Forty years of genetic recombination in bacteria

Between April and June 1946, Joshua Lederberg and Edward L. Tatum carried out a series of experiments that proved that bacteria can exchange their genes by sexual crossings. The experiments were reported in Nature just 40 years ago. In the following pair of articles, Joshua Lederberg first provides a personal reminiscence of the circumstances of the discovery and then, together with Harriet Zuckerman, considers it as a possible case of "postmature" scientific discovery.

A fortieth anniversary reminiscence

Joshua Lederberg

In September 1941, when I started as an undergraduate at Columbia University, the genetics of bacteria was still a man's-land between the disciplines of genetics and (medical) bacteriology. The question whether "bacteria have genes, like all other organisms" was still unanswered, indeed rarely asked. My own thoughts at that moment lay elsewhere. I looked forward to a career in medical research applying chemical analysis to problems like cancer and the malfunctions of the brain. Cytotoxicology then appeared to be the most promising approach to cell biology. It was Francis J. Ryan (d. 1963) who turned my attention to the sharper tools of genetics.

Ryan had spent 1941-42 as a postdoctoral fellow at Stanford University, where he had met G. W. Beadle and E. L. Tatum (d. 1975), and had become fascinated with their recent invention of nutritional mutations in Neurospora as a tool for biochemical genetic analysis. Although working on a fungus like Neurospora did not go down smoothly in a Department of Zoology as at Columbia, where Ryan had accepted an instructorship, he established a laboratory to continue these studies. In January 1943 I was reassigned to begin my studies at Columbia Medical School; but I continued working with Ryan at the Morning-Side Heights campus.

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**Discovery**

The important biological discovery of that year, by Avery, MacLeod and McCarty, was the identification of DNA as the substance responsible for the Pneumococcus transformation. This phenomenon could be viewed as the transmission of a gene from one bacterial cell to another; but such an interpretation was inevitably clouded by the obscure understanding of bacterial genetics at the time. Avery's work, at the Rockefeller Institute in New York, was promptly communicated to Columbia biologists by Theodosius Dobzhansky (who visited Rockefeller) and by Alfred Mirsky (of the Rockefeller faculty) who was a close collaborator of Arthur Pollister in the Zoology Department. The work was the focus of widespread and critical discussion among the faculty and students. Mirsky was a vociferous critic of the purported chemical identification of the transforming agent, while applauding the central importance of the work. For my own part, the transcendent leap was simply the feasibility of knowing the chemistry of the gene. Whether this was DNA or protein would certainly be clarified quickly, provided the Pneumococcus transformation could be securely related within the context of gene transmission. I read the Avery, MacLeod and McCarty paper on 20 January 1945, prompted by Harriett Taylor (later Ephrussi-Taylor) a graduate student in Zoology who planned to pursue her postdoctoral studies with Avery. My excited response is recorded as... "unlimited in its implications... Direct demonstration of the multiplication of transforming factors... Viruses are gene-type compounds."

At once, I thought of attempting similar transformations by DNA in Neurospora. This organism had a well understood life cycle and genetic structure. The biochemical mutants opened up by Beadle and Tatum also allowed the efficient detection of nutritionally self-sufficient (prototrophic) forms, even if these were vanishingly rare. This would facilitate the assay of transformational events.

Between January and May, 1945, I shared this idea with Francis Ryan; in June, he invited me to work on the subject with him. Our discovery was soon discovered that the leucine-minus Neurospora mutant would spontaneously revert to prototrophy, leaving us with no reliable assay for the effect of DNA in mediating genetic change in Neurospora. Questions about the biology of transformation would remain inaccessible to conventional genetic analysis if bacteria lacked a sexual stage. But was it true that bacteria were asexual? René Dubos' monograph, The Bacterial Cell, footnoted how inconclusive the claims were for or against any morphological exhibition of sexual union between bacterial cells.

My notes dated 8 July 1945 detail hypothetical experiments both to search for mating among Monilia (medically important yeast-like fungi) and to seek genetic recombination in bacteria (by the protocol that later proved to be successful). These notes coincide with the beginning of my course in medical bacteriology. They were provoked by the contrast of the true
tional teaching that bacteria were *Schizosaccharomyces*, asexual primitive plants, with an appreciation of sexuality in yeast, which was represented at Columbia by the graduate research work of Sol Spiegelman and Harriett Taylor.

Dubos' cited many unclear, and two clear-cut negative results for sexuality in bacteria using genetic exchange methodology. But these two studies had no selective method for the detection of recombinants and so would have overlooked the process had it occurred less often than perhaps once per thousand cells. With the use of a pair of nutritional mutants, say A'B' and A B', one could plate out innumerable cells in a selective medium and find a single A'B' recombinant. In early July, I began experiments along these lines. In the first instance I used a set of biochemical mutants in *Escherichia coli*, which I began to accumulate in Ryan's laboratory. To avoid the difficulty that had arisen in our *Neurospora* experiments, a spontaneous reversion from A'B' to A'B in *E. coli*, the strategy would be to use a pair of double mutants, A B'C'D' and A'B'C'D'. Sexual crossing should still generate A B'C'D' prototroph recombinants. These would be unlikely to arise by spontaneous reversion which, in theory, requires the coincidence of two rare events; A' → A' and B' → B'. Much effort was devoted to control experiments to show that double reversions would follow this model, and so occur at a negligible frequency in the cultures handled separately. Thus the occurrence of prototrophs in the mixed cultures would be presumptive evidence of genetic recombination.

**Experimental luck**

1. We have learned that *E. coli* strain K-12 itself was a remarkably lucky choice of experimental material: only about one in twenty randomly chosen strains of *E. coli* would have given positive results in experiments designed according to our protocols. In particular, strain is, which has become the standard material for work on bacteriophage, would have been stubbornly unfruitful. Tatum had acquired K-12 from the routine stock culture collection in Stanford's microbiology department when he sought an *E. coli* strain to use as a source of tryptophanase in work on tryptophan synthesis in *Neurospora*. The same strain was then in hand when he set out to make single and then double mutants in *E. coli*.

In 1946, I was very much aware of strain specificities and was speculating about recombination types (as in *Neurospora*), and then no way to say how many other strains would have been tried, or in how many combinations, had the June 1946 experiments not been successful.

2. An equally important piece of luck was that, the selected markers Thr (threonine) and Leu (leucine) are found almost at the origin of the *E. coli* chromosome map. The cognoscenti will recognize that in a cross B M T'L'. F × B T'L'. F, the configuration used in June 1946, these chromosome localizations offer almost a maximum yield of selectable recombinants. We were therefore led stepwise into the complexities of mapping.

**Long shot**

Meanwhile at Stanford, Ed Tatum, whose doctoral training at Wisconsin had been in the biochemistry of bacteria, was returning to bacteria as experimental subjects, having published two papers on the production of biochemical mutants in *E. coli*, including double mutants like those described here. During the summer of 1945 Francis Ryan learned that Tatum was leaving Stanford to set up a new program in microbiology at Yale. He suggested that, rather than merely ask Tatum to share these new strains, I apply to work with him and get the further benefit of his detailed experience and general wisdom. Tatum agreed and suggested that I arrive in New Haven in late March, to give him time to set up his laboratory. He hinted that, in addition to his previous concerns of the group that Lwoff had raised, I should look in mid-April for major results. This was dedicated to the study of asexual primitive plants, with emphasis in Great Britain by Totemographic Limited, Basingstoke, Hampshire