The Ribonucleic Acids

Second Edition

Edited by
P. R. Stewart and D. S. Letham

Springer-Verlag
New York · Heidelberg · Berlin

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With Contributions by

J. M. Adams · G. D. Clark-Walker · L. Dalgarno
D. S. Letham · H. Naora · G. M. Polya · R. Poulson
J. Shine · P. R. Stewart · R. E. H. Wettenhall
P. R. Whitfeld

With 55 Figures

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Library of Congress Cataloging in Publication Data
Main entry under title:
The Ribonucleic acids.
Includes bibliographies and index.
1. Ribonucleic acid. I. Stewart, P. R. II. Letham, D. S.
QP623.R5 1977 574.8'732 77-4899

9 8 7 6 5 4 3 2

Printed in the United States of America.

ISBN 0-387-90281-3 Springer-Verlag New York
ISBN 3-540-90281-3 Springer-Verlag Berlin Heidelberg

Preface

The central role of the ribonucleic acids (RNA) in mediating the expression of information encoded in DNA in living cells is now well established. Research in this area of biology continues at a remarkable rate, and new and significant information appears almost daily in a wide range of journals, published symposia and specialist reviews.

The diverse nature of this information makes it difficult for the newcomer to the field of RNA biochemistry to obtain a general view of established concepts, current activity, and new advances. Moreover, the reviews available are frequently concerned with insular aspects of these ubiquitous molecules, or in the case of textbooks, the subject is treated as part of a general outline of properties of nucleic acids and thus may be superficial.

The authors of the chapters in this collection attempt to provide a comprehensive, though not overly detailed, outline of the biological roles of RNA. They have written for students with basic training in biochemistry, but otherwise with a wide variety of biological interests—plant physiology, virology, organelle biochemistry, genetics, cell biology, differentiation and development. Viral RNA, which was dealt with as a separate chapter in the first edition, has been deleted from this edition because it is an unmanageably large single topic, and at the same time is addressed in a number of ways in many different places in the book.

The collection is not meant to be a completely comprehensive, up to the minute account of the latest findings in the biology and chemistry of RNA. It aims to provide a grounding in established facts and concepts in most aspects of the function of RNA species. Newer developments that seem to be important and are likely to result in a greater understanding of the role of RNA in living cells are also dealt with.

P. R. STEWART
D. S. LETHAM

Canberra, July 1977

Contributors

ADAMS, J. M., Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Melbourne, Australia

CLARK-WALKER, G. D., Department of Genetics, Research School of Biological Sciences, Institute of Advanced Studies, Australian National University, Canberra, Australia

DALGARNO, L., Department of Biochemistry, Faculty of Science, Australian National University, Canberra, Australia

LETHAM, D. S., Department of Developmental Biology, Research School of Biological Sciences, Institute of Advanced Studies, Australian National University, Canberra, Australia

NAORA, H., Molecular Biology Unit, Research School of Biological Sciences, Institute of Advanced Studies, Australian National University, Canberra, Australia

POLYA, G. M., Department of Biochemistry, La Trobe University, Bundoora, Victoria, Australia

POULSON, R., Department of Biochemistry, Faculty of Medicine, University of British Columbia. Vancouver, Canada

SHINE, J., Molecular Biology Unit, Research School of Biological Sciences, Institute of Advanced Studies, Australian National University, Canberra, Australia

STEWART, P. R., Department of Biochemistry, Faculty of Science, Australian National University, Canberra, Australia

WETTENHALL, R. E. H., Department of Biochemistry, La Trobe University, Bundoora, Victoria, Australia

WHITFELD, P. R., C.S.I.R.O. Division of Plant Industry, Canberra, Australia.

Principal Abbreviations

rDNA	DNA coding for ribosomal RNA
cRNA	complementary RNA
ChRNA	chromosomal RNA
dsRNA	double-stranded RNA
HnRNA	heterogeneous nuclear RNA
LnRNA	low-molecular-weight nuclear RNA
mRNA	messenger RNA
rRNA	ribosomal RNA
tRNA	transfer RNA
A*	adenine or adenosine
C*	cytosine or cytidine
G*	guanine or guanosine
T*	thymine or thymine riboside
U*	uracil or uridine
AMP, CMP, GMP, TMP, UMP	5′-phosphates of corresponding nucleosides
polyA	polyadenylic acid
polyU	polyuridylic acid
polyC	polycytidylic acid
Py	pyrimidine
Pu	purine

Minor and modified nucleosides found in RNA—a list of abbreviations is given on p. 137.

aa	aminoacyl
CAP	catabolite gene activator protein
CRP	cAMP receptor protein (c = cyclic)
CTA	cetyltrimethylammonium
DEP	diethyl pyrocarbonate
DMSO	dimethyl sulfoxide
IPTG	isopropylthiogalactoside
MAK	methylated serum-albumin-coated kieselguhr
mRNP	messenger ribonucleoprotein
SDS	sodium dodecylsulfate

* In current accepted international nomenclature these symbols designate nucleosides. In this book they have been applied to both the base and nucleoside but their meaning is clear from the context in which they are used.

Contents

CHAPTER 5

CHAPTER 6

CHAPTER 7

CHAPTER 8

CHAPTER 1

RNA in Retrospect

D. S. LETHAM and P. R. STEWART

The molecular basis of gene expression whereby organisms develop inherited characteristics and the associated regulatory mechanisms that control this development are of fundamental significance to almost every area of modern biology. Today it is accepted that gene expression can be equated with protein formation and function. Growth, differentiation, and reproduction all depend on chemical reactions mediated by enzymes, a special class of proteins. The polypeptide chains of proteins are polymers of 20 different amino acids, and all proteins have unique amino acid sequences; however, the living cell can reproduce a particular sequence precisely. Protein synthesis involves three types of ribonucleic acid (RNA): (a) messenger (mRNA), (b) ribosomal (rRNA), and (c) transfer (tRNA). Our present knowledge of the structure and function of RNA is derived from studies involving many disciplines, including genetics, biochemistry, organic chemistry, and physics. In this introductory chapter an attempt is made to outline the development of our knowledge of the basic structural features and the functions of RNA.

Yeast served as the principal source of RNA for the initial structural studies. Hydrolysis of RNA (termed "pentose nucleic acid" by early workers) with alkali yielded an approximately equimolecular mixture of four nucleotides termed "adenylic," "guanylic," "cytidylic," and "uridylic" acids. In each nucleotide, a heterocyclic base was joined to a pentose sugar monophosphate. Principally as a result of the endeavor of the organic chemist, P. A. LEVENE and his co-workers in New York over the years 1908–1936, the following conclusions were reached regarding ribonucleotide structure: (a) the heterocyclic bases are adenine, guanine, cytosine and uracil, (b) the pentose sugar in all nucleotides is D-ribose, (c) the sugar moiety has a furanose ring structure and is attached to position 9 of purine bases and to position 3 (position 1 in modern nomenclature) of pyrimidines, but the stereochemical configuration of the linkage remained unknown, and (d) the phosphate group is attached to either position 2′ or 3′ of the sugar moiety. For a lucid, detailed account of such early achievements the reader is referred to the review by TIPSON (1945). By ion-exchange chromatography, COHN (1950) showed that each nucleotide was a mixture of two isomers that were later identified as the 2′- and 3′- phosphates (BROWN et al., 1954a). These arose by cleavage of a 2′:3′-cyclic phosphate intermediate formed during alkaline hydrolysis. The configuration of the base-sugar linkage was not defined until DAVOLL et al. (1946) demonstrated that the dialdehyde produced by periodate oxidation of adenosine is identical to that obtained by similar treatment of 9-β-D-

glucopyranosyladenine, the configuration of which was established by unambiguous synthesis. By similar methods the β configuration of other nucleosides was established.

In the union of ribonucleotides to form polyribonucleotides, several types of linkage are theoretically possible. However, electrometric titration studies by LEVENE in 1926, and also by later workers, were consistent with the internucleotide bond being a phosphodiester linkage between ribose moieties. These studies also eliminated other likely possibilities, namely, pyrophosphate, phosphoamide, and ether linkages. In 1935 LEVENE and TIPSON proposed a 2′:3′ phosphodiester linkage, but the actual location of the bond was not established unequivocally until 1954. Degradation of RNA with snake-venom phosphodiesterase yielded the 5′-phosphates of all four nucleosides (COHN and VOLKIN, 1953). A spleen nuclease, shown to hydrolyze specifically phosphodiester groups located at the 3′ position of ribonucleosides, was found to degrade RNA to the nucleoside 3′-phosphates without formation of a cyclic phosphate intermediate (BROWN et al., 1954b; HEPPEL et al., 1953). Hence enzymic degradation established that the internucleotide bond in RNA is a phosphodiester linkage joining the 3′ position of one nucleotide with the 5′ position of the adjacent nucleotide. No unequivocal evidence for branching in RNA has been presented; RNA molecules appear to be entirely linear polymers of mononucleotides.

RNA molecules can possess secondary and tertiary structures. The macromolecular properties of polynucleotides are greatly influenced by the negatively charged electrostatic field arising from the phosphodiester groups and by the ability of bases to interact to form helical structures. These two forces tend to oppose each other and consequently conformation depends on ionic strength, temperature, and pH (COX, 1968). Three conformations of single-stranded polyribonucleotides have been characterized and are as follows (COX, 1068): (a) an amorphous form in which the bases have no preferred orientation with respect to one another, (b) a "stacked" conformation in which the flat purine and pyrimidine rings tend to pile one upon another to form a single helix, and (c) a "hairpin-loop" conformation stabilized by hydrogen bonding between complementary bases to give a double-helical structure below the loop. In such a conformation, sequences that cannot find appropriate bases for pairing tend to be "looped out."

Double helices also form between RNA strands. The resulting structure resembles that of DNA in that it consists of two antiparallel polynucleotide chains stabilized by WATSON–CRICK-type base-pairing. Natural double-stranded RNA occurs in certain viruses. Without doubt, conformation greatly affects the functional properties of RNA.

About 1950, as the elucidation of the basic structure of RNA neared completion, biochemists began to study actively the mechanism of protein synthesis. These studies eventually established the role played by RNA in *de novo* synthesis of proteins, the principal function of RNA. RNA may also be involved in memory and learning phenomena (GLASSMAN, 1969) but this topic is beyond the scope of the present volume. Prior to 1950 by use of histochemical techniques, BRACHET and CASPERSSON independently established that cells active in protein synthesis possessed a high RNA content. The suggestion was made that there

was an intimate connection between protein synthesis and RNA. It was not until cell-free protein-synthesizing systems were developed, however, that real progress was made in establishing the role of RNA in the synthesis of polypeptide chains. In 1954, ZAMECNIK and associates in Boston had developed such a system from rat liver. Essential components of this cell-free system were amino acids, ATP, GTP, the ribonucleoprotein fraction of the microsomes (i.e., the ribosomes), and factors including enzymes in the 105,000-g supernatant. By use of this system it was soon established that the ribosome was the probable site of synthesis of peptide chains (KELLER et al., 1954; LITTLEFIELD et al., 1955) and that formation of "activated" amino acids, aminoacyl adenylates, was the first step in the synthesis of protein from amino acids (HOAGLAND et al., 1956). In 1957 and 1958, again by use of the *in vitro* protein-synthesizing system, the ZAMECNIK group made a dramatic advance that established the second step in the biosynthetic pathway. The activated amino acids were shown to become covalently bound to a type of RNA, termed "soluble" RNA (sRNA), in the 105,000-g supernatant. Next it was demonstrated that aminoacyl-sRNA substituted for free amino acid in the cell-free system and that the transfer of amino acid from sRNA (later termed "transfer" RNA) to the peptide chain on the ribosome was dependent on GTP (HOAGLAND et al., 1958). Evidence soon followed that tRNA was a complex mixture of polynucleotides and that each tRNA species was specific for a particular amino acid.

Although elucidation of the function of sRNA was a great achievement, a considerable gap still existed in our knowledge of protein synthesis in that the link between the genetic information in DNA and the final amino acid sequence in a protein was not established. Prior to the elucidation of the function of tRNA, it was thought by some that DNA might act directly as a template for assembly of amino acids into protein, a concept introduced by GAMOW (1954) after consideration of the WATSON–CRICK double-helical structure for DNA. Each amino acid was considered to make a direct steric fit with a sequence of bases. Later it was proposed that amino acids were held directly on RNA templates and then linked enzymically. The significance of these early concepts is elegantly discussed by WOESE (1967). Unlike many of his contemporaries, CRICK (1955) rejected these concepts and proposed that "each amino acid would combine chemically, at a special enzyme, with a small molecule which, having a specific hydrogen-bonding surface, would combine specifically with the nucleic acid template."

This became known as the "adaptor hypothesis." The molecules, or adaptors, to which the amino acids became attached were considered to be mediators between the encoded information of the nucleic acid and the extremely variable chemical structures of amino acids. The discovery of tRNA and its specificity completely vindicated the adaptor hypothesis; tRNA possessed all the properties of CRICK's adaptor. Complementarity between polynucleotides and amino acids then became a concept of purely historical significance.

By 1958 the ribosome was established as the site of cytoplasmic protein synthesis, and many assumed that ribosomal RNA was a carrier of genetic information transcribed from DNA. It was also considered that rRNA was the template that combined, not with amino acids, but, by hydrogen bonding, with

specific groups of bases on the tRNA moiety of aminoacyl-tRNA molecules. By 1961, however, this hypothesis was generally discarded. It was replaced by a new concept supported by the elegant experiments of BRENNER et al. (1961). These workers found that after infection of the bacterium *Escherichia coli* with T2-bacteriophage no new ribosomes were formed for synthesis of viral protein, but in fact a new RNA species with a rapid turnover and a base composition complementary to that of phage DNA was produced and attached to pre-existing *E. coli* ribosomes. Such RNA with template function, termed "messenger" RNA, was postulated by JACOB and MONOD (1961) in their theory of protein synthesis. Ribonucleic acid species that labeled very rapidly, attached to ribosomes and possessed a DNA-like base composition were soon detected in normal uninfected bacterial cells (GROS et al., 1961).

Further support for the mRNA concept was provided by the classic *in vitro* experiments of NIRENBERG and MATTHAEI (1961) who, using a cell-free protein-synthesizing system from *E. coli,* showed that addition of synthetic polyuridylic acid resulted in the formation of polyphenylalanine. This observation, together with similar experiments using other synthetic polymers as templates, convincingly established the mRNA concept and also provided important information regarding the now familiar genetic code. In recent years the existence of mRNA has been demonstrated unequivocally and in this connection the work of LOCKARD and LINGREL (1969) merits special mention. Treatment of mouse reticulocyte polysomes with detergent yielded an RNA species (9S) that sedimented between tRNA and rRNA in sucrose gradients. Although several properties of this RNA were consistent with it being the mRNA for globin, demonstration of ability to direct the synthesis of the globin chains was required to establish this unequivocally. LOCKARD and LINGREL showed that the 9S RNA from mouse polysomes did direct the formation of mouse globin β-chains in a rabbit reticulocyte cell-free protein-synthesizing system. It has also been demonstrated that an RNA fraction from rabbit reticulocytes can direct synthesis of globin in a cell-free system from *E. coli* (LAYCOCK and HUNT, 1969).

The concept that mRNA carries information in the form of nucleotide sequences from the gene to the protein-synthesizing mechanism is now clearly established. On the ribosome the codons (sequences of three adjacent nucleotides that code for an amino acid) of mRNA pair sequentially with the anticodons of aminoacyl-tRNA molecules, and, since each tRNA is specific for a particular amino acid, amino acid sequence is dictated. In eukaryotic cells the mRNA synthesized on the DNA template passes from the nucleus to the cytoplasm for translation into protein by cytoplasmic ribosomes, and transcription and translation are essentially independent processes. However, in bacteria mRNA appears to be translated by a cluster of ribosomes that follow closely behind the RNA polymerase involved in transcription. Thus in bacteria both transcription and translation of an mRNA chain occur simultaneously, and the two processes may be coupled (MORSE et al., 1969; IMAMOTO and KANO, 1971).

The structural features of mRNA molecules are now becoming clear. Whereas eukaryotic mRNAs are monocistronic, the typical prokaryotic mRNAs are

polycistronic. The polycistronic mRNAs for which there is now considerable structural information are the RNAs from certain related bacteriophages. An outstanding achievement was the determination of the entire sequence of the 3569 nucleotides in MS2 bacteriophage RNA (FIERS et al., 1976). Information regarding the structure of eukaryotic mRNAs is limited. However, it is noteworthy that such mRNAs usually possess a variable number (50–200) of adenylic acid residues (a polyA sequence) at the 3′-terminus and also a unique 5′-terminal structure in which 7-methylguanosine is linked through its 5′-carbon by a triphosphate group to the 5′-carbon of a 2′-*O*-methyl nucleoside.

During the past five years, knowledge of tRNA biosynthesis, structure, and function has advanced greatly and merits special comment. Our understanding of tRNA exceeds that of any other RNA species. The development of cell-free systems for tRNA biosynthesis, the first being devised by ZUBAY et al. (1971), contributed a great deal to our understanding of tRNA transcription and the processing of tRNA precursors. These may contain several potential tRNA molecules linked by oligonucleotide segments and several tRNA precursors have been completely sequenced (ALTMAN, 1975). The first functional tRNA molecule was sequenced by HOLLEY et al. (1965); today about 80 tRNA sequences are known, and the list includes tRNAs for all 20 amino acids except asparagine. The determination in 1975 of the three-dimensional (tertiary) structure of crystalline yeast phenylalanine tRNA by X-ray-diffraction analysis at 2.5-Å resolution was a monumental achievement of great significance (LADNER et al., 1975; QUIGLEY et al., 1975). A diversity of techniques including laser Raman and nuclear magnetic resonance (NMR) spectroscopy indicate that basically the same structure exists in solution (CHEN et al., 1975; ROBILLARD et al., 1976). The four common nucleosides in tRNA undergo sequence specific enzymic modification to yield a diversity of minor nucleosides, 43 of which have been identified unequivocally. This extensive nucleoside modification distinguishes tRNA from all other RNA species. Of special interest are certain N^6-substituted adenosines, such as N^6-(3-methylbut-2-enyl)adenosine, which are located adjacent to the anticodon and possess plant-hormone (cytokinin) activity, although they occur in tRNA from animals, plants, fungi, and bacteria. These and closely related compounds occur free in plants as phytohormones, of which zeatin was the first to be isolated in a crystalline form. Zeatin has been assigned the structure 6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)purine (LETHAM et al. 1964, 1967).

In recent years it has become clear that tRNA has multiple functions and is not merely an adaptor molecule in protein synthesis. In addition to certain biosynthetic roles that do not involve ribosomes but result in transfer of amino acids (SOFFER, 1974), tRNAs appear also to have certain regulatory functions. The best understood of these is the repression by aminoacyl–tRNAs of enzymes involved in amino acid metabolism. The first significant work in this area was the observation by SCHLESINGER and MAGASANIK (1964), which indicated that the concentration of charged histidine tRNA, not free histidine, controlled repression of histidine biosynthetic enzymes in *Salmonella typhimurium*. The function

of some polynucleotide polymerase systems, including reverse transcriptase, appears to depend on tRNAs as cofactors (PANET et al., 1975). The regulatory significance of tRNA is now beginning to unfold.

Similarly, it now seems that rRNA is not simply an inert structural component of the active ribosome. The recent identification of sequences in the 3′-termini of the rRNA of the small ribosomal subunit in prokaryotes that are partly complementary to initiation sequences within ribosomal binding sites in mRNA (SHINE and DALGARNO, 1975a,b) indicates that interaction between rRNA and mRNA may be important in determining the relative translation efficiencies of different mRNAs, including individual cistrons of polycistronic mRNA.

In the nucleus there are some species of low-molecular-weight RNA (4–8S) that are unique to this organelle and have no obvious relationship to those RNA species that proceed from the nucleus to the cytoplasm (BUSCH et al., 1971). The role of this nuclear RNA has been the subject of speculation in relation to the regulation of gene expression. In the nucleus, DNA is also transcribed to yield precursors of all RNA species required for the synthesis of proteins on cytoplasmic ribosomes. This precursor RNA transcribed from chromosomal DNA is invariably larger and chemically unmodified compared with the functional species of RNA that associate, together with proteins, to form the active ribosomal or polyribosomal protein synthetic complex. The features of the cleaving, trimming, and modifying reactions that result in the ultimate formation of functional species of rRNA, tRNA, and mRNA are now beginning to be understood. These reactions will undoubtedly be of considerable significance in a fuller understanding of the regulation of gene expression.

The other major organelles of the eukaryotic cell, the mitochondria and chloroplasts, synthesize, process, and employ RNA in a way that represents a microcosm of corresponding events occurring elsewhere in the cell. The identification of these partially autonomous genetic systems in mitochondria and chloroplasts with all the essential features of the now classical flow of information (DNA → RNA → protein) has been an important milestone in studies of RNA biosynthesis and function in the past 15 years. Although early studies pointed to striking similarities between gene expression in eukaryotic organelles and that occurring in the simple prokaryotes, there is now a greater tendency to view mitochondria and chloroplasts, irrespective of their phylogenetic origins, as separate and distinct systems in their own right. Insight into the interdependence and interactions between organelle and nuclear genetic systems may provide us with new models in the search for an understanding of the general integrative features that underlie cellular growth and differentiation.

Transfer of information from DNA to RNA to protein is an established basic concept, a keystone in the structure of molecular biology. However, the important work of TEMIN and MIZUTANI (1970) has established that flow of information from RNA to DNA also occurs. An RNA-dependent DNA polymerase is present in the virions of certain RNA tumor viruses. This polymerase, termed "reverse transcriptase," has been used to synthesize *in vitro* DNA copies of mammalian globin mRNA (VERMA et al., 1972; KACIAN et al., 1972). Our present knowledge of transfer of information between DNA, RNA, and protein

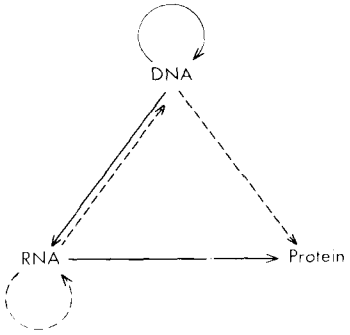

Fig. 1. Flow of information between macromolecules. Solid arrows indicate general transfers; dotted arrows indicate special transfers. (Arrows do not represent flow of matter, but the direction of flow of encoded information)

can be represented diagrammatically (Fig. 1) as suggested by CRICK (1970). General transfers probably occurring in all cells are: (a) DNA → DNA, (b) DNA → RNA, and (c) RNA → protein. Mammalian reticulocytes, because they are enucleate, probably lack the first two transfers and may constitute an exception. Special transfers that do not generally occur are: (a) RNA → RNA, (b) RNA → DNA, and (c) DNA → protein. The first two transfers are known to occur in certain virus-infected cells; the third occurs in a special cell-free system containing single-stranded DNA and a streptomycinoid antibiotic (MCCARTHY and HOLLAND, 1965).

At the beginning of the 20th century, MORGAN and others established the chromosomal basis of heredity. Elucidation of the molecular basis of heredity and gene expression then became a challenging concept in the mind of man. Even in 1940, knowledge of this field was meager indeed. It should be remembered that it was not until 1944 that AVERY and co-workers unequivocally established the genetic role of DNA; in 1956 and 1957 experiments with tobacco mosaic virus (TMV) RNA proved the same role for viral RNA (GIERER and SCHRAMM, 1956; FRAENKEL-CONRAT et al., 1957). Today, as evidenced by the contributions to the present volume concerning RNA, our understanding of the molecular basis of gene expression is very extensive and undoubtedly represents one of the most significant achievements of modern scientific endeavor.

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