Molecular Biology in Physiology

Editor

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Preface

The aim of this book is to introduce recent developments in molecular biology to physiologists working at cellular and organ-system levels, so that these new approaches can be applied to physiological research.

The first two chapters cover basic concepts and commonly used terminology in molecular biology, thus providing individuals who have little prior knowledge with the background information needed to follow the remainder of this book. This volume illustrates how molecular biology is applied to elucidate physiological functions in example systems. The topics discussed include the molecular biology of cell membrane transport proteins (the anion transport protein band 3 and proton ATPase), humoral agents regulating body fluids and cardiovascular functions (renin and atrial naturietic factor), autonomic receptors and ionic channels (adrenergic receptors, acetylcholine receptors, and sodium channel), and long-term memory.

These areas should interest a broad spectrum of scientists working on various systems including membrane transport, body fluids and electrolytes, kidney, circulation, endocrine system, nerve-muscle, autonomic nervous system, and central nervous system. There is diversity as well as interrelationship among the topics. It is my belief that this book will contribute to the opening of new horizons for physiological research by making use of the rapid advances in molecular biology as we enter the second hundred years of APS history.

Shu Chien, M.D., Ph.D.
March 1988
Editor

Acknowledgments

In celebration of the Centennial of the American Physiological Society (APS), a Symposium on Molecular Biology in Physiology was organized in Washington, D.C. during the 1987 Annual Meeting of the Federation of American Societies for Experimental Biology.

I wish to express my sincere thanks to the Cell and General Physiology Section (Chairman: Dr. Louis Reuss; Program Committee Chairman: Dr. Paul DeWeer) and the Program Executive Committee (Chairman: Dr. Carl V. Gisolfi) of APS for sponsoring this Symposium. I would like to thank Dr. Franklyn Knox, the APS President during the Centennial year, members of APS Council, Dr. Alfred P. Fishman, Chairman of the Centennial Committee, and Dr. Martin Frank, the Executive Director, for their encouragement and support. I wish to thank the speakers (authors) for the excellent contributions to this Symposium-monograph. I am particularly grateful to Dr. J. Jay Gargus for his guidance in organizing this symposium and co-chairing the Symposium. Special thanks are expressed to Ms. Micheline Faublas for her excellent editorial assistance.

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Contents

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AN INTRODUCTION TO MOLECULAR BIOLOGY FOR PHYSIOLOGISTS

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INTRODUCTION

The dramatic advances in molecular biology in recent years have opened new ways to probe the structural and functional bases of physiological events at the molecular level (1,2). The application of modern molecular biological techniques to biomedical research has led to the development of new methods for the study of cellular properties and interactions, the elucidation of the mechanisms by which hormonal factors affect cellular functions, the production of new biologically active compounds, and the design of innovative diagnostic procedures. These scientific advances resulting from the application of molecular biological approaches have been particularly notable in research on several areas of cellular physiology, e.g. nerve, muscle, and endocrine system.

In physiological research we study the structure and function of the body at various levels, ranging from subcellular components to cells, tissues, organs, systems and the whole body. A major determinant of the structural and functional characteristics of biological organisms is the protein composition of their components, which in turn is controlled by molecular mchanisms. In the final analysis all physiological phenomena have a molecular basis. Therefore, the utilization of molecular biological approaches to study physiological problems should contribute to the understanding of the mechanisms of normal and pathophysiological processes at the molecular level. Since most physiologists were trained before the advent of modern molecular biology, many may not be familiar with these new developments.

The aim of this Chapter is to present a very elementary introduction to molecular biology, which will serve as a starting point for readers unfamiliar with the field to facilitate their reading of this book. Some of the materials presented in this Chapter are amplified in greater detail in the next Chapter on Tools for the Molecular Analysis of Gene Structure and Function (3). These two chapters together provide a

background framework of the principles and techniques which are applied in the research reported in the remainder of this book, additional information on such background material is available in several textbooks (e.g. 4,5).

The specific areas covered in this monograph are the molecular biology of band 3 membrane protein (6), proton ATPase (7), atrial natriuretic factor and renin (8), adrenergic receptor (9), ionic channels (10) and long-term and short term memory (11). These topics are selected because of their relevance to physiological functions and the recent developments in these areas that exemplify the application of molecular biology to physiological systems. The information contained in these chapters is probably of interest to physiologists working on transport, body fluids and electrolytes, acid base balance, kidney, endocrine system, cardiovascular system, autonomic nervous system, nerve–muscle, and higher functions of the central nervous system.

A BRIEF SURVEY OF MOLECULAR BIOLOGY

DNA and RNA

The genetic information is stored in deoxyribonucleic acids (DNA). The genetic code is first transcribed to ribonucleic acids (RNA) and then translated to amino acids during protein synthesis. In this manner, the types of proteins synthesized by the cell are genetically controlled. The transcription from DNA to RNA and the translation from RNA to proteins constitute the central dogma in molecular biology (Fig. 1). Quantitative and qualitative changes in protein synthesis during physiological and pathophysiological conditions are effected by alterations in transcriptional, translational or post-translational events.

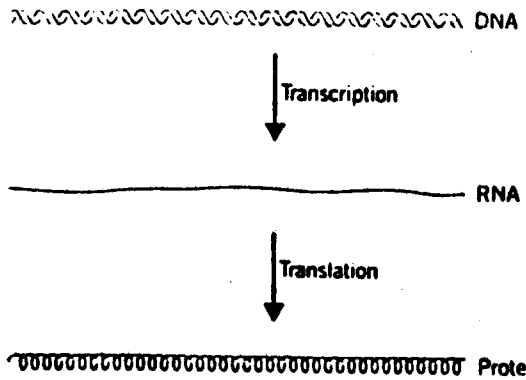

Fig. 1 The central dogma in molecular biology, showing the transcription of DNA to RNA and the translation of RNA to protein.

Deoxyribonucleic acids are linear polymers of nucleotides that are composed of four types of bases (Fig. 2), i.e. two types of purine: adenine (A) and guanine (G), and two types of pyrimidine: cytosine (C) and thymine (T). These bases are linked together by a sugar–phosphate backbone in which the phosphate and the deoxyribose sugar form 3' and 5' phosphodiester bonds, giving rise to a 5' end and a 3' end of the DNA molecule (Fig. 3).

In the cell DNA molecules are packed in the chromosomes, and they are referred to as chromosomal DNA or genomic DNA. In cells with a well defined nucleus (eukaryotes), DNA molecules are paired to form double strands, with the bases on opposite strands held in precise register by hydrogen bonding, i.e. A–T and G–C form complementary base–pairs (Fig. 4). In cells without a defined nucleus (Prokaryotes), DNA are often single stranded and they may be circular. The enzyme DNA polymerase catalyzes the replication of DNA; the replication process involves the use of one of the strands as a template to synthesize a complementary strand by adding nucleotides according to base pairing. The nucleotide sequence in DNA and the specific base pairing provide the mechanisms for the storage and replication of genetic information.

Ribonucleic acids are also linear polymers of nucleotides that contain four types of bases; three of these (A, C, G) are the same as those in DNA, but the fourth is uracil (U) instead of thymine. RNA also differs from DNA in that the sugar in the RNA backbone is ribose rather than deoxyribose (Fig. 2). RNA is generally single stranded.

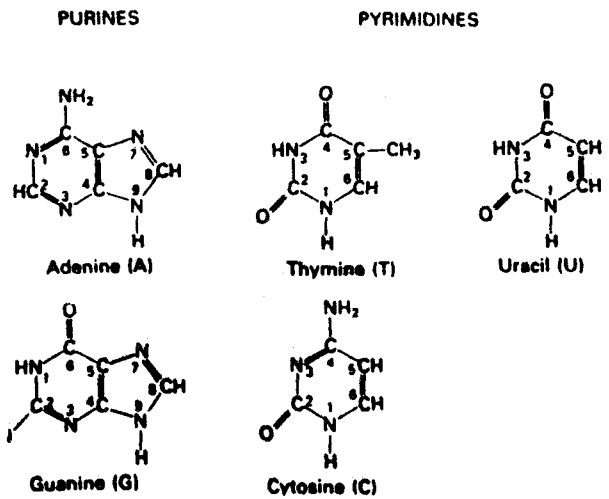

Fig. 2 The purine and pyrimidine bases making up DNA and RNA.

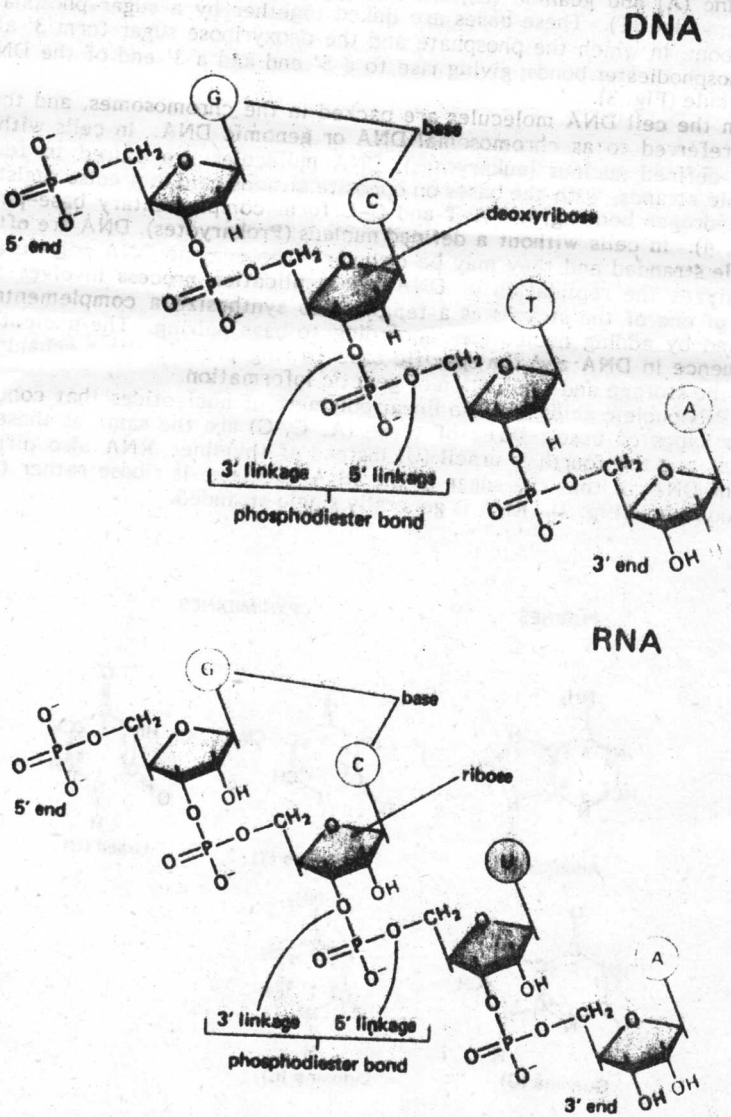

Fig. 3 The sugar–phosphate backbone and attached bases for A: DNA (top) and B: RNA (bottom).

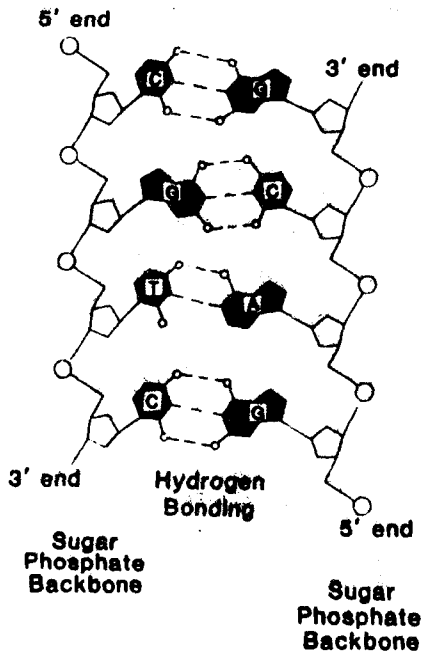

Fig. 4 Base pairing of two DNA strands. The pentagon represents the deoxyriboses in the sugar–phosphate backbone.

Transcription from DNA to RNA

In most eukaryotes, DNA is packaged with the positively charged histone proteins to form chromatin (Fig. 5) and is less accessible to activation and transcription than that in prokaryotes. The transcription of DNA is catalyzed by RNA polymerases (Fig. 6), which can search for a site on the promoter region of the DNA molecule for the initiation of transcription. Eukaryotes have three types of RNA polymerases (I, II and III). In the transcription process, a portion of the DNA double strand is split into two single strands; each strand can serve as a template for transcription. The nucleotides complementary to the DNA template strand are added sequentially to the RNA chain which grows in a 5' to 3' direction (Fig. 7). The elongation of the RNA chain is followed by its separation from the DNA template. The transcription process is terminated when the RNA polymerase encounters the terminator portion of the DNA molecule (Fig. 8).

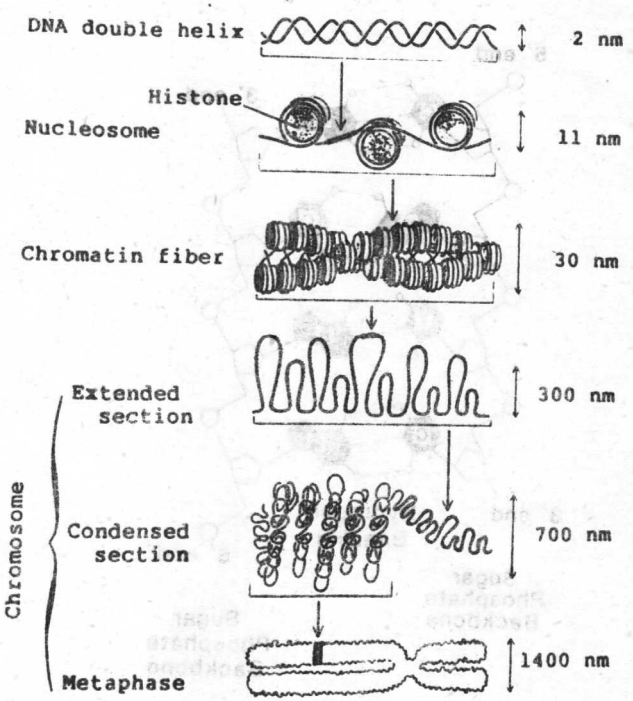

Fig. 5 Schematic diagram to show the packaging of DNA with histone to form nucleosome, chromatin fiber, and chromosome. Drawings from bottom to top show serial enlargements.

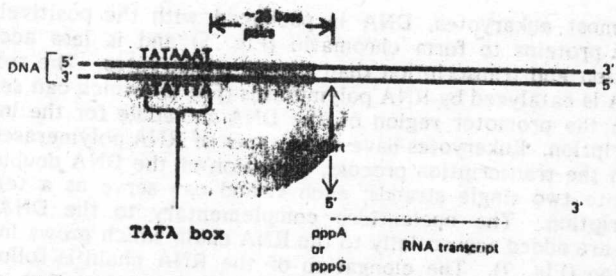

Fig. 6 A polymerase II (round figure with an opening on the right) bound to the TATA box of the promoter region of a double stranded DNA to start the formation of an RNA transcript.

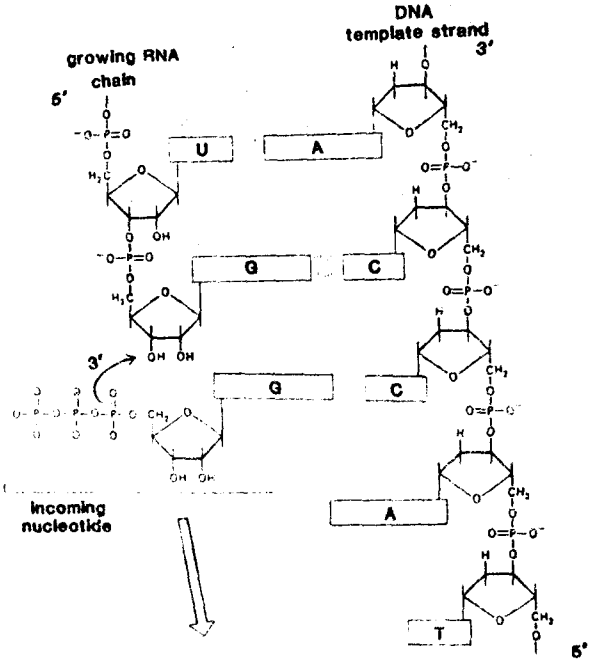

Fig. 7 The transcription process showing the growing of an RNA chain from the 5' to the 3' direcion by adding an incoming nucleotide which forms base pairing with the DNA template strand.

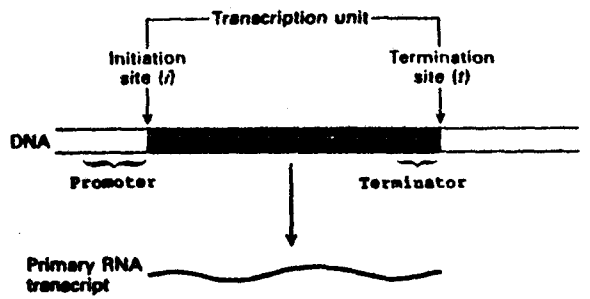

Fig. 8 The transcription unit in DNA. It begins with the initiation site, which is preceded by the promoter region, and ends with the termination site, which is preceded by the terminator region.

In the prokaryotes, which have no nucleus, the RNA transcript does not undergo any modification, and transcription is directly coupled with translation. In contract, in eukaryotes transcription proceeds in the nucleus, and the primary RNA transcript molecules are modified before their exit through the nuclear membrane into the cytoplasm as messenger RNA (mRNA) (Fig. 9). These modifications include the capping with methyl guanosine at the 5' end (m^7Gppp in Fig. 9) and usually the addition of a poly(A) tail (containing a large number of adenine nucleotides) at the 3' end. The primary RNA transcript is composed of exons interspersed with introns. Exons are the regions preserved in the mature mRNA which exit into the cytoplasm, introns are the regions spliced out and removed from the mRNA during maturation (Fig. 10). The exons contain not only the protein-encoding regions which are translated, but also the 5' and 3' control regions which are not to be translated into peptides. The functions of introns are not well understood. The structural organization and the RNA synthesis in prokaryotes and eukaryotes are compared in Table I.

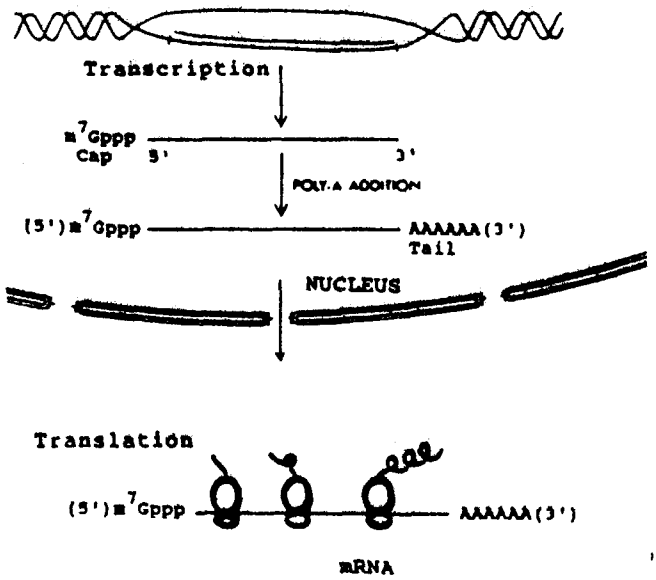

Fig. 9 Transcription in eukaryotes showing that RNA is modified in the nucleus before its exit into the cytoplasm.

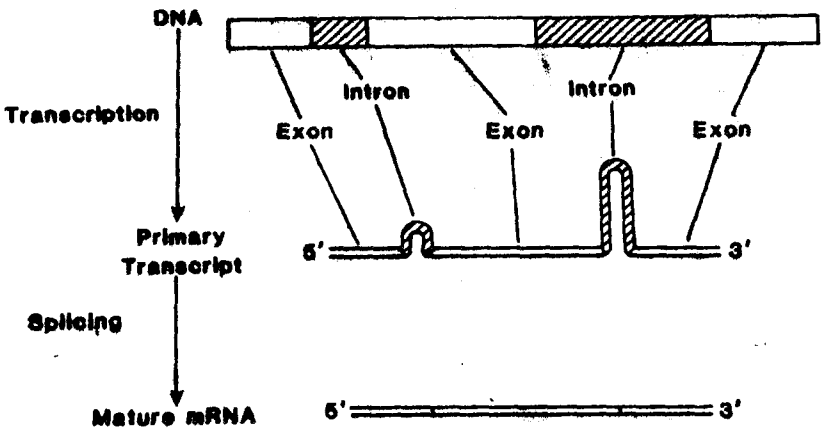

Fig. 10 Schematic drawing to show the removal of introns and the retention of exons when the primary RNA transcript is spliced to form mature mRNA.

Table I. Comparison of Prokaryotes and Eukaryotes

	PROKARYOTES	EUKARYOTES
Cell Structure		
Nucleus	No defined nucleus	Contains a true nucleus
Membrane-bound organelles	Generally none	Mitochondria, etc.
Cytoskeleton	No	Actin filaments, centrioles
DNA Organization	Mostly single circular, naked DNA	Mostly double stranded, associated with histones
RNA Synthesis		
RNA Polymerase	Single	Multiple
Transcription & Translation	Coupled	Separated
Posttranscrip. Modification	Rare	Yes
Stability of mRNA	Short ($T_{1/2}$: sec–min)	More stable (usu. hours)