Just another recent item.

Perspectives

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Genetic Recombination in *Escherichia coli*: Disputation at Cold Spring Harbor, 1946–1996

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THE Cold Spring Harbor Symposium in July, 1946 was a celebration of the postwar reunion of international genetic science. It was devoted to the genetics of microorganisms; apart from the phytopathogenic fungi (MOULTON 1940), this was virtually *terra incognita* before World War II. Only within the most recent few years had any geneticists schooled in the main-line organisms (Drosophila, maize) made any serious contact with the microbes. One of those, importantly, was MILISLAV DEMEREC, who had found drug- and phage-resistance mutations in *Escherichia coli* to be ideal for his own favorite interests in chemically induced mutation. As Director of the Cold Spring Harbor Laboratory, he had given great nurturance to LURIA and DELBRÜCK and, among other newly rising stars, to EVELYN WITKIN. He was also the organizer of the symposium. The international contingent numbered such celebrities as EPHRUSSI, F. KAUFFMANN, LATARJET, LWOFF, MONOD, PIRIE, PONTECORVO, and M. J. D. WHITE. Scarcely any American even remotely interested in the area was absent; I can only think of G. W. BEADLE, who was just moving to Caltech and whose work in Neurospora biochemical genetics was represented by his recent collaborators, EDWARD L. TATUM and DAVID BONNER.

ED TATUM (LEDERBERG 1990), just recently moved from Stanford to Yale, had been my own lab chief since mid-March, when I had come up from Columbia to join him at the behest of FRANCIS RYAN. My status at Yale was a temporary research fellow (of the Jane Coffin Childs Fund), a medical student on brief elective leave from Columbia Medical School (P&S) after the grinding schedules of wartime education under the Navy V-12 training program.

Since the summer of 1945 I had been working with RYAN on a fanciful project, namely the search for sexual processes in bacteria, more precisely genetic recombination in *E. coli*. This had been motivated by the 1944 report from the Rockefeller Institute (AVERY *et al.* 1944) on transformation in pneumococcus mediated by DNA. To my mind, that report had all the earmarks of being the foundation of a new molecular genetics, as indeed turned out to be the case (LEDERBERG 1994). One catch was, could one really speak of "genes" in bacteria when there was no experimental procedure to see them segregate and reassort, no Mendelian paradigm? Among those who thought about the matter at all, there were plenty of skeptics who took a more holistic view of the bacterium, including such giants as HINSHELWOOD (1946) and HUXLEY (1942) who saw no reason to impute more fine-grained genetic structure within the bacterial cell. It was looked upon as a dynamic reaction network. Mendelian genetics was a battleground of political ideologies as well, with its suppression in the Soviet Union under the banner of LYSENKO, enforced by STALIN's police state, who nevertheless found many sympathizers among intellectuals not actively involved in experimental genetics research.

After a half-hearted and for then futile effort to achieve transformation in Neurospora with extracts (which may or may not have had any DNA aboard), I concluded that these investigations with DNA would have to be pressed with bacteria. I searched the historic literature, but found no compelling evidence, pro or con, to reject sexuality as part of the life history of bacteria. Never mind that LEEUWENHOEK and most reputable microscopists since had failed to see any couplings of the kind readily observable, *e.g.*, with Paramecium; and never mind that the class name "Schizomycetes" virtually defined bacteria by their chastity. In this agnosticism, I was greatly encouraged by RENÉ DUBOS's (1945) extraordinarily insightful *The Bacterial Cell*, which fulfilled the expectations of its title in offering a very broad biological perspective on bacteria as organisms, not merely as malicious agents of putrefaction and disease. In this work, which appeared late in the summer of 1945, he remarks, "If bacteria do really reproduce by sexual methods, it should be possible to cross closely related species and strains and

Dedicated to the memory of ANDRÉ LWOFF, 1902–1994 (see JACOB 1994).

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to determine something of their genetical behavior . . . . most workers have reported only failure . . . it has not yet been proven that the inheritance of characters in bacteria follows the Mendelian pattern." I took this as strong encouragement that the question was still open, and a bolstering of the experiments I had already begun with RYAN's critical oversight.

Furthermore, looking at the natural history of bacteria, I was struck by the combinatorial patterning of the cell envelope and the flagellar antigens in Salmonella serovars (KAUFFMANN 1941); this would have its most ready explanation if some mechanism of genetic exchange did operate in that genus. We were helped by the mystique that denominated each new serovar with a new species name, like *S. durban, S. newport,* etc., occasioning a formal published report and periodic recompilation of the names already apportioned. My expectations were also bolstered by the experience I had under RYAN's tutelage with the life history of Neurospora, and my reading of the complex life histories, including sexual stages, of many other microfungi, algae, and protozoa (CALKINS 1926; HARTMANN 1943). They were reinforced by my personal experience as a parasitology technician in a naval hospital, where my main duty was to diagnose malaria (*Plasmodium falciparum vs. P. vivax*) in blood smears from the First Division Marines returned from Guadalcanal in 1943.

My proposed experimental design was derivative of my experiments with RYAN on auxotrophic mutants of Neurospora, that these could be subjected to stringent selection for reverse mutations by plating large numbers of cells in minimal medium (RYAN and LEDERBERG 1946). Similar things happen with mutants of *E. coli*; but could one nevertheless find additional outcomes from the interaction of two complementary strains? I felt that could only be settled by using pairs of double mutants, each strain then being pragmatically perfectly stable even when billions of cells were subjected to stringent selection. The trouble was, with the methods of those days, mutants were hard to come by, and needed a lot of tedious handpicking of colonies. But ED TATUM had made that investment—he already had gotten on to using presterilized toothpicks, which saved the step of flaming a nichrome wire needle—and he had already reported getting double auxotrophs (TATUM 1945).

I wrote to TATUM asking if he had exercised those double mutants in the direction of seeking recombination; if not, might he either make them available to me or, my even fonder hope, allow me to work on the project in his own laboratory. TATUM knew RYAN well, from the latter's postdoctoral experience at Stanford in 1941–1942, prior to his return to Columbia to find me (an eager sophomore) camped on his doorstep imploring him for a place in his lab. So RYAN's recommendation carried a lot of weight. Many years later, after RYAN's early death in 1963 (RAVIN 1976), a mutual friend told me of one of RYAN's deeper motives in arranging that liaison, in behalf of my long-term academic career interests. He foretold I would face serious obstacles as a brash New Yorker, and a Hebraic one to boot, without an established champion. An Ivy League stamp might help ameliorate that. In fact, in his letters of recommendation for my first academic position, TATUM took pains to argue that my research qualifications far outweighed the impediments "of . . . personality and . . . race."

So I did arrive via the New York-New Haven-Hartford railroad on the 18th of March. The first question was to find an affordable place to live. I doubt if it had anyone's formal approval, but I was able to camp in the medieval tower of the Osborn Botanical Laboratory, a ladder's climb up from the third-floor laboratory bench assigned to me. For a couple of weeks, I was glad to have the company in my encampment of ART GALSTON, just arrived from Caltech and looking for an apartment so that his family could join him in his appointment to the Yale faculty. ART still chides me for my ravings about crossing bacteria, when he was trying to get some rest. It was pretty lonely there after he left, but the Tower was a convenient location for getting in two or three shifts of experiments a day with zero distractions.

I felt the main task was to get all of the controls in place, before I dared do a crossing experiment. I was worried about syntrophy or cross-feeding of complementary auxotrophs, an interchange of metabolites through the medium which might confuse a finding of interchange of genes between cells (LEDERBERG 1946). Single mutants would often do this, as could be shown by the diffuse growth seen when agar layers heavily seeded with one mutant were superimposed with the other. BERNIE DAVIS made very constructive use of asymmetrical syntrophy in ordering metabolic pathways (DAVIS 1955). As expected, this was greatly reduced with double mutants. It was also important to isolate more mutants, and I worked out an elementary way to do this: just look for the small colonies, or the late-appearing ones, on marginally supplemented agar medium. Most of these were phenocopies, but it did reduce the tedious picking of thousands of bacterial colonies at random. There was still plenty of motivation to develop more efficient procedures later on (LEDERBERG and ZINDER 1948; LEDERBERG 1989). Above all, I remonstrated with myself, be sure that the double mutants live up to their reputation and show no measurable reversion to wild type (prototrophs), imputedly a two-step process, even under stringent selection of large populations (10^8 or 10^9 at one blow).

My notebooks show the first clear-cut positive finding on Sunday, June 2, 1946. By the 19th I had already repeated it a dozen times, and while visiting HARRIETT TAYLOR, later EPHRUSSI (RAVIN 1968) at the Rockefeller Institute, I wrote, "Still working to clinch the evidence . . . it may take another week more." (HARRIETT had

been an important herald at Columbia of the work in AVERY's lab at the Rockefeller; and it was her reprint of the 1944 paper that actually propelled me on the trail.) And on the 21st, I had written to Dean AURA E. SEVERINGHAUS pleading for a more extended leave from medical school, and excusing it in the following terms: "compelling evidence not as yet conclusive for the existence of a primitive sexuality in bacteria . . . importance epidemiology, chemotheraphy, . . . gene action and growth in general."

I had had one false start: shaken, aerated cultures did not work well. I could not anticipate how fragile the mating pairs were, as was beautifully exploited later by JACOB and WOLLMAN (1961), nor that aeration that promotes vegetative growth actually inhibits the fertility function. In fact, the simplest design worked beautifully: just spread a drop each of broth cultures of the two parents on a minimal agar plate. The residual nutrient carry-over is negligible, may even be helpful, and there is a high enough cell density to allow for undisturbed mating contacts.

By the end of June, three and a half months after arrival, I had also incorporated unselected, segregating markers into the crosses, namely resistance to phage T1 as well as additional auxotrophies. It was reassuring that a variety of recombinant types for unselected markers could be found among the selected prototrophs. To use today's terminology, *bio met ton* × *thr leu* gave prototrophs, some *ton*, some *ton*$^+$. In other experiments where biotin selection was relaxed, one could also find *bio*$^+$ *ton* and *bio ton*$^+$ among recombinants selected as *met*$^+$ *thr*$^+$ *leu*$^+$. This was feasible because *met* proved to be absolutely stable against reversion (probably a deletion).

So these lab results came to a head just as the Cold Spring Harbor symposium loomed. ED TATUM had been scheduled to give a talk on chemical and ultraviolet mutagenesis in *E. coli* (TATUM 1946); he helped me get into the audience as a graduate student. Somewhat archly, he mentioned, "The main attribute lacking in bacteria which would make them ideal material for combined genetic and biochemical investigation is their apparent lack of a sexual phase . . ." We were just not sure whether the time was ripe for the announcement of my recent findings. CARL LINDEGREN did pick up the cautious wording about "apparent lack," saying "[Tatum] was somewhat more cautious than Dr. Dubos, Dr. Lwoff, and Dr. Luria all of whom deplored the fact that 'there is no sexual mechanism in bacteria'." He then voiced the parable that the sexual phase of a red bread mold (namely Neurospora) was unknown for a hundred years. TATUM's discussion did then refer to my experiments, and he negotiated with DEMEREC for an exception from the published program to permit me to present them. We were also motivated by A. D. HERSHEY's (1946) presentation of the first data on genetic recombination in bacteriophage. TATUM's care to be sure that I would get full credit (or blame?) was characteristic of his fairness and generosity to his younger colleagues. I was grateful that he did append his name, for that surely enhanced the credibility of a 21-year-old making his first appearance before the scientific establishment.

So, there was a presentation, by LEDERBERG and TATUM (1946a), entitled "Novel genotypes in mixed cultures of biochemical mutants of bacteria." Besides the publication and my own recollections, I have no written record of that day. The date was probably Thursday, July 11, and I would be most grateful (as I have asked before) for any more detailed documentation.

I do recall most vividly a protracted debate, notably with ANDRÉ LWOFF, who was not at all convinced that I had demonstrated prototrophy in single-cell clones despite my care with conventional plating, and the bolstering evidence of some clones being *ton*, some *ton*$^+$. I rejoined that, in addition to my repeated replatings, a *ton*$^+$ clone, fully sensitive to phage T1, could hardly be a mixture. LWOFF had been an early pioneer in bacterial nutrition and had discovered the requirements for hemin and for nicotinamide on the part of various Hemophilus species (LWOFF 1971). He was well aware, as indeed I was, of a prototypic example of syntrophy, the cross-feeding of *Hemophilus canis* and *H. parainfluenzae* (VALENTINE and RIVERS 1927). Perhaps my "prototrophs" were no more than cross-feeding mixtures. I insisted I had taken full account of that possibility; he persevered. After a while we were talking past each other, and MAX ZELLE offered to assist in the technology of single-cell isolation to put the matter to rest. The opportunity for that critical debate was a boon I did not appreciate for many years (ZUCKERMAN and LEDERBERG 1986; LEDERBERG 1987a). Hard questions had been fully argued in front of an informed audience; that forum undoubtedly led to an earlier acceptance among geneticists than simply floating a publication, which would have allowed a diffuse skepticism or diffidence. One could not have participated in that critical discourse, amongst so many peers, without reaching a conclusion one way or another.

The term "sex" does not appear in the Cold Spring Harbor paper: several experiments remained to be done to show that the genetic recombinants entailed a cell-to-cell interaction, which would be a hallmark of any process that deserved the term sexual.

There was one hiccup: LURIA promptly tried to emulate the findings, using phage resistance markers in *E. coli* strain B. He failed, and it soon became apparent that *E. coli* K-12 was an especially lucky selection; only about one strain in twenty would have worked with the protocol used (LEDERBERG 1951). This is just one more example of ED TATUM's notorious serendipity. But LURIA's failure was made much of at Caltech, though greatly mitigated later when AARON NOVICK and LEO SZILARD at Chicago were able to advise the grapevine

that they had no trouble reproducing my results with the K-12 strains I had furnished them. LURIA himself was wholly congenial and encouraged me to stand up to the few old-timers whose critical rationality, he said, might be complicated by envy. And I have similar, warmest recollections of avuncular encouragement from TRACY SONNEBORN, CURT STERN, and H. J. MULLER, not to mention my prime mentor FRANCIS RYAN. I have been so fortunate: I could name dozens of others who have stood as wonderful role models of science as a sharing community devoted to truth, and supportive even of the callowest youngsters.

The main competitor to a sexual model was that of DNA-mediated transformation, as in the pneumococcus, though our knowledge of this was still confined to the single marker for the polysaccharide capsule (LEDERBERG 1994). After the symposium, that was the first order of business. Sterile filtrates of single and of mixed cultures had no gene transfer capability. The symposium itself would not be published for many months, so in mid-September we submitted a paper for *Nature*, "Gene recombination in *Escherichia coli*," a far less reticent title (LEDERBERG and TATUM 1946b). And this did recite "cell fusion" and "sexual process" as the prime candidates of interpretation. This was published on October 19, 1946, and was the first exposure in print of these claims. Subsequently, thanks to MACLYN MCCARTY, I could test his crystalline deoxyribonuclease and found it had no influence on genetic exchange. Then BERNARD DAVIS introduced the "bundling board," a sintered glass filter separating the cultures of would-be mating cells; this did frustrate genetic exchange and lent further credence to cell-cell interactions (DAVIS 1950).

Beguiled by copulating paramecia and gamete fusion in yeast, I was, however, wrong about "cell fusion." As we know from the later work of JACOB and WOLLMAN (1961), the transfer of DNA is progressive. It may take up to 100 minutes for the entire chromosome to be transmitted, and the process is easily disrupted by mechanical shaking, greatly simplifying the construction of linkage maps. While "sex pili" play an indispensable role in the cohesion of mating cells, the fine structural details of the DNA transfer remain enigmatic (FIRTH *et al.* 1996).

Most of the following year was devoted to the recruitment of additional markers, notably *lac*, and the elaboration of the first linkage maps (LEDERBERG 1987b). This work was retrospectively designated as my Ph.D. dissertation, and armed with that credential, I had to face the agonizing decision whether to complete my medical studies or embark on a new academic research position at the University of Wisconsin. This would not have happened without the staunch support of the late R. A. BRINK (OWEN and NELSON 1986), heading the Genetics Department there, who had to overcome many ramparts of prejudice in sponsoring a New Yorker, 22 years old, in a new branch of genetics, and bringing no farm experience whatsoever, for an appointment as assistant professor in a college of agriculture.

The wisdom of my choice for the latter option in September, 1947 was warmly confirmed when an editor of this series, JAMES F. CROW, joined the same faculty; so I have the occasion to commemorate a 50-year friendship as well.

In later years, I came to wonder why such simple experiments as came to fruition in 1946 had not been concocted, say in the wake of the rediscovery of MENDEL's laws in 1900. That might have set microbiology ahead by a half-century. In pondering this issue, HARRIET ZUCKERMAN's sociological and historical insights have been invaluable. We have jointly posited a class of what we term "postmature discoveries" (ZUCKERMAN and LEDERBERG 1986). This may be a bit like asking why evolution took four billion years, not three, to come up with *Homo sapiens*; others might marvel or lament that it could have that consummation at all. Indeed, earlier history of science was vastly less saturated with the expertise and energy than prevail today. We concluded nevertheless that the differentiation of disciplines played a crucial role: bacteriology was mainly the province of medically oriented people, whose task was the eradication of evil infection. In this task, the long way round, the surest way, is getting involved with the target organism and understanding pathogenetic mechanisms as part of its way of making a living. Among historic figures in bacteriology, we could locate very few candidates who might have transcended the disciplinary boundaries. MARTIN BEIJERINCK was one, but he was busy enough as one of the pioneers of bacterial physiology. Today's academic and grantmaking structures still propagate that constraint. The joint M.D.-Ph.D. curriculum is one rare counteravenue towards achieving a broader interdisciplinary education. But it is immensely costly in time and in money, for both the student and the school. With a truncated *de facto* experience, I got the best of both worlds at a far more affordable price. However, no university would countenance that as a designed plan.

Another impediment is the fallacy of the name. In a wonderful taxonomic clarification, FERDINAND COHN categorized the Class Schizomycetes, distinguishing bacteria from other microbes. But in labeling them "fission fungi" he institutionalized the perception that they must lack a sexual phase.

Finally, it may be argued that science today is so much more densely populated—some regions are a highly competitive jungle—that no stone will be left unturned. But who dares today to undertake risky experiments, even for high stakes, when interruption of external grant support is tantamount to the guillotine, and our universities are on too tight a tether to provide their own shelter? We can foresee many wonderful fruits from the rather obvious and virtually risk-free paths of exploration of the human genome, with indus-

trial as well as governmental enthusiasm—and the more highly automated the better. Will we ever know whatever still more revolutionary redirections we will have missed, or will they eventually be recounted as the postmature discoveries of another era?

LITERATURE CITED

AVERY, O. T., C. M. MACLEOD and M. MCCARTY, 1944 Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J. Exp. Med. **79:** 137–158.

CALKINS, G. N., 1926 *The Biology of the Protozoa.* Lea & Febiger, Philadelphia.

DAVIS, B. D., 1950 Nonfiltrability of the agents of recombination in *Escherichia coli.* J. Bacteriol. **60:** 507–508.

DAVIS, B. D., 1955 Biochemical explorations with bacterial mutants. Harvey Lectures, Series L, 1954–1955, pp. 230–257.

DUBOS, R. J., 1945 *The Bacterial Cell.* Harvard University Press, Cambridge, MA.

FIRTH, N., K. IPPEN-IHLER and R. A. SKURRAY, 1996 Structure and function of the F factor and mechanism of conjugation, pp. 2377–2401 in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, Vols. 1 and 2, Ed. 2, edited by F. C. NEIDHARDT, American Society of Microbiology, Washington, DC.

HARTMANN, M., 1943 *Die Sexualität.* Gustav Fischer Verlag, Jena.

HERSHEY, A. D., 1946 Spontaneous mutations in bacterial viruses. Cold Spring Harbor Symp. Quant. Biol. **11:** 67–77.

HINSHELWOOD, C. N., 1946 *The Chemical Kinetics of the Bacterial Cell.* Clarendon Press, Oxford.

HUXLEY, J. S., 1942 *Evolution: The Modern Synthesis.* Harper Press, New York.

JACOB, F., 1994 André Lwoff (1902–1994). Nature **371:** 653.

JACOB, F., and E. L. WOLLMAN, 1961 *Sexuality and the Genetics of Bacteria.* Academic Press, New York.

KAUFFMANN, F., 1941 *Die Bakteriologie der Salmonella-Gruppe.* Munksgaard, Copenhagen.

LEDERBERG, J., 1946 Studies in bacterial genetics. J. Bacteriol. **53:** 503.

LEDERBERG, J., 1951 Prevalence of *Escherichia coli* strains exhibiting genetic recombination. Science **114:** 68–69.

LEDERBERG, J., 1987a Genetic recombination in bacteria: a discovery account. Annu. Rev. Genet. **21:** 23–46.

LEDERBERG, J., 1987b Gene recombination and linked segregations in *Escherichia coli.* Genetics **117:** 1–4.

LEDERBERG, J., 1989 Replica plating and indirect selection of bacterial mutants: isolation of preadaptive mutants in bacteria by sib selection. Genetics **121:** 395–399.

LEDERBERG, J., 1990 Edward Lawrie Tatum. Biographical Memoirs, National Academy of Sciences **59:** 357–386.

LEDERBERG, J., 1994 The transformation of genetics by DNA: an anniversary celebration of AVERY, MACLEOD and MCCARTY (1944). Genetics **136:** 423–426.

LEDERBERG, J., and E. L. TATUM, 1946a Novel genotypes in mixed cultures of biochemical mutants of bacteria. Cold Spring Harbor Symp. Quant. Biol. **11:** 113–114.

LEDERBERG, J., and E. L. TATUM, 1946b Gene recombination in *Escherichia coli.* Nature **158:** 558.

LEDERBERG, J., and N. D. ZINDER, 1948 Concentration of biochemical mutants of bacteria with penicillin. J. Am. Chem. Soc. **70:** 4267–4268.

LWOFF, A., 1971 From protozoa to bacteria and viruses. Fifty years with microbes. Annu. Rev. Microbiol. **25:** 1–22.

MOULTON, F. R. (Editor), 1940 *The Genetics of Pathogenic Organisms.* Science Press, Lancaster, PA.

OWEN, R. D., and O. E. NELSON, JR., 1986 ROYAL ALEXANDER BRINK (1897–1984). Genetics **112:** 1–10.

RAVIN, A. W., 1968 HARRIETT EPHRUSSI-TAYLOR. April 10, 1918–March 30, 1968 Genetics **60,** Suppl. **24** (unpaginated).

RAVIN, A. W., 1976 FRANCIS JOSEPH RYAN (1916–1963). Genetics **84:** 1–15.

RYAN, F., and J. LEDERBERG, 1946 Reverse-mutation and adaptation in leucineless Neurospora. Proc. Natl. Acad. Sci. USA **32:** 163–173.

TATUM, E. L., 1945 X-ray induced mutant strains of *E. coli.* Proc. Natl. Acad. Sci. USA **31:** 215–219.

TATUM, E. L., 1946 Induced biochemical mutations in bacteria. Cold Spring Harbor Symp. Quant. Biol. **11:** 278–284.

VALENTINE, F. C. O., and T. M. RIVERS, 1927 Further observations concerning growth requirements of hemophilic bacilli. J. Exp. Med. **45:** 993–1001.

ZUCKERMAN, H. A., and J. LEDERBERG, 1986 Forty years of genetic recombination in bacteria. Postmature scientific discovery? Nature **324:** 629–631.