HANDBOOK OF MOLECULAR CYTOLOGY

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General preface

The aim of the publication of this series of monographs, known under the collective title of '*Frontiers of Biology*', is to present coherent and up-to-date views of the fundamental concepts which dominate modern biology.

Biology in its widest sense has made very great advances during the past decade, and the rate of progress has been steadily accelerating. Undoubtedly important factors in this acceleration have been the effective use by biologists of new techniques, including electron microscopy, isotopic labels, and a great variety of physical and chemical techniques, especially those with varying degrees of automation. In addition, scientists with partly physical or chemical backgrounds have become interested in the great variety of problems presented by living organisms. Most significant, however, increasing interest in and understanding of the biology of the cell, expecially in regard to the molecular events involved in genetic phenomena and in metabolism and its control, have led to the recognition of patterns common to all forms of life from bacteria to man. These factors and unifying concepts have led to a situation in which the sharp boundaries between the various classical biological disciplines are rapidly disappearing.

Thus, while scientists are becoming increasingly specialized in their techniques, to an increasing extent they need an intellectual and conceptual approach on a wide and non-specialized basis. It is with these considerations and needs in mind that this series of monographs, '*Frontiers of Biology*' has been conceived.

The advances in various areas of biology, including microbiology, biochemistry, genetics, cytology, and cell structure and function in general will be presented by authors who have themselves contributed significantly to these developments. They will have, in this series, the opportunity of bringing together, from diverse sources, theories and experimental data, and of integrating these into a more general conceptual framework. It is unavoidable, and probably even desirable, that the special bias of the individual authors will become evident in their contributions. Scope will also be given for presentation of new and challenging ideas and hypotheses for which complete evidence is at present lacking. However, the main emphasis will be on fairly complete and objective presentation of the more important and more rapidly advancing aspects of biology. The level will be advanced, directed primarily to the needs of the graduate student and research worker.

Most monographs in this series will be in the range of 200–300 pages, but on occasion a collective work of major importance may be included exceeding this figure. The intent of the publishers is to bring out these books promptly and in fairly quick succession.

It is on the basis of all these various considerations that we welcome the opportunity of supporting the publication of the series '*Frontiers of Biology*' by North-Holland Publishing Company.

E. L. TATUM
A. NEUBERGER, *General Editors*

Preface

Cytology has made great advances during the past decade. At the turn of the century, the rediscovery of Mendel's laws aroused particular interest in the nucleus, more especially in the chromosomes which were sought as the carriers of hereditary factors. Rapid expansion of chromosome studies led to a great development in cytology, which for several decades consisted mainly of the study of the nucleus. The application of the techniques of electron microscopy to the cytoplasmic organelles after World War II changed this trend, making the cytoplasm an equally important field of research in cytology. Investigation in this last area expanded so rapidly that workers concerned with the nucleus and those concerned with the cytoplasm had only limited points of scientific contact. However, with the development of molecular biology there has been a rapid increase in our knowledge of the molecular organization of both cytoplasmic and nuclear components, and of molecular interactions between cellular structures. Molecular cytology became common ground for the two groups of cytologists.

The gathering of contributions from the diversified fields of molecular cytology into a handbook is the result of the pressing need for a deeper understanding of the molecular interactions within the cell.

In planning the Handbook it was found appropriate to have about half of the chapters written by European authors and the other half by American colleagues. The same principle was applied in choosing the Advisory Editorial Board which consisted of: W. Beermann, W. Bernhard, H. Fernández-Morán, K. Porter, and H. Swift. An attempt was also made to have the nuclear structures represented by about the same number of chapters as the cytoplasmic organelles, to permit a better understanding of the macromolecular interactions between the two cellular compartments.

It is apparent that in a number of cytological fields our knowledge has not yet reached the molecular level. Cytology is, however, coming closer to this stage every day. We are near the threshold of a synthesis that embraces the information from the light microscopic studies, ultrastructural analysis and the biochemical data. The purpose in preparing the Handbook has been to bring together this knowledge at a moment when it has not yet crystallized into a coherent body of molecular organization and interaction. By having in one book most of the information available on the evolution of DNA, the ultrastructure and biochemistry of chromosomes, and the ultrastructure and biochemistry of cytoplasmic organelles, the reader may be in a better position to get a general picture of the molecular interactions within the cell, to see the areas which are least developed, and to find out where new and significant trends in research lie.

DNA is present not only in the nucleus, but also in the mitochondria and the chloro-

plasts. This is confirmation that there are three genetic systems in the cell. A pressing problem is to find out how the three genetic systems interact and their degree of independence. Extra DNA copies are released from the chromosomes and find their way to the cytoplasm in the oocytes of several species of invertebrates and vertebrates. This phenomenon brings the nuclear and the mitochondrial DNA's closer and one wonders in what way mitochondrial DNA activity may be affected by the presence of nuclear DNA in the cytoplasm.

The study of the evolution of DNA is the basis of the understanding of the evolution of the chromosome from viruses to higher organisms.

In the Handbook the treatment of the evolution of DNA starts with the study of the evolution of base sequences in this macromolecule (McCarthy). It is followed by the occurrence of repetition of nucleotide sequences in chromosomes of lower and higher organisms (Britten and Kohne). Special attention is paid to the highly repetitive DNA of rodents (Walker, Flamm and McLaren) and to the doublet pattern of the nucleic acids of viruses since this study has a direct bearing on their origin (Subak-Sharpe).

The pattern of DNA in viruses leads to the study of the viral chromosome (Kleinschmidt) and to the organization of the chromosome in bacteria (Caro). At this point it seemed appropriate to insert three chapters on the fine structure of the bacterial cell (Van Iterson).

The next step in the evolution of the chromosome leads us to the molecular organization of chromosomes in higher organisms. This subject is first treated by Ris, and is followed by two chapters on the organization of heterochromatin at the molecular level: one on the biochemistry and molecular biophysics of hetero- and euchromatin, which describes the interactions between histones and DNA (Frenster), and the other on the DNA replication and gene amplification in heterochromatin, which deals mainly with the occurrence of ribosomal cistrons in this chromosome material (Lima-de-Faria). The problem of crossing-over has not yet completely reached the molecular level but Henderson discusses the information available at this level. The relation between this phenomenon and DNA replication has become clearer by the finding that DNA synthesis not only occurs at the interphase of meiosis but also at the early stages of the meiotic prophase (see the chapters by Stern and Hotta, Callan, and Lima-de-Faria).

Only by combining our knowledge of the ultrastructure with the study of the biochemical activity can we expect to reach an understanding of the function of the nucleus and of the chromosomes. The information on the ultrastructure of the interphase nucleus, spermatids, mitotic and meiotic chromosomes has been assembled by Kaye, Lafontaine and Lord, and Sotelo, respectively. The treatment of the biochemistry of the interphase nucleus and of mitotic and meiotic chromosomes starts with the nuclear protein fractions (Fambrough), is followed by the DNA, RNA and protein synthesis during the mitotic cell cycle (Monesi), and the biochemistry of meiosis (Stern and Hotta). Due to their importance, the biochemical activity of polytene and

lampbrush chromosomes is treated separately (Berendes and Beermann, and Callan, respectively). Chromosome movements have long been a subject of speculation but at present ultrastructural and biochemical data are yielding information on the molecular mechanisms involved in this process (Forer).

Special attention is paid to the nucleolus due to its central role in the production of ribosomes. The ultrastructure and function (Miller and Beatty) and its synthetic activity (Perry) are discussed in detail.

Differentiation, which for many years has been one of the least developed areas of cytogenetics, seems to have entered a new period of expansion. New data are emerging on the regulation of DNA and RNA synthesis in cells, and the work of Gurdon, Tarkowski and Beatrice Mintz receives special attention in the chapter by McLaren. Cell hybrids induced by viruses and other aspects of nuclear function are described in the chapter by Ringertz.

Cancer is a central problem in cell differentiation, but its study has only partly reached the molecular level. However, the interaction between viruses and chromosomes (Nichols) is one of the fields of research which has brought this problem to the level of macromolecular interactions between these two types of structures. The relation between chromosome abnormalities and carcinogenesis as well as the ultrastructure of the cancer cell are an integral part of this problem (Levan, and Bernhard, respectively). Within the general context of chromosome abnormalities are those which involve the sex chromosomes and the autosomes in man and mammals. It was felt that these should not be left out due to their importance in differentiation and their medical interest (Hamerton, and Taylor, respectively).

The study of the structure and function of the nuclear envelope closes the section of the Handbook dealing with the nucleus (Stevens and André).

Mitochondria and chloroplasts fulfil basic functions in the cell, but the discovery of nucleic acids, and in particular DNA, in these organelles has further increased their functional significance. Five chapters written by Munn, Borst, Kroon, Swift and Wohlstenholme, and Lefort-Tran are concerned with the ultrastructure, biogenesis, biochemistry and function of these organelles.

Polysaccharides are synthesized and coupled in the Golgi apparatus (Favard) with proteins coming from the cavities of the endoplasmic reticulum (Goldblatt). This last cell structure is the main site of protein synthesis where ribosomes play a basic role (De Man and Noorduyn). However, information is largely lacking about the molecular arrangement of the membranes of the endoplasmic reticulum, associated enzymes, and even the structural arrangement of the ribosomes on the endoplasmic reticulum.

Lysosomes are known for their nutritive role in the cell, their active part in the defence against various pathogenic bodies and their role in lytic phenomena leading to the disappearance of intracellular organelles or structures during differentiation (Wattiaux). On the other hand peroxisomes are a special type of respiratory particle

with the ability of reducing oxygen to water by a two-step mechanism involving hydrogen peroxide as an intermediate (Baudhuin).

Specialized cell structures involved in chromosome and cell movements are the centrioles (Pitelka), the cilia and the flagella (Afzelius, and Sleigh). These organelles are intimately interrelated since centrioles can produce cilia. Their ultrastructure, biochemistry and physiology are treated in three chapters.

The Handbook closes with seven chapters on the cell surface, pynocytosis, cell membranes and walls. Although this is the last part of the book it is not the least significant. Our knowledge of these structures and their function is in many ways rudimentary, but this is also an area that is expanding rapidly as the methods of molecular biology are being applied more extensively to these structures. This problem is treated by Bennett (two chapters), Schoffeniels (two chapters), Stockem and Wohlfart-Bottermann, Robertson, and Kreger.

A Handbook of this kind can make no claim to be complete or exhaustive. It is only intended as an introductory work to Molecular Cytology.

It is a great pleasure to acknowledge the collaboration of the Advisory Editorial Board and of other colleagues who have made valuable suggestions in the preparation of the Handbook. These are: W. Beermann, E. L. Benedetti, W. Bernhard, M. Birnstiel, J. Bonner, P. Borst, H. Burström, J. E. Cummins, H. Fernández-Morán, H. Holter, T. Laurent, D. Mazia, M. J. Moses, A. Neuberger, D. S. Parsons, K. Porter, R. D. Preston, J. Prichard, H. Swift, and E. L. Tatum.

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Lund, May 30, 1969

A. LIMA-DE-FARIA

Contents

For a detailed list of contents the reader is referred to the first page of each chapter.

Part XI – Ultrastructure and biochemistry of mitochondria and chloroplasts

Part XII – Ultrastructure and biochemistry of ribosomes, endoplasmic reticulum and Golgi apparatus

Part XIII – Ultrastructure and biochemistry of lysosomes and peroxisomes

Part XIV – Ultrastructure and biochemistry of centrioles, flagella and cilia

Part XV – Cell surface and function

Part XVI – Cell membranes and walls

PART I

Evolution of DNA

CHAPTER 1

The evolution of base sequences in nucleic acids

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Contents

1. Introduction and methods of approach

Although there is an almost complete lack of detailed sequence information for DNA or RNA, a considerable body of data exists relating to the overall base composition and to differences and similarities among the base sequences of DNAs of related organisms. A large number of determinations of base composition have been made on a variety of organisms. This has proved useful for taxonomic purposes especially in micro-organisms where the base composition is highly variable. In higher plants and animals only small differences in base composition exist so that such measurements are not useful for comparative purposes.

Chemical analyses of DNA samples show the equivalence of A to T and G to C. Thus, the overall base composition of DNA may be described in terms of the % G + C. Chemical analyses of the relative amounts of the four bases present are essential where the possibility exists of the presence of unusual bases. Otherwise, this analysis has been largely replaced by less tedious methods. The two most popular of these indirect methods involve measurements of the buoyant density in cesium chloride (Schildkraut et al. 1962a) or the mean thermal denaturation temperature, T_m (Marmur and Doty). The great advantages of the former method derive from the fact that only 1 μg is required and adequate determinations may be made on a crude homogenate without purification of the DNA.

More detailed sequence data have been obtained only with the smallest DNA molecule known, that of the coliphage ΦX174 (Hall and Sinsheimer; Sedat and Sinsheimer). Since there are no known deoxyribonucleases of sufficient specificity for sequence work, the most useful degradative methods are chemical procedures selectively destroying the purines or the pyrimidines. Such procedures allow the estimation of purine and pyrimidine sequences of various lengths (Burton; Hall and Sinsheimer; Sedat and Sinsheimer). Using these methods, Burton and co-workers have shown that sequences of polydeoxythymidylic acid exist in higher than statistically expected proportions in all DNAs, whereas corresponding polydeoxycytidylic acid sequences were very rare.

Measurements of nearest neighbor or dinucleotide frequences in DNA may be made by means of *in vitro* copying of DNA by DNA polymerase (Josse et al.). Using a given DNA primer and the four 5′-deoxyribotriphosphates, one of which is labeled with P^{32}, a faithful copy of the primer is made. Degradation of the synthesized DNA leading to 3′-nucleotides leads to the 'nearest neighbor' pattern of that particular base residue. By repeating the analysis with the other three labeled nucleotides, the frequencies of all sixteen dinucleotide sequences may be established. This kind of analysis may be extended to larger nucleotide sequences by allowing the incorporation of a mixture of ribonucleotides and deoxyribonucleotides in the presence of Mn^{2+} (Berg et al.). Degradation with alkali leads to oligonucleotide fragments. This is a promising, but yet largely unexplored approach to detailed sequence data.

Thus, the problem of complete sequence determination of DNA is far from solution

and indirect means must be used to obtain comparative sequence data. At the present time a very productive indirect approach is offered by the use of annealing reactions between nucleic acids. Following the discovery that the two strands of DNA may be physically separated and reassociated (Doty et al.), the possible value of this reaction for comparisons of related nucleic acids became apparent. The formation of heteroduplex DNA molecules by the separated DNA strands originating from two related organisms is a function of their base sequence divergence. These reactions may be studied by the CsCl density gradient method if one of the DNAs is labeled with a heavy isotope such as N^{15}, C^{13} or H^{2}. Other procedures, such as the DNA agar method, (McCarthy and Bolton; Hoyer and Roberts) or Denhardt's adaptation of the membrane filter method of Gillespie and Spiegelman demand only radio-labeled DNA and are consequently much more convenient. Recent developments of this methodology allow the quantitative estimation of the extent of nucleotide sequence divergence (McCarthy 1967).

2. *The DNA content of various genomes*

The evolution of the more complex from the simpler forms of life has been accompanied by a large increase in the total amount of DNA per cell nucleus. This is consistent with increase in the total information content necessary for more developmental, structural and behavioral complexity. A selection of some of the available data for a variety of organisms is given in Table 1. The total complement of DNA increases by a factor of about 10^3 from bacteria to mammals. Viruses contain even smaller amounts of DNA. In general, the amount of DNA per haploid cell nucleus increases systematically with the complexity of the organism although several exceptions exist. The *Urodela* contain much larger amounts of DNA per cell nucleus than do the other amphibia, the subclass *Anura*. The values for all amphibians are higher than those for mammals. Other exceptions to this general rule exist in the plant kingdom, some of which may be explained by polyploidization (Stebbins). Within a closely related group of organisms, such as placental mammals, reptiles or birds, the amounts of DNA per cell nucleus differ by no more than 10% (Atkin et al.).

Several possible mechanisms may be proposed to account for this steady increase in the amount of DNA per genome (McCarthy 1965). The simplest is 'end addition' of nucleotides to an intact DNA molecule (Kornberg). It is possible for a DNA molecule to increase in length during replication through this end addition. This process would create random sequences upon which selection might act. Another possibility involves the acquisition of genetic material from another organism. This process occurs in bacteria through the transfer of episomes and by viral transduction. It is conceivable that viruses may play a part in genetic transfer in eucaryotic organisms. However, gene duplication appears to be the most important process at the present time. Many examples of the operation of this process have been suggested from work on amino