

Perspectives

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Forty Years Ago: The Discovery of Bacterial Transduction

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FORTY years ago we published a paper describing bacterial transduction (ZINDER and LEDERBERG 1952). The work was done during the previous two years in JOSHUA LEDERBERG's laboratory at the University of Wisconsin where I was attempting to extend his discovery of bacterial conjugation. Since over many years one's memory can play tricks as well as be influenced by external circumstances, I've reconstructed the discovery from my notes. Although they are for the most part readable, we must remember that they were written at a time when concepts such as phage, lysogeny, and even the gene were obscure.

Getting started: With the discovery of the penicillin enrichment technique for isolating auxotrophic bacterial mutants (DAVIS 1948; LEDERBERG and ZINDER 1948), a number of strains of *Salmonella typhimurium* were marked so as to permit tests for bacterial conjugation. Marking the strains involved the isolation of two sets of nonoverlapping double mutants, most having amino acid requirements. Tests were done by mixing two cultures, plating on a minimal medium, and looking for prototrophic colonies. At that time, similarly marked mutant strains of *Escherichia coli* K-12 would give between 10^{-6} and 10^{-7} prototrophic recombinants. It is also important to note that the *E. coli* data strongly suggested that the bacteria were haploid (TATUM and LEDERBERG 1947).

This work really began when 22 phage-typed *S. typhimurium* strains arrived from LILLEENGEN in Sweden (LILLEENGEN 1948). From each of these strains large numbers of auxotrophic mutants were obtained by the penicillin enrichment procedure. Two mutagenesis protocols were used involving two UV dosages. Trying to reconstruct why I chose these dosages, my guess is that some of the strains carried UV-inducible phages and were readily killed but not readily mutagenized, necessitating a larger UV dose. There are no interpretable data in this part of my notes other than that the lower dose reduced the

viability of the test strain to 10^{-3} while the larger dose reduced it to 10^{-5} . This strain was probably noninducible, but at the time, of course, I knew nothing of phage induction. Over a period of months I accumulated a collection of mutant strain pairs and started intrastain crosses.

Desperate moves: By June, 1950 I noted that all self-crosses had failed. That is, every cross within a strain for which I had a complementary pair of mutant markers failed to give prototrophs. I guess that out of desperation because there was little theory then, I started crossing different strains. Were I to get a cross to work between two different strains, it would already differ from the findings with *E. coli* K-12, because at that time none of the other laboratory strains would mate with K-12 while as far as tested, all K-12 strains were interfertile. There were many interstrain *Salmonella* crosses to try, a 20×20 set. They could only be done slowly and had to be analyzed in detail because there were always contaminants and partial revertants on the plates (false positives) that kept one busy. Crosses were done by washing overnight broth cultures and spreading 10^8 bacteria of each parental type on the surface of minimal agar Petri dishes. There were similar unmixed control plates. With double auxotrophs it was rare to find any real revertants, but during the four-day incubation contaminants would often appear. However, real signs soon appeared that some of the interstrain crosses were indeed producing prototrophs.

Discovery and confusion: We now come to the fall of 1950. On October 5 I did a cross between two of the LILLEENGEN strains, LT-2 and LT-22. It yielded recombinants at a frequency even higher than previously observed for *E. coli* crosses. Colonies appeared at a frequency of about 10^{-5} . This was exciting. We crossed all of our LT-2 and LT-22 derivatives with each other and with other mutant strains to try to understand the phenomenon. Slowly it became clear

that the important element was that one of the parents be a particular mutant of LT-22, a double mutation in the pathway of aromatic amino acid synthesis. Next we compared the nature of the progeny with those from *E. coli* crosses. The *E. coli* recombinants not only had a prototrophic phenotype, that is, the four wild-type alleles of two pairs of markers being selected, but they also segregated a number of unselected markers such as for lactose fermentation and for phage resistance. In the Salmonella case I tested nine different markers. None of them segregated; all matched the LT-22 parent. What then seemed all the more remarkable was that one could always set up the cross in such a way as to select one of the previously unselected markers of the same LT-22 strain, and it would also give 10^{-5} recombinants while none of the other markers segregated, including previously selected ones. Again the markers recovered were always those of strain LT-22. It was an asymmetric recombination that involved only one marker at a time. It resembled pneumococcal transformation and this is why we decided to find out whether or not it required cell-to-cell contact for "mating" to occur. To decide this we did a U-tube experiment. This was first done by BERNARD DAVIS (1950) to ask the same question about *E. coli* K-12 conjugation. Two different cultures were grown in the separate arms of a glass U-tube. Between the arms was a sintered glass filter with pore size small enough to prevent bacterial passage. The medium was flushed back and forth between the arms as the bacteria grew, and those in each arm were sampled periodically for prototrophs. The LT-2 parent was an auxotroph requiring methionine and histidine while the LT-22 parent was a phenylalanine (tyrosine) and tryptophan auxotroph. Prototrophs were found among the LT-22 bacteria but not among the LT-2 bacteria.

Over the succeeding months the following observations were made. Supernatants from the separate cultures were without effect. When the two cultures were grown together, however, the filter-sterilized supernatant would convert, proportional to the amount used, about 10^{-6} of LT-22 mutants to prototrophy while being without effect on the LT-2 mutations. Some months later this could be understood. LT-22 carried a phage (PLT-22, now P22) that crossed the filter, grew on the LT-2 strain, and then returned through the filter, now carrying genes able to replace the fortuitously linked *arom* mutations in LT-22. At the time we floundered for an explanation. JOSH recalled the many stories in the bacterial literature about filterable L-forms (DIENES and WEINBERGER 1951; KLINEBERGER-NOBEL 1951), which looked like what we now call spheroplasts and which supposedly were induced when bacteria were stressed. Because they could regenerate, they must have had

genetic material and were somehow involved in what we were observing.

To confound matters, the effective lysates did indeed contain structures that looked like L-forms and that eventually turned out to be membranes from bacteria lysed by phage.

With the LT-22 strain as our assay, we found that when many of the Salmonella strains were stressed, for instance with chemicals such as crystal violet, by aging, or by the growth of certain Salmonella phages, varying amounts of an activity were produced which we called FA (filterable agent). The range of the effect was also extended. In fact, any single selectable marker from almost any Salmonella strain could be transduced (but this name was not proposed until the fall of 1951). We had clearly been using precisely the wrong procedure, a random collection of double markers, to find this phenomenon. Only two serendipitous occurrences allowed us to detect transduction at all. First, the original markers in LT-22 with which we found transduction were both in the pathway of aromatic amino acid biosynthesis and were probably linked so that they cotransduced. Second, the LT-22 strain carried a lysogenic transducing phage that could propagate on LT-2.

Cold Spring Harbor, 1951: The spring of 1951 brought little further insight into transduction as the notion of L-forms clouded our vision. Nevertheless we set off to the 1951 Cold Spring Harbor Symposium. I note, for those interested in the history of genetics, the most extraordinary volume documenting this symposium (Cold Spring Harbor 1951). If ever a science was in a prerevolutionary crisis (KUHN 1970) it was genetics in 1951. The symposium was opened by RICHARD GOLDSCHMIDT, who proclaimed that there was no such thing as a gene, rather that the chromosome was the unit of function and mutations were no more than analogs to stops on the string of a violin. This was in some ways supported by the mystifying studies of position effect pseudoallelism by LEWIS in *Drosophila* and STADLER in corn. This is also the Symposium that spawned the myth that McCLINTOCK's description of transposition was not understood. There was in fact a separate meeting with BARBARA to discuss the details of her experiments. Moreover, R. A. BRINK, a corn geneticist, was there and had obtained similar results with what he then called *Mp*, but which later turned out to be homologous to McCLINTOCK's *Ac*. As BRINK's colleagues from Wisconsin, we were certainly aware of transposition because we were all drafted to plant and pollinate his corn. For those who still believed in the gene, there were such as J. H. TAYLOR, who said, "I wish I could say something in behalf of the recently deceased, the gene, but . . ." On the other hand some beautiful experiments by HOROWITZ and LEUPOLD provided

substantial evidence for the one-gene, one-enzyme hypothesis. Still others accepted the one-gene, one-enzyme relationship but choked on the "one" in both phrases. EPHRUSSI presented a reasonable paper on cytoplasmic genes (mitochondria) in yeast while SPIEGELMAN confused all with his discussion of long-term adaptation and plasmagenes in yeasts. LEDERBERG gave a paper from our laboratory which I believe has won all competition for incomprehensibility. He spoke for more than six hours. Only H. J. MULLER even began to follow it. There was also a lot on mutagenesis and particularly the new chemical mutagenesis, although most of the analysis was not directed toward the chemical nature of the gene but rather to chromosome damage and segregation effects. I've never found out why, but the Symposium closed with a defense of F. MOUWUS, the Chlamydomonas geneticist, by no less than T. SONNEBORN. MOEWUS's work was at best incompetent, at worst fraudulent.

For me personally, all of 22 years old, it was a revelation. I tried to believe everything I heard, which of course was impossible and left me confused. Still, when HARRIETT EPHRUSSI-TAYLOR said that transduction was due to a phage with some DNA stuck onto it, I knew what I had to do when I got back to the laboratory. From that moment on even a glance at my notes reveals that the experiments became highly focused.

Figuring it out: In addition to the idea that the FA was intimately related to the phage found in all my preparations, the many papers at Cold Spring Harbor on mutagenesis by a variety of mutagens raised the question whether FA was itself a mutagen rather than gene-like. We had so far done no experiment showing that the genetic events we detected were determined by the genotype of the donor bacteria. From August through October of 1951, experiments were done to clarify the nature of both the transduction event and the transducing particles. With the resolution of these questions we were able to give a cogent name to this phenomenon.

A search of phage stocks already in the laboratory revealed several with FA activity. Moreover, the transducing potential of any preparation reflected the genotype of the last host on which the phage had grown. For example, phage grown on a histidine-requiring mutant strain could transduce all genes except histidine. A further experiment showed that it was the genotype and not the phenotype that was determinant. A set of galactose-negative mutations was prepared and phage were grown on each one. Two groups of mutants were found which could mutually transduce each other to galactose fermentation, establishing that transduction reflected the underlying genotype and that there were at least two genes for galactose fermentation.

To this point, all of our transductions had been from mutant back to wild type. Using streptomycin resistance, a marker known to result from a recessive mutation in *E. coli*, we quickly showed that there was no mutant/wild-type directionality in the phenomenon. Additional clarification of the genetic nature of transduction came from studies of the Salmonella flagellar antigens. Different strains have characteristic antigens: *S. typhimurium* antigen i and *Salmonella paratyphi* A antigen a. With serum selection, *S. paratyphi* A could be transduced to carry flagellar antigen i, losing the a. Many other such antigens could be interchanged. The stability of the transductants, the recessive nature of streptomycin resistance and the interchangeability of flagellar antigens all pointed to gene replacement on a haploid background rather than gene addition as the genetic event of transduction.

While putting the genetics of transduction on a solid footing, we sought procedures to determine the nature of the particle involved. HOTCHKISS graciously provided us with purified DNase and we found it to be without effect against FA; however, our only control for DNase activity was its ability to decrease the visible viscosity of an *E. coli* DNA preparation. Antibody was prepared by injecting rabbits with an FA preparation. Neutralizing activity for plaque-forming ability and for transducing activity were both measured; they fell off at the same rate. Also at this time the lab had a large collection of different Salmonella strains. Those that adsorbed the phage also adsorbed FA and *vice versa*, and adsorption correlated with the presence of Salmonella somatic antigen XII.

Both FA and phage were retained by membrane filters with an average pore diameter of 0.1 μm , although both readily passed through bacteriological filter candles. Both were resistant to protease, nucleases, or chloroform treatment. Only UV irradiation inactivated plaque-forming activity at a much faster rate than transducing activity. In retrospect, we know that P22 requires many times as much genetic material to function as does the average bacterial gene, about 30 to 1. FA was obviously bacterial genetic material in a phage particle. Recall that this is all before the HERSHEY-CHASE experiment and the WATSON-CRICK model for DNA. Even LWOFF's critical experiments on the lysogenic state for some phages were just beginning to become known. Still, by the standards of 1951, we were convinced that we had a reasonable explanation for the Salmonella phenomenon and LEDERBERG suggested that we call it "transduction." Other words such as "entrainment" were considered and wisely rejected.

That fall BRUCE STOCKER joined the laboratory and we worked on the transduction of motility and flagellar antigens. The first clear example of a linked transduction then turned up. A nonmotile strain of *S.*

paratyphi B was transduced to motility. The flagella of some clones had the characteristic b antigen of para B while a third of the clones had the i antigen of the motile *S. typhimurium* donor. Evidently, some of the flagella mobility genes were linked to flagella antigen genes. Transduction progeny tests proved this point (STOCKER, ZINDER and LEDERBERG 1953).

Afterward: Shortly thereafter, I went to a meeting of the Society of American Bacteriologists (now the ASM) in Boston. Among those present were FRANCIS RYAN, ED TATUM, SOL SPIEGELMAN and others who were familiar with our findings. A place was created for me to talk at one of the symposia. I introduced a large and appreciative audience to phage PLT-22 and its works. What probably sold the group's true bacteriologists on the reality of what I said was the transduction of *Salmonella typhi* (agent of typhoid fever) from its classical IX, XII:d- (monophasic) to IX, XII:i (monophasic), something never seen in nature. To this day, however, I have never understood the quick acceptance of transduction as a phenomenon when the majority of the audience still believed in a Lamarckian mechanism for bacterial mutations, if they believed in bacterial genes at all, and only a few accepted the existence of conjugation in *E. coli*.

Within a few years of its discovery, transduction was used to study the linked genes specifying the enzymes of intermediate metabolism (operons) as well as the fine structure of a gene. "Transformations and transduction which deal directly with fragments of genetic material acting upon large populations might then provide the tools for genetic analysis at precisely the level wherein the analysis of higher forms become difficult" (ZINDER 1953). So in that strange way that

science can absorb and quickly integrate that which it finds most useful, transduction became classical within a few years. Today it includes the cell-to-cell transfer of foreign genes by any virus, whether the laboratory constructs made by recombinant DNA technology or natural oncogenes in viral oncogenesis. "The ability of viruses to act in so many ways in the bacterial systems as bacterial genes resolves the intellectual difficulties of the mutation theory of the etiology of cancer and the viral theory, but in no way as yet ameliorates the medical problem" (ZINDER 1960).

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