Perspectives

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Fifty Years Ago: The Neurospora Revolution

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THIS year marks the fiftieth anniversary of the publication of one of the pivotal works of modern biology, the first Neurospora paper of BEADLE and TATUM (1941). This brief paper, revolutionary in both its methods and its findings, changed the genetic landscape for all time. Where previously there existed only scattered observations (albeit with some acute insights) on the relation between genetics and biochemistry, this paper established biochemical genetics as an experimental science, one in which progress would no longer be limited by the rarity of mutants with biochemically knowable phenotypes, but where such mutants would be generated at will and where findings could be repeated and hypotheses explored, as in other experimental sciences. This paper was the first in a series of fundamental advances in chemical genetics that by 1953 had bridged the gap between genetics and biochemistry and ushered in the age of molecular biology.

I have explained in a recent memoir of BEADLE (HOROWITZ 1990) how the Neurospora investigation arose from his earlier study of the genetics of eye-color synthesis in Drosophila with BORIS EPHRUSSI, and I will not repeat this history here.

The methodological innovations of the BEADLE-TATUM paper were twofold. First, the authors introduced what was for most geneticists a new kind of experimental organism–a microorganism that was ideally suited for classical genetic studies but which differed from the classical organisms in that it grew readily on a medium of defined chemical composition. It was actually superior in some ways to the usual experimental species because the entire meiotic tetrad could be recovered and cultured. This novel creature was the filamentous ascomycete *Neurospora crassa*. (*Neurospora sitophila* was also used in the early studies, but was abandoned before long in favor of *N. crassa*.) It is well known that the investigations that led to the development of molecular genetics largely employed microorganisms, but it should be pointed out that the Neurospora discoveries first described in the 1941 paper were crucial for making bacteria genetically useful.

BEADLE had learned of Neurospora at a lecture by B. O. DODGE given at Cornell University in 1930, when the former was a graduate student. DODGE, a mycologist at the New York Botanical Garden, was a strong advocate of Neurospora as a genetic organism. It was he who found that the ascospores–the products of meiosis–required heat shock to induce germination. (He had made this discovery originally in Ascobolus, by accident, after setting down some plates of ascospores in a sterilizing oven that he thought was turned off.) This finding made Neurospora available for genetic studies, and DODGE worked out the basic genetics of the organism. Among other things, he investigated the inheritance of mating type, albinism, and other monogenic characters. He showed that the eight ascospores of an ascus display a perfect Mendelian ratio (4:4). By isolating and culturing the ascospores in the linear order in which they occur in the ascus, he discovered the patterns of first- and second-division segregations. DODGE also understood the benefits that haploidy offered for genetic studies. When combined with the other features of Neurospora, it convinced him that this ascomycete was the ideal genetic organism. He frequently pointed this out to his friend T. H. MORGAN, arguing that it was actually superior to Drosophila (ROBBINS 1962).

As its second methodological innovation, the BEADLE-TATUM paper introduced a procedure for recovering an important class of lethal mutations, namely those blocking the synthesis of essential biological substances. These were expressed in the organism as new nutritional requirements. These mutations were crucial for understanding the biochemistry of gene action. They displayed in a most convincing manner the central importance of genes in biochem-

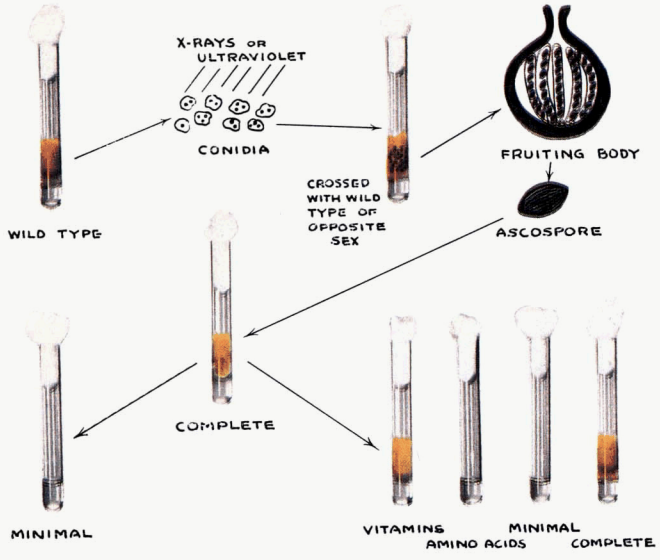


FIGURE 1.—BEADLE's lantern slide explaining the procedure for isolating biochemical mutants of Neurospora.

istry and ended forever the idea that the role of the genes in metabolism was somehow a subordinate one. Genetics, which before the Neurospora revolution had been notably isolated from the physical sciences, now found itself in the mainstream of biochemistry. Or, more correctly, genetics and biochemistry were now seen to be different aspects of the same thing.

A diagram of the BEADLE-TATUM procedure is shown in Figure 1. This figure is reproduced from a lantern slide drawn and lettered by BEADLE, one of a set that he used in lectures in the 1940s. As the slide suggests, BEADLE favored the word "sex" rather than "mating type" in his writing and speaking about Neurospora. It should be noted that the test tube labeled "vitamins" also contained nucleic acid components.

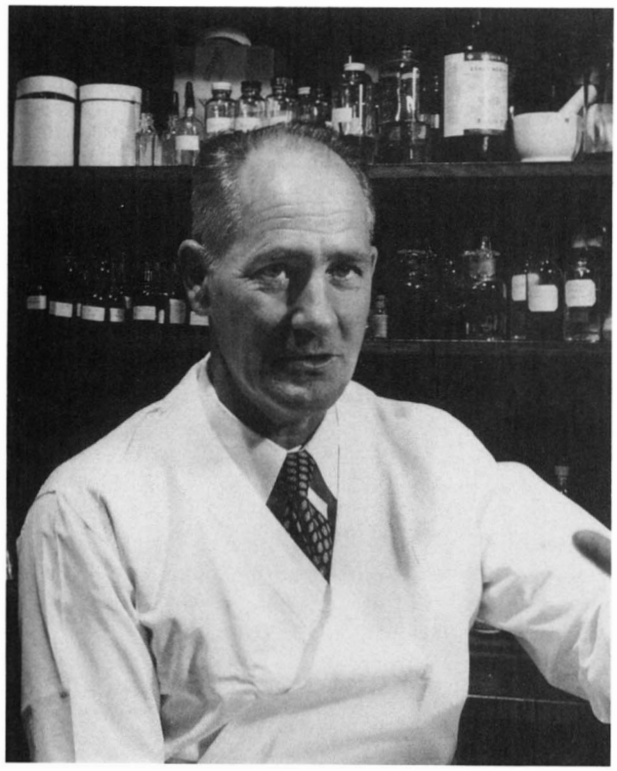
The essential character of the substances whose syntheses were affected in the Neurospora mutants–amino acids, purines, pyrimidines, vitamins–suggested that similar mutations should occur in other microbial species. This proved to be the case. In the first important extension of the Neurospora findings, GRAY and TATUM (1944) showed that "biochemical mutations" could be induced in bacteria. This result solved a fundamental difficulty that had long prevented progress toward a genetics of bacteria–that is, the lack of suitable markers–and led directly to the demonstration of genetic recombination in *Escherichia coli* by TATUM's student JOSHUA LEDERBERG. Biochemical mutations were induced later in yeast and other microorganisms. Modern microbial genetics is to a large extent based on mutations of the type first described by BEADLE and TATUM in their 1941 paper and on temperature-sensitive alleles of these and other essential genes. The discovery of temperature-sensitive mutants followed directly from the 1941 paper, as will be shown later.

Aside from its revolutionary methods, the BEADLE-TATUM paper was remarkable for the results it reported. It described three X-ray-induced mutants that grew on "complete medium" (a complex, undefined mixture containing yeast extract), but that failed to grow on "minimal medium" (a mixture consisting of the minimal nutrients capable of supporting the growth of wild-type Neurospora). The presumption was that the mutations expressed in these cultures affected genes needed for the production of growth-essential compounds present in complete, but not minimal, medium. A systematic search revealed that each of the mutants required a different substance. The three substances were pyridoxine, thiamine and *p*-aminobenzoic acid, and the inability to synthesize them was eventually shown, in every case, to be inherited as a single-gene defect. (The 1941 paper reported on the genetics of only the "pyridoxineless" mutant.) The thiamine-requiring mutant was found to respond to the thiazole moiety of thiamine by itself, implying that the mutant could synthesize the pyrimidine half of the molecule and showing that genes were limited in the range of their individual chemical effects.

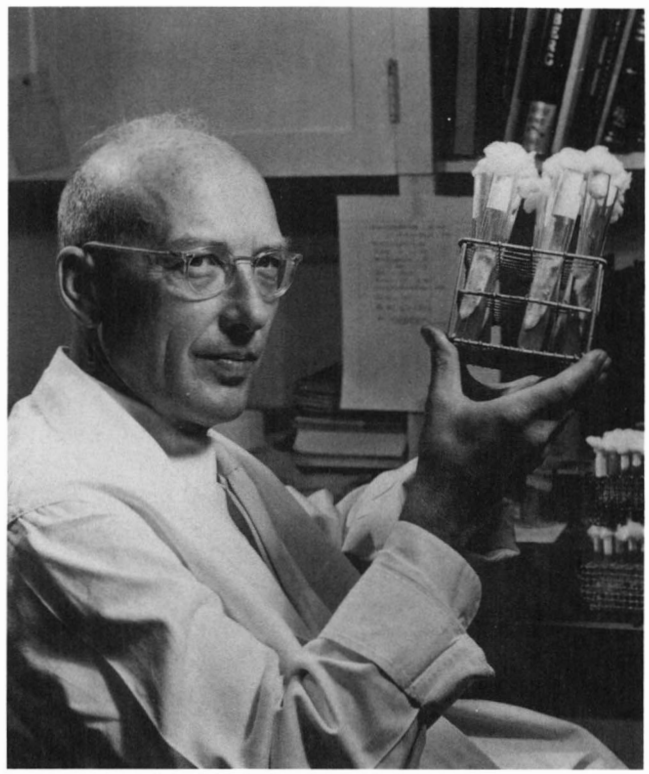
The fact that the first three mutants found by BEADLE and TATUM were vitamin auxotrophs reflects, at least in part, the relatively high frequency of such mutants recovered by their method of mutant selection. [See BEADLE and TATUM (1945), Table 5, for a listing of all Neurospora mutants identified and cited in the literature up to that time.] In this method, ascospore descendants of irradiated conidia were isolated and cultured separately (see Figure 1), a procedure that recovers even mutants with trace requirements. The mass selection procedures that came later are biased against such mutants because of cross-feeding.

The pyridoxineless mutant, No. 299, is of special interest. This was the first mutant found by BEADLE and TATUM, and it was one of the few that were recovered in *N. sitophila*. It was, so to speak, the breakthrough mutant, the one that vindicated their ideas about a new kind of genetics. But its importance did not end there. Soon after the 1941 paper was published, BEADLE received a letter from an acquaintance at the Merck Research Laboratory requesting a culture of No. 299 for the purpose of developing an assay method for pyridoxine. BEADLE sent a transfer, as he invariably did once a mutant had been referred to in print. BEADLE firmly believed that this policy was in the best interest of science, a belief that was certainly confirmed in this case because, in the course of their investigation, the Merck group discovered that No. 299 would grow without pyridoxine if the pH of minimal medium was raised to 6 from its normal value of 5 (STOKES, FOSTER and WOODWARD 1943).

I recall first hearing of this unexpected result at an



GEORGE BEADLE



EDWARD TATUM

afternoon tea-break in the BEADLE lab at Stanford University. In the ensuing discussion, it was decided to learn if other environmental variables–temperature, in particular–might also affect the phenotype of mutants in a specific way. The mutant hunt that ran more or less continuously in the lab was accordingly modified to include an incubation step at 35° in addition to the usual one at 25°. Soon the first temperature-sensitive mutants were found. The first published description of one of these was by MITCHELL and HOULAHAN (1946). These mutants turned out to be very important–arguably more important than the nonconditional auxotrophs. By modifying the gene in such a way that its activity was abolished in only part of the organism's normal temperature range, temperature-sensitive mutants were essentially unselected. That is, mutants which in the ordinary course of events would be lost because of the impermeability, instability, or unavailability for any other reason of the genetic end-product, can be recovered as temperature-sensitive alleles. Regarding them in the light of present-day knowledge, we can see that any gene whose end-product is a protein should be recoverable as a temperature-sensitive mutant. This attribute made them useful in an early test of the one gene-one enzyme hypothesis (HOROWITZ 1948, 1950; HOROWITZ and LEUPOLD 1951). The utility of temperature-sensitive mutants for problems of this kind was rediscovered years later by EDGAR, who has written a perceptive essay on the rediscovery (EDGAR 1966).

In another, and different, early application, a temperature-sensitive mutant of *E. coli* was used to demonstrate that genes determine the molecular properties, as well as the presence or absence, of enzymes (MAAS and DAVIS 1952).

The Neurospora mutants, as everyone knows, opened a new approach to the study of biosynthetic pathways, the cumulative results of which led to the one gene-one enzyme theory (BEADLE 1945). This theory had already been foreshadowed in the first paragraph of the 1941 paper, where the authors suggest the possibility that genes may act "by determining the specificities of enzymes" with the further possibility of "simple one-to-one relations" between genes and chemical reactions. These ideas doubtless grew out of the authors' earlier work on Drosophila eye colors. In his Nobel lecture, BEADLE, in an oft-quoted passage referring to one gene-one enzyme, said, "In this long, roundabout way, first in Drosophila and then in Neurospora, we had rediscovered what GARROD had seen so clearly so many years before" (BEADLE 1959; GARROD 1909). BEADLE was without doubt sincere in this characteristically generous remark, but was he right? And if he was right, does this diminish the importance of the BEADLE-TATUM accomplishment? The answer to both questions is, I think, "No."

In a penetrating discussion of the first question,

SCRIVER and CHILDS (1989) raise the question of whether, at this date, we can actually know what was in GARROD's mind when he wrote his great works on human hereditary disease. These authors show that GARROD's understanding of genetics appears not to have extended beyond 1910 (he lived until 1936). They suggest that "His words could have meant one thing to him when he uttered them and something else to us who are tempted to freight them with contemporary significance." They conclude that GARROD could hardly have had BEADLE's "one gene-one enzyme" idea in mind. It is hard to disagree with them when one considers the state of genetics and biochemistry at the time. The year 1909, when GARROD's famous book was published, was the same year that JOHANNSEN introduced the word *gene* into the language. The chromosome theory of inheritance was still in the future. Biochemistry was also in an embryonic state. In a monograph published in 1914, W. M. BAYLISS considered it necessary to defend the idea that enzymes could be assumed to be definite chemical compounds, "at all events until stronger evidence has been brought to the contrary." The one thing that seemed clear was that enzymes were not proteins (BAYLISS 1914). This question was not settled until SUMNER crystallized urease in 1926.

The same considerations must apply with equal or greater force to the most prescient of all writings about genes and enzymes, those of the French geneticist LUCIEN CUÉNOT. In 1903, CUÉNOT discussed his celebrated experiments on the inheritance of coat color in mice in terms of *mnémons* (genes), enzymes, and a chromogen (see WAGNER 1989). Sadly, CUÉNOT gave up genetics and discouraged his students from entering the field; see BURIAN, GAYON and ZALLEN (1988).

There were, of course, later antecedents of the one gene-one enzyme principle in the writings of WRIGHT, HALDANE and others, where unfamiliarity with modern science does not enter in. But while these works were correct in deducing that genes must act through their effects on enzymes (and other proteins), none of them succeeded in persuading geneticists of the classical era that a direct relation between genes and proteins was real and important and was, in fact, the key to understanding the organization of living matter. According to STURTEVANT (1965), geneticists were disinclined to accept simple ideas of gene action because they were convinced that development was too complex a process to be explained by any simple theory. Not long before he died, STURTEVANT told me that especially E. B. WILSON's position on gene action had carried much weight. WILSON, a cytologist, was one of the most influential figures in American biology. Although he died in 1939, the third edition of his monumental book, *The Cell in Development and Heredity*, published in 1925, is still in print. Usually very clearheaded, Wilson took what can only be described as an exceedingly murky view when, regarding the role of the genes, he wrote:

> In what sense can the chromosomes be considered as agents of determination? By many writers they have been treated as the actual and even as the exclusive "bearers of heredity"; numerous citations from the literature of the subject might be offered to show how often they have been treated as central, governing factors of heredity and development, to which all else is subsidiary . . . Many writers, while avoiding this particular usage, have referred to the chromosomes or their components [WILSON rarely used the word "gene"] as "determiners" of corresponding characters; but this term, too, is becoming obsolete save as a convenient descriptive device. The whole tendency of modern investigation has been toward a different and more rational conception which recognizes the fact that the egg is a reaction-system . . . and that (to cite an earlier statement) "the whole germinal complex is directly or indirectly involved in the production of every character" (WILSON 1925).

In an obvious and not very interesting sense, the foregoing statement is correct; but in another and much more important one, it is altogether wrong. With the Neurospora revolution, musings of this sort on the nature of gene action faded away. The evidence for a one-to-one relation between genes and enzymes (actually proteins, later modified to polypeptides) now became clear, abundant and undeniable. The individual gene in some way determined the specific enzyme, although it was not yet seen how. The efforts of the pre-Neurospora workers to understand gene action had been made with systems often not suited for both biochemical and genetic studies. BEADLE and TATUM changed this by founding a new science based on an organism and an experimental protocol designed to be maximally useful for the purposes of biochemical genetics. In doing so, they transformed biology, and that is the reason we remember this fiftieth anniversary.

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