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The Origins of Molecular Genetics: One Gene, One Enzyme

Norman H. Horowitz

Summary

Roots *presents articles on landmark discoveries that laid the basis for contemporary molecular and cellular biology. In this article, N. H. Horowitz, Professor Emeritus at the California Institute of Technology, and a former associate of George Beadle's, reviews the work that led to the one gene–one enzyme hypothesis.*

Found and Lost: The Secret of Gene Function

The discovery that individual genes represent individual polypeptides was made twice. In 1902, Archibald Garrod, an English physician, published a paper in which he called attention to the fact that alcaptonuria, a rare disease of man characterized by the lifelong excretion of homogentisic acid, is hereditary. He cited William Bateson, the leading British proponent of the just-rediscovered science of Mendelian genetics, as authority for the conclusion that the disease was transmitted as a simple Mendelian recessive.

By 1908, Garrod was able to show that homogentisate is a normal product of tyrosine and phenylalanine metabolism that is destroyed by normal persons but accumulated by alcaptonurics. The alternative view, widely accepted at the time, was that homogentisate is an abnormal product made by infecting bacteria. Garrod pointed out that the latter hypothesis forced one to assume that alcaptonurics are exceptional both in possessing homogentisate and in being unable to destroy it. He proposed instead that the disease is caused by lack of an enzyme, present in normal individuals, that opens the ring of homogentisic acid. Direct confirmation of this inference was obtained by La Du and coworkers in 1958.[1] Garrod summarized his findings in his classic treatise, *Inborn Errors of Metabolism* (1909), along with evidence suggesting that certain other diseases of man had a similar basis.

Thus, practically at the outset of modern genetics, Garrod put his finger on the relationship between genes and enzymes, thereby revealing a major fundamental feature of the organization of living systems. But despite the importance of Garrod's discovery – in retrospect second only to that of Mendelism itself – it had no impact on genetics. Garrod shares with Mendel the distinction of being the father of a science that became aware of him only after he had passed from the scene and after his work had been repeated independently by others. In Garrod's case, at least, it is clear that his discoveries came too early to be appreciated. In 1909, the year the word 'gene' was first used, genes were abstractions about which little was known beyond their formal rules of hereditary transmission. It was not yet agreed that they could be identified with any material substance in the cell, a demonstration that was not made until 1915, when Morgan and his students presented the full chromosomal theory of Mendelian inheritance. As late as 1916 Bateson could still say, in opposing the chromosome theory: '...it is inconceivable that particles of chromatin or of any other substance, however complex, can possess those powers which must be assigned to our factors [i.e. genes]' (quoted by Sturtevant).[2]

The state of knowledge regarding enzymes was almost equally cloudy. In a monograph published in 1914, W. M. Bayliss[3] considered it necessary to defend the position that enzymes could be assumed to be definite chemical compounds, 'at all events until stronger evidence has been brought to the contrary'. The one thing that seemed clear was that enzymes were not proteins. This error was not corrected until 1926, when Sumner crystallized urease.

By the time biological science reached the stage where Garrod's results might have been meaningful, the genetic aspects of his work had been forgotten. Although his findings regarding the metabolism of phenylalanine and tyrosine were taught in biochemistry textbooks of the thirties, the implications of these same findings for the problem of gene function were ignored. Geneticists had by then become convinced that genes were 'pleiotropic' in their action; that is, that each gene had multiple primary effects. As I have pointed out elsewhere,[4] this conviction originated in De Vries' early studies of mutations in the evening primrose, *Oenothera lamarckiana*, and in a theoretical position adopted by E. B. Wilson in his highly influential book, *The Cell in Development and Heredity*. Both arguments were faulty. The *Oenothera* mutations were not single-gene mutations, as has long been known, and in any case the relation between morphological end-effects and the primary action of genes was and remains problematic. The biochemical traits studied by Garrod were far more revealing in this regard. The failure of geneticists to appreciate this fact can be attributed to their ignorance of biochemistry, testified to by Sturtevant.[2] Wilson's somewhat metaphysical argument (quoted in reference 4) arose more from his contemplation of the complexities of animal development than from genetics; it is actually ambiguous on the question of primary gene action.

In any case, Garrod's findings had no influence on the development of biochemical genetics. When, in time, the problem of gene action became a pressing one, the relationship between genes and enzymes had to be rediscovered. This time, the attack was made through experimental species in which the results could be repeated at will and explored in depth.

The Modern Era: The *Drosophila* Eye-Color Investigation

The modern study of gene action began in 1935 with a paper by Ephrussi and Beadle describing experiments on the transplantation of eye discs in *Drosophila melanogaster*. In holometabolous insects, the organs of the adult exist as primordia (discs) in the larva. Development into the adult state occurs during pupation. The eye of *Drosophila* contains two pigments, red and brown. Mutants of the vermilion series lack the brown pigment, those of the brown series lack red, and white-eyed mutants lack both pigments. Ephrussi and Beadle transplanted eye discs from larvae of one genotype into the abdomens of larvae of a different genotype in order to test the effect of the new environment on the development of pigment in the transplanted eye. Their inspiration for this experiment was an

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early observation by Sturtevant showing that in mosaic flies made up of wild-type and genetically vermilion tissues, the eyes were frequently normal in color even when they were known, from the presence of linked marker genes, to be genotypically vermilion. Ephrussi and Beadle were attracted by the opportunity that this case offered for a study of the chemical basis of gene function.

The transplantation experiment confirmed the 'non-autonomous' nature of the vermilion mutation: genetically vermilion eyes growing in wild-type hosts develop normal pigmentation. This result was interpreted to mean that a diffusible substance, called v^+ substance, was formed in wild-type under the influence of the normal allele of the vermilion gene and caused vermilion eyes to produce brown pigment. By the transplantation method, Ephrussi and Beadle carried out a search for other non-autonomous eye colors among 26 eye-color mutants of *Drosophila*. Just one more was found – cinnabar, also deficient in brown pigment. The question now was whether the same diffusible factor was missing in both mutants, or whether a separate cn^+ substance was implicated in cinnabar. The question was answered by reciprocal transplants, which showed that cinnabar eye discs in vermilion hosts remain mutant, but vermilion discs in cinnabar hosts become wild type. The relevant exchanges are summarized in Table I, from Ephrussi.[5]

TABLE I. Results of eye-disc transplantations

Genotype of implant	Host	Phenotype of implant
Vermilion	Wild-type	Wild-type
Cinnabar	Wild-type	Wild-type
Wild-type	Vermilion	Wild-type
Wild-type	Cinnabar	Wild-type
Vermilion	Cinnabar	Wild-type
Cinnabar	Vermilion	Cinnabar

The transplantation results were taken to imply the existence of the following reaction pathway:

$$\text{precursor} \rightarrow v^+ \text{ substance} \rightarrow cn^+ \text{ substance} \rightarrow \text{brown pigment.}$$

The pathway is blocked before the v^+ substance in vermilion mutants and before the cn^+ substance in cinnabar. The next step, identification of the substances, was achieved by Butenandt and coworkers after Ephrussi and Beadle and their research groups had obtained evidence indicating that tryptophan was the common precursor. Systematic testing of known tryptophan metabolites led Butenandt to conclude that the v^+ substance is kynurenine and, later, that the cn^+ substance is 3-OH-kynurenine. References can be found in Ephrussi[5,6] and in Beadle's Nobel Address.[7] More recent work has shown that the brown eye pigment is a phenoxazinone formed by condensation of two molecules of 3-OH-kynurenine.[8]

The *Neurospora* Investigations

The short sequence of gene-controlled reactions revealed by the eye-color experiments implanted in the investigators' minds the idea that gene action could be analysed in terms of discrete biochemical steps, and it provided the first glimmer of what later became known as the one gene–one enzyme theory.[7] To explore this idea, however, would require a new approach, since no more non-autonomous mutants were known in *Drosophila*. Beadle has said that the idea of using a microorganism for this purpose came to him while attending a lecture on comparative biochemistry by his associate, Edward L. Tatum, at Stanford University. Microbial species were known to differ in their nutritional requirements, despite the fact that they all used the same basic substances in their metabolism. If, Beadle argued, the observed nutritional diversity reflected inherited differences in biosynthetic capacities, it should be possible to generate new growth-factor requirements in an organism by inducing mutations in genes controlling the synthesis of essential metabolites. This approach, if it worked, would produce mutations affecting the synthesis of known biochemical substances, thereby simplifying the problem of identifying genes with their cognate biochemical reactions – the most difficult part of the *Drosophila* eye-color study and the problem that had blocked biochemical analysis of the morphological mutations of classical genetics.

To qualify for such a program, the microorganism had to meet two requirements: it had to be workable genetically and it had to grow on a chemically defined medium. In 1941, when the crucial test was undertaken, only one organism met the conditions: *Neurospora*, a filamentous fungus ('red bread mold'), the basic genetics of which had been elucidated by B. O. Dodge and C. C. Lindegren. Two species, *crassa* and *sitophila*, formed the subject of the first publication by Beadle and Tatum,[9] but later all the work was done with *crassa*. Both these species are haploid and heterothallic. When the two mating types are crossed, they form a diploid zygote that quickly undergoes meiosis to form ascospores. The ascospores are thus the sexual progeny of the strains that entered the cross. They germinate to form a mycelium. Besides a carbon source and the usual inorganic salts, the only growth requirement of wild-type *Neurospora* is biotin, concentrates of which had become available at about the time Beadle and Tatum started their experiments.

The plan was to irradiate wild-type conidia (asexually formed spores) to induce mutations, cross them to the opposite mating type, isolate random ascospores, and germinate the latter on a 'complete' medium – a rich mixture (containing yeast extract) calculated to satisfy the nutritional requirements of the maximal number of potential mutants. The resulting cultures would then be transferred to 'minimal' medium, consisting of the bare essentials needed for growth of wild-type, in order to determine whether any new growth-factor requirements had been induced. The two collaborators were so unsure of the outcome of the experiment that they agreed at the outset to test 5000 ascospores before giving up the project.

Success came with spore 299, a mutant that grew on complete but not on minimal medium unless pyridoxine were added. This mutant was followed by two others requiring thiamine and *p*-aminobenzoate, respectively. They all showed simple Mendelian inheritance in crosses to wild type.[9] Mutants requiring amino acids, purines, or pyrimidines followed in short order. Amino acid-requiring mutants were especially numerous, and their analysis formed much of the evidence for the one gene–one enzyme theory. Descriptions of most known *Neurospora* mutants, with an extensive bibliography, have been given by Perkins *et al.*[10]

It soon became clear that the biosynthesis of a given molecular end-product was governed by a constellation of non-allelic genes. Study of the growth requirements of individual mutants, their response to known or surmised intermediates in the blocked biosynthetic pathway, and identification of intermediates that accumulated in blocked pathways (identical in principle to the accumulation of homogentisic acid in alcaptonuria) made it clear that the range of action of each gene was restricted to a single step in the reaction chain. The inference followed that the gene had an essential role in the production of the enzyme specific for

that step. In 1945, Beadle suggested that the role was that of a 'master molecule or templet'.[11]

The first direct demonstration of an enzymatic deficiency in a *Neurospora* mutant was made in 1948.[12] However, the 'master molecule' theory predicted that mutations would produce qualitative changes in protein molecules as well as quantitative changes in activity. In 1949, Pauling *et al.*[13] showed that such was the case with sickle-cell hemoglobin, which differed from normal hemoglobin in its isoelectric point. Mutant enzymes showing alterations of thermostability were shortly afterwards found in *E. coli*[14] and *Neurospora.*[15]

Single-gene mutants that required two or more growth factors were occasionally encountered and were of special interest, since they represented potential contradictions of the one gene–one reaction rule. On investigation, however, they turned out to be exceptions that proved the rule. Early examples were a *Neurospora* mutant that required methionine + threonine for growth and another that required isoleucine + valine. The first mutant was found to grow on homoserine, a previously unrecognized precursor of methionine and threonine.[16] The second mutant, requiring isoleucine and valine, involved not a common precursor, but a common enzyme used in two separate biosynthetic pathways.[17]

A subtle question concerning the generality of these findings was raised by Max Delbrück. Delbrück argued that the procedure for detecting nutritional mutations was biased against the recovery of mutants with complex, multiple requirements. The mutants on which Beadle's theory rested might well be a highly selected sample. To examine this question, Leupold and I[18] studied the growth requirements of temperature-sensitive mutants of *Neurospora* and *E. coli.* Since these mutants grow like wild-type at the permissive temperature, they are recoverable even when their growth requirement is not satisfied by the complete medium. This study showed that while mutants unable to grow in standard complete medium do indeed occur, they are in the minority; the simple nutritional deficiency mutants recovered by the Beadle–Tatum procedure represent typical genes, not rarities.

Bacterial genetics was an offspring of the *Neurospora* studies. It was shown early by Tatum[19] that the procedure used for obtaining nutritional mutants in *Neurospora* produces the same kinds of mutants in bacteria. One of the bacteria he experimented with was, by chance, the F-episome-bearing strain of *E. coli*, K-12. The stage was thus set for the birth of bacterial genetics. The final confirmation of the one gene–one enzyme theory eventually came through studies of mutants of *E. coli* and coliphage T4 by, respectively, Yanofsky *et al.*[20] and Sarabhai *et al.*[21] Their demonstrations of colinearity between gene and protein sequences essentially concluded the long story. The later revelation of non-coding regions in eukaryotic DNA did not violate the one gene–one polypeptide principle, which does not require that all the genetic material within the 'gene' be represented in the polypeptide. The finding of viral DNAs that can be read meaningfully in different reading frames to produce more than one protein[22] *is* a violation of the principle, but this is manifestly a special adaptation to life as a small virus. As clearly as does anything in biology, the discoveries about gene action of the last 50 years illustrate Albert Einstein's aphorism: 'The most incomprehensible thing about the universe is that it is comprehensible.'

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NORMAN H. HOROWITZ *is Professor Emeritus at the Division of Biology, 156-29, California Institute of Technology, Pasadena, California 91125, USA*