GENETIC RECOMBINATION IN BACTERIA: A DISCOVERY ACCOUNT

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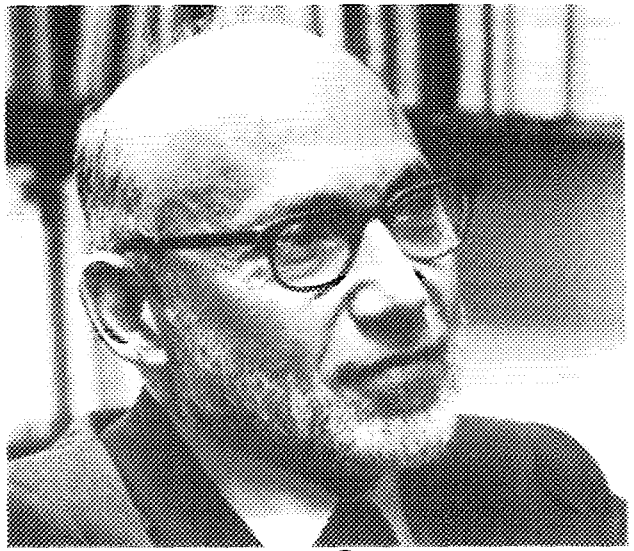
For the past four decades, bacteria have been favored objects for molecular genetic research. Along with bacteriophages and other plasmids, they have also been instrumental in the contemporary revolution in biotechnology. The importance of bacteria as agents of infectious disease was clearly established by 1876, but this motivated little interest in their fundamental biology until about sixty-five years later. For most of that interval, the genetics of bacteria was a particularly neglected no-man's-land between the disciplines of genetics and of medical bacteriology. Bacteria could not be adopted as models for genetic research until there was some substantiation of the view that they had a genetic system like other organisms. On the contrary, Julian Huxley had once suggested of bacteria that "the entire organism appears to function both as soma and germ plasm and evolution must be a matter of alteration in the reaction system as a whole" (34). Other influential figures like Hinshelwood (32) and Darlington (15) voiced similar views. (Darlington and Huxley, but not Hinshelwood, quickly embraced a more modern perspective when new evidence emerged.)

The question reached closure in 1946 with the demonstration of sexual crossing in the bacterium *Escherichia coli* strain K-12 (66). A brief reminiscence has been published for the fortieth anniversary of that publication (60). That article was joined with some reflections on whether this was a postmature discovery and whether the same inquiry might have been made at a much earlier historical epoch, perhaps promptly after the rediscovery of Mendelism at the turn of the century (103).

The present account concentrates on the scientific milieu and convergent personal histories of Francis J. Ryan (1916–1963) (76, 80), Edward L. Tatum (1909–1975) (59, 61), and myself, Joshua Lederberg (1925–) at Columbia University and Yale, culminating in the 1946 publication. If I have any one message to convey, it is an account of my debts: to the individuals who gave so much of themselves as parents, teachers, colleagues, and friends, and to a system that has offered extraordinary nurture to whatever talent and ambition I could bring. That system, the social milieu of science, is under the microscope today, scrutinized for every aberration and pathology. Taken for granted, and thereby overlooked in the presentation of the scientific career to younger people, are its positive aspects of community and of the traditional (and reciprocal!) bonds of teachers and students, not to mention the unique thrills of discovery and the gratification of its application for human benefit.

The pivot of my account is September 1941, when I enrolled as an entering undergraduate at Columbia College in New York City. Although I was born



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in Montclair, New Jersey, my early education was framed by the New York City public school system. A cadre of devoted and sympathetic teachers went far beyond their duty in encouraging a precocious youngster, despite his taunting them with questions they could not always answer. The culmination was Stuyvesant High School, which specializes in science. Stuyvesant also offered unusual opportunities for practical work in machine shops and analytical laboratories. Most important of all, it attracted a peer group (then unfortunately limited to boys) of the keenest young intellects: for the first time, I had a few intellectual sparring partners. The laboratory opportunities offered at Stuyvesant were augmented by the American Institute Science Laboratory, a forerunner of the Westinghouse Science Talent Search. Instead of offering prizes for the most elegant posters, the AISL offered facilities (in space donated by IBM in the shadow of the Empire State Building) for the conduct of original research, after school hours and on weekends. Here I began to look at the chemical basis of histological fixation and staining: cytochemistry seemed the most challenging point of entry into fundamental biological questions. The New York Public Library was another important element of an efficient and calculated system of Americanization, and of social mobility for first-generation immigrant youth.

My earliest recollections aver an unswerving interest in science, as the means by which man could strive for understanding of his origin, setting, and purpose, and for power to forestall his natural fate of hunger, disease, and death. [Since 1945 the power to destroy has weighed in negative balance on the scientific conscience: we are no longer assured that net human benefit will

be achieved as an automatic consequence of the enhancement of knowledge (57, 58). We are not abandoning the enterprise; the global competition, if nothing else, forfends a halt. Weighing the benefit of scientific research has become more complicated.]

The books that engaged me most deeply as a youth, before more advanced texts were accessible, were Eddington and Jeans on physics and inspirational works like Jaffe's *Crucibles* in chemistry. Wells, Huxley & Wells's encyclopedic *The Science of Life* was the most influential source of my perspective on biology and man's place in the cosmos, seen as evolutionary drama. De Kruif's *Microbe Hunters* turned my entire generation toward a career in medical research. Albert Einstein and Chaim Weizmann were towering culture heroes. The ambitions they inspired were reinforced by a popular culture that idealized the medical scientist with novels and movies like *Arrowsmith, The Magic Bullet, The Life of Louis Pasteur,* and *The Symphony of Six Million.* In a mood born of the Great Depression, however, many of these works painted a bleak picture of the personal life of the scientist: marriage and family were expected to be Baconian "hostages to fortune" (3).

Actual medical textbooks were not so readily available; nevertheless, I was able to read histology, microbiology, and immunology while in high school. Immunology, as then presented, was almost impenetrable to my efforts at orderly, scientific integration. (It took me two decades to realize that the fault was not mine.)

The library book that most impacted my further scientific development was Bodansky's *Introduction to Physiological Chemistry* (7). The copy I received as a Bar Mitzvah present (1938) stands on my bookshelf today, the print almost worn off the pages. This text is medically oriented but covers intermediary metabolism thoroughly, as well as the structure of amino acids and proteins. It also gives an excellent account of Garrod's work on inborn errors of metabolism, a premonition of the founding of biochemical genetics by Beadle & Tatum in 1941 (6). With respect to nucleic acids, nothing is said about their biological function. They are purported to be complexed with protein (by unspecified linkages) to form nucleins. Yeast nucleic acid (also found in plants) contains ribose; thymus nucleic acid contains desoxyribose. Both are tetranucleotides. (All of course quoted from Phoebus Levene.) A second treasured possession was E. B. Wilson's magisterial work, *The Cell in Development and Heredity* (97), a gift for my sixteenth birthday. Published in 1925, this book is probably the most authoritative documentation of pre-1940s biological thought on the cell-biological and biochemical bases of heredity and their relationship to development. Misled by the fluctuating appearances of stained chromosomes at varying stages of compactness, Wilson did attribute the genetic continuity of chromosomes to their oxyphilic (nonnucleic acid) constituents (97a). If he was derailed on this item, we should not overlook Wilson's clarity in seeking explicit mechanistic chemical interpretations in an era that was still shadowed by thoughts of a mystical, life-endowing protoplasm.

With these cardinal inspirations, my entry to Columbia that fall was motivated by a passion to learn how to bring the power of chemical analysis to the secrets of life. I looked forward to a career in medical research where such advances could be applied to problems like cancer and the malfunctions of the brain.

As it turned out, Columbia was the most fortunate of choices and opportunities. At the time I applied, I doubt if I knew more about Columbia than of its general academic reputation and that Wilson had been on its faculty. The clincher was the award of a tuition scholarship, in the amount of $400 per year, from the Hayden Trust. This, together with commuting from my parental home, made college financially feasible.

My curriculum at Columbia was somewhat topsy-turvy. As soon as a dubious bureaucracy would permit a freshman to do so, I registered in a number of graduate courses in the Department of Zoology. Not until my last senior term did I find the time or maturity to profit from a rounding of my humanistic education at the hands of teachers like Lionel Trilling and James Gutman.

Professor H. Burr Steinbach, who taught the introductory Zoology 1 course, helped arrange a laboratory desk in the histology lab where I could pursue some small research of my own. I had become interested in the cytochemistry of the nucleolus in plant cells the year before, at the AISL. I soon heard of Marcus Rhoades's and Barbara McClintock's cytogenetic research, especially her work on the nucleolar organizer in maize (73a). This introduced me to the uses of genetic analysis in cell biology, and I was soon able to enlist them as helpful counselors.

Professor Franz Schrader's course in cytology introduced me to some of the problems of mitosis (87). I became curious about how the drug colchicine interferes with the mitotic spindle. Herein was my first (albeit trivial) "discovery" in cytotoxicology: an apparent gradient of susceptibility to colchicine down the onion root meristem; but I had no way to answer whether this difference was intrinsic in the cells, or was a transport problem.

This work led to two other starts: (*a*) an effort to induce chromosome aneuploidy in mice by the application of limiting concentrations of colchicine during spermatogenesis, and (*b*) a broader inquiry into the effects of narcotics and other specific inhibitors on the mitotic process. It was easy to disrupt spermatogenesis with colchicine; I saw giant (aneuploid and polyploid) spermatids, but I had no evidence of their successful maturation and functioning in fertilization. It remains, nevertheless, a prototype of potential teratogenesis from anesthetics and other environmental agents. The cytological preparations of colchicine-inhibited mitosis and meiosis were remarked upon by my professors as being strikingly clear for chromosome counts. Had we understood that the karyotype of *Homo sapiens* was problematical, we might have accelerated the recognition (93) that $2n = 46$ (not 48) by over a decade. Salome Waelsch may or may not have approved of my "project," but she was most encouraging and helpful in providing mice, sometimes to the discomfiture of Professor Schrader in his supervision of the cytology laboratory.

The puzzles of the cytophysiology of mitosis led me to look for courses in cell physiology. However, at that time they were focused on energy metabolism rather than on macromolecular synthesis and fiber assembly. Mendelian genetics seemed to have little relationship to the biology of the cell, presented as it was in the form of combinatorial checkerboards.

I first met Francis Ryan in September of 1942. He had just returned from his postdoctoral fellowship at Stanford University, with E. L. Tatum, to become an instructor in Zoology at Columbia. He brought back the new science of *Neurospora* biochemical genetics and a gift of inspired teaching that was to be a decisive turning point in my own career. I had limited contact with him in formal courses, but by January 1943 I was working in his laboratory assisting in the preparation of media and handling of *Neurospora* cultures. For the first time I was able to observe significant research as it was unfolding and to engage in recurrent discussions with Francis, and with an ever-widening group of graduate students in the department, about *Neurospora,* life, and science. A very cheerful presence in the laboratory was Elizabeth Wilkinson Ryan, who worked (83) alongside Francis through the war years. Lillian Schneider (now Professor Wainright) was Ryan's principal technician after 1943, and also helped enormously to nurture youngsters in the lab and still keep Ryan's research on track.

Ryan had worked with Lester G. Barth at Columbia, in close company with Arthur Pollister and John A. Moore, on the temperature relations of rates of embryological development in frogs. This research was in the tradition of W. J. Crozier and the Chicago school of biophysical physiology. On completing his doctoral dissertation in 1941 (81), Ryan sought a postdoctoral fellowship at Stanford with Douglas Whitaker, with support from the National Research Council, in quest of simpler experimental material, namely *Fucus*. When he arrived at Stanford that fall, Beadle and Tatum had just reported their first findings on biochemical mutants in *Neurospora,* genetically blocked in the biosynthesis of any of a multitude of specific growth factors (5, 6). Ryan implored them to accept him in their lab and was finally accepted, as their first postdoctoral fellow. This was Ryan's own conversion to the power of genetic analysis in the dissection of problems in cellular and general physiology, a zeal he was soon to pass on to me. His work with *Neurospora* began with effects of temperature (and other environmental variables) on growth and on convenient methods of measuring it (84).

Upon his return to Columbia, he extended these methods to the use of *Neurospora* mutants for bioassay of leucine and other nutrients (11, 82, 85). He also began studies on the nutrition, physiology, and chemotherapy of *Clostridium septicum* infection (gas gangrene), which was an important complication of traumatic wounds (83). This work was supported by the Rockefeller Foundation (one more credit to Warren Weaver's historic program in molecular biology) and by the Office of Scientific Research and Development, as part of the mobilization of US science for war-related projects. That support gave Ryan some of the resources that enabled him to take me on as another part-time laboratory helper.

For my own part, I had enlisted in the Navy V-12 college training program upon reaching my seventeenth birthday. The V-12 curriculum for medical officers was designed to compress premedical training to about eighteen months of instruction, and the four-year MD curriculum into three calendar years. My subsequent months at Columbia College were alternated with spells of duty at the US Naval Hospital, St. Albans, Long Island. Here I was assigned to the clinical pathology laboratory, supervised by Commander Harry Zimmerman, a distinguished neuropathologist in his later career at Albert Einstein Medical College. The practical use of my previous training in cytology was the examination of stool specimens for parasite ova and the routine examination of blood smears for malaria among the US Marines returning from the Guadalcanal campaign. This gave me the opportunity to look for the chromosomes of *Plasmodium vivax*. The "chromosomes" were so tiny and the Feulgen staining so faint that it is difficult to insist on the reality of those observations. However, this experience informed me of the sexual stages of the malaria parasite and undoubtedly sensitized me to the possibility of cryptic sexual stages in other microbes (perhaps even bacteria).

In October 1944 I was reassigned to begin my medical course at Columbia College of Physicians and Surgeons (P & S). As a medical student, I continued research on the control of mitosis: namely a search for a hypothetical humoral factor that promoted the rapid regenerative growth of the liver after partial surgical excision (cf. 79). A fellow student, Anthony Iannone, and I had some encouraging responses to parabiosis. However, neither the available assay methods nor our surgical skills and facilities approached what was needed for the task. First-year medical students at P & S were actually discouraged from research, and my intellectual and social environment continued to center on the Morningside Heights campus.

The important biological discovery of 1944 was the identification by Avery, MacLeod & McCarty, at the Rockefeller Institute, of the substance responsible for pneumococcal transformation (1). This phenomenon, which Fred Griffith had stumbled on in 1928 (28), appeared to be the transmission of a gene from one bacterial cell to another; but this interpretation was inevitably obscured by the poor general understanding of bacterial genetics at that time (52). That vagueness was confounded by two outstanding misinterpretations: (*a*) that the transmissible agent was the polysaccharide itself [It is sometimes overlooked that Griffith understood the distinction well enough. Better than many of his followers, he had at least the germ of a genetic theory: "By S substance I mean that specific protein structure of the virulent pneumococcus which enables it to manufacture a specific soluble carbohydrate" (28a).] and (*b*) that the agent was a "specific mutagen." For example, Dobzhansky wrote that "we are dealing with authentic cases of induction of specific mutations by specific treatments—a feat which geneticists have vainly tried to accomplish in higher organisms" (19). This formally correct attribution, from a most influential source, obfuscates the idea that the agent is the genetic information.

In retrospect, it is difficult to give proper credit to the logical validity of a large range of alternative interpretations, and to reconstruct the confusions

about what was meant by "gene" and "genetic." Recall that until 1951, the only marker observed in transformation was the capsular polysaccharide, the biosynthesis of which was itself subject to many conjectures (e.g. about the role of starter fragments in self-assembly). Avery, undoubtedly somewhat intimidated by Dobzhansky's authority, was reluctant to put his speculations about the genetic significance of transformation in print; his famous letter to his brother surfaced only years later (33, 38, 73, 77). As late as 1948, so distinguished a geneticist as G. W. Beadle still referred to the phenomenon as a "first success in transmuting genes in predetermined ways" (4).

On the other hand, Avery's actual findings were accurately and promptly communicated to Columbia by Dobzhansky (who visited the Rockefeller) and by Alfred Mirsky (of the Rockefeller faculty), who was a close collaborator of Arthur Pollister. The Rockefeller work was the focus of widespread and critical discussion among the faculty and students there. Mirsky was a vocal critic of the chemical identification of the transforming agent. I believe he was quite persuaded that this was an instance of gene transfer, but the more reluctant to concede that the evidence to date settled so important a question as the chemical identity of the gene as pure DNA (versus a complex nucleoprotein). 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 in short order, provided the pneumococcal transformation could be securely retained within the conceptual domain of gene transmission. When biologists of that era used terms like *protein, nucleic acid,* or *nucleoprotein,* it can hardly be assumed that the words had today's crisp connotations of defined chemical structure. Sleepwalking, we were all groping to discover just what was important about the chemical basis of biological specificity. It was clear to the circle I frequented at Columbia that Avery's work was the most exciting key to that insight.

My own information about the Avery group's work was word of mouth until January 20, 1945 when Harriett Taylor (later Ephrussi-Taylor) lent me her reprint of Avery et al's article (1). At that time she was a PhD candidate, working at Columbia on the kinetics of growth in yeast; she had already arranged to pursue her postdoctoral studies with Avery at the Rockefeller Institute. My immediate private response to reading the 1944 paper was that the research was "unlimited in its implications. . . . Direct demonstration of the multiplication of transforming factor. . . . Viruses are gene-type compounds [sic]. . . ."

What could be done to incorporate this dramatic finding into the mainstream of biological research; how could one further advance these new hints about the chemistry of the gene? These questions suggested to me the merits of attempting a similar transformation by DNA in *Neurospora*. Not only did this organism have a well-understood life cycle and genetic structure; it also had the advantage of being amenable to selection for rare nutritionally self-sufficient (prototrophic) forms that would facilitate the assay for the transformed cells. And Ryan was working with it in the lab.

In mid-spring 1945, I brought this suggestion to Francis Ryan, who welcomed it as my first research project under his direction. As a brief vacation was looming (to follow rigorous examinations in Anatomy), we agreed to begin in June. However, we soon discovered that the *Neurospora* mutant *leucineless* (allocated to him by Beadle out of the Stanford library) would spontaneously revert to prototrophy. We did not therefore have a reliable assay for the effect of DNA in *Neurospora*. However, the genetic analysis of the reverse-mutation phenomenon resulted in my first scientific publication, with Ryan (86).

Questions about the biological significance of transformation in bacteria would then continue to fester so long as bacteria remained inaccessible to conventional genetic analysis for lack of a sexual stage. But was it true that bacteria were asexual? The standard reply was to mock the fantasies of polymorphisms that were purported exhibitions of sexual union between bacterial cells (60, 103). Most of these surely were attributable to contaminated cultures. Some of the more sophisticated textbooks, and especially Dubos's monograph, *The Bacterial Cell* (20), indeed had footnotes indicating the inconclusive status of claims for sexuality, and pointed out that there had been little genetic testing of this hypothesis. Another important input to this intellectual confrontation was an appreciation of sexuality in yeast, popularized at Columbia via the research work of Sol Spiegelman and Harriett Taylor. Yeast is at least superficially a microbial cousin to bacteria. Gene segregation and recombination in yeast had been demonstrated in 1937 by Winge & Laustsen (98) and then further exploited for physiological genetic analysis by Lindegren (69) and Spiegelman (89). These successes only dramatized the importance of finding a sexual stage, if it existed, in a variety of microbes. If bacteria could be crossed, a new repertoire of biological materials for experimental analysis would be available to physiological genetics and biochemistry. This work might also have important practical applications for vaccine improvement and the understanding of virulence—a latter-day extension of Pasteur's primitive techniques. The compelling motive was to allow the exploitation of DNA transformation in an organism with manifest genetic structure, to further the launching of what is today called "molecular genetics." These were high stakes to justify what was obviously a very long gamble on success (103). Besides having little to lose (I did not need a successful research dissertation for an MD degree), I sensed that no journey on that uncharted ocean would be totally fruitless; even an unsuccessful pursuit of recombination would turn up other phenomena of interest. Such indeed had been my experience with reversion in *Neurospora*, and I have rarely been disappointed since. One cannot be so sanguine today about the opportunity for exploration of new territories, under the pressure for precisely predicted performance that has become pathologically associated with the project system of federal research support.

Some of my notes dated July 8, 1945, articulate, on neighboring pages, hypothetical experiments involving (*a*) a search for mating between the

medically important yeastlike fungi, the monilia and then (*b*) the design of experiments to seek genetic recombination in bacteria (by the protocol that later proved to be successful). These notes also coincide, within a few days, with the beginning of my course in medical bacteriology at medical school. They may have been provoked by the repeatedly asserted common wisdom that bacteria were "Schizomycetes," that is, asexual, primitive plants. The basic protocol of these speculative notes entailed the use of a pair of nutritional mutants, say A^+B^- and A^-B^+. If crossing occurred, one could plate out billions of cells in a selective medium if need be: one should be able to find even a single A^+B^+ recombinant. This experimental design was encouraged by Beadle & Coonradt's report of nutritional symbiosis in *Neurospora* heterokaryons (5a). Their speculations [which preceded the finding of recombination in viruses (18, 30)] on the role of heterokaryosis in the evolution of sexual reproduction, offered the bonus that we might find heterokaryosis in bacteria, if not full-blown sexuality. In any event, we would have to be quite attentive to a wide spectrum of possible modes of physiological and genetic complementation.

Dubos's monograph (20) was published and appeared in the Columbia library at a most propitious time, shortly after these speculative ruminations. It furnished an exhaustive and critical review of prior efforts to assess sexuality in bacteria, mainly by morphological and also by genetic methods. Most of these attempts were muddled, but two were more clearheaded (26, 88), albeit with negative findings. But these latter two lacked any selective method for the detection of recombinants. Therefore, the investigators would have overlooked such a process if it occurred in perhaps fewer than one per thousand cells. All in all, Dubos's analysis substantiated the outlook that the question had never been critically tested.

The principal encouragement to think about genes in bacteria had come from Luria & Delbrück's (1943) experiments on the statistics of mutation in *E. coli* (71). These results supported the view that hereditary adaptive changes, specifically to virus resistance, occurred by spontaneous mutations filtered by selection (i.e. with the bacterial virus). In this respect, at least, there was some evidence that bacteria had "genes," although these experiments do not reach the particulate basis of heredity; they had more to do with a Darwinian than a Mendelian perspective.

One of the principal obscurations to genetic thinking in bacteriology had been the idea that bacteria reacted holistically to environmental insult, that drug or virus resistance was some kind of physiological adaptation that could then become genetically fixed. This anti-Darwinian view was also very much at odds with the gene concept as it had emerged in *Drosophila* studies; but it persuaded many to argue that bacteria did not share the Mendelian organization of their hereditary particles seen in higher organisms. This "last stronghold of Lamarckism" (70) was undoubtedly sustained by sympathy for Lysenko's anti-Mendelism campaign in the USSR. It achieved considerable prestige by being supported by Sir Cyril Hinshelwood, a Nobel-laureate

physical chemist and President of the Royal Society, well into the late 1950s. He had the admirable goal of modeling the bacterial cell as a metabolic network, without needing recourse to a specialized store of genetic information. Holistic adaptation, could it but be experimentally verified, would have fitted neatly into his theoretical scheme (32; compare Delbrück, 17).

It is difficult to find a clear instance of a scientific revolution in the history of biology, in the strict sense of a paradigm shift barely coupled to experimental evidence, as enunciated by Kuhn in 1962 (42). The Darwinian revolution comes very close, especially in its application to microbiology. For several decades, the concept of holistic adaptation in bacteria was entertained in the absence of any evidence for it and despite its contradiction to the conceptual framework of population analysis that had emerged for the rest of biology. Today's "DNA revolution" is no less important, but it is related to experimental data more than to such a failure of confrontation.

More explicit encouragement for the possibility of gene recombination in the natural history of bacteria was presented by taxonomic tables of the species or serotypes of *Salmonella* (40). The importance of these bacteria in food poisoning, typhoid fever, and other enteric infections had led to their being studied in a painstaking way to identify antigens helpful in tracking strains through epidemics. As a further consequence, every antigenic strain difference was allowed to attract a novel binomial name, e.g. *Salmonella newport,* which helped commemorate a place—and extend the author's bibliography. A beneficial side effect of this luxuriant publication was the accessibility of synoptic data that would have been otherwise buried. My reading this literature prompted the speculation that the numerous combinations of somatic and flagellar antigens were generated by some recombinational mechanism.

[As soon as I had my own laboratory and the collaboration of other immunologists and of graduate students, I determined to verify this. That enterprise had the happiest results: the discovery with Norton Zinder of phage-mediated transduction (101), and a series of analyses of the genetics of *Salmonella* antigens with P. R. Edwards, Bruce Stocker, and T. Iino (62, 63, 90). These in turn have furnished exciting models of switches of gene expression based on segmental DNA inversions (10, 12, 35). But all this was to come later.]

The speculation about natural recombination in *Salmonella* also bolstered the idea of looking for it in *E. coli,* as these are very close relatives. For the time being *E. coli* had the advantage of being nonpathogenic (at least for our laboratory strains), and as we shall see, a further advantage was that some nutritional mutants had already been secured in *E. coli.*

Within a few days I set out on my own experiments along these lines—using in the first instance a set of biochemical mutants in bacteria that I laboriously began to accumulate in Ryan's laboratory. None of the well-honed shortcuts we have now (16, 64, 68) were then available, and this was a painstaking process. I was quickly able to get methionine-dependent mutants

by selection with sulfonamides, as had been reported by Kohn & Harris (41) (the process is still not really understood). However, the same difficulty as in the *Neurospora* experiments, a spontaneous reversion from A^-B^+ to A^+B^+, had to be accounted for. 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 reversions. In theory their occurrence requires the coincidence of two rare events: say $A^- \rightarrow A^+$ and $B^- \rightarrow B^+$. Much effort was devoted to control experiments to verify that double reversions would follow that expectation, and not interfere. The need for double mutants posed a tedious prospect of strain development.

Had a broader range of antibiotics been available, I might already have used selection for multiple drug resistance as an index of crossing (46). However, it was important to use markers closely analogous to those already validated as gene effects in *Neurospora,* namely clear-cut blocks in biosynthesis.

Meanwhile at Stanford Ed Tatum, whose doctoral training at Wisconsin had been in the biochemistry of bacteria, was returning to bacteria as experimental objects, having published two papers on the production of biochemical mutants in *E. coli* (27, 92). During that summer of 1945 Ryan learned that Tatum was about to move from Stanford University to set up a new program in microbiology at Yale. He suggested that rather than ask Tatum merely to share some of his bacterial strains, I should apply to work directly with him and get the benefit of his detailed experience and general wisdom. The war was nearing a victorious conclusion; civilian life and academic schedules might soon be renormalized and make such a visit possible. With Ryan's encouragement, I then wrote Tatum of my research plan (Figure 1) and applied for such an accommodation. Tatum congenially agreed and suggested that I arrive in New Haven in late March 1946, to give him time to set up his laboratory. He had looked into support on my behalf from the Jane Coffin Childs Fund. I had some hint that he may have been formulating similar experimental plans, but these were never elaborated to me. This arrangement suited him by leaving him free to complete the rebuilding of his laboratory, continue his current work in the biochemistry of *Neurospora,* and still follow up the long-shot gamble in looking for bacterial sex.

Once I was at New Haven, my lab efforts were devoted to rechecking the stability of Tatum's existing double-mutant strains, like 58-161 and 679-183 (biotin-methionine and threonine-proline, respectively). Then, additional mutations such as resistance to virus T1 were also incorporated to allow segregation of unselected markers among the prototrophs selected from the mixed cultures on minimal agar medium. It took about six weeks from the time the first serious efforts at crossing were set up in mid-April to establish well-controlled, positive results. By mid-June, Tatum and I felt that the time was ripe to announce them.

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