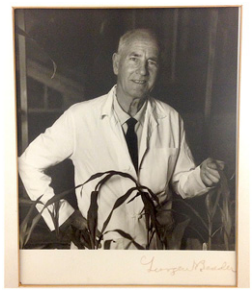
Biochemical Genetics and Molecular Biology: The Contributions of George Beadle and Edward Tatum



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> It will concern us particularly to take note of those cases in which men not only solved a problem but had to alter their mentality in the process, or at least discovered afterwards that the solution involved a change in their mental approach (Butterfield 1962).

SEVENTY-FIVE years ago, George Beadle and Edward Tatum published their method for producing nutritional mutants in Neurospora crassa. Their study signaled the start of a new era in experimental biology, but its significance is generally misunderstood today. The importance of the work is usually summarized as providing support for the "one gene–one enzyme" hypothesis, but its major value actually lay both in providing a general methodology for the investigation of gene function and in suggesting a simple biochemical relationship between genes and the characters they control. I discuss the reasons for this misunderstanding and the role of Beadle and Tatum in the development of molecular biology.

This year (2016) marks the 75th anniversary of an article by George Beadle and Edward Tatum that ushered in a new era in experimental biology (Beadle and Tatum 1941a). Their work is properly seen as a major foundation stone of what is now called molecular biology, yet today its precise significance is largely misapprehended. It is worthwhile revisiting the biological scene at the time of their work, considering what they accomplished, and investigating the factors that may have led to what was really their major contribution being relatively overlooked.

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Genetics in the Early 1940s

By the end of the 1930s, geneticists had developed a sophisticated, self-contained science. In particular, they were able to predict the patterns of inheritance of a variety of characteristics, most morphological in nature, in a variety of organisms although the favorites at the time were clearly *Drosophila* and corn (*Zea mays*). These characteristics were determined by mysterious entities known as "genes," known to be located at particular positions on the chromosomes. Furthermore, a variety of peculiar patterns of inheritance could be accounted for by alteration in chromosome structure and number with predictions as to inheritance pattern being quantitative and statistical. The state of genetics in this period was described in a textbook by Alfred Sturtevant and George Beadle, *An Introduction to Genetics* (Sturtevant and Beadle 1939), which remains one of the best statements of the field at that time.

Notwithstanding the predictive powers of the theory, genetics remained innocent of significant contact not only with (bio) chemistry but also with embryology. An original goal, understanding the mechanism of development, had essentially been abandoned in favor of pursuit of the more easily understood mechanics of inheritance. The chemical nature of the gene was especially elusive. Indeed, it was not clear whether genes were single molecules or complex organelles, and the steps that must exist between gene and character were completely unknown. The only clue to the nature of the gene was that radiation experiments and target theory gave size estimates for a very large molecule. It was not even clear to all geneticists that individual genes, as distinct from chromosomes, had a separable existence. Richard Goldschmidt in *Physiological Genetics* (Goldschmidt 1938) proposed that only the whole chromosome could be

considered as the unit of inheritance (a point of view from the early 20th century that could once again be defended). We knew about "genes" as such only because mutation of a segment changed the phenotype, but that did not show that there was a normal segment that by itself produced the wild-type version. Sturtevant and Beadle (1939) devoted only a few pages at the end of their text to genes and phenotypes. They did mention the studies of anthocyanin pigments and noted that "the fact that an oxygen atom difference between pigment molecules is gene controlled, while it does not solve the problem of the relation between gene and character, is at least one step in the desired direction" (p. 356). There is no mention, however, of Archibald Garrod's *Inborn Errors of Metabolism* (Garrod 1909), discussed below. Goldschmidt, mentioned above, had spent his career in studying gene action, and he had concluded (Goldschmidt 1938) that genes act "by changing the rates of partial processes of development." Nevertheless, the processes that Goldschmidt and others studied (wing development in *Drosophila* as an example) were complex and not accessible to biochemists.

Although genetics developed in large part in an effort to explain development, the problem was too complex for solution by the biochemical methods of the late 1930s and early 1940s. "The theory of the gene" was in large part the product of the work of a former embryologist, Thomas Hunt Morgan, who had initially been intent on accounting for the mysteries of development, and his students. By the mid-1930s, however, the attempt to reconcile genetics and embryology seemed to be at a dead end. The following anecdote illustrates the situation. After reading T. H. Morgan's book *Embryology and Genetics* (1934), Boris Ephrussi said to Morgan that he seemed not to have integrated the two fields adequately. Morgan replied, with a smile, "You think the title is misleading. What is the title?" "*Embryology and Genetics*" Ephrussi answered. "Well," Morgan countered, "is there not some embryology and some genetics?" (Ephrussi 1958).

Protein Biochemistry in the Early 1940s

It is so obvious to us today that proteins are linear polymers of amino acids arranged in a specific sequence and connected by peptide bonds that it is difficult to put oneself in the mindset of a student of biochemistry in the years around 1940. To do so, it is instructive to look at successive editions of standard textbooks written in this period (Bodansky 1938; Harrow 1935, 1940, 1943, 1947, 1950, 1954, 1958, 1962, 1966). Not only were there some odd notions, to our eyes, about the nature of protein structure, but also many assumed that the genes themselves were proteins. The second (1940) edition of the text by Harrow discusses the structure of proteins in terms of the Bergmann–Niemann hypothesis of repeating 288 amino acid units. They supposed that the proteins were constructed along simple lines with a limited number of amino acids repeating at regular intervals. Also seriously considered was the "cyclol" hypothesis of Dorothy Wrinch, which supposed the proteins to be made up of a series of hexagonal structures composed of amino acids. Stanley's crystallization of tobacco mosaic virus (TMV) was discussed as an example of the genetic role of protein. The Bergmann hypothesis was still discussed in the third (1943) edition of Harrow although the cyclol hypothesis had been discarded. TMV was recognized as a nucleoprotein but not much was made of the nucleo portion. The fourth edition (1947) did not change much. The fifth (1950) edition no longer discusses either the Bergmann or the cyclol hypothesis.

The importance of hydrogen bonds in determining the three-dimensional structure of molecules, in particular proteins, had been recognized, however. In 1942, Linus Pauling published an article in the *Journal of Experimental Medicine* claiming the formation of specific antibodies by denaturing and renaturing globulin around an antigen (Pauling and Campbell 1942). As late as 1954, *Chemical Reviews* could publish an article on the "Microheterogeneity of Proteins" (Colvin *et al.* 1954).

Why was the connection between metabolic reactions and genetic factors not given more attention? One explanation might be that it was unclear what to do about it. That is, it was not obvious how the problem could be approached experimentally. After all, alkaptonuria and the other conditions discussed in Archibald Garrod's book *Inborn Errors of Metabolism* (Garrod 1909) were rare and, as he stressed, not life threatening. It was not clear how these observations on a metabolic oddity fit into a general pattern linking biochemistry and genetics, especially since it was uncertain whether the "gene" was a molecule or an organelle and the basis of enzyme specificity could not even be guessed, given the then current knowledge of protein structure.

The Contribution of Beadle and Tatum

In the late 1930s, George Beadle and Boris Ephrussi were studying the production of eye color in the fruit fly *Drosophila* using a demanding technique of transplanting the imaginal discs of developing mutant flies into larvae of different genetic types. Boris Ephrussi was a Russian émigré embryologist who had come to T. H. Morgan's Department at Caltech on a Rockefeller fellowship to gain a genetic perspective on his studies. [According to the Wikipedia entry on him, Ephrussi was part of the Russian branch of a Jewish family of major bankers and art collectors thriving at the end of the 19th century with branches in Paris and Vienna and who are the subject of an equally fascinating but unrelated story (de Waal 2010).] There he met George Beadle who was a postdoctoral fellow at the time, studying crossing over in *Drosophila*. The two decided to work together on the problem of reconciling genetics and embryology (see Beadle's biography by Berg and Singer 2003 for details). According to Beadle, they decided to study the development of the eye-color mutant vermilion because of an earlier observation of Sturtevant that vermilion eyes fail to develop autonomously;

that is, they can be affected by the genetic nature of the surrounding tissue (Beadle 1958), with correction of the mutant defect, evidently by some diffusible factor from that tissue. Such transplantation of organs to study the origin of insect eye pigments, however, had first been accomplished by Ernst Caspari in the flour moth *Ephestia* (Caspari 1933), as described in Grossbach (2009). Caspari was a German Jew and 1933 was a bad time for such individuals to be starting an academic career in Germany! As a result, Caspari was unable to follow up the work although his boss Alfred Kuhn did. [Kuhn seems to have behaved as well as possible under the circumstances (Grossbach 2009).] Caspari went to Turkey at first and then later to the United States. After an initially difficult time, he had a very good career. He later headed the Biology Department at Rochester, was President of the Genetics Society of America, and edited this Journal from 1968 to 1972 (Grossbach 2009).

Beadle and Ephrussi moved to Ephrussi's laboratory in Paris where they honed their transplantation technique—which proved to be a more demanding task in the small *Drosophila* larva, as compared to that of *Ephestia*. The publications describing this work do acknowledge the earlier technical contribution of Caspari. By transplanting the imaginal discs of developing mutant flies into larvae of different genetic types, they were able to demonstrate that some combinations resulted in the development of wild-type eye pigment in the mutant imaginal discs. Beadle and Ephrussi deduced a sequence of reactions dependent on the production of gene-controlled soluble "hormones," but the biochemistry of these "hormones" eluded them. Their use of the term "hormone" is certainly in accord with the definition of the term, but they eventually realized that what they meant was a diffusible metabolic intermediate. The identification of kynurenine as such an intermediate, however, was first made by a student of Butenandt in collaboration with Alfred Kuhn. Kuhn had begun this collaboration with Adolf Butenandt, a Nobel Prize winner in chemistry for studies on sex hormones, in the mid-1930s. The identification of kynurenine was made by Wolfhard Weidel, a student in Butenandt's laboratory (Rheinberger 2000). There was clearly a heated race to determine the structure of the vermilion and cinnabar intermediates with Beadle and Tatum both cooperating and competing with Ephrussi and with both competing with Kuhn's group. It is something of a historical jest that the hormone/intermediate should finally have been identified by the group in which the first observation (by Caspari) had been made.

Being "scooped" in this way undoubtedly added to Beadle's dissatisfaction with the complexities of the fly system for biochemical studies and added impetus to his search for a new and simpler system. According to Berg and Singer (2003), he had been "disturbed" by the outcome of the race although I would guess this to be a minimal description. According to Beadle, it was at this point, during one of Tatum's lectures on comparative biochemistry, that he hit upon the idea of reversing the procedure of attempting to elucidate the biochemical effect of known mutations and instead to look for mutations that controlled known biochemical reactions (Berg and Singer 2003). They (it was almost certainly Beadle) hit on the ascomycete fungus *Neurospora* as appropriate experimental material. The basic genetics of *Neurospora* had been worked out by Carl Lindegren, a graduate student at Caltech in T. H. Morgan's department, while Beadle was a postdoctoral fellow there. Beadle had first heard about *Neurospora* and its life cycle while a graduate student at Cornell from a seminar by B. O. Dodge. Two features about this organism made it suitable for Beadle's plan. First, it has a manageable sexual cycle, which means that one can make and analyze the results of crosses. This makes it possible to demonstrate that a mutant differs from the wild type by a change in a single gene. Second, its nutrition is simple. The mold grows on a medium (minimal medium) of inorganic salts and one vitamin, biotin. As a result, any change in requirements is easy to detect.

Beadle's idea seems obvious, but it revolutionized biology (Beadle and Tatum 1941a). Instead of waiting for spontaneous mutant organisms with traits that could be analyzed, an investigator could mutagenize an organism and *select* a strain with the desired trait. Beadle and Tatum X-irradiated (haploid) spores and germinated them on a complex medium. At the time, X-irradiation was the generally accepted treatment for inducing mutation. They then tested the cultures of the resulting mutants for ability to grow on either a complex or a minimal medium. Mutants would grow on the former, but not the latter. It was then possible to determine which constituent (or constituents) of the complex medium were necessary to permit growth. The results of their first experiments were exhilarating. Three mutants that each required either vitamin B6, B1 (thiazole), or *p*-aminobenzoic acid to grow at an essentially normal rate were obtained. Genetic analysis indicated that the mutants differed from the wild type by change in just a single gene. By 1945, the Beadle group had isolated ~500 mutants with requirements for amino acids and vitamins representing an (estimated) 100 genes (Horowitz *et al.* 1945).

One can only imagine the excitement that these results produced in the Beadle/Tatum laboratory. The results were quickly followed up with the isolation of numerous amino acid and vitamin-requiring mutants. The few cases in which a mutant required multiple factors were explained by common intermediates or by inhibitory interactions between the compounds involved. The finding of so many cases in which a mutation resulted in the requirement for a single substance was used as evidence for the "one gene–one enzyme" hypothesis. Beadle himself, however, believed that this hypothesis antedated the *Neurospora* experiments and provided the impetus for the work rather than being the other way round. He states that the hypothesis itself "was the product of gradual evolution beginning with Garrod and contributed to by

many" and that it later had been given its "most explicit formulation" by Horowitz and Leupold (1951) (Beadle 1958). Beadle occasionally modified its statement to "one gene–one reaction"(Beadle 1945), but the hypothesis in either formulation has not stood the test of time. The developing understanding that single stretches of DNA can code multiple peptides as a result of multiple start sites and patterns of exon use and of the nature of protein structure make it clear that single peptides may participate in numerous reactions and that single proteins may participate in numerous developmental processes. In retrospect, the investigators were fortunate in choosing unicellular fungi and bacteria without numerous introns or multiple start sites for their experiments. This recognition of the complex relationship between single genes and phenotypes may have diminished the perceived importance of Beadle's contribution. One might recall, however, a statement of Einstein's about classical physics: "No fairer destiny could be allotted to any physical theory, than that it should of itself point out the way to the introduction of a more comprehensive theory, in which it lives on as a limiting case" (Einstein 1921, pp. 90–91).

Although the "one gene–one enzyme" hypothesis led directly to our current understanding of gene action, it is arguably not the most important part of Beadle and Tatum's contribution. In fact, that contribution is twofold. First, they provided an experimental tool, a general methodology, for the investigation of a wide variety of biological phenomena. Production and selection of mutants has provided material for studies ranging from metabolic pathways to the analysis of development (Nusslein-Volhard and Wieschaus 1980) and behavior (Benzer 1967). The success with *Neurospora* prompted Tatum to extend the work to *Escherichia coli* (Tatum 1945) providing the markers used by Lederberg (Lederberg and Tatum 1946) to demonstrate sexuality in bacteria, a discovery that in turn led to the demonstration of linkage in these organisms. Not only was what was true for *E. coli* also true for elephants [to paraphrase Jacques Monod (Friedmann 2004)], but also the reverse: bacteria, like elephants, had chromosomes.

The most significant aspect of this work, however, may have been its simplicity. The results of the experiment suggest that there is a straightforward, and understandable, connection between gene and character. The experiments are easy to describe, easy to perform, and easy to interpret, notwithstanding the physical labor involved. So simple that in 1947 a new graduate student could be given the advice: "If you need statistics to interpret an experiment, do another experiment" (N. H. Horowitz, personal communication). Contrast this with the problems in interpreting the effect of gene dosage on wing-vein development in *Drosophila* (Goldschmidt 1938) or the "Regulation of Regenerative Growth and Patterning in Drosophila"—the title of a recent (2015) seminar in my department. Contrasted with the attempt to account for complex morphological features, the problem of connecting hereditary information with biochemical (metabolic) transformations suddenly seemed like something that could be approached experimentally with the hope of obtaining real answers. Looking at the course of biochemical investigation following the Beadle/Tatum experiment supports the contention that this realization persuaded a generation of biologists to look for molecular explanations. In particular, I suppose that the demonstration that the analysis of biological phenomena might lead to straightforward molecular answers was a major factor in inducing a new generation of physical scientists to consider taking up such studies.

Beadle was remarkably effusive in ascribing credit for the initial recognition of the relationship between genes and enzymes to Garrod (Beadle 1945, 1958). In particular, he attributed to Garrod's studies on *Inborn Errors of Metabolism* (Garrod 1909) the beginnings of biochemical genetics. Beadle's tribute is so fulsome, however, that one might be tempted to think of Garrod's work as analogous to that of Mendel, and of Beadle and Tatum's as analogous to the rediscovery of Mendel's laws. This interpretation, however, was later effectively criticized by Joshua Lederberg in his official memoir of Tatum (Lederberg 1990). Lederberg concluded "that while Garrod understood how genetic anomalies could assist in the unraveling of metabolic pathways. . .. he had no comprehensive theory of gene action."

It is certainly true, however, that Garrod's insight was remarkable. In his 1909 Croonian lecture, he states "We may further conceive that the splitting of the enzyme ring in normal metabolism is the work of a special enzyme, that in congenital alkaptonuria this enzyme is wanting, whilst in disease its working may be partially or even completely inhibited" (Garrod, p. 50). It is also clear that he understood metabolism as consisting of a series of linked reactions and that a reaction that was blocked would lead to the accumulation and excretion of the blocked intermediate. We should not make the mistake, however, of ascribing our interpretation of his statement, made with our current knowledge, to that of a scientist working in the early 1900s. Lederberg claims that Garrod himself "never made the leap from the anomaly provoked by the mutant gene to the positive functioning of its normal allele. Nor did he recognize enzymes as the direct products of genes in their normal function but rather referred to mutational anomalies as freaks or aberrations to be compared with the effects of infection or intoxication" (Lederberg 1990). Garrod's own words (above) seem to contradict that claim, but it does seem clear that Garrod was thinking of a quantitative relationship that could also be affected by disease rather than a determinant of specificity. Furthermore, Garrod takes pains to point out that these inborn errors are for the most part harmless, and he seems to consider them as the extremes of a normal distribution of human variation. This may be a reflection of the view, current during the period, that genes determined only "superficial" traits.

A survey of genetics and biochemistry textbooks of the period confirms this view. There is no mention of Garrod's work in Goldschmidt's (1938) monograph, *Physiological Genetics*. [In a reminiscence Beadle (1966) reports that Goldschmidt

told him that he had known of Garrod's work and referred to it earlier and that "he could not understand" how he had not cited it.] Sturtevant and Beadle's 1939 textbook *An Introduction to Genetics* does not mention alcaptonuria in the chapter on "Genes and Phenotypes" (Sturtevant and Beadle 1939), although Beadle does refer to the work in a 1941 paper (Beadle and Tatum 1941b). The reference is incidental and peculiar ["the failure of alcaptonuric individuals to oxidize homogentisic acid (Garrod and others) and the incomplete transformation of uric acid in the Dalmation coach hound. . .."] and is not accompanied by a citation of Garrod in the list of references. Some biochemistry textbooks of the period 1938–1940 do mention Garrod's work, however. Bodansky (1938), for example, both cites *Inborn Errors of Metabolism* and points out that alcaptonuria "appears to be hereditary" although without any statement as to what that might mean. Harrow, the author of a series of textbooks that went through nine editions, states in the 1940 version that "in the rare disease known as alcaptonuria, the urine blackens on standing," but there is no mention of genetics. It does seem clear, however, that the retrospective recognition of Garrod's important work (Judson 1980) has diminished the importance ascribed to the contribution of Beadle and Tatum.

The Rise of Molecular Biology

Molecular biology as we define it has several origins. Beadle was able to keep the basic work on *Neurospora* going throughout the period of the United States' participation in the World War II. Beadle's work was funded by the Rockefeller Foundation, whose Director for the Natural Sciences was Warren Weaver. Weaver plays an important role in this story since he is likely the inventor of the term "molecular biology" used to describe the reductionist science he wanted to support (Weaver 1970; Kay 1993). Weaver was a major supporter of both Pauling and Beadle, whose work was not connected, while the Rockefeller Institute provided increased support for Beadle's fundamental studies during the war (Kay 1993). It seemed likely that proteins (or nucleoproteins) could be gene-like and that investigations of protein structure would be fruitful, particularly after the crystallization of tobacco mosaic virus.

The end of World War II coincided, and was probably speeded up by, one of the great technical developments of scientific knowledge, the atomic bomb. There followed a series of discoveries critical for the development of molecular biology. In particular, the development of the analytical methods of column and paper chromatography (Martin and Synge 1941; Moore and Stein 1951) made the quantitative analysis of both proteins and nucleic acids possible for the first time. It was these developments that made it possible for Fred Sanger to determine the structure of insulin (Sanger and Tuppy 1951a,b) and for Erwin Chargaff to analyze the base compositions of nucleic acids (Chargaff 1950). One can only agree with an argument of Judson (1993) that Fred Sanger's demonstration of the unique amino acid sequence of insulin, and to a lesser extent, the Hotchkiss (1948) and Chargaff (1950) demonstration of the unique composition of different nucleic acids, made it possible to understand how the specific structure of the genes could relate to the specific structure of proteins. In this connection, two other investigators deserve more attention than they have received. While Sanger showed that amino acid sequence was fixed and that this primary protein structure was crucial, it was Vernon Ingram who demonstrated the ability of a single mutation to change a single amino acid in the primary sequence, thereby resulting in a protein with different and pathogenic properties (Ingram 1956). And it was Charles Yanofsky who demonstrated that the linear structure of the gene, as demonstrated in genetic experiments, coincided with the linear structure of a protein (Yanofsky 1967). Yanofsky's achievement is usually reported as eclipsed by Crick and Brenner's earlier insightful use of the *r* mutants of bacteriophage to demonstrate the triplet nature of the code (Crick *et al.* 1961), but the demonstrations are different in their nature, Yanofsky's being based on biochemistry.

A second attractive view as to the origins of molecular biology ascribes a major role to the influx of physicists and especially of Max Delbruck to biology. Delbruck was a trained physicist who had selected bacteriophage as a possible simple system that would make possible the analysis of fundamental biological processes without the distractions introduced by cellular systems. As importantly, he was the intellectual leader of a group of talented physical scientists making their entry into biology (Fischer and Lipson 1988).

These explanations start with the influence of a lecture by Niels Bohr in 1933 in which Bohr suggested the possibility of special physical laws applicable to biological systems (Bohr 1933). Much of the career of Max Delbruck was taken up in the search for such laws. Another physicist, Erwin Schrodinger, made Delbruck visible in his highly influential little book *What Is Life*: *The Physical Aspect of the Living Cell* (Schrodinger 1944) in which he wrote about a model of the gene Delbruck had published. Schrodinger's book helped persuade a generation of young physical scientists that biology was a field in which new laws could be discovered, echoing Niels Bohr's suggestion. There is no doubt that Schrodinger's book and the Delbruck "school" recruited major talent to molecular biology. Delbruck's goal, however, of finding new physical principles special to biology was never realized. The critical blow was probably the discovery of the structure of DNA itself and the fact, as the discoverers noted, that it directly suggested a mode of DNA replication based on well-established biochemical principles (Watson and Crick 1953). This discovery meant that much in biology would, for the near future at least, be explicable by biochemistry.

The realization that Beadle's experiments introduced a simplifying element into biology is my explanation for Max Delbruck's instinctive opposition to the "one gene–one enzyme" hypothesis (comment to Bonner in Bonner 1946).

Delbruck's objection made sense: the Beadle methodology necessarily selected for mutations resulting in only a simple requirement. Any mutations resulting in multiple changes, or in changes involving unknown developmental processes, could not be rescued by growth on the complex medium so that it would necessarily appear, from the Beadle–Tatum methodology, that mutations generally resulted in single requirements. This argument, however, was later experimentally countered by the use of temperature-sensitive (conditional lethal) mutants by Horowitz and Leupold (1951). Their approach depended on the hypothesis that simple and complex mutations should have temperature-sensitive alleles in equal proportions. One could then isolate temperature-sensitive mutations and determine what proportion had simple growth requirements under nonpermissive conditions. The results showed that a majority of isolated mutants could be rescued on complex medium as required by the hypothesis. Today one might argue that by asking the question of unicellular organisms the investigators were foreordaining the answer. A multicellular organism might have given a different result if there were a way of doing the experiment. But at the time it was an effective response.

The particulars of Delbruck's objection are understandable, but it is also the case that his program involved looking for new laws of physics that applied to biology (Delbruck 1949). From that perspective, the Beadle and Tatum experiments implied too straightforward a relationship between chemistry and biology. It is clear from looking at the 1951 Cold Spring Harbor Symposium on Genes and Mutations that Delbruck was not alone in his skepticism. The known complexities of developmental mutations made many of the participants suspicious of supposing any simple relationship between gene and character. It required new developments in biochemistry to make it clear how genes could determine both protein specificity and the timing of their production, which in turn would contribute to an understanding of trait development.

A third set of proposed origins of molecular biology starts with the publication by Avery in 1944 of his demonstration that the "transforming principle" was DNA (Avery *et al.* 1944). It took approximately a decade, however, for the general recognition that the genetic material was indeed DNA. It is not that the work was unknown. Although the fourth edition (1947) of the Harrow general biochemistry text does not mention Avery's work on transforming principle, by the fifth (1950) edition there appeared the statement "striking evidence that the nucleoprotein and the gene are intimately related comes from the work of Avery. . ." (Harrow 1950). Why did it take so long to remove the protein from "nucleoprotein"? Alternatively, one might ask whether a decade is really a long time, considering the state of nucleic acid chemistry at the time? Gunther Stent claimed Avery's discovery was a case of prematurity in science (Stent 1972). A generally accepted argument is that because of a misconceived earlier proposed structure of DNA as a repeating tetranucleotide, it was hard to see where the necessary specificity would reside. The demonstrations by Hotchkiss (1948) and Chargaff (1950) of the variability between (bacterial) species of DNA base composition and finally the illumination of the Watson–Crick structure for DNA (Watson and Crick 1953) were landmarks in the understanding of the role of DNA. The Hershey–Chase experiment (Hershey and Chase 1952) is often cited as a determinant, but in fact their data are no more (and possibly less) convincing than was Avery's and may well have seemed acceptable because by then the (molecular) genetics community was ready to be convinced of the importance of DNA.

I would like to suggest an additional, experimental reason why Avery's work was not followed up more actively. Pneumococcal transformation is not an easy experimental procedure. Work with pneumococcus required microbiological skills not possessed by the new recruits to biology. A variety of serum factors was required, the organism had complex growth requirements, the assay for transformation was not quantitative, and the ability of the organisms to respond to the DNA (competence) was hard to control. Only a few laboratories had the expertise to work with this system. It was probably the work of Hotchkiss (Hotchkiss 1951; Hotchkiss and Marmur 1954) in providing selectable markers that made work on pneumococcal DNA transformation more generally accessible.

Neglected Revolutionaries

What is the evidence that the Beadle and Tatum contribution is undervalued? After all, Beadle and Tatum were awarded the Nobel prize in 1958 (shared with Lederberg) "for their discovery that genes act by regulating definite chemical events" (http://www.nobelprize.org/nobel_prizes/medicine/laureates/1958/). But Horace Judson, one of the most popular historians of molecular biology (Judson 1979), summarizes their contribution as follows: "In 1940, George Beadle and Edward Tatum using a mould that grows on bread first put to effective work Garrod's realization that what a gene does is specify an enzyme" (Judson 1980, p. 399). A usual response to a question about their contribution is the formulation of the "one gene–one enzyme" hypothesis. Correspondingly, their major contributions—providing a methodology for investigating gene function relationships and the demonstration that gene action might be described in simple biochemical terms—tend to be forgotten. The real nature of their contribution was well stated by a noted microbiologist, C. B. Van Niel: "It is true that the germs of this hypothesis" (*i.e.*, one gene–one enzyme) "can be found in the scientific literature of the first quarter of this century and that the current interpretation of biosynthetic mechanisms had been clearly formulated in a general manner by Kluyver in 1930. *But the concept of Beadle and Tatum derives its fundamental importance from the fact that it provided a general methodology that made exact experimentation on biosynthesis feasible*" [italics mine] (Van Niel 1955). In addition, their contribution was made near the beginning of the field. A "time line" puts their article early on in what we can recognize as molecular biology.

There are other contributing factors. After his discovery, Beadle retired from experimental science, and it was not

until after retiring from the presidency of The University of Chicago, much later, that he returned to the problem of the origin of corn (*Z. mays*) (Berg and Singer 2003). This was a study he could pursue alone in a cornfield and in his basement without financial support. It was generally thought among students at Caltech in the late 1940s that if Beadle couldn't come first in honest competition, whether Ping-Pong or mountain climbing, he wasn't much interested in participating and the fact was that the biochemical genetics that he helped create was not amenable to his experimental talents. Instead, he turned to administration and to fostering the work of others. It is often noted that Delbruck moved to Caltech, but it is not often noted that Beadle was chair of the department to which he came and played a prime role in the move. Beadle also cultivated the image of a simple Nebraska farm boy, an image that served him well in his public relations as president of the University of Chicago but made his status as an intellectual suspect (at least to some of the Chicago faculty[1]).

Almost all of us, when thinking about molecular biology, think of DNA. There is little point in arguing about the significance of understanding the DNA structure. In fact, it is difficult for anyone brought up in science after 1953 to even conceive of how one thought about gene action before Watson and Crick. But I submit that there is another critical factor involved. If one considers the cast of characters in the development of molecular biology, one has to include James Watson and Francis Crick, Francois Jacob, a wounded war hero, and Jacques Monod, a hero of the French resistance, plus an equally interesting group of supporting actors. These players all had charisma as well as having had the extraordinary advantage of recruiting marvelous literary talent, both from their own ranks (for example, Watson's *The Double Helix*) and from writers serving their apprenticeship at *The New Yorker* (Horace Judson). That's a combination that would have been hard for a Nebraska farmer to beat.

Acknowledgments

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[1] A personal anecdote: On the occasion of the 100th anniversary of The University of Chicago, I was asked by Edward Shils to write a biography, based on personal reminiscence of Howard Ricketts (discoverer of the Rickettsiae) for a volume on distinguished Chicago professors. I replied that I was a little young to have known Ricketts who died in 1914, years before I was born, but I would be happy to write about Beadle. "Oh no," replied Shils,"Beadle hadn't done anything intellectual while at Chicago!"

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