CELL BIOLOGY

A Comprehensive Treatise

Edited by Lester Goldstein
and David M. Prescott

VOLUME 1

Genetic Mechanisms of Cells

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Genetic Mechanisms of Cells

Edited by

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Preface

Almost two decades have passed since the appearance of the first volume of "The Cell" edited by Jean Brachet and Alfred E. Mirsky (Vols. I–VI, 1959–1964, Academic, New York), the only comprehensive treatment of cell biology to date. In the intervening years this field has advanced enormously. Cell biology is now a truly mature science; research that a decade or two ago appeared to be aimless gropings into the unknown has now to a great extent been replaced by theoretically well-grounded investigations that provide useful answers to questions on cell function. Moreover, contemporary investigations can draw on a large resource of well-documented facts about cells. Knowing all this, we agreed that this was a propitious time to compile an up-to-date comprehensive treatise on cell biology that would serve for the next decade or two as a single source of information on many areas of this discipline.

We planned this multivolume treatise as a primary source of fundamental knowledge for graduate students, investigators working in peripheral areas, and for anyone else in need of information on some particular phase of cell biology. Thus, we asked authors to write chapters emphasizing reasonably well-established facts and concepts, but not to attempt the more traditional up-to-the-minute reviews that investigators working in specialized fields count on. A measure of the maturity of cell biology also became evident from the fact that it has been a relatively simple matter to construct each volume around a single, unified theme.

As a reflection of the fundamental role of genetics in cell biology, as in most areas of biology, the introductory volume of this treatise is devoted to genetic mechanisms. Since it was impossible to cover the full range of these mechanisms in one volume, we have focused on subjects that have, or will have, particular relevance for cell biology and that are not likely to be dealt with adequately in traditional textbooks. The emphasis in these contributions has been on the nonmolecular aspects of genetics, but obviously no one can do justice to any aspect of genetics without some discussion of some of the molecular features of the mechanisms under consid-

eration. Fuller discussions of the molecular biology of gene function will appear in subsequent volumes. Because of what we consider to be a certain degree of neglect of nonnuclear aspects of cell heredity in most other general treatments of genetic mechanisms, we have placed what some might consider to be a disproportionate emphasis on these subjects. However, we expect that the average reader will have had a solid background in the more traditional areas of genetics.

We are saddened to note that prior to the appearance of this volume Gordon Tomkins, of our Advisory Board, and Spencer W. Brown, coauthor of Chapter 4 of this volume, died prematurely. Both were making valuable scientific contributions at the time of their deaths; they will be sorely missed by the scientific community.

Lester Goldstein
David M. Prescott

Titles of Other Volumes

Volume 2 The Structure and Replication of Genetic Material

Volume 3 Genetic Expression: The Production of RNA's

Volume 4 Genetic Expression: Translation and the Behavior of Proteins

Volume 5 Cell Growth and Reproduction

Volume 6 Cell Differentiation

Volume 7 Specialized Cells and Organelles

Volume 8 Cellular Interactions

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Defining the Gene by Mutation, Recombination, and Function

E. D. Garber and M. S. Esposito

I. INTRODUCTION

Within four decades after the rediscovery of the Mendelian principles of heredity, geneticists had erected an elaborate theoretical structure of the gene based on mutation, recombination, and function. Although these approaches to understanding the nature of the gene often yielded confused or contradictory pictures, it was possible to attribute certain characteristics to the gene. By the fourth decade, the assault on the gene was stalled, indicating that new experimental organisms and methods would be required (Muller, 1945). Transmission (Mendelian) genetics was confronted by three major problems whose common denominator was the mysterious gene: (1) the physicochemical organization of the genetic material, (2) the origin and nature of genic mutation, and (3) the cellular machinery involved in transforming genotype into phenotype. The convergence of several developments was responsible for the explosive breakthrough and activity that characterized the next two decades of genetic research: (1) the introduction of microbial species, particularly prokaryotes, into the study of transmission genetics, (2) the availability of isotopically labeled precursors of two types of macromolecules, proteins and nucleic acids, (3) new physical and chemical techniques for extracting, separating, and characterizing these molecules, and (4) an active collaboration among geneticists, biochemists, and molecular biologists. Cell biologists were the beneficiaries of the new genetics, and their contributions resulted in a better understanding of the basic unit of life, the cell.

The double helix model for the structure of deoxyribonucleic acid (DNA), previously identified as the hereditary material, represents the talisman of genetics (Watson and Crick, 1953a,b). The model provided a basis for understanding and integrating the empirically determined characteristics of the gene and a springboard for the rational investigation of protein synthesis. The recently trained generation of cellular biologists did not experience the impact of the double helix model on the course of genetics. The torrent of publications and symposia devoted to the new genetics that appeared during the decade following the Watson–Crick publications indicates frenetic activity and headlong progress.

It has become fashionable to equate transmission genetics with classical genetics and molecular genetics with modern genetics. The terminology and concepts of transmission genetics, however, are imbedded in molecular genetics and used by cell and molecular geneticists. Transmission genetics produced models which did not demand molecular evidence to merit serious consideration. Mutation, recombination, and function represent the three operational methods to define the gene. Cell biologists and molecular geneticists provided the structural and molecular foundations for the concepts and terminology of transmission genetics.

II. MUTATION

Mendel (1866) explained the inheritance of contrasting characters in the garden pea by proposing two forms, later termed *alleles*, of a hereditary factor, later termed *gene*, which segregate during the formation of pollen and eggs. He did not seem to be concerned with the origin of alleles or the physicochemical nature of the gene. The Mutation Theory proposed by de Vries (1901) accounted for the abrupt appearance of a relatively few individuals with an altered, heritable character in large populations of the evening primrose *(Oenothera lamarckiana)*. While the mutants appeared to explain the origin of alleles, the theory was based almost completely on the presence of an extra chromosome and not on mutated genes in the mutant plants. The sudden appearance of a transmissible altered phenotype constitutes a mutation. Mutations *in sensu lato* can result from an altered gene, structural or numerical chromosomal aberrations, or an extranuclear event involving such organelles as chloroplastids and mitochondria, or plasmids and episomes. Consequently, mutation is an operational term and must be defined in each case by Mendelian ratios, cytological observations, and the consequences of reciprocal crosses between mutant and nonmutant parents. Intrachromosomal aberrations, particularly minute missing chromosome segments, termed *deficiency* or deletion, can simulate point (genic) mutations, as determined by breeding tests.

The early transmission geneticists obtained mutant alleles by screening many progenies from parents collected from the wild or by noting occasional spontaneous mutants in progenies from homozygous wild-type parents. Early attempts to induce mutations by physical or chemical agents failed because the experimental design could not distinguish spontaneous from induced mutations. Muller (1927) used X-rays as the mutagenic agent to induce sex-linked lethal mutations in the X chromosomes of *Drosophila melanogaster* males which were then mated to females. This ingenious experimental design relied on the sex-ratio in progeny from females with one X-rayed X chromosome and a sex-linked lethal mutation in the other X chromosome. The control progeny had twice as many females as males; a female with an induced sex-linked lethal mutation, however, gave no male progeny. Many of these sex-linked lethals were probably chromosome deficiencies, but genic mutations have also been obtained by X-ray treatment. Auerbach and Robson (1947) induced mutations in *D. melanogaster* by chemical mutagens, indicating that point mutation could involve a chemical alteration in the genetic material. Microbial species eventually provided the appropriate experimental material for mutagenic studies. Large populations of unicellular individuals are treated with an agent that readily reaches the genetic material, and the occasional mutant

can be easily detected. When the microbial species has a sexual stage or its equivalent, mutants can be tested to determine whether the mutation is genic.

Transmission geneticists developed empirical criteria to characterize genic mutation: (1) relative stability of the mutant phenotype, (2) finite occurrence, measured as mutation rate or mutant frequency, and (3) mutability to other alleles, spontaneously or after treatment with mutagens. These criteria were supported by breeding data and cytological observations whenever the organism possessed chromosomes suitable for study by light microscopy. By a remarkably fortuitous coincidence, the exquisite banding of the polytene chromosomes in the salivary glands of larvae of *D. melanogaster*, the genetically best studied organism until the 1950's, provided suitable material to detect minute deficiencies that could not be detected in other species.

Chemical mutagenesis was expected to furnish insight into the nature of the gene. Although these studies failed in their purpose, they provided techniques to obtain mutants and, later, to test models explaining genic mutation at the molecular level. Transformation in pneumococcus by extracted DNA was viewed as an interesting mutational phenomenon but failed to excite contemporary geneticists (Beadle, 1948). Although mounting circumstantial evidence from diverse sources implicated DNA as the genetic material (Olby, 1974), Hershey and Chase (1952) presented the convincing evidence from experiments with phage T2. They used radioactive tracers to follow DNA and protein from cell infection to lysis and the release of phage particles, demonstrating that DNA and not protein was indeed the genetic material.

A. Circumstantial Evidence for Triplet Codons

The now familiar double helix of polynucleotide chains furnished the key in understanding the gene as the unit of mutation, recombination, and function. The four bases along the polynucleotide chain provide the units that code the 20 amino acids in proteins. Combinations of two adjacent bases are insufficient to code for 20 amino acids; three adjacent bases yield more than enough, 64 combinations. Serious attempts to formulate a code of bases for the different amino acids centered in base triplets. The question of overlapping or nonoverlapping triplets was raised and received theoretical and experimental answers, favoring nonoverlapping triplets. Colinearity between nucleotide sequences in the polynucleotide chain and amino acid sequences in the corresponding polypeptide was a reasonable but unproved assumption when Brenner (1957) analyzed the known sequences of amino acids in a number of polypeptides. The diversity of

amino acids preceding or following a particular amino acid demanded 70 different overlapping triplets to account for the 29 amino acids, that is, more than the available 64 triplet combinations, later termed codons. Furthermore, one nucleotide substitution in one triplet would be expected in certain cases to yield three amino acid replacements for overlapping triplets. Ingram (1956) had already demonstrated the genic mutation for sickle cell hemoglobin was responsible for a single amino acid replacement in the β-globin chain.

Experimental evidence for a nonoverlapping triplet code emerged from an investigation of proflavin-induced frame-shift mutations in the *B* cistron of the *r*II locus in phage T4 (Crick *et al.*, 1961). Proflavin causes the addition or subtraction of a nucleotide during the replication of the polynucleotide chains of the double helix of the phage, thereby altering the sequence of nucleotides from the site of the added or deleted nucleotide. Proflavin-induced mutations in the *r*II locus are detected by the large, sharply defined plaques on strain B of *Escherichia coli* and by their ability to kill, but not lyse cells of strain K (Benzer, 1955). Furthermore, these frame-shift mutations can be assigned to a segment at one end of the *B* cistron by appropriate complementation and recombination tests. One proflavin-induced *r*II*B* mutant gave spontaneous revertants which lysed K cells but did not yield precisely the same type of plaques as the wild-type phase. The pseudowild-type revertants were crossed with wild-type phage and the progeny included, wild–type, pseudowild-type and mutant phage. The mutant phage, however, represented mutations at two different but nearby sites in the *B* cistron: one site for the original proflavin-induced mutation and the second site for the spontaneous mutation, termed an *intragenic suppressor*. Because the second mutation behaved as an intragenic suppressor in combination with the proflavin-induced *r*II*B* mutation, it was reasonable to argue that the intragenic suppressor was also a frame-shift mutation. According to this argument, the first mutation was responsible for the addition (+) of a base pair and the second mutation for the loss (−) of a base pair. If the reading of the cistron to produce the corresponding polypeptide begins from a fixed point and proceeds along the putative, nonoverlapping triplets to the end of the cistron, then the addition (+) of a base pair would alter the triplet sequence from the site of the addition. In the pseudowild revertant, the deleted (−) base pair restores the proper triplet sequence from the site of loss, thereby yielding a functional polypeptide. If the interval between the added and deleted base pairs had been too long, the polypeptide would not likely be functional and a pseudowild revertant would not have occurred. Finally, repeated treatment of mutant phage with proflavin eventually yielded pseudowild revertants. Crosses between such revertants and wild-type phage gave progeny

with mutations at three close sites in the *r*II*B* cistron, indicating that the pseudowild revertants resulted from three added (+++) or deleted (−−−) base pairs from successive frame-shift mutations.

Terzaghi *et al.* (1966) determined the amino acid sequence for the lysozyme produced by wild-type phage T4 and in a plus-minus revertant and found a difference of five amino acids in sequence at positions 36 to 40, thereby confirming the view that the intragenic suppressor restores the frame reading of the cistron.

B. *In Vitro* Evidence for Codons

Nirenberg and Matthei (1961) used a cell-free protein-synthesizing system from *E. coli*, labeled amino acids, and a synthetic polyribonucleotide. Polyribonucleotides containing only uracil produced labeled polypeptide from phenylalanine monomers. One codon or triplet of three nucleotides, therefore, could be identified as UUU = phenylalanine before the evidence for a nonoverlapping triplet code was available. Furthermore, codons were assigned to mRNA which is concerned with translation, the synthesis of the polypeptide coded by the corresponding segment of DNA (cistron). The codons in mRNA are translated for polypeptide synthesis by the appropriate anticodon in tRNA which recognizes the mRNA codon. Synthetic polyribonucleotides assembled from different proportions of two nucleotides yielded polypeptides with different ratios of specific amino acids that were statistically correlated with the ratios for the different possible triplets so that it was possible to assign triplets to specific amino acids (Lengyel *et al.*, 1961). The *in vitro* search for codon assignments to the 20 amino acids produced the genetic code (Nirenberg and Leder, 1964; Khorana *et al.*, 1967): 61 of the 64 triplets were associated with specific amino acids. Certain amino acids are coded by more than one triplet, a phenomenon unfortunately termed code degeneracy. The three unassigned triplets were later identified by *in vivo* studies as polypeptide chain terminators.

C. *In Vivo* Confirmation of the Genetic Code

The demonstration of an amino acid substitution at a specific position in one polypeptide of sickle cell hemoglobin as a consequence of a genetic mutation in man (an unlikely experimental subject in an era of microbial experimental organisms) was a significant contribution to the *in vivo* confirmation of the genetic code.

Ingram (1956) was confronted with the formidable task of detecting and identifying the proposed replaced amino acid in sickle cell hemoglobin.

The technical problems were evaded by the development of the fingerprint technique. Normal and sickle cell globin was digested with trypsin to produce comparable peptide fragments which were subjected to paper chromatography and then high voltage electrophoresis at right angles to the plane of chromatography. A pattern or fingerprint of about 30 peptide fragment sites was obtained. Different sites for peptide fragment 4 distinguished the two globins. Sequencing amino acids in the different fragments indicated that the sixth amino acid, glutamic acid, in the fragment from normal globin had been replaced by valine in the fragment from sickle cell globin. The replacement site was eventually assigned to the sixth amino acid in the β-chain.

Mutant polypeptides from several microbial species furnished *in vivo* confirmation of the code: coat protein of tobacco mosaic virus (Tsugita and Fraenkel-Conrat, 1962; Wittman and Wittman-Liebold, 1967); the A polypeptide of tryptophan synthetase from *E. coli* (Guest and Yanofsky, 1965; Yanofsky *et al.*, 1967b); human hemoglobin (White, 1972); and iso-1-cytochrome *c* from yeast (Sherman *et al.*, 1975). In every case, a nucleotide substitution in the resident codon for the wild-type amino acid gave the appropriate codon for the substituted amino acid as exemplified in polypeptide A of *E. coli* tryptophan synthetase. Guest and Yanofsky (1965) showed that the amino acid substitutions at a specific position in the A polypeptide of triptophan synthetase of *E. coli* in revertants or from recombination between mutants with substituted amino acids for the same resident amino acid were readily explained by a single nucleotide replacement within one codon (Fig. 1).

D. Molecular Basis of Genic Mutation

The consequences of a nucleotide replacement in one codon can be predicted from the genetic code in terms of the codons assigned to different amino acids but not necessarily in terms of the enzymatic or other activity of the resulting polypeptide. Moreover, because the code is degenerate, a nucleotide replacement need not result in an amino acid substitution. Finally, 3 of the 64 possible codons do not correspond to an amino acid. These codons were termed *nonsense codons* to contrast with the other codons which make sense, that is, correspond to an amino acid. The substitution of one amino acid for another so that a mutant phenotype results has been termed a *missense mutation.*

Watson and Crick (1953b) suggested that spontaneous genic mutations might result when a base in the polynucleotide chain assumes one of its tautomeric forms during DNA replication. In such a situation (Fig. 2), a "wrong" base would be inserted into this position in the complementary