THE MECHANISM OF PROTEIN SYNTHESIS AND ITS REGULATION

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Editors' 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, especially 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 theses 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 somewhat 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

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CHAPTER 1

Introduction

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Biosynthetic processes that lead to the formation of nucleic acids and proteins are complicated because of their requirements for preexisting information. Besides energy and matter, a biological template is necessary to direct the arrangement of different monomers in a specific linear sequence prior to the formation of covalent bonds. In the case of nucleic acid synthesis, where biological template and product both are polynucleotides, the intricacies are small compared to those encountered with the translation of messenger RNA into polypeptide. The protein synthesizing machinery is believed to comprise some 200 distinct macromolecules and although their interplay in the formation of peptide bonds under the direction of the genetic message has become clear in rough outline, accurate knowledge of the mechanistic details of their interaction is still lacking.

In particular this is true for the constituents of the ribosome that enable this particle, provided that sufficient free energy is available, to perform a great number of actions such as movement along the messenger, translocation of peptidyl-tRNA, accommodation of a variety of macromolecular components like initiation, elongation and termination factors, and dissociation into subunits. Thanks to increased efforts during the last few years, we are rapidly gaining information concerning the chemical and physical properties of these ribosomal components, their structure, and even their topography inside the particle. The assembly of the various constituents into a biologically active particle has been achieved. Nevertheless our insight into the functional aspects of the ribosomal organisation is still rudimentary. It is not surprising, therefore, that the ribosome stands in the limelight of current interest.

Notwithstanding the fact that our knowledge of the protein synthesizing system is still superficial, the accumulation of detailed information is enormous and justifies the appearance of a book like the present one. It is true that a number of excellent reviews has appeared recently but the abundance of data now collected makes it attractive to publish a multi-author volume in which various scientists actively engaged in the field can express personal views in relation to the status quo and to possible future research.

Since the development of cell-free systems, capable of amino acid incorporation in vitro, investigations of polypeptide synthesis have gone through a number of stages. The various components essential for the biosynthetic system, like activating enzymes, tRNA and ribosomes were recognized and their function and the energy requirements were described in some detail in the fifties. These investigations received a very strong impetus when it became possible to program cell-free bacterial systems with synthetic and natural messengers. Problems related to coding could then be approached by direct in vitro experiments and the polypeptide products synthesized in a cell-free system under the direction of a natural (viral) messenger proved to be identical to authentic viral protein. The deciphering of the genetic code became a fact in the sixties and evidence for the universality of the code throughout nature was presented soon thereafter. The enzymology of the biosynthetic process was also studied in more detail and the primary structure of some 28 different species of tRNA from various origins was elucidated during the last decade.

In the near future structural studies will continue to provide indispensable information about the nature of the macromolecules involved in protein biosynthesis and their mode of action. All sequences of the tRNA species elucidated so far can be arranged in the well-known clover leaf model by base paring. When the problem of the three-dimensional structure of these small RNAs is solved new light may be shed on some of the most intriguing questions of molecular biology, those related to protein-nucleic acid interaction.

Other examples of the fact that structural investigations are beginning to bear fruit are the sequencing of the 16S RNA derived from *E. coli* ribosomes, which is now well underway, and the determination of the entire nucleotide sequence of the coat cistron of the bacteriophage MS_2-RNA completed in 1971. A specific recognition between rRNA and proteins takes place during the assembly of the ribosomal

particles, and characterization of the structural details of the rRNAs is essential for delineating protein-RNA recognition sites of the rRNA chains. The elucidation of the nucleotide sequence of the MS_2 coat cistron not only enables us to make certain proposals concerning the secondary structure of this large RNA segment, but it also suggests explanations for a number of biological phenomena such as polarity and the regulation of phage RNA translation. As the amino acid sequence of the viral coat protein has been known for some time, this is the first instance that the primary structures of both the template and the biosynthetic product of a reasonably sized naturally occurring macromolecule can be directly correlated. It will not be long before the complete primary structure of one or more of the phage RNAs is known. This may also answer interesting questions concerning RNA-protein interactions.

Structural investigations of the various proteins implicated in polypeptide formation will give the necessary complement to the studies of RNA structure. In particular, aminoacyl-tRNA synthetases are attractive study objects as they are available in sufficient quantities in the cell, their purification has been worked out in a great number of cases and the primary structure of many isologous tRNAs is known.

The mechanism of polypeptide synthesis and its regulation are intimately related. Although there are various influences from outside or from other parts of the cell that may effect this process in situ, recent mechanistic investigations have revealed a wealth of subtle and elegant control mechanisms which are the direct consequence of the admirable organization of the biosynthetic machinery per se. It is the purpose of the present book to deal with the latter aspects of translational control in particular.

In principle regulatory devices for polypeptide synthesis are numerous because most steps of the process have the potential of regulation. This is explicitly clear in the case of chain initiation, where initiation factors have been shown to be capable of messenger and cistron selection, but control mechanisms have also been connected with other phases of protein biosynthesis like elongation and with the ribosome cycle. Furthermore control functions have been ascribed to the end products of the biosynthetic process. For example viral coat protein and phage RNA synthetase act as repressors of the synthesis of non-coat proteins. A very striking regulatory mechanism exerted by the secondary and the tertiary structure of the phage messenger itself has

been mentioned above. It remains for future investigations to study the universality of these controlling factors. It is not unreasonable to assume that these factors are numerous and that each polycistronic messenger has its own, possibly unique, translational control mechanism.

So far no basic differences have been detected between protein synthesis in prokaryotic and eukaryotic cells. Evidently the higher organisation of the latter implies that polypeptide formation can occur at different places and in different organelles. A further consequence is that in the eukaryotic cell we are confronted with different classes of ribosomes and with soluble enzymes that may not be compatible with each class of ribosomes. Nevertheless the differences do not seem essential. This does not mean that the eukaryotic system is less instructive. On the contrary, the almost boundless variability of the higher organisms provides us with very useful systems, which sometimes are equipped with a rather homogeneous population of messenger RNA molecules. Studies of protein biosynthesis in eukaryotic cell-free systems have entered a new era in the last few years with the availability of natural messengers in pure form, both from cellular and viral origin. This will undoubtedly contribute significantly to our understanding of protein formation in these systems. The occurrence of mammalian messengers in association with protein, although far from understood, presumably represents a new aspect of the messenger function. It is the investigation of the animal cell which has revealed the existence of these non-ribosomal ribonucleoprotein particles. Post-translational cleavage of giant polypeptides to generate the individual biosynthetic proteins, as observed in mammalian cells infected with a virus, is a feature which has not yet been detected in the prokaryotic cell. It is for these reasons that ample attention is given in this book to the biosynthetic activities of the eukaryotic cell and the last four chapters are entirely devoted to problems of this type.

For various reasons, completeness cannot be reached, even in a volume of this size. Manuscripts were delivered to the editor in the period of August to November 1971. Since then numerous articles have been published in the literature, which are highly relevant to the subjects covered in this book. Nevertheless, it is hoped that this volume will be of value both to those research workers who are already familiar with aspects of protein biosynthesis through their own work, but desire a general treatment of the problems, and to investigators and postgraduate students not yet actively engaged in the field.

CHAPTER 2

Aminoacyl-tRNA synthetases

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2.1. Introduction

Peptide bond formation during protein synthesis requires the activation of each amino acid and its precise positioning on a specific ribosomal site: positioning which is directed by ribosome-bound messenger RNA. ATP and two families of macromolecules are involved in these two reactions: aminoacyl-tRNA synthetases and transfer RNAs. For each of the twenty amino acids present in proteins there is, in prokaryotic organisms, only one specific synthetase and at least one tRNA. Each enzyme catalyzes two successive reactions which lead to the formation of an ester bond between the 2'-3' hydroxyl group of the terminal adenosine of tRNA and the α carboxylic group of the amino acid.

$$\text{Amino acid} + \text{ATP} + \text{Enzyme} \rightleftharpoons \text{Aminoacyladenylate-Enzyme} + \text{PPi} \quad (1)$$

$$\text{Aminoacyladenylate-Enzyme} + \text{tRNA} \rightleftharpoons \text{aminoacyl-tRNA} + \text{AMP} + \text{Enzyme} \quad (2)$$

The first reaction which requires the presence of ATP leads to the formation of an enzyme bound aminoacyladenylate and of free pyrophosphate. This reaction during which an anhydride bond between the phosphate of AMP and the carboxylic group of the amino acid is formed, is often called activation reaction. During the second reaction the amino acid is transferred from the AMP to the transfer RNA and the products are aminoacyl-tRNA and AMP. The potential energy of the ester bond formed is high and similar to that of the anhydride bond