

C H A P T E R 9

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From Flies to Molds

Once Beadle and Tatum had crystallized the putative *v* substance, they were reasonably confident that they would soon solve its chemical structure. However, unknown to them when they went to Edinburgh, someone else had beaten them to it. Adolf Butenandt, an eminent German chemist working closely with Alfred Kühn on the meal moth (*Ephestia*) eye-color hormone, was probably aware of Tatum's report describing the production of the *v* substance from tryptophan by bacterial action¹ and tested a variety of known intermediates of tryptophan metabolism for *v* substance activity. One, kynurenine, which had previously been identified as the first product in the breakdown of tryptophan in mammals, restored normal eye color to both *Drosophila* and *Ephestia* eye-color mutants.² Tatum had missed identifying kynurenine because the crystalline material he obtained from the *Bacillus* cultures was kynurenine combined with sucrose. Removal of the sucrose did not affect the *v* substance activity, and he quickly confirmed that the resulting product was, indeed, authentic kynurenine as the German group had reported.³ As little as one-hundredth of a microgram of isolated kynurenine injected into a *vermillion* pupa fully restored normal eye color in the emerging fly.

Beadle contacted Professor Clarence P. Berg at the University of Iowa for samples of authentic kynurenine. Berg had been involved in the compound's initial identification, and his kynurenine samples were chemically identical to the material Tatum had isolated. Ironically, it was not until Berg read about Beadle's fame in the *Wahoo Democrat* of January 9, 1947 that he learned that Beadle was the same person who grew up in Wahoo. Berg's family had settled in Wahoo in the 1930s, and his father, a harness maker, knew the Beadle farm and probably his father.⁴ Isolation of kynurenine directly from pupal tissues of *cn* mutants, where it was presumed to accumulate, provided the final proof of the chemical nature of the *v* substance.⁵ It wasn't until 1949 that the *cn* substance was identified as a previously unknown derivative of kynurenine, 3-hydroxykynurenine.⁶

Thus, the substances that Beadle and Ephrussi believed were *Drosophila* hormones are not unique to insects; rather, they are produced in bacteria and

mammals during the normal breakdown of tryptophan. Today we know that the *vermilion* gene codes for an enzyme that converts tryptophan to *N*-formylkynurenine, after which another enzyme produces kynurenine. The *cinnabar* gene product is the enzyme responsible for the transformation of kynurenine into 3-hydroxykynurenine. The enzyme activities are absent from the respective *Drosophila* mutants.⁷

Butenandt's subsequent studies of the chemistry of 3-hydroxykynurenine confirmed that two molecules are used to form a product that polymerizes into the brown pigment, each step being performed by a genetically specified enzyme.⁸ In contrast, virtually nothing is known about the series of reactions forming the red pigment, although there are numerous mutations that block that pathway. The *white* mutation, which produced Morgan's famous white-eyed fly, prevents the formation of both the red and brown pigments, most probably by blocking a step that is common to the formation of both pigments.

The failure of the Beadle and Ephrussi team's five-year effort to be first to determine the chemical identity of the *v* and *cn* substances was a disappointment. The frustration at having been scooped was galling, especially since the German group's identification of the *v* substance as kynurenine had relied on clues they had discovered. The Paris and Stanford groups both despaired that their efforts had been for naught. Beadle was disconsolate and considered Butenandt's action to be unseemly, even referring to it as "bitchy." Although he was clearly disturbed, it's unclear whether Beadle believed that Butenandt had been unethical in using their findings as a stepping stone to the identification of the *v* substance. But Butenandt was already engaged in chemical studies of tryptophan-like metabolites and through his association with the Kühn group was not only aware of Tatum's clue but also in a position to test his compounds straight away. One must wonder why the Beadle-Ephrussi groups failed to test a variety of chemical derivatives of tryptophan. They knew that tryptophan itself was not the *v* substance and that it was converted to active material by bacteria. Perhaps, they failed to consider that possibility or were unaware of compounds metabolically derived from tryptophan or lacked access to them.

It's uncertain how Ephrussi learned about Butenandt's discovery or what his reaction was. At the time of the German's publication, Ephrussi was more concerned about finding a way for his family to escape from France than he was about science. Ephrussi was mobilized almost immediately on his return to Paris from Edinburgh. His family stayed in Orléans while he was stationed at a laboratory in the neighboring countryside where he was assigned work that was quite unrelated to *Drosophila*. While in Orléans, he urged Beadle to send reprints and other scientific news to his laboratory address, planning to

retrieve them on occasional undercover trips into Paris.⁹ During the next half-year, he managed to go to Paris once a week, but all he found were empty laboratories. Khouvine and Mme. Auge, another of Ephrussi's assistants, stowed his flies, although he doubted that he would see them again.¹⁰ Just as in most of Europe, genetic research in France ground to a halt. Many of the scientists were leaders of the resistance operating underground. Early in February 1940, Beadle and Marion offered to have Irène Ephrussi, then only a child, come to live with them to ensure her safety, but being relatively safe at the time, Ephrussi and Raja decided they should all stay together. Ephrussi responded: "I will nevertheless keep in mind your proposal and consider it as valid in the future. And we are grateful for having thought of it, anyway, [*sic*] to both of you."¹¹

Sometime near the end of 1940, Ephrussi determined that it was becoming increasingly precarious for a Jewish family to remain in the German-occupied region of France. He communicated with Demerec and others about the prospects for a position or financial support should they be able to escape. During January of 1941, Ephrussi was warned that the Gestapo was planning to arrest him and his family. Early the next day, in complete secrecy and without any of their belongings, the Ephrussis fled, accompanied by Boris's father, Mrs. Louis Rapkine, and her daughter.¹² They traveled by night train from Paris to Bayonne, hoping that no one would ask for their papers, which identified them as Jewish. On their arrival in Bayonne, one of Ephrussi's colleagues put him in touch with a man who guided their trek from occupied into unoccupied France. From Clermont-Ferrand, they made their way to Juan-les-Pins on the Riviera where, after several months and considerable uncertainty, Louis Rapkine arranged for them to receive visas to enter and stay in the United States. The party traveled legally from Marseille to Barcelona and through Spain to Lisbon, where they sailed for New York on the steamer *Excalibur*. After a brief stay with the Dobzhanskys for part of the summer at Cold Spring Harbor, Ephrussi settled in Baltimore. The Rockefeller Foundation had arranged a two-year fellowship for him in the department of genetics at Johns Hopkins University beginning September 1, 1941. In the 1942–1943 academic year, Ephrussi was appointed associate professor of genetics.¹³

Ephrussi remained at Johns Hopkins for three years, focusing on the composition, properties, and measurements of the amount of the *Drosophila* eye pigments rather than the genetics of their development. The experience in Baltimore served as a "holding operation" until he could leave for London to join the Free French forces where he helped evaluate the results of allied bombing operations during the invasion of Normandy. When the war ended, he returned to Paris and was appointed the first professor of genetics in

France. Soon thereafter, Ephrussi and Raja were divorced and he married Harriet Taylor, an American geneticist. Years later, he lamented how the war and the disruptions it caused had “robbed” him of precious time to pursue the research he and Beadle had begun.

Beadle’s plenary lecture at the Edinburgh Genetics Congress summarized the progress that he and Tatum, and Ephrussi and Khouvine, had made in revealing the genetic control of *Drosophila* eye-color development. The lecture¹⁴ began by citing the work of others, notably R. Scott-Moncrieff, J.B.S. Haldane, and their collaborators at the John Innes Horticultural Institution, on the formation of plant pigments that account for the various colors of ornamental flowering plants,¹⁵ and A.E. Garrod’s analysis of alcaptonuria, an inborn inability to metabolize the amino acids phenylalanine and tyrosine beyond homogentisic acid.¹⁶ Beadle inferred that “the defect or deficiency in the enzyme systems concerned with these processes...can be attributed to a change resulting from a single gene substitution.” Beadle was evidently aware of the gene–enzyme connection in the systems he cited, but he seemed not to regard them as sufficiently robust for pursuing that connection.¹⁷

Regarding *Drosophila* eye-color development, Beadle concluded “that the process proceeds by a system of reactions occurring in series and in parallel, each of which is gene controlled.” At the end of the lecture, he acknowledged the tentativeness of the scheme and the likelihood that it would be modified as more information became available, but he thought that it was essentially correct. His and Tatum’s proposal that “genes acting through the intermediation of enzymes” were responsible for the formation of *Drosophila* eye pigments was, he admitted, a “purely gratuitous assumption” for which there was no direct knowledge of the enzyme systems involved. Nevertheless, in defense of the proposition, he added that “we know,” that “in any such system of biological reactions, enzymes must be concerned in the catalysis of the various steps, and since we are convinced by the accumulating evidence that the specificity of genes is of approximately the same order as that of enzymes, we are strongly biased in favor of the assumption.” Speculating further, Beadle was more explicit in stating “that the immediate products of many genes may be enzymes or their protein components.” But, he added, “at the present time the facts at our disposal probably do not justify the elaboration of hypotheses based on this assumption.”¹⁸ That speculation is important, he concluded “not because it tells us much about what genes do but rather because it may indicate a method of attack that we may hope will become increasingly useful to both geneticists and biochemists.” Earlier, he had suggested such an approach: “There are many ways in which genetics can aid biochemistry and perhaps even more ways in which the geneticist can profit from co-operation

with the biochemist.”¹⁹ Although he lacked a formal background in biochemistry, Beadle was nevertheless convinced that biochemical and genetical approaches were complementary, a view he reiterated on many later occasions. Perhaps the lesson of having missed out on the identification of the v substance as kynurenine, a known metabolic product of tryptophan, had persuaded him of the importance of biochemistry.

The lasting achievement of the Beadle–Ephrussi collaboration was the realization that genes controlled individual steps in a metabolic pathway. Having missed out on the identification of the v substance only reinforced Beadle’s belief that neither the *Drosophila* system nor any other system being studied anywhere was optimal for exploring the broader problem of the physiology of gene action, or, as he referred to it, biochemical genetics. Beadle recognized that a more amenable biological system and experimental strategy were needed, one in which mutations would affect easily recognizable, already known, physiological functions. Logically, it seemed, it would be considerably easier to assess the physiologic function of a gene if mutations affected the production of known compounds rather than unknown ones, as was the case with the *Drosophila* eye-color pigments.

While he and Tatum were struggling with how best to pursue the gene-enzyme paradigm experimentally, Beadle was struck by something Tatum said during one of his lectures in a course on comparative biochemistry given during the winter quarter of the 1940–1941 academic year. Many years later, he recalled the circumstances: “Sitting in on one of Tatum’s lectures...and observing him writing sequences of reactions on the blackboard, I suddenly realized how stupid we had been all these years. Here were all those enzymatic reactions already worked out by competent biochemists. If our gene-enzyme concepts were correct, then we ought to be able to identify the genes immediately responsible for specifically known enzyme-catalyzed reactions. So why not reverse the approach? Instead of looking for reactions by enzymes controlled by known genes, why not look for genes that control already known chemical reactions and thus make the chemistry far easier? How? It should be easy. Start with an organism capable of carrying out reaction chains for a number of known end products. Then find genes that control the specific steps in the chains of reactions.” Beadle’s logic was elegant in its clarity and simplicity. But the idea had to be translated into an explicit experimental approach: “Since essentially all processes in an organism are biochemical, each should be resolvable into a series of specific reactions.... If all such syntheses are gene controlled, it should be possible to block the production of specific substances in the cell by inducing mutations in the genes controlling these syntheses. We might then expect to find mutations...characterized by an

inability of the organism to synthesize essential diffusible substances such as vitamins, amino acids and other building blocks of the cell's protoplasm."²⁰ Beadle was confident that this approach would succeed.

What exactly in Tatum's lectures triggered Beadle's epiphany? Fortunately, the contents of the lectures were preserved in a set of handwritten notes by Carleton Schwerdt, a young faculty member in the department of medical bacteriology.²¹ Throughout January and well into February 1941, Tatum presented a comprehensive review of what was known at the time about the intermediary metabolism of microbes, plants, and animals. Tatum implied that the wide variety of nutritional requirements of microorganisms for vitamins, amino acids, purines, pyrimidines, etc. reflected the organism's inability to make these metabolites from simpler precursors. More specifically, he proposed that complex nutritional requirements reflected the loss of one or more genes responsible for the production of a required nutrient, a view that had been developed by others.²²

As the course neared its end, Tatum asked rhetorically "What do genes do?" He concluded that genes control metabolic reactions and that mutations accumulated over evolutionary time cause a loss of function of some particular metabolic function. He provided numerous examples in which microbes differ in their ability to synthesize amino acids, vitamins, etc.; even humans, he speculated, were unable to make a variety of amino acids and vitamins because reactions involved in their synthesis were blocked probably by the loss of the corresponding genes. But Tatum failed to provide any insight as to how genes control metabolic reactions or, for that matter, any property that is determined by a gene.

Beadle recognized immediately that if mutations were found which caused known nutritional deficiencies, they could link gene action to metabolic processes. However, to succeed they needed an organism that had as few nutritional requirements as possible. The organism would also have to be mutable by radiation or other mutagenic agents. Additionally, it should be amenable to rapid genetic analysis so that any nutritional deficiency could be attributed to a single gene, preferably one that could be mapped to a particular chromosomal location. Tatum scouted around in the nearby medical bacteriology department for a microorganism that might meet their needs but got little help from that group.²³ One candidate was the ascomycete, *Neurospora*, a fungus whose properties and genetics Beadle was already familiar with.

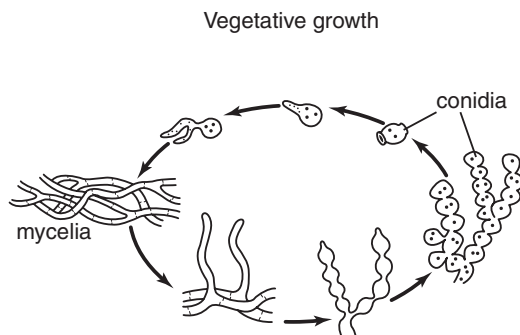
The organism had attracted the attention of the famous Dutch botanist, F.A.F.C. Went, during his travels in Java, and he cataloged many different species. The mold is ubiquitous in nature, its most obvious feature being a bright salmon-orange-colored "fuzz" covering the surface of various forms of

vegetation. The year 1927 marked the birth of the genus *Neurospora* and the beginning of its use in genetic studies. What made *Neurospora* attractive to Beadle was that it could be propagated asexually and could also be induced to undergo a sexual cycle. Like *Drosophila*, *Neurospora* exists in two mating types, and when these mate the products of crossing-over during the meiotic divisions are preserved in the resulting spores. In a practical sense, just as with *Drosophila*, the segregation pattern of genetic markers in the spores provides a way to determine the order of genes in the chromosome. For Beadle's purposes, however, the important aspect of *Neurospora*'s sexuality was that mutations causing a change in nutritional requirements could be verified as being single gene changes and assigned a specific chromosomal location.

Beadle first heard about *Neurospora* as a graduate student when B.O. Dodge, a noted plant pathologist at the New York Botanical Gardens, had visited Cornell and presented a seminar on the mold's cytogenetics.²⁴ More than any other individual, Dodge laid the foundation for *Neurospora* genetics by working out much of its life cycle and mating behavior, particularly details of the meiotic events leading to spore formation. His serendipitous discovery that spores produced in the sexual phase of the life cycle could be stored and then germinated after heating facilitated the fungus's genetic analysis.

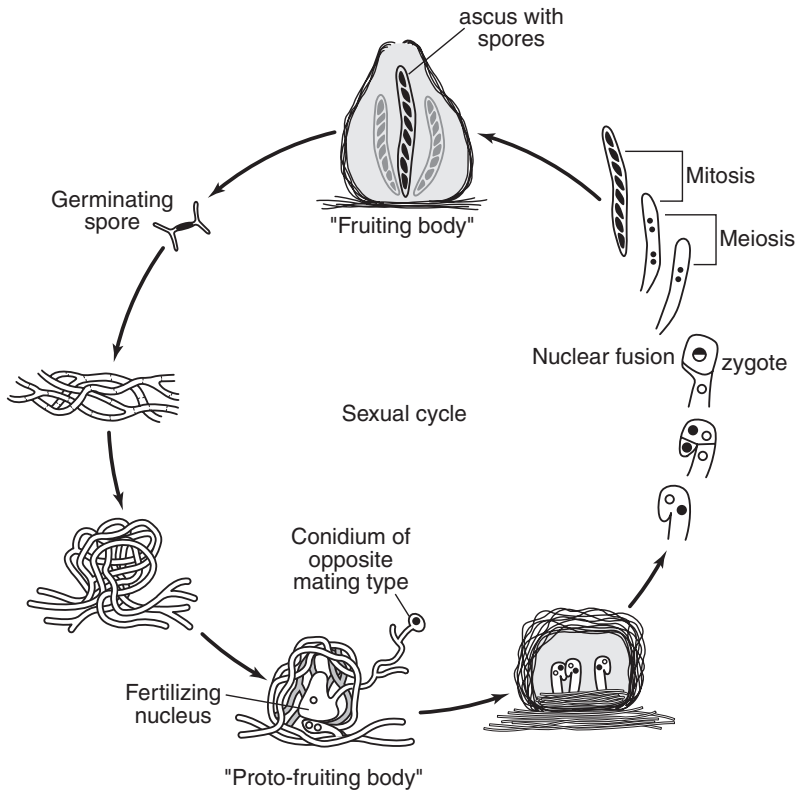
Dodge did not graduate from high school until age 20 because he needed to help out on his father's Wisconsin farm. After 15 or so years teaching high school and attending the University of Wisconsin intermittently, he obtained a doctorate in mycology at Columbia University. Only after he was appointed principal pathologist at the New York Botanical Garden at age 56 did Dodge do his most notable research with *Neurospora*.²⁵ Dodge believed that *Neurospora* deserved to share the genetics limelight with *Drosophila* and convinced T.H. Morgan, then a close friend, to take some *Neurospora* cultures with him to Pasadena. But Morgan's attempts to work with *Neurospora* in the new laboratories resulted only in getting them contaminated with bacteria and *Penicillium*, the common bread mold, both of which were frequent inhabitants of the *Drosophila* culture medium used in the lab. When Carl C. Lindegren joined Caltech's new biology division as a Ph.D. student, Morgan suggested that he work with the fungus for his thesis. With considerable help and advice, principally from Sturtevant and Bridges, he advanced the basic genetics of *Neurospora*; new genes were found, methods were developed for examining the progeny of genetic crosses, and a good start was made toward mapping the chromosomes.²⁶ Beadle was well aware of the mold's potential usefulness because Lindegren was still working on *Neurospora* in 1931 when he arrived at Caltech, and he and Sturtevant had reviewed its genetics in their textbook.²⁷

About the time Tatum's lectures were addressing the question of the function of genes, Beadle wrote to tell Dodge that "Dr. Tatum and I are interested in doing some work on the nutrition of *Neurospora* with the eventual aim of determining whether the requirements might be dependent on the genetic constitution." He asked Dodge for different isolates of the fungus so that "if preliminary experiments prove to be encouraging we will be interested in trying out the available *Neurospora* species and various 'wild-types.'"²⁸ Two weeks later, Beadle wrote Dodge that Tatum's survey of the nutritional requirements of three different strains of *Neurospora* from different sources revealed that none of them would grow without the newly discovered B vitamin, biotin.²⁹ As it happened, Tatum was familiar with biotin; he had been in Utrecht with Professor F. Kögl at the time the vitamin was isolated and identified as a required growth factor for yeast and fungi.³⁰ Coincidentally, Nils Fries, a Swedish research fellow who shared Tatum's lab in Utrecht, was exploring the requirements of a wide range of fungi for biotin. In the process, Fries developed a very simple diet for growing *Neurospora*: It consisted of a sugar (several different ones could serve), a source of nitrogen (either ammonia or a nitrate salt), phosphorus (in the form of a phosphate salt), several minerals, and biotin. This diet, named for Fries, is still used in *Neurospora* research. Although biotin was somewhat scarce at the time, Tatum's past association with the Kögl lab assured him a ready supply.³¹



Neurospora's growth behavior and its elementary genetic properties were central to Beadle and Tatum's plan for obtaining mutants with readily identifiable nutritional deficiencies. On a solid surface containing all the essential nutrients, *Neurospora crassa*, the species they adopted, propagates asexually as a tangled mat of highly branched, tube-like filaments referred to as mycelia or hyphae. Septa divide the filaments into irregular-sized segments, within which nuclei are distributed among the other intracellular cytoplasmic organelles. Growth occurs by extension at the tips of the filaments; the rate at which the

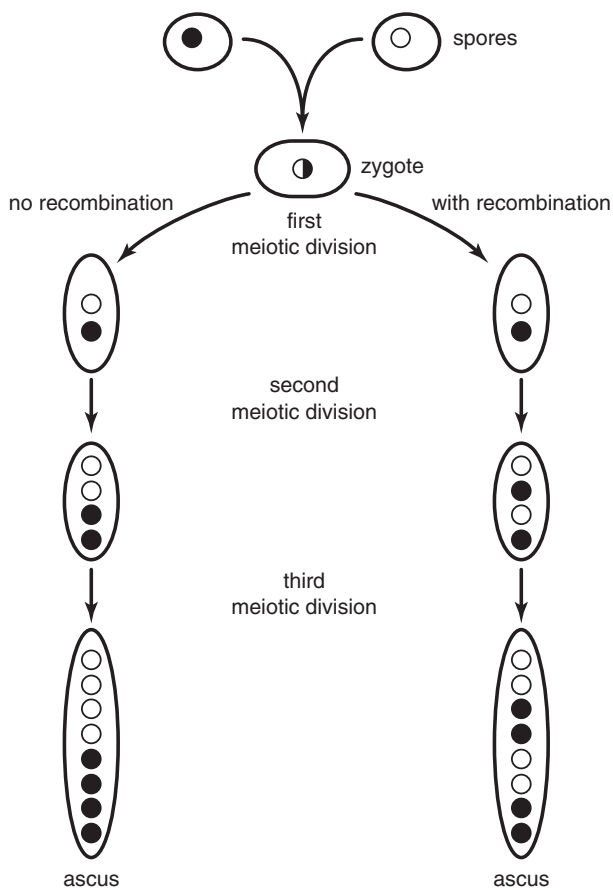
tip extends is a measure of the fungus's growth, a feature that Beadle used to good advantage. Occasionally, specialized aerial or surface filaments differentiate into spherical salmon-colored structures called conidia that are easily spread by mild air currents. Under suitable conditions, the dispersed conidia germinate, initiating new sites of filament growth. In this phase, referred to as vegetative growth, the nuclei of both filaments and conidia are haploid; they contain one copy of each of *Neurospora's* seven chromosomes.



Neurospora was especially attractive for Beadle because of its ability to enter a sexual phase. Haploid cells exist in either of two mating types, referred to as **A** or **a** types. When starved, portions of the mycelium differentiate into a "fruiting body" within which haploid nuclei of **A**- and **a**-type conidia fuse to form cells containing the two types of nuclei. After the nuclei fuse, the cells elongate to form asci, sac-like structures within which the diploid nuclei undergo two successive meiotic divisions. In the process, four haploid spores are formed, and each undergoes a single mitotic division to yield eight or four

pairs of spores. At this point, walls are laid down around each of the spores.

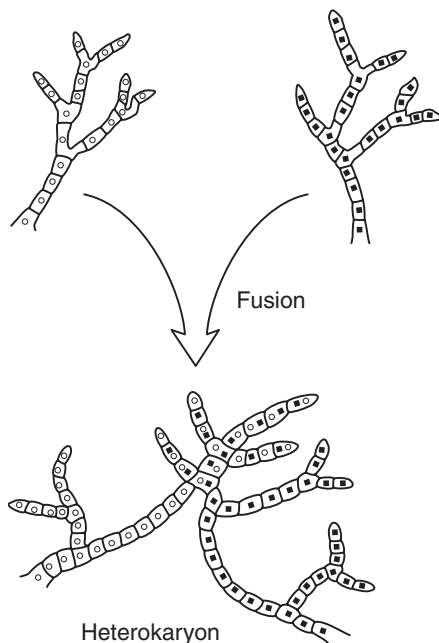
The two spores produced after the first meiotic division lie side by side, and when each divides in the second meiotic division, the resulting four spores are arranged as side-by-side pairs. Each of the four spores then undergoes a single mitotic division yielding eight spores. Consequently, the four spores in the top originate from one of the two spores formed at the first meiotic division and the four in the bottom half of the ascus originate from the other spore produced at that stage. Most important, the products of the successive divisions occupy a specific spatial arrangement in the asci, specifically, in the linear order of the four pairs of spores. To analyze the spores without disrupting their order, *Neurospora* geneticists have over the years adapted dissecting needles to pry open individual asci and remove the spores in order, one by one.



Genetic analysis of the four pairs of spores in each ascus allows inferences about the genotypes of the two haploid conidia that initiated the process as well as what transpired during the meiotic divisions. For example, because the difference between the **A**- and **a**-type mating forms depends on alternative alleles of a single gene, four spores in the asci will be of the **A** type and four of the **a** type. Similarly, when a wild type and a nutritional mutant are mated, four spores will have the wild-type allele and four the mutant allele. If more than one mutation is responsible for the altered phenotype, the arrangement and types of spores will deviate from the above outcome.

The arrangement of spores in the ascus also reflects whether recombination occurred during meiosis. If recombination does not occur, the ascus will contain four spores of one type adjacent to four spores of the other type (4-4). However, if recombination occurs during meiosis, the spores are arranged in pairs, one pair of one type adjacent to a pair of another type (2-2). The fraction of asci with the 2-2 spore arrangement relative to those with the 4-4 array is a measure of the frequency of recombination during meiosis. The genotypes and phenotypes of the four pairs of spores in multiple asci can confirm that the two mating forms differ by a single genetic difference. For example, Beadle could determine whether a nutritionally deficient isolate resulted from a single mutation by examining the spore pattern in a mating with the wild type. If he obtained four mutant spores and four wild-type spores in such a mating, he could be certain that the nutritional requirement was the consequence of a single mutation. Two isolates with an identical nutritional requirement would be considered to be alleles in the same gene if they produced the same ratios of 4-4 and 2-2 spore arrangements after mating with the wild type. If two mutants with the same nutritional requirement produced distinctly different ratios of the two spore arrangements in different asci, they were said to be affected in different genes.

Another property of *Neurospora* that proved invaluable is the ability of hyphae of the same mating type to fuse. In this case, the two haploid nuclei remain separate in the cytoplasm. If the resulting hyphae contain nuclei that have different genotypes, they are referred to as heterokaryons. In a genetic sense, heterokaryons are the equivalent of diploid animal or plant cells that contain two different haploid genomes in the same nucleus. If heterokaryons formed from a mutant and a wild-type strain have properties of the wild type, the mutation is recessive. If, however, the heterokaryons have a mutant phenotype, the mutation is dominant. Heterokaryons formed from strains with mutations in different genes generally have a wild-type phenotype because each haploid nucleus is able to provide the wild-type allele missing in the other. Heterokaryons formed with strains whose muta-



tions are in the same gene have the mutant phenotype because no normal allele is available.

Beadle and Tatum could, therefore, characterize their mutants in two ways: by the arrangement of ascospores when a mutant was mated to the wild-type parent, and by the properties of heterokaryons resulting from fusions between mutant and wild type or between two mutants. They could readily determine whether independent isolates of mutants displaying the same nutritional requirement reflected a change in the same gene or in different genes. They could also localize mutations to a particular chromosomal location.

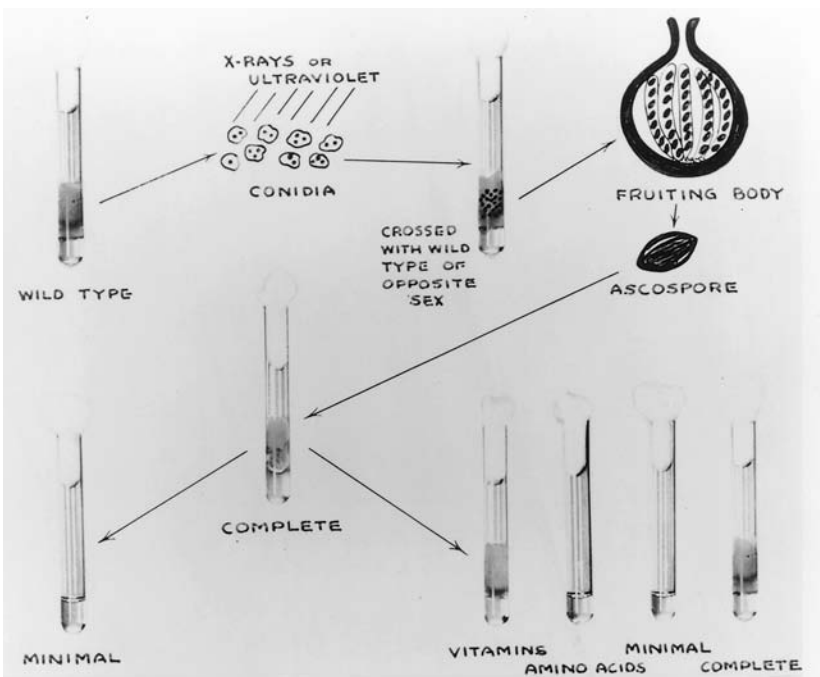
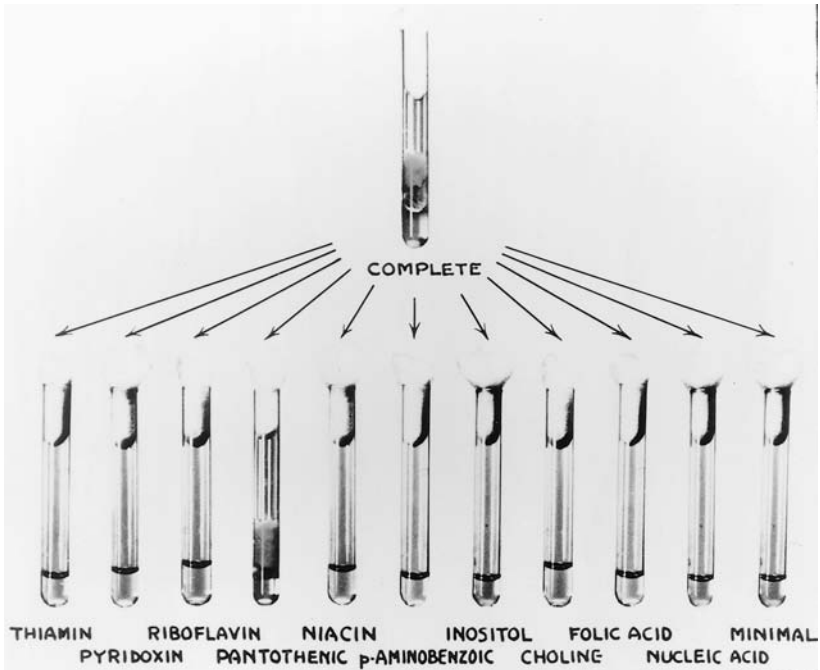
Neurospora was, therefore, perfectly suited for discovering and characterizing mutations that create identifiable nutritional requirements by determining what each mutant required to restore its ability to grow in Fries' simple culture medium. Beadle and Tatum's confidence that this strategy would work was bolstered by their belief that single mutations affecting *Drosophila* eye color blocked what appeared to be single reactions. Most geneticists, however, if they were aware of the experiments that were in the offing, would have been skeptical, because a direct connection between a gene and a single biochemical function seemed unlikely. This view was predicated on the notion that multiple genes were required to determine any one trait or biochemical

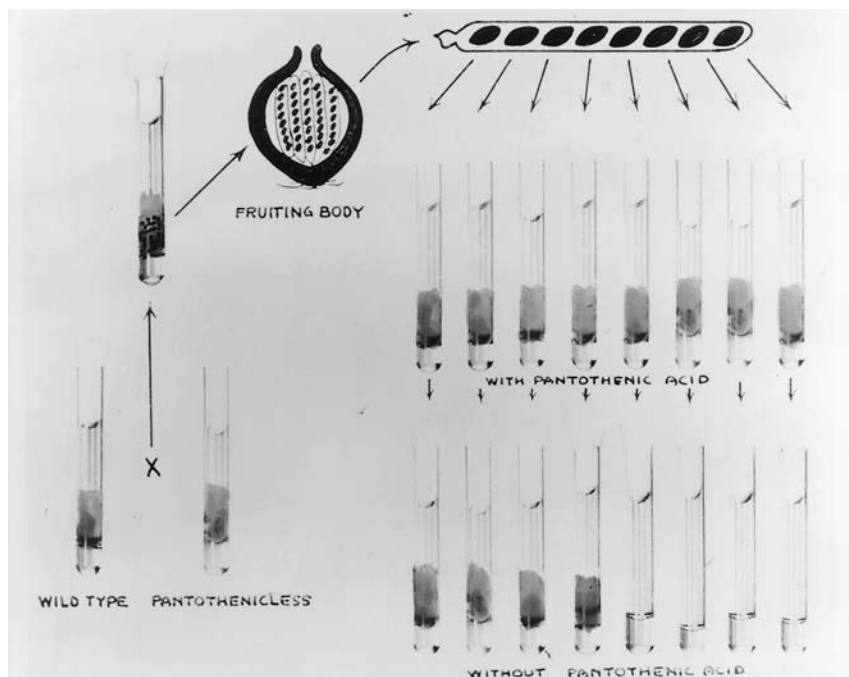
function. There seemed little reason to doubt that individual genes had multiple functions, and therefore mutations were likely to have more substantial consequences, probably causing multiple deficiencies. That view had been expressed by Muller more than 10 years earlier: "When we find, for example, that a certain gene difference results in the presence or absence of a particular enzyme, we have not proved that the gene directly produced the enzyme; it may merely have caused, through a series of intermediate processes, the production of an acid that inactivated or destroyed that enzyme, the acid having in turn been produced by another enzyme, and that activated by a co-enzyme, and that produced by a protein—when the latter was ionized by the gene! Who can tell, in this house that Jack built?"³² Others were even more pessimistic, believing that an understanding of gene action was beyond experimental dissection. Even 5 years after these experiments were initiated, Muller remained vague about the nature of the gene–enzyme relationship. But Beadle and Tatum had little doubt that they were on the right track. Their lone uncertainty was whether the frequency of mutations would be too low for them to find the mutants they sought.

They generated mutations in *Neurospora* by irradiating spores with various intensities of X rays. The physics department provided a homemade X-ray machine that was surrounded by hammered-down lead plates because "it leaked radiation like crazy."³³ Exposing spores to ultraviolet light also produced mutants. They collected only one spore from each irradiated culture to ensure that each mutant arose by an independent event.³⁴

To avoid being needlessly discouraged, Beadle and Tatum collected and stored 5000 irradiated spores before systematically searching for mutations. The irradiated spores were germinated, and portions of each culture were deposited on agar surfaces containing a rich mix of every known component of proteins, nucleic acids, sugars, and all of the vitamins. Both mutant and wild-type *Neurospora* grew on such a rich medium and produced the characteristic filamentous mycelia. Samples of each of the mycelia from the rich medium were then placed on separate agar surfaces containing the simple Fries' diet. Wild-type *Neurospora* grew out normally on the simple diet, but any mutant that required a missing nutrient failed to grow. It remained to determine that required nutrient.

Substances known to be present in the rich nutrient mixture were added back to Fries' simple mixture, first in combinations and then singly, to determine which one restored a mutant's ability to grow. For example, mutants were tested for their ability to grow on the simple mixture to which all the amino acids or all the known vitamins were added. If the simple mixture containing all the vitamins supported growth, the mutant would be tested with





groups of vitamins at a time and, where growth occurred, the vitamins were tested individually. In this way, mutants could be identified as requiring a single vitamin, a single amino acid, or even a metabolic precursor to one of these. When cultures grew in the rich medium but failed to grow when any of the defined supplements was added to the simple medium, the mutants were presumed to be unable to make some unknown but essential compound. Because the unknown compound was contained in the rich mixture, it could be isolated and identified. Candidate mutants were then mated with the wild type of the opposite mating type to determine whether they had a single gene difference. Thus, if crosses between the putative mutant and the wild type of opposite mating type yielded four haploid spores that grew on the simple medium and four that did not, the mutation was judged to result from a single genetic change.

Beadle and Tatum found their first mutant after analyzing only 299 spores. Within about three months after receiving the *Neurospora* cultures and deducing their requirements for growth, Beadle reported to Lindegren that he had "rather good luck with the *Neurosporas*," adding that he "always knew they were fine bugs to work with but never appreciated all their advantages"³⁵; the same news was reported to Dodge two weeks later.³⁶ Within two

months, they published a paper describing their method and success in obtaining three nutritionally deficient mutants.³⁷ One mutant required vitamin B6 (pyridoxin), another could not grow without *p*-aminobenzoic acid (a component of folic acid), and the third needed vitamin B1 (thiamin). They knew that vitamin B1 is made up of two parts—a thiazole and a pyrimidine—and had already determined that this mutant was unable to make the pyrimidine portion. Judging from the fact that the paper was submitted for publication on October 8, 1941, and appeared in print in the November issue of the *Proceedings of the National Academy of Sciences*, the manuscript must have received accelerated treatment, probably because it was viewed as a scientific “bombshell.”

Beadle knew that the new discovery could unite genetics with biochemistry in a way that had defied them in the *Drosophila* eye-color work. The unique properties of the *Neurospora* system provided an experimental wedge to explore not only the genetic control of morphological features, but also the control of metabolism; in short, the mechanism of gene action seemed within their grasp. To pursue that goal vigorously, he would need to increase the size of the research group substantially, particularly adding more people with biochemical training. Caltech seemed a fertile recruiting ground for bright young biologists, and no sooner had the paper been submitted for publication than Beadle went there looking for people to join him at Stanford.

Those who attended the seminar he gave during his visit were stunned. The title, if there was one, gave no clue about what was to come. As far as any of the attendees knew, it was the usual general biology seminar held in Kerckhoff. One vivid description of the occasion recalled that “the talk lasted only half an hour, and when it was suddenly over, the room was silent. The silence was a form of tribute. The audience was thinking nobody with such a discovery could stop speaking after just 30 minutes—there must be more. Superimposed on this thought was the realization that something historic had happened. Each one of us, I suspect, was surveying, as best he could, the consequences of the revolution that had just taken place. Finally, when it became clear that Beadle had actually finished speaking, Professor Fritz Went, whose father had carried out the first nutritional studies on *Neurospora*, got to his feet and, with characteristic enthusiasm, addressed the graduate students in the room. This lecture proved, said Went, that biology is not a finished subject—there are still great discoveries to be made!”³⁸ As usual, there were questions, many of which Beadle had anticipated. For some he had answers and for others he said that experiments were under way. It was a “tour de force” both scientifically and by way of showmanship. Lacking any description of the lecture other than what is quoted above, it’s uncertain

whether Morgan's criticism of his earlier presentations had improved Beadle's style. It seems more likely that the substance of his electrifying presentation overcame any stylistic shortcomings.

As word spread of the Beadle–Tatum discovery, even Stanford's first- and second-year medical students were thrilled by its implications.³⁹ Ephrussi, who by this point was working at Johns Hopkins University on the *Drosophila* eye-color problem, wrote in his usual effusive style, "I want to congratulate both you and Tatum. I believe that these first results leave no doubt that you are entering an unexplored field of most promising possibilities."⁴⁰ In their ensuing correspondence, Ephrussi expressed some concern about their collaboration on the *Drosophila* eye-color project, but Beadle's replies spoke mostly of the latest successes in obtaining additional *Neurospora* nutritional mutants. His response to Ephrussi's question about some data and the disposition of several fly cultures was "unless I can find a student to interest in it, i.e. *Drosophila*, I'm not sure I'll ever get back to it. The *Neurospora* work is constantly accelerating and I find I'm more and more tied to it. This state of affairs seems to apply to all the 'fly lab' workers so *Drosophila* seems to be at least temporarily out of luck at Stanford."⁴¹ Beadle never returned to *Drosophila*, although Ephrussi continued to characterize the eye pigments for a while and then he, too, moved on to other areas.

At the time, Beadle's research was supported from the 10-year \$200,000 grant to Stanford from the Rockefeller Foundation. His share of those funds was, however, insufficient to support the increased pace of activity and the personnel he knew had to be added. The American Philosophical Society's Penrose Fund, which had a history of providing limited amounts of money to support innovative research, had provided incremental funding for Beadle and Tatum's initial experiments, but only the Rockefeller Foundation could provide a substantial increase in support. A week or two after the initial paper appeared in print, Beadle wrote to Weaver at the Rockefeller Foundation, advising him of the breakthrough and including a detailed description of the principle and methods underlying their strategy. By then, he and Tatum had accumulated a dozen or so confirmed mutants. Beadle emphasized that "we have an approach not only useful in biochemical genetics but of great potential value"; he foresaw that simple and reliable vitamin and amino acid assay methods would be a by-product of the work.⁴² This was the first of many times that Beadle stressed the advantages of their mutants for assaying vitamins and amino acids more specifically and rapidly than possible with existing methods.

Hoping that Rockefeller would increase its support for his research, Beadle offered to fly to New York in mid-December, 1941, to discuss that pos-

sibility.⁴³ Characteristically, he was not deterred from his planned meeting with Weaver by Japan's attack on Pearl Harbor the week before and the ensuing declaration of war. Barring blackouts, the possibility of Japanese invasions, and such, Beadle was intent on making it to New York. There is no hint from the communications between Beadle and the Rockefeller Foundation in the months immediately following the entry of the U.S. into the war that either of them had changed their priorities. Nevertheless, both recognized that the war effort would impose certain limitations on basic research in the life sciences. Beadle was determined, however, to drive the new research forward even as he realized that ways had to be found to juggle basic and applied investigations to keep his research on track.

Before he met with Weaver, officials from Merck indicated that they were prepared to fund his entire program in return for exclusive patents on any discoveries his group made that they deemed important. Merck believed that the *Neurospora* work could facilitate their own efforts to identify new growth factors and that they could enhance Beadle's work by providing him with raw materials and considerable expertise in the isolation of natural products. Although Merck was prepared to provide substantial funding for his operation, Beadle sought Weaver's advice about the advisability of entering into a collaborative agreement with Merck. Stanford President Wilbur and his department chairman, Taylor, encouraged Beadle to explore the Merck connection. But Beadle was concerned that such an arrangement, while assisting the research, might bring with it problems concerning manufacturing and patent rights. He was also well aware that Stanford University policy was opposed to its faculty seeking patents on research. In that event, Merck officials suggested the possibility of some sort of collaboration, one that precluded patents but assured Merck scientists an exclusive "early look" at the laboratory's findings.

During his meeting with the Rockefeller staff, Beadle told Hanson that he had "no interest in patents nor any personal profit for himself but, on the other hand, must find outside assistance to push this work rapidly." He also told Hanson that besides the interest expressed by Merck, he had also received an offer of \$10,000 from the Research Corporation of America (RCA) to fund his research program. RCA was a nonprofit organization that held university-generated patents for the purpose of using their royalties to support promising research. However, Beadle confessed to Hanson that he was leery of both these propositions because of potential complications. In his report of their meeting, Hanson noted that Beadle's first preference "would be a grant from the Rockefeller Foundation that would free him of all obligations other than to work hard and publish freely his results. His second choice would be the Research Corporation and third, Merck."⁴⁴

Hanson had long regarded Beadle as “one of the most promising men of his age in biology.”⁴⁵ He recognized the enormous significance of the *Neurospora* breakthrough and agreed to increase Beadle’s annual support. At the time, Beadle was receiving \$20,000 per year from the biology department’s Rockefeller Foundation grant. Hanson notified him that the Foundation would provide a supplement of \$7500 in 1942 and the same amount for each of the 2 succeeding years.⁴⁶ Beadle asked that the Rockefeller grant be reviewed on a yearly basis so that his needs could be periodically assessed; he was concerned about a possible reduction of his funding needs should he lose some of his group to the draft. Although the Research Corporation never funded his research, Beadle was reassured by their promise to do so should the funding from Rockefeller prove to be inadequate.⁴⁷

Soon after the Pasadena seminar, and with funding in hand, Beadle invited two recent Caltech Ph.D.s, Norman H. Horowitz and David M. Bonner, to join the *Neurospora* project. Years before, Beadle had put in a “good word” for Horowitz when, in reviewing his application to Caltech for graduate work, he learned that Horowitz had already published work in transplantation as an undergraduate at the University of Pittsburgh. A native of Pittsburgh, Horowitz arrived at Caltech in the fall of 1936 expecting to earn a Ph.D. in genetics, but Morgan assigned him to work with Albert Tyler on the embryology of marine organisms. His thesis research explored the nature and temporal aspects of respiration as they pertain to morphological development in the eggs of two marine invertebrates. Horowitz received his degree in 1939, after which he was awarded a National Research Council fellowship to study abroad. But the war in Europe forced him to change his plans, and he went to Stanford to continue his research with Professor Douglas Whitaker in embryology. While there, he renewed his connection with Beadle, who was then deeply involved in the final stages of the *Drosophila* eye-pigment work. At the end of the year, in need of a job, Horowitz was persuaded by Professor Henry Borsook to return to Caltech as a postdoctoral fellow to work on the enzymology of tooth formation, a project that was being funded by a local dentist. Not long afterward, Horowitz attended Beadle’s electrifying lecture on the initial *Neurospora* findings and was thrilled when Beadle offered him a postdoctoral position in his group at Stanford. Accepting without hesitation, Horowitz delayed his arrival at Stanford until mid-1942, when he and his family rented a house at the edge of the campus, an easy ten-minute bicycle ride or brisk walk to the lab. Cars were still a luxury, especially with wartime gasoline rationing.

David Bonner had also just completed his Ph.D. research when he heard Beadle’s seminar and he, too, eagerly accepted the invitation to work on the

Neurospora project. Bonner had grown up in a large Mormon family in Salt Lake City, Utah, where his father was a professor of chemistry at the university. After completing an undergraduate chemistry degree in 1937, he came to Caltech, where the oldest of his four brothers, James, was already one of the biology division faculty. At the time, the hot topic in plant physiology was how various plant growth factors, auxins, stimulated growth of only certain parts of the plant; e.g., roots, shoots, or flowers. Working initially with F.H. Went, and then with A. Haagen-Smit, Bonner identified growth factors that specifically affected leaf development. Subsequently, he examined the relationship of the leaf auxin to those that affect other plant tissues. Because of his chemical experience and interest in metabolism, Bonner seemed well suited to characterize the metabolic deficiencies caused by individual *Neurospora* mutations. He and Horowitz arrived at Stanford at about the same time and worked in the part of the basement adjoining Tatum's labs.

Herschel K. Mitchell turned up at Stanford uninvited after he heard about the *Neurospora* experiments and pleaded with Beadle to hire him. A native Californian and chemistry graduate from Pomona College, Mitchell had already had two notable scientific successes. While still a master's degree student at the University of Oregon, he had worked out the structure of the vitamin pantothenic acid and then a few years later, as part of his Ph.D. thesis research with R.J. Williams at the University of Texas, he identified folic acid. Mitchell's experience with enzymes won Beadle over, although funds to pay him were not yet in hand. He never regretted the decision. Mary Houlahan, whose husband had just taken a position in San Francisco, was another "walk-on." She brought with her strong recommendations from Cold Spring Harbor for her work in radiation biology. Told that she was a wonderful worker and that he would not be sorry if he hired her, Beadle was persuaded to add her to the team. Several years later, she and Mitchell divorced their respective spouses and married.

Besides the comparatively well-trained postdocs, there were many graduate students who wanted to be part of the new venture. But Beadle had to find money for their support. Fortunately, realizing that *Neurospora* mutants with defined and specific nutritional deficiencies could be used for bioassays, the Nutrition Foundation, with the blessing of the Rockefeller Foundation, agreed to fund several graduate fellowships for work along those lines. Over the ensuing years these were held by Adrian M. Srb, August H. (Gus) Doermann, Frank C. Hungate, Taine T. Bell, Verna Coonradt, and David Regnery.

Like Beadle, Srb had grown up on a small Nebraska farm, attended the university in Lincoln, majored in agronomy, and been introduced to research by Professor Keim, Beadle's undergraduate mentor and friend. Sent by Keim

to study with Beadle, Srb arrived in 1941 expecting to work on the *Drosophila* project, but “the bulk of workers in the laboratory were manipulating bits of ‘orange fuzz,’ an organism not yet recognized in the Agronomy Department at Nebraska”⁴⁸ or for that matter hardly anywhere else. Doermann, an Illinois native, had studied biology at Wabash College and the University of Illinois, and arrived at Stanford just as the *Neurospora* work was picking up steam. Regnery had been a student in Beadle’s undergraduate genetics class, but not being enthusiastic about continuing to work on the *Drosophila* eye-color problem as a graduate student, he went to Caltech. He too was overwhelmed by Beadle’s seminar on the *Neurospora* work and returned to Stanford to join the *Neurospora* group. Soon after, he was called for military service and did not return to complete his graduate research until 1945.

Beadle believed strongly that students should generate their own research problems. It was the way he was raised at Cornell and at Caltech. He had been expected to identify and chart an experimental program of his own design while being assured of help along the way. But the “mutant-hunting operation,” manned primarily by undergraduates and technicians, was turning up mutants at a great rate, and they all needed to be studied. Graduate students, generally working with the more senior postdoctoral fellows, selected groups of mutants whose nutritional requirements needed to be identified. They were to characterize the mutants they selected, establish whether the deficiencies were due to single mutations, pinpoint their chromosomal locations, and identify the affected biosynthetic reaction.

Beadle and Tatum opened the way for a new assault on the problems that had eluded geneticists from the beginning: What exactly do genes do and how do they do it? They believed they had developed an experimental approach that could lead to an answer to the first question. Their experiments pointed to a role for genes in specifying the production of enzymes that catalyze the myriad reactions that comprise the organism’s metabolic and developmental capabilities. How genes and enzymes were related remained to be determined, and the mechanism by which genes “guide” the production of enzymes, not at all indicated by these early experiments, remained a matter for speculation and debate. Nevertheless, together with the very talented and devoted efforts of the team that Beadle assembled so quickly, and the wisdom of the Rockefeller Foundation in providing the needed resources, he and Tatum were able to build on their breakthrough on the gene–enzyme connection. Although they had provided a way to explore the functional relationship between genes and enzymes, an understanding of how genes were involved was not achieved for another 20 years.