might have a tangible reality and second that they may have an internal structure. Imagine suggesting to an ancient Greek that an atom might have internal structure! But by the end of this article, Benzer will have removed the quotation marks and proposed a functional definition for a gene... except that he uses the term "gene" nowhere else in the article outside of the first paragraph!

SQ1. To what in Morgan's work does Benzer refer when he says "linear arrangement of hereditary elements"?*

SQ2. Is there any reason to believe from classical genetics that genes have internal structure?*

Paragraph 2: Benzer feels the need to justify working with *E. coli* and viruses, anticipating the question of why he doesn't use fruit flies or corn.

SQ3. Could the structure of a gene be elucidated using fruit flies? Consider that genes in fruit flies were mapped using recombination, just as Benzer will use recombination to map within the gene. Fruit fly geneticists describe distances between genes in units of centiMorgans, where a centiMorgan is the genetic distance large enough to allow recombination in 1% of crosses. We now know that a centiMorgan corresponds to about a million nucleotides of DNA and that a gene corresponds to about a thousand nucleotides. How many flies would you need to examine to observe recombination within a gene?

Paragraph 3: Benzer continues to address the classical geneticist. We needn't worry about the niceties of negative interference, and Benzer tells us so much. He says that in this article, he will dispense with any consideration of quantitative distances and look only at the topological arrangement of the elements within a gene. What does he mean by "topology"?

We have burned into our brains the notion that DNA is a linear molecule, hence genes must be linear as well, but this was by no means established at the time Benzer wrote his article. The famous X-ray diffraction image of Franklin and Wilkins that was used by Watson and Crick for their model could easily accommodate branches in the DNA at rare intervals. For all anyone knew, genes could be linear, or they could consist of many independent circular bits of DNA, or they could contain many branches of DNA hanging off of a central trunk. Benzer's article was the first to offer experimental results pertinent to this question.

SQ4. Consider the 26 letters below topologically. They can be arranged into a large group of 12 letters, a group of four letters, plus three groups of two letters each, and the rest singlets, according to their topologies. What letters are in the largest group? The second largest?

ABCDEFGHIJKLMNOPQRSTUVWXYZ

SQ5. Draw at least three possible topologies of a gene, according to what was known prior to Benzer's article.

Paragraph 4: This is a well-written article. The **Introduction** section ends with a statement of the experiment to be described, flowing logically from the discussion that precedes it.

The Material and the Method

Usually the section is entitled **Materials and Methods**, but in this refreshing case there is just one set of materials and only one method!

Paragraph 1: I hope Benzer's explanation of the T4 system is now familiar to you!

* If you're not familiar with classical fruitfly genetics, just blip over these questions.

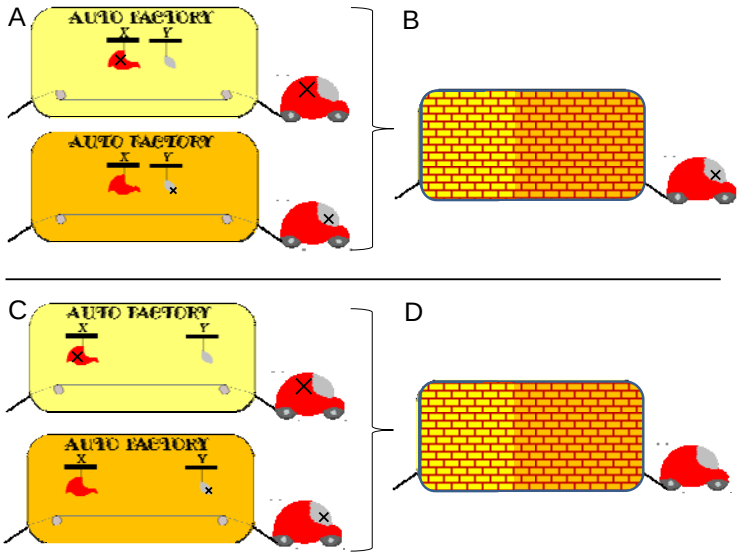

Figure 4. Recombination between two equivalent car factories. Two factories have identical assembly lines, except for different defects. The insides of the factories are shown in worlds **A** and **C**, but they are behind brick walls and can't be seen from the outside. The two buildings are cut blindly into two pieces and recombined to form a single factory. (**A** and **B**) Machines **X** and **Y** are close to each other on the assembly line. It is difficult to cut the buildings precisely between the two machines. The recombined factory still has the defective **Y** and makes defective cars. (**C** and **D**) Machines **X** and **Y** are distant from one another. It's easy to cut between the two machines. The recombined factory has a good **X** from one factory and a good **Y** from the other, producing good cars.

Paragraph 2: Here Benzer explains the power offered by his accidental discovery concerning the failure of T4 rII mutants to grow on E. coli K12. It is an easy matter to control how many phages are mixed with how many *E. coli* cells. When multiple phages enter the same cell, recombination – the exchange of genetic material – may take place between them. The frequency of recombination can tell you the distance between two genetic loci.

If you're unfamiliar with recombination, try this: Suppose that there are two car factories in the same city. They are equivalent, that is, they manufacture exactly the same car in exactly the same way… except that neither factory is operational. In the first factory, the machine in the assembly line that adds the top of the car is defective, and in the second the machine that attaches the windshield doesn't work. There is no source of replacement machines, so the only hope is for the two factories to share resources somehow. It would be nice if the body-attaching machine could be brought over from factory #2 to factory #1, but that's not possible, because the machine is physically attached to the assembly line. Since you can't move the machinery out of the factories, an alternative solution is found – to break the two assembly lines and reattach the good portion of one to the good portion of the other. A huge factory knife is brought in, but outside the walls of the factory, it is difficult to judge where to cut. In the end, the cut is done at random. **Fig. 4** presents two alternative worlds. In one, the two machines in question lie close together on the assembly line. In the second, they lie far apart.

SQ6. With random cut-and-pasting, which world is more likely to end up with a functional recombinant factory?

SQ7. Translate the part of the paragraph talking about "blemishes affecting the same part of the structure" into the language of car factories.

SQ8. Why does Benzer grow the rII mutants in strain B before plating on cells of strain K12?

SQ9. It's easy to plate several million infected *E. coli* cells on a single plate. Is this enough to detect recombination within a gene? (refer back to SQ3)

Paragraph 3 (summary): …

SQ10. Why does Benzer describe the experiment as providing a yes/no answer?

Paragraphs 4 through 6 (the two postulates): …

SQ11. Describe each of the two postulates in the language of car factories.

Effect of the topological nature of the structure

Enough of car factories! On to real genes! … well, first Benzer has a couple of metaphors of his own.

Paragraph 1 (packs of cards): …

SQ12. Give a specific example of two mutant packs of cards that can recombine to form a complete pack of cards.

Paragraph 2 (music tape): …

SQ13. Using your favorite song (one with words), give a specific example of two mutant songs derived from it that cannot recombine to form a complete song.

SQ14. Take the very short song "*Apathy is killing me and nobody cares*" (that's it, there is no more), and consider the smallest segment to be a single word. How many mutants can you make of this song that are simple in the sense described by Benzer?

Paragraph 3: Now finally to real genes, although in a highly abstract form. It is important to understand the relationship between Benzer's Fig. 1a and 1b and the possible relationship of both to real genes. Referring to Fig. 1a, suppose that mutant 1 is crossed with mutant 2. Mutant 1 is defective because something is wrong with elements **c** through **k** (symbolized by **c'** through **k'**). Mutant 2 is defective because of bad elements **e** and **f**. Could any recombination between them lead to a complete gene? Since the answer is no, the position at row 1 column 2 shows a **O**.

SQ15. Benzer's Fig. 1b is symmetric – you could fold it along one of the diagonals and O's will always touch O's. What property of recombination makes this true?

SQ16. Why is Fig. 1b said to be in dictionary (i.e. O before 1) order? If you were given the table jumbled up, could you place the elements in dictionary order?

SQ17. Consider the last sentence of the paragraph (we'll call this the *contiguity rule*). Why is it true? Suppose you changed Row 1, Column 4 from O to | … How would that change Fig. 1b?

Paragraph 4 (Conversely…): …

SQ18. Is this true? Play with Figs. 1a and 1b until you've satisfied yourself one way or the other.

Paragraph 5 (Discussion of Fig. 2): Fig. 2a may not be easy to understand, so I've redrawn it (**Fig. 5)**. Whether you prefer the multicolor circle or Benzer's original, Mutant 1 cannot recombine with Mutant 3 to form a complete circle, and so forth.

SQ19. Check each of the tables in Benzer's Fig. 2b to verify that they are all consistent with Fig. 2a. Are they?

SQ20. Point to the defect in each case that prevents the Fig. 2b table from satisfying the contiguity rule.

Paragraph 6 (weird structures): …

SQ21. Confirm that Benzer's Fig. 3a is consistent with Fig. 3b by mentally doing some of the crosses.

SQ22. Does Fig. 3b follow the contiguity rule? Can you rearrange the lines so that it does?

SQ23. Draw/visualize a structure requiring three dimensions.

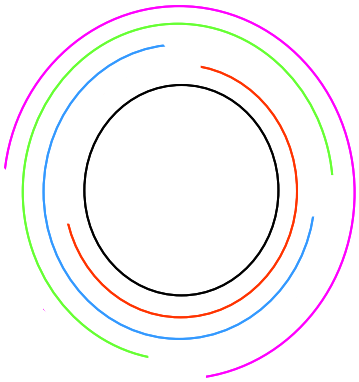

Figure 5. Redrawing of Benzer's Fig. 2a. Each line symbolizes a version of a circular gene, with empty spaces representing deletions: wild-type (black), Mutant 1 (red), Mutant 2 (blue), Mutant 3 (green), Mutant 4 (magenta).

Experimental

Paragraph 1: By "…the structure in phage T4 that controls its ability to multiply in K,…" Benzer means the RII gene(s). He is trying to avoid the use of the word "gene". Note that at the beginning of this section, Benzer restates the main purpose of his work. Isn't this article a joy to read?

Paragraph 2: Benzer describes the experiment for at least the third time, but this time using the language of T4 phage.

SQ24. Recast each sentence in this paragraph in terms of one of the preceding metaphors (factories, decks of cards, music).

Choice of non-reverting mutants

Paragraph 1: Benzer considers the problem of spontaneously reverting mutants. We shouldn't be surprised that some mutant T4 phages spontaneously go back to normal. After all, the mutants arose by a spontaneous process. But it would be deadly to his experiment if he used mutants capable of spontaneous reversion.

SQ25. Suppose you had multiple decks of cards that were lacking a recognizable queen of spades and multiple decks lacking a recognizable ace of hearts. Not being experienced in cards, you're not sure whether the queen of spades is actually the same card as the ace of hearts. So you mix two defective decks together and put them inside an Automated Complete Deck Checker to see if they can productively recombine. And just in case, you repeat the experiment hundreds of times. Suppose that the defect on the queen of spades is just a flattened cockroach. But the roach is still alive, and every so often, it picks itself up and walks away. How would that affect your conclusions?

SQ26. Describe the experiment Benzer must have done that enabled him to write the last sentence of this paragraph.

Paragraph 2: Let's agree not to worry about this paragraph. I suspect Benzer thought of it because it turned out that the non-revertible mutants often had large alterations. Logically, however, it doesn't stand up. One can imagine a world where small mutations are non-revertible, and, indeed, some mutants of this sort were soon discovered by others.

Paragraph 3: 145 mutants…

SQ27. To test every nonreverting mutant against the other, how many crosses would Benzer have had to perform? (this question has a perhaps subtle connection to SQ14)

Fortunately, Benzer found a somewhat easier way, which he called spot test, permitting him to do many crosses on a single plate. Nonetheless, he wrote to Sydney Brenner after finishing the experiments of this paper: "I have been crossing mutants until I feel groggy."[†]

Results

Paragraph 1: Benzer is kind enough to give us a subset of his data to chew on before dropping the entire 145x145 table on our heads. The results of his Fig. 4 are described in the text as "listed in the order in which the mutants were isolated" and in the figure legend as "in arbitrary order". Which is it? There is no mistake. The mutants were isolated in no particular order ***with respect to their positions within rII***, so in the sense pertinent to Fig. 4, the order ***is*** arbitrary.

Paragraph 2: All of the a, b, c', d's have led us up to this point in the article. Fig. 4 is the results as Benzer saw them. If they can be arranged in a dictionary order, then the mutations are consistent with a

[†] Judson HF (1996). *The Eighth Day of Creation*. Cold Spring Harbor, p.299.

linear gene. If not, then they're inconsistent, and we're forced to think about branches and worse. Unfortunately, at this point, he did not know the order of the mutations and therefore could not arrange them from prior knowledge into a dictionary order. But he says that it is not difficult to ***find*** an arrangement of the mutants that works, and proves this by showing one to us (Fig. 5).

SQ28. If it's so easy, then you do it! Without considering Benzer's Fig. 5, rearrange the order of the mutants shown in Fig. 4 to find an order that observes the contiguity rule. It is too much work to do this completely by hand, so I've provided a computer program to do the drudge work (see Analysis Tool on the calendar or DNA unit page). It will instantaneously move mutants around according to your wishes. At first, you may have no idea what to move, but after some playing around, you should get the hang of it. Use the program to find an order different from that of Fig. 5. How do you reconcile your order with that of Fig. 5?

Then Benzer somehow transforms Fig. 5 into Fig. 6. How? First of all, let us remind ourselves that Fig. 6 is a ***topological*** map. It is supposed to be accurate with respect to which deletions lie between which other mutations, but there is no claim that it is quantitatively correct. For example, it may well be that the seemingly small deletion of mutant 455 is actually huge. So long as its hugeness fits between the deletions of mutants 215 and C51, the map remains correct.

To translate Benzer's Fig. 5 into Fig. 6, it is useful to process each line of information, one at a time, from top to bottom (i.e. from left-most deletion to right-most deletion). I've started the process in my **Fig. 6**. Fig. 5 tells me that mutant H23 can not make viable recombinants with any of the other mutants. Therefore its deletion must overlap with all the others. To represent this, I drew a line, representing the length of the deletion, starting at the left-most point. I'm certain of the left boundary, because it's the first gene in dictionary order, but I'm not certain of the right boundary. It is possible that other deletions extend beyond the end of the H23 deletion. I've represented this uncertainty with a dashed line.

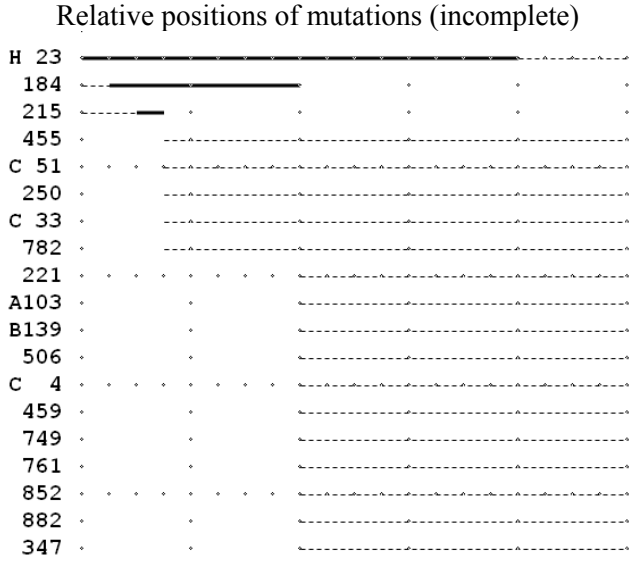

Figure 6. Interpretation of Benzer's Fig. 5 (incomplete). Thick solid lines represent the certain breadth of a mutation. Thin dashed lines represent possible extensions. Dots form a grid to facilitate alignment.

Now on to the second line, representing mutant 184. Fig. 5 tells me that it is the second in dictionary order. Therefore, it ***may*** begin at the same left boundary as H23, but no other deletion besides that of H23 begins further to the left. I start its deletion with a dashed line to represent the uncertainty. Its right boundary must lie to the left of the right boundary of H23, because there are some mutants, mutants 221 through 347, with which 184 forms viable recombinants. The deletions of these 11 mutants can't overlap with the deletion of mutant 184, therefore their right boundary of 184 must lie to the left of the left boundary of the others. This is shown with 11 dashed lines.

SQ29. Complete the partially worked out map.

SQ30. Suppose you tried to make a map not with Fig. 5 but with Fig. 4, i.e. a list of mutants not in dictionary order. Follow the same process as you did ins SQ29. Where do you run into a contradiction?

SQ31. From your experience, explain why the contiguity rule is simply a graphical restatement of what "dictionary order" means.

Paragraph 3 (summary): …

SQ32. Give specific examples from Figs. 4 through 6 to support Benzer's conclusions. Give a result that should have been observed if rII behaved according to the pack of cards model.

SQ33. Suppose that the rII region actually looks like what's diagrammed in my Fig. 7, i.e. separate domains of DNA connected to each other by a non-DNA core. Suppose that deletions can take place anywhere in a DNA branch or within the core (losing any attached branches). What results would you expect from Benzer's experiments? Can his results exclude this model?

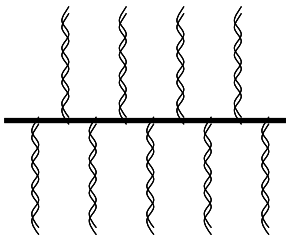

Figure 7. Hypothetical view of rII region. Segments of DNA (wavy lines) are connected to a common core (thick line)

Paragraph 5: Now as sort of a denouement, we get the full table (Fig. 7) and its interpretation (Fig. 8). He doesn't draw any conclusions from this, because all the conclusions have already been drawn from the smaller table.

SQ34. Are the two tables (Figs. 5 and 7) compatible with each other? Pick out a few mutants from Fig. 5 and find them in Fig. 7. Bring your magnifying glass.

Reflection

If you read about this experiment in a textbook, you'd get something like "From the observation that mutations within the *rII* region form a self-consistent, linear recombination map, [Benzer] concluded that a gene is composed of a continuous linear sequence of nucleotide pairs within the DNA." But you read the paper.

SQ35. Does the sentence above capture what you read? Summarize what you've learned and why you believe it to be true.

Of course it turns out that many genes are ***not*** composed of a continuous linear sequence of nucleotide pairs. Introns were quite a shock when they were discovered. By storing the experiment alongside the conclusion, you maintain a direct connection with the Truth. Benzer's observations are and always will be true, no matter how much future experiments deepen our understanding of gene topology.

SQ36. Suppose that the rII region contained introns (some phage genes do!). What would results would you expect from Benzer's experiment?

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