Neurospora and the Beginnings of Molecular Genetics[1]

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I first heard of biochemical mutants in Neurospora at a memorable seminar given by George Beadle early in the fall of 1941 at Caltech. Beadle had come to Pasadena to recruit one or two postdoctoral fellows to join him and Tatum at Stanford in the investigation of these new mutants.[2] His lecture consisted of a presentation of the results that shortly afterward appeared in the first publication by Beadle and Tatum on Neurospora, in the Proceedings of the National Academy of Sciences. 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 talking about it after just thirty minutes -- there must be more. Superimposed on this thought was the realization that something historic had happened. Each one of us, I suspect, was mentally 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, Prof. Frits Went -- whose father had carried out the first nutritional studies on Neurospora in Java at the turn of the century -- 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!

In spite of some valiant attempts to bring them together, there had been little contact between genetics and biochemistry before 1941. A few fragmentary biochemical descriptions of mutant phenotypes were known. There was, for example, the brilliant study of the vermilion and cinnabar mutants in Drosophila that had been initiated by Ephrussi and Beadle. There was also the genetic study of anthocyanin pigments in ornamental flowering plants carried out by the group at the John Innes Horticultural Institution. Most remarkable of all was Garrod's analysis of human alcaptonuria -- which, however, was unknown to most geneticists, although it was a standard item in biochemistry textbooks; in the latter, of course, the interest was not in gene action, but in the pathway of phenylalanine-tyrosine metabolism.

Although they were individually interesting, none of these investigations had sufficient depth to give a clear idea of how genes functioned, nor sufficient breadth to give generality to their conclusions. Furthermore, there was a bias among geneticists against "deterministic" models of gene action. A. H. Sturtevant once remarked to me that Garrod's idea of gene action was resisted among geneticists because they were convinced that gene function was basically pleiotropic. This conviction was founded, according to Sturtevant, on De Vries' mutations in Oenothera and on a theoretical argument by E. B. Wilson. I believe that the argument Sturtevant referred to was the following, from the 3rd edition of The Cell:

> "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' .... Many writers, while avoiding this particular usage, have referred to the chromosomes, or their components 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 towards 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.' Genetic research is constantly bringing to light new cases of the cooperation of several or many factors in the production of single characters; and it is possible that all the chromosomes, or even all of the units which they contain, may be concerned in the production of every character."

In a sense, this argument is perfectly correct; but in another, and equally valid sense, it is totally wrong. It was a long time before these different ways of viewing gene action could be sorted out and understood. In the meantime, Garrod's important discovery was forgotten.

But if the chemistry of gene action was in a weak state, the chemistry of the gene itself was still weaker. The assumption that, of the substances known to be present in chromosomes, protein, rather than nucleic acid, was probably the genetic material, was perfectly reasonable in the light of existing knowledge. To hold any other opinion, one would have had to be either perverse or omniscient. Much harder to understand is the favor that the protein theory continued to enjoy in the '40s and '50s in the face of mounting evidence in favor of DNA. But that is another story.

In short, genetics in the fall of 1941 was still in that state of splendid isolation from other biological disciplines and from other sciences that Sturtevant and Beadle had described in the Preface to their Introduction to Genetics, published in 1939:

> "Physics, chemistry, astronomy, and physiology all deal with atoms, molecules, electrons, centimeters, seconds, grams -- their measuring systems are all reducible to these common units. Genetics has none of these as a recognizable component in its fundamental units, yet it is a mathematically formulated subject that is logically complete and self-contained."

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1. For my old friend Beets on his 70th birthday.
2. David Bonner and I were the fortunate two.

The 1941 paper of Beadle and Tatum truly marks the beginning of the end of this isolation of genetics from the physical sciences. Their recovery of single-gene mutants in which specific biosynthetic pathways were blocked opened entirely new dimensions in the study of gene action. In place of the chemically undefinable morphological alterations that up to that time had formed the vast majority of known mutations, there was now a wealth of fascinating metabolic defects, the analysis of which could be based directly on contemporary biochemical knowledge. Where this analysis would lead, no one attending Beadle's seminar that afternoon could tell, but that it would lead far seemed certain.

The period of 12 years commencing with the paper of Beadle and Tatum and ending with the 1953 Watson-Crick paper on DNA has been called the Romantic Period of genetics by Gunther Stent in his delightful book of scientific and social commentary, The Coming of the Golden Age. The romance, of course, was the search for the physical gene. Besides the Neurospora approach, two other important lines of attack on the physical nature of the gene got under way in the early '40s: the attack via the phage-E. coli system and that via the Pneumococcus transforming principle. The Neurospora approach was typically that of a geneticist -- by the analysis of segregating mutant characters. The phage approach was that of a physicist (Max Delbrück) who saw self-duplication as the central problem of genetics and who chose the simplest self-duplicating entity in order to investigate it. Study of the transforming principle was the route of microbiologists. It actually led to the isolation and correct identification of genetic material, but for years the significance of this discovery was clouded by doubts as to whether transformation really involved a transfer of gene material (might it not be directed mutation, or the selection of rare spontaneous mutants among the treated cells?) and, if so, whether this material was nucleic acid or contaminating protein.

The genetic method of analysing biosynthetic pathways proved to be so versatile and powerful that much of the Neurospora work in the '40s and '50s concerned itself with biochemical questions that seemed to have little relation to the problems of genetics. There was some criticism of the Neurospora group for that. The fact is, however, that out of these biochemical investigations came the body of detailed knowledge that showed -- often contrary to first appearances -- that a simple relation exists between genes and metabolic reactions. One example will illustrate the point. In the early '40s, Bonner and Tatum found a mutant that grew on hydrolysed casein, but not on any single amino acid or a synthetic mixture of them. All tests led to the conclusion that the mutant required a hitherto unidentified amino acid. A new amino acid in casein was a prize worth working for, and Bonner and Tatum eventually isolated a crystalline material which analysed like a mixture of C-5 and C-6 amino acids. After much more work, it was found that the crystalline material was a mixture of valine, isoleucine, and leucine, of which the mutant required the first two for growth. Inhibition by certain other amino acids had prevented this from being recognized earlier. The requirement for two amino acids was difficult to understand, however, and for years an interesting, but erroneous, hypothesis was in vogue. The truth, worked out by Wagner and others, was even more interesting: the same enzymes catalyse the last few steps in the synthesis of isoleucine and valine.

The cumulative biochemical evidence thus led Beadle to the one-gene-one-enzyme hypothesis. In his Nobel address, Beadle, with characteristic generosity, gives credit to Garrod for having first perceived the direct relation between genes and enzymes:

> "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. By now we knew of his work and were aware that we had added little if anything new in principle. We were working with a more favorable organism and were able to produce, almost at will, inborn errors of metabolism for almost any chemical reaction whose product we could supply through the medium. Thus we were able to demonstrate that what Garrod had shown for a few genes and a few chemical reactions in man was true for many genes and many reactions in Neurospora."

Actually, as this passage suggests, the methods worked out for Neurospora were just as important in ushering in the new genetics as were the results. In particular, the discovery of auxotrophic mutants in Neurospora led Tatum to attempt to induce similar mutations in E. coli (strain K-12, by chance). His success led, in turn, to the demonstration of sexual recombination in E. coli and all of the developments that came from that. Earlier attempts to detect recombination in bacteria had failed owing to inadequacy of the available markers as well as, possibly, to the unlucky choice of strains.

The first temperature-conditional markers were also obtained in Neurospora. I recall that the decision to incorporate a search for temperature mutants into the mutant hunt that ran more or less continuously in Beadle's laboratory at Stanford was made after Stokes, Foster, and Woodward, at Merck & Co., found that the original pyridoxinless mutant of Neurospora would grow in minimal medium if the pH were brought to 5.8 or higher. It was soon found at Stanford that a corresponding temperature-sensitive class of mutations could be generated in which the mutant phenotype was expressed at 35° but not at 25°. Mitchell and Houlahan published the first descriptions of these mutants. Later it occurred to me that by means of such mutations one could, in principle, recover mutants that would be lethal and therefore undetectable in their fully inactive form; an objection that Delbrück had raised to the one-gene-one-enzyme hypothesis might thereby be tested. In pursuing this idea, Leupold and I found that temperature-sensitive mutants were readily obtainable in E. coli, as well. It was recognized at an early stage that the temperature-sensitive phenotype might result from the production of thermolabile enzymes. Maas and Davis first showed this to be the case in a pantothenicless mutant of E. coli.

All this is ancient history now and a little boring to all but the ex-participants. Even the one-gene-one enzyme hypothesis, so fiercely debated 25 years ago, raises no eyebrows any more. It is now seen to be a special case of the general relation: one-polynucleotide-one-polypeptide. The objections that were formerly advanced against it either are encompassed in this formulation, or they are seen in retrospect to have been overly sophisticated extensions of E. B. Wilson's doctrine. Living matter does, in fact, operate on simple principles. The scientific problem is to discern the simplicity amid the confusion. The psychological problem is to accept the simple solution when it is found. George Beadle has always understood these things.