Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

GENE RECOMBINATION AND LINKED SEGREGATIONS IN *Escherichia coli*

AN article with this title was published in GENETICS just 40 years ago[1] (LEDERBERG 1947), following soon after the first discovery of recombination in *Escherichia coli* strain K-12 (LEDERBERG and TATUM 1946). Its appearance coincided with my arrival at the University of Wisconsin to become an assistant professor of genetics. The work had been completed in E. L. TATUM's laboratory at Yale University between March 1946 and June 1947. I then spent the summer at the Marine Biological Laboratory at Woods Hole writing my Ph.D. dissertation and the 1947 article[2] (which was its most important chapter). These studies had begun at Columbia University (in the Zoology Department!) in FRANCIS J. RYAN's laboratory in July of 1945. Although his name does not appear in the authorship, I had benefited enormously from his tutelage, encouragement and discipline. Homage to Francis has been expressed more fully in my own reminiscences (LEDERBERG 1986, 1987) and by others as well (MOORE 1964; RAVIN 1976). I was equally fortunate to have had TATUM take me in his laboratory and share the then hard-won auxotrophic mutants of *E. coli* K-12 that greatly facilitated the experiments (LEDERBERG 1977, 1988).

Our first presentation about crossing in K-12 had been to the Symposium on Heredity and Variation in Microorganisms at Cold Spring Harbor in July, 1946. Although it elicited many critical questions, I was most fortunate to have had such an extraordinary forum in which to respond to them. With the notable intransigence of MAX DELBRÜCK aside, I encountered little further entrenched skepticism about the result, except from some few who had not participated in that debate. What could be reported that July included:

1. The production of prototrophic recombinants from the admixture of various auxotrophs. Stringent selection allowed the detection of as few as one per million recombinants and these occurred even from parents doubly marked to forfend occasional spontaneous one-locus reversions.

2. The segregation among selected recombinants of unselected markers, including auxotrophy (*e.g.*, proline-less in proline-supplemented medium) and resistance to phage T1.

The fact that T1 resistance segregated among prototrophs was an important datum, for it seemed to rule out additive cell-mixture or nuclear-mixture (heterokaryosis) as an artefact. If the prototrophic isolate was pure and stably sensitive, it could hardly contain resistant cells. If it were a heterokaryon, it might be either sensitive or resistant (in fact sensitivity is dominant), but one would not expect a sharp segregation into two stable categories of prototrophs, pure sensitive and pure resistant. However, not everyone at Cold Spring Harbor was so persuaded by these genetic arguments, and I was compelled to promise to do explicit single-cell isolations. MAX ZELLE helped me to learn that technique, and it was to do good service in later studies (ZELLE and LEDERBERG 1951; LEDERBERG 1956, 1957; NOSSAL and LEDERBERG 1958).

So many new questions were now opened up by these thrilling observations! How to react? For one thing, I had to seek another year's leave from medical school; who in his right mind would leave the problem at that stage? The entire project had been motivated by AVERY, MACLEOD and McCARTY's discovery (1944) of DNA as the transforming principle in the pneumococcus. This could not be assimilated as the chemistry of the gene without a broader base of genetics in bacteria. We were disappointed, however, not to find a way to use DNA directly in *E. coli* genetics. (It took some years before a witch's brew was concocted to condition the cells.) Deoxyribonuclease did not influence the K-12 crosses, arguing for some direct cell-to-cell interaction—perhaps like conjugation in ciliates, so brilliantly investigated by T. M. SONNEBORN (1947; *cf.* WENRICH 1954). But it was hard to design direct approaches to the physical mech-

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[1] It will be awkward to cite original sources at every point of historical attribution: see especially LEDERBERG (1987), BACHMANN (1983), IPPEN-IHLER and MINKLEY (1986), and JACOB and WOLLMAN (1961) for comprehensive reviews. An important overview of *E. coli* and *Salmonella* genetics has just been announced (NEIDHARDT 1987).

[2] It is time to correct a typographical error in my 1947 paper: N. T. J. BAILEY has pointed out to me that "203" on the first line of Table 5 should be "303."

anism of crossing when it remained such a rare, sporadic phenomenon. Even with hyperfertile strains, this remains something of a difficulty today, compared to the massive synchronization that facilitates kinetic studies of viral infection.

The main issues that could be addressed at that point, and which would contribute to the "Mendelization" of bacteria, were:

1. What is the range of markers that participate in crossing? and

2. Are they organized in linkage groups or chromosomes?

The work of 1946–1947 was then devoted to accumulating a wider panoply of markers and to improving the methods for handling them. Besides the auxotrophs and virus resistance, sugar fermentation mutants were particularly attractive: they could be acquired by visual inspection of the colonies on indicator media (such as eosin-methylene blue agar), and similar methods could be used to score the segregants in numbers. The relevant enzymes, especially the disaccharases, would also be most readily amenable to further studies. [The β-D-galactosidase of E. coli K-12 (LEDERBERG 1950) has certainly made its contributions to our understanding of gene action!]

Table 1 of the 1947 paper lists eight markers, in addition to another eight auxotrophies used for recombinant selection. The former all segregated, and recombined in every imaginable fashion, but not at random. The statistics of co-segregation implied a single linkage group, and permitted the figuring of the first map, substantially consistent with today's very nearly complete mappings of over a thousand markers (BACHMANN 1983) and the expectation that E. coli K-12 will have its DNA completely sequenced (about 5 megabase pairs) within this decade. Reverse crosses were used to show that the segregation ratios were intrinsic to the locus rather than to the physiology of the allele. Four-strand crossing-over was also looked for as prototroph colonies containing two clones of different crossover classes. These were rare, partly because of the stringencies of selection. A later study on microscopically isolated zygotic pairs corroborated the loss of chromosome segments and gave evidence of recurrent cycles of recombination in the exconjugant clone (LEDERBERG 1957; ANDERSON and MAZÉ 1957). Needless to say, these efforts to graft the classical concepts of chromosomal behavior in meiosis onto recombination in bacteria have been overtaken by molecular genetic perspectives.

A great and continuing puzzle was the failure to find chromosomal aberrations even in heavily irradiated stocks, in contrast to the results typical of Drosophila and other eukaryotes. Yes, deletions (COOK and LEDERBERG 1962) and, of course, insertions are now well known, but inversions are rare indeed, apart

from those involved in adaptive gene regulations (BORST and GREAVES 1987). I am not aware of any systematic study of the production of inversions in bacteria by physical or chemical mutagens. I had thought at the time that bacteria might lack explicit enzymatic machinery for heterologous translocational repairs of broken DNA. One also had to think of differences in chromosomal organization: there was no evidence of histones in bacteria. Later, the discovery of species-specific repeated-sequence DNA in eukaryotes opened the possibility that this might furnish homologous stretches for reunion modelled on crossing-over. (Repetitious DNA is far less abundant in bacteria.) I have looked in vain for published reports on the distribution of chromosome rearrangements in interspecific somatic cell hybrids that might test that hypothesis; it is, however, supported by the correlation of chromosome breakpoints in Drosophila rearrangements with repetitious DNA (LEE 1975; cf. DAVIS, SHEN and JUDD 1987). The matter was of some consequence in our efforts, starting in the late 1960s, to design ways of splicing foreign DNA into the bacterial genome (LEDERBERG 1969; CIFERRI, BARLATI and LEDERBERG 1970; SGARAMELLA 1972; EHRLICH, SGARAMELLA and LEDERBERG 1977; HARRIS-WARRICK and LEDERBERG 1978). To this day we rely mainly upon interaction of homologous sequences in designing for DNA integration. The relative paucity of inversions may also be a perspective of scale: there is little intergenic spacing in E. coli. Most pairs of breaks would do potentially lethal intragenic damage in two places (not to mention the problems of reversing the direction of transcription). SCHMIDT and ROTH (1983) have discussed these and other contingencies in connection with the rarity of inversions in Salmonella.

The overall maps of distantly related species, like Salmonella and E. coli, are remarkably well conserved despite their large divergence in DNA homology. It has then been proposed (RILEY and ANILIONIS 1978) that the large-scale organization of the map in bacteria may be functionally constrained, as it surely is in viruses.

Our original linear map started to fall apart when additional markers were recruited, especially xylose and maltose fermentation and streptomycin resistance. They just did not fit: in 1951, I published a map needing three branches (LEDERBERG et al. 1951). Despite my admonition at the symposium, and in the caption, that "This diagram is purely formal and does not imply a true branched chromosome," the model was taken as a concrete proposal rather than as a portent of problems. J. D. WATSON and W. HAYES (1953) thought that three chromosomes would suit better than a branched monstrosity. Fortunately, WOLLMAN and JACOB (1958; reviewed by JACOB and

WOLLMAN 1961) soon resolved these and other confusions with their kinetic studies of progressive chromosome transfer, sometimes interrupted in midpassage, and the now accepted circular map. In my 1947 paper, the selective markers had provided operational termini for the map, as noted in the discussion.

There are some points of tenderness here. Other data (on persistent heterozygotes with hemizygous reaches, NELSON and LEDERBERG 1954) implied that chromosome segments might be deleted from either parent. For some time (LEDERBERG 1955), I believed that this post-zygotic elimination (shades of SCIARA or the MARY LYON effect!) was an alternative to progressive transfer. That was plainly wrong-headed; but the data still pertain, although interruptable progressive transfer is clearly the first-order mechanism. Very probably, both processes operate: the issue has been virtually forgotten and has not been cogently addressed for almost 30 years. (I left K-12 behind when I moved from Wisconsin to Stanford in 1959, and there concentrated on DNA-amenable systems like *Bacillus subtilis*.)

It is many years since WOLLMAN and JACOB illuminated the kinetic mechanism of DNA transfer. We are approaching the completion of the *E. coli* map in a way that portends the sequencing of the human genome. *E. coli* today is perhaps exploited almost as much for its technological potential as for fundamental studies. Nevertheless, some elementary aspects remain unsettled, in particular the precise physical conduit of the DNA strand as it is progressively passed from one cell to the other, and what happens to it on its way to the formation of recombinants.

JOSHUA LEDERBERG
The Rockefeller University
New York, New York 10021-6399

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