

Adventures in the rII Region

Writing a paper about genetic fine structure means overcoming two powerful blocks imposed upon me by Max Delbrück. One was laid down in 1955, when Delbrück said that the problem would keep me occupied for ten years. He was right: in 1965 my interest suddenly turned off post-hypnotically, and it is now more than I can do even to think about the subject. The second block was laid down more recently, when several papers of mine happened to appear at about the same time. To a letter from his wife to mine, Delbrück appended a footnote: "Dear Dotty, please tell Seymour to stop writing so many papers. If I gave them the attention his papers *used* to deserve, they would take all my time. If he *must* continue, tell him to do what Ernst Mayr asked his mother to do in her long daily letters, namely, *underline what is important.*" It is very difficult for me now to think of anything worthy of being underlined.

Delbrück first entered my life in the form of the chapter heading "Delbrück's Model" in Schrödinger's book, "What is Life?" I read that book at an impressionable age, while still a graduate student in pre-transistor solid state physics at Purdue University. Not long afterward, at a meeting of the American Physical Society at Bloomington, Indiana, a friend took me to visit the home of a former coed classmate of his. Her husband not only knew Delbrück personally, but even pulled a snapshot of him out of a drawer. I could not have been more impressed. The husband's name was Salvador Luria, and it was not long before he had persuaded me to enroll in the phage course at Cold Spring Harbor. Thus I was suddenly plunged into the biology business.

After spending an initial postdoctoral year in A. Hollaender's newly organized Biology Division at Oak Ridge, I had a choice of going to Luria's or to Delbrück's laboratory and asked Luria's student James Watson for advice. Watson thought that it depended on what I wanted. Luria, he said, would be likely to ask me every day what I had done, whereas I might not see Delbrück for a week at a time. I chose to join Delbrück at Caltech. That was sixteen years ago. I have just returned to Caltech and many of the old

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memories seem quite fresh. But this time it is to work on neurobiology with Roger Sperry, who also has the virtue of disappearing for a week at a time.

At Caltech in 1949, Jean Weigle and I shared a room. Since he was a "lark," rising at 4 a.m., and I was an "owl," we could do round-the-clock phage experiments together. I would put the plates into the incubator before retiring and he would take them out a few hours later, record the results and do the next experiment. When I arrived, we could do a further experiment together, and then I would do one more experiment after he went to bed. This system broke down on Mondays, however. There was always a shortage of pipettes on that day, since the kitchen staff did not work on weekends. Complaints to Delbrück were in vain—he insisted that it was good to have one pipetteless day during which one was obliged to think.

I recall one experiment that Weigle and I tried. We thought that one might be able to transfer genes from one bacterium to another via a temperate phage. Delbrück said that the idea was crazy and bet us (50 milkshakes to one) that our experiment would not work. And he won his bet. But the experiment failed not because the idea was crazy, but because it failed to incorporate what Delbrück calls "the principle of limited sloppiness." To keep the background of spontaneous mutants low, we used as donor and recipient a pair of bacterial strains that differed in *two* genes, and scored, as Lederberg and Tatum had done in the discovery of bacterial conjugation, only cases in which *both* genes were transferred. Not long afterward, Zinder and Lederberg did discover phage-mediated transduction, but found, of course, that only genes that are very closely linked (the ones we used had not been) are transferred jointly.

Others working in Delbrück's group at Caltech at that time were Renato Dulbecco, Gunther Stent, Elie Wollman, Wolfhard Weidel, and, as graduate students, Armin D. Kaiser and George Bowen. The urge to do experiments was always 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 Biology 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 every one had a completed paper. That was how my paper on UV irradiation of intracellular phage came to be written.

Delbrück deprecated biochemistry, and this influenced some of us to avoid it. In fostering this attitude, he was assisted by Weidel, the only biochemist in the Caltech phage group at that time, who, when he became impatient with some of our purely formal discussions, would annoy us by saying things like, "Now let a biochemist tell you what this is *really* all

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about." Weidel, after some months of work, isolated the "phage-receptor substance" from *E. coli*, which seemed very impressive until he looked at his "molecules" in the electron microscope and saw that they were lovely, empty, complete cell walls. Though by means of these studies Weidel was ultimately to elucidate the structure of the bacterial cell wall, this dénouement simply confirmed our prejudice as to the despicability of biochemistry, a comforting prejudice to justify our ignorance. Time has changed that for most of us, however. Even Delbrück himself recently spent time studying *lipid* biochemistry in connection with his *Phycomyces* work.

Sometimes Delbrück would proclaim Wednesday and Thursday as a weekend, to avoid crowds and highway traffic on camping trips. The first camping trip in which I participated, into the Anza desert, was fairly typical. We just kept driving until the car got stuck in the sand, and that determined the campsite. Most of the following day was spent in digging the car out. Such visits to the desert were (and still are) the favorite means of entertaining visitors, although some people, like Luria, will not come to Caltech unless guaranteed immunity from camping. It was on one such trip in 1950 that André Lwoff invited me to spend a year in his laboratory at the Institut Pasteur in Paris.

In Lwoff's laboratory I shared a room with François Jacob. It was a lively year. In addition to the other regulars like Jacques Monod, Elie Wollman and Pierre Schaeffer, there was a fine crew of long- and short-term transients: Melvin Cohn, Louis Siminovitch, Annamaria Torriani, Germaine Cohen-Bazire, Roger Stanier and Gunther Stent.

I first discovered the rII phenomenon in Paris, but did not then recognize its significance. As Pasteur would say, "my mind was not prepared." My research project in Paris was inspired by a remark made by Roger Stanier in a review on "Enzymatic Adaptation," in which he had said that while it would be interesting to know whether all bacteria in a culture adapt simultaneously to fermentation of a new carbon source, that would be "almost impossible" to find out. That statement was a challenge for me, since the Luria-Latarjet experiment of UV irradiation 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. For instance, in a bacterial population "half-adapted" to lactose, all cells might have half the maximal level of

galactosidase, or half the cells might have the full enzyme complement. 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.

Once I had started to work in Paris, however, I hit on a more direct non-radiobiological approach to this problem. Monod and Wollman had shown three years earlier that in the bacterial strains they used galactosidase synthesis is arrested by phage infection and that the enzyme is released intact upon the eventual lysis of the infected cells. Thus, if prior to phage infection, a cell population were homogenous in its galactosidase content, a plot of cells lysed vs. enzyme released at various times after phage infection, in a medium containing lactose as only carbon-energy source, would give a straight line. But if the population were heterogenous such a plot would give a curve, its initial slope corresponding to the cells with the highest enzyme level, provided that the enzyme content of each cell were the limiting factor for the rate of phage development within it.

It seemed only natural for me to use one of the bacterial strains then under study in Lwoff's laboratory, a K12 derivative of *E. coli* K12 lysogenic for phage lambda, inducible for galactosidase formation, and sensitive to phage T2. To avoid complications arising from re-infection by early-released phages leading to "lysis inhibition" I chose an *r* (rapid lysis) mutant of T2. But I could not make the experiment work; for some reason the T2-infected cells did not lyse—even when they had been fully induced in lactose medium prior to infection. I checked the phage stock titer again, plating as usual for plaque formation on *E. coli* strain B, and there seemed to be nothing wrong with the stock. That was, of course, the first discovery of the *r*II phenomenon, namely the inability of *r*II mutants of T-even phages to grow on *E. coli* strains carrying the lambda prophage. Rather than trying to figure out at that point what was going on in the system I had chosen, I shifted to the bacterial strain and phage combination used by Monod and Wollman in the first place. This promptly worked and made it possible to demonstrate that, under certain conditions of adaptation of an *E. coli* culture, there is striking heterogeneity in the amount of induced enzyme appearing in individual cells.

That was in 1952, the year of the Hershey-Chase experiment showing the germinal role of the phage DNA. The next year brought the Watson-Crick model, and now DNA was really *in*. Upon my return to Purdue University, I was invited to give a genetics seminar and chose the topic "The Size of the Gene." It was largely based on a review that had recently been published by G. Pontecorvo in *Advances in Enzymology*. The article

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made the point that the various definitions of the gene were not necessarily equivalent and that high resolution genetic mapping would be required to distinguish them. High resolving power requires detection of small numbers of recombinants, so that to resolve details on the level of the size of a gene it would be necessary to apply selective techniques to large mating populations.

The second time I encountered the *rII* phenomenon my mind *was* prepared. 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 phage 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 T2*r* and T2*r*⁺ on the strains I had on 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 T2*r* and T2*r*⁺ 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 this result was now obvious at once; here was a system with the features needed 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.

It soon became evident that this plating behavior was shown only by certain r mutants, namely those belonging to one of the map "clusters" found by Alfred Hershey. These mutants all mapped within a few per cent recombination of each other, but were located at a large number of distinct sites. A unique order of the sites could be established, and the map could be sharply divided into two contiguous segments or *cistrons*, as I later called them, such that any mutant located within one cistron would functionally complement any located within the other cistron. Certain mutants were anomalous in the sense that they would not yield recombinants when crossed with two or more other mutants that did give recombinants with each other. These "deletions" turned out to be especially useful for later work.

The state of thinking in genetics at that time can perhaps be judged by the following experience. In April, 1954, at a meeting at Oak Ridge, I asked a geneticist who was very familiar with Hershey's cluster of mutations and the rII region what he thought was their significance. He said he thought that all were really the same mutation, but located at different places in the heterochromatin.

In that summer of 1954 the Hersheys generously lent their Cold Spring Harbor house to my family. There I met Sydney Brenner. Brenner later described this encounter in a talk at a Brookhaven Symposium: "I was carrying around a book on sequence analysis in proteins and Seymour was carrying around a map of the rII region—consisting of two mutants that mapped in a straight line." The future seemed to us quite straightforward— isolate the rII protein from various mutants, then establish the colinearity of alterations in amino acid sequence with the locations of the mutations in the genetic map. If we could somehow identify the DNA bases, we could even solve the genetic code.

I wrote up the story of the rII mutants and showed the manuscript to Delbrück in Amsterdam later that summer. One of his typically succinct comments was: "Delusions of grandeur." Delbrück knocked the paper so badly that not until a visit to Caltech the following spring did I dare to approach him with another version. He submitted it to the *Proceedings of the National Academy of Sciences*, and it contained an appropriate acknowledgment of his "moderating influence."

Alan Garen came to Purdue and embarked on the isolation of "the rII protein." He began by finding that an extract from r^+ infected cells could stimulate development of rII mutants in $K12(\lambda)$. To identify the active principle, he tried inactivating it with specific enzymes. But adding deoxyribonuclease to the principle greatly *enhanced* the effect, and he soon found that this was due to the Mg^{++} that had been added to activate the enzyme. From then on, his time at Purdue was spent in analyzing the Mg^{++} effect

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on growth of *rII* mutants in K12(λ). Since I had bet that the active principle was a protein, I paid off a bottle of champagne on that one. Strictly speaking, however, Garen never really established that the activity in the original extract was due to traces of Mg^{++} , and it is conceivable that the bottle of champagne may have to be paid back someday. The tradition established by Garen of not finding the elusive *rII* protein was later valiantly continued at Purdue by Masayasu Nomura, F. Robert Williams, and Mutsuo Sekiguchi, and elsewhere by others, using a variety of techniques. Some interesting facts, nonetheless, have emerged from these studies. In spite of the lack of success so far, there is reason to believe that an *rII* protein actually *does* exist because suppressors that act to produce structural changes in other proteins will also suppress some *rII* mutations.

Mapping the *rII* region was more rewarding. It was an example of what we called "Hershey Heaven." This expression comes from a reply that Alfred Hershey gave when Garen once asked him for his idea of scientific happiness: "To have one experiment that works, and keep doing it all the time." Making use of the properties of overlapping deletions, it was possible to divide the map into segments that fitted a strictly linear topology. The *rII* region also had striking differences in local topography, as could be shown by the different frequencies of occurrence of mutations at various "hot spots."

The results brought into focus the distinction between the various definitions of a gene and led me to propose the names *cistron*, *recon*, and *muton* for the units of function, recombination, and mutation.

Ernest Freese came to Purdue about that time. He had gone to Caltech from Germany, to do physics, but became interested in biology. Delbrück invoked his "moderating influence" to talk him out of going into biology, which was, of course, a very effective seduction technique. At Purdue, Freese followed up on the discovery by Rose Litman and Arthur Pardee that one can induce mutations in T2 phage with 5-bromouracil. This chemical mutagenesis had appeared to them as "nonspecific," because the pyrimidine analogue induced mutations in many different phage genes. By mapping the induced *rII* mutants, however, we found that the mutation rate at certain *rII* sites was raised 10,000-fold, while remaining unchanged at some other sites, giving a completely different site distribution for induced and spontaneous mutations. This finding opened the field of relating mutagenic specificity to DNA structure, which Freese and his associates pursued vigorously in the following years.

In 1957, I went to Cambridge for a year to join up, at last, with Sydney Brenner to finish that little problem of colinearity of gene and protein structure. George Streisinger and Sewell Champe joined in. By this time, several other laboratories were in the race. Instead of trying to find the *rII*

protein, we tried to find a gene to fit a phage protein that could be isolated. We shifted our choice from phage heads to tails to tail sheaths and back again to heads. That was the heyday of fingerprinting, which had recently been developed by Vernon Ingram, and we had the privilege of working under the supervision of the Master. It was always easy to find structural differences between the corresponding proteins of T₂ and T₄, but in that year we never did find a difference that could be related to a single mutation. Renato Dulbecco, who was also spending that year at Cambridge, was a spoilsport. He maintained that the result would be interesting only if the genetic map of a cistron and its polypeptide product were *not* colinear. In the end, it was Charles Yanofsky, who, several years later, achieved the first demonstration of colinearity for the tryptophan synthetase of *E. coli*.

The year at Cambridge was hardly wasted, however. In addition to analyzing mutant hemoglobins with Vernon Ingram and building models with Francis Crick (in a very drafty tower room at the Cavendish), it was that year that Brenner, Barnett, and I mapped the *r*II mutations induced by proflavine, finding that its specificity of action was utterly different from 5-bromouracil. The significance of this observation was not evident at the time, but important developments grew out of it later, when it occurred to Crick that proflavine might cause insertions or deletions in the DNA that would shift the reading frame for codons in protein synthesis.

Returning to Purdue, I resumed the project of running the map into the ground with the aid of all the new mutagens that had been found. But I was jarred out of this by the discovery of ambivalent mutants. Irwin Tessman at Purdue had been isolating *r*II mutants in connection with his studies on mutagenesis, and I asked him to give me samples of any *r*II mutants that indicated previously unobserved map sites. Some of those he gave me did not behave like *r*II mutants at all—they multiplied happily on my strain of K₁₂(λ). It turned out that Tessman's K₁₂(λ) strain was different from mine, and that some of my "good" *r*II mutants would lyse *his* strain. This immediately suggested to me that there might be differences in the genetic code in the two bacterial strains. Sewell Champe and I then demonstrated, by genetic experiments, that one such suppressor mutation in a bacterium could modify the genetic code of the cell in such a way that a nonsense codon in the phage genome would be changed to sense.

The place to look for the physical basis of these changes in the code was in the sRNA and the amino acid-activating enzymes. So I became, at last, really embroiled in biochemistry. Soon I was grinding up cells and extracting sRNA and getting involved with various combinations of collaborators, such as Bernard Weisblum, Gunter von Ehrenstein, Robert Holley, Fritz Lipmann, and François Chapeville.

It turned out that sRNA and activating enzymes were, indeed, subject

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to genetic modification, and that an organism may contain several sRNA varieties that accept the same amino acid. The validity of Crick's idea that sRNA acts as an adaptor in protein synthesis was demonstrated in two ways. The first was done by attaching an amino acid A to its normal sRNA, then changing it, while still attached, to another amino acid B. When transferred into protein, amino acid B went into the position where A normally belonged. The second proof was that a given amino acid, when attached to two different sRNA molecules, would go into different positions in protein. The latter experiment also established a physical basis for degeneracy in the code.

This all got more and more exciting, until it dawned on me how many people were doing the same things. I had almost gone down the biochemical drain. Delbrück saved me, when he wrote to my wife to tell me to stop writing so many papers. And I did stop.