BOOKS: MOLECULAR BIOLOGY

In the Beginning, There Was RNA

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About 30 years ago, it was suggested that prior to the emergence of proteins and DNA, self-replicating RNA molecules served both information storage and catalytic functions in the cells of the earliest life-forms. In the early 1980s, the discovery of ribozymes, RNA molecules with enzymatic activity, demonstrated that RNA catalysis is still prevalent in present-day organisms. This unexpected finding inspired the first edition of *The RNA World*, edited by Ray Gesteland and John Atkins, which appeared six years ago. The enormous progress made since then has prompted the editors, now joined by Tom Cech, to produce an impressive second edition. About half of the 24 chapters update contributions to the first edition; the remainder are entirely new chapters, also written by eminent experts.

The RNA World
2nd ed.
Raymond F. Gesteland, Thomas R. Cech, and John F. Atkins, Eds.
Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1999. 735 pp. $129. ISBN 0-87969-561-7.

In the first of the volume's three sections, contributions examine the prebiotic origins of RNA and the evolution to a hypothetical "RNA only" world. Chapters in the middle part consider aspects of RNA structure and function, and those in the final section discuss the transition to an "RNP [ribonucleoprotein] world" and beyond. Pervading the book are the ideas that remnants of an RNA-only stage of life can be found in the replication machinery, the components involved in RNA processing, and the translation apparatus of all contemporary organisms and that some reactions associated with these processes are catalyzed by RNA, not proteins. An additional theme is that scenarios for the evolution of RNA-catalyzed reactions and RNA-based replication systems can be formulated from attempts to mimic chemical reactions under prebiotic conditions and from studies on the in vitro evolution of RNA molecules.

Although S. J. Mojzsis, R. Krishnamurthy, and G. Arrhenius note that there are still many uncertainties in trying to understand the geophysical and geochemical conditions prevailing on early Earth, they present the important conclusion that cellular life has existed on Earth for well over 3.85 billion years—which leaves an interval of no more than 600 million years for the emergence and early evolution of life. Our knowledge of the chemical synthesis of compounds required to assemble simple oligonucleotides (as precursors to self-replicating RNA molecules) remains incomplete. G. F. Joyce and L. E. Orgel lead us into the RNA world with a description of the difficulties in achieving the direct synthesis of nucleosides and nucleotides from prebiotic precursors and conclude that the de novo appearance of oligonucleotides on primitive Earth amounts to a "near miracle." They also discuss the possibility that alternative genetic systems, based on the pyranosyl analog of ribose (p-RNA) or on peptide nucleic acid (PNA), might have preceded the RNA world, and they present scenarios for the emergence of an RNA replicase ribozyme from a mixture of random polynucleotides.

D. P. Bartel notes that all versions of the RNA world hypothesis require an RNA replicase. Methods using iterative in vitro amplification and selection from large pools of RNA with randomized sequences can generate molecules resembling RNA replicases. These landmark experiments demonstrate the intrinsic ability of RNA to catalyze RNA polymerization. Some of these ribozymes can copy a short RNA-primer template and can synthesize a segment of RNA from ribonucleoside triphosphate monomers. It may soon become feasible to generate a replicase ribozyme that can carry out extensive polymerization with high fidelity. The selection of self-acylating ribozymes from pools of RNA with internal randomized sequences that catalyze the covalent attachment of amino acids to their 3′ ends is reviewed by M. Yarus and M. Illangasekare. Such ribozymes have striking similarities to modern aminoacyl tRNA synthetases; they use activated adenylate as cofactor and produce the same terminally 2′(3′)-aminoacylated RNA as do their protein counterparts.

Several chapters illustrate what can be learned about the evolution of tRNA, ribosomal RNA (rRNA), and ribonuclease P (RNase P) by tracing "molecular fossils" and by comparative analysis of RNA sequence, structure, and function. Taking rRNA and the RNA component of RNase P as paradigmatic examples, N. R. Pace, B. C. Thomas, and C. R. Woese demonstrate the power of comparative analyses to predict structural and functional elements and to establish phylogenetic relationships among organisms. N. Maizels and A. M. Weiner show that tRNA and tRNA-like sequences ("genomic tags") are involved in a wide variety of replicative processes including replication of single-stranded RNA viruses, DNA plasmids of fungal mitochondria, retroviruses, and telomeres of modern chromosomes. They argue that tRNA-like structures played a central role in the earliest replicative systems (early in an RNA world) and that the two key components of the translation apparatus, tRNA and tRNA aminoacylation activity, first evolved as components of the replication apparatus and were subsequently co-opted by the translation machinery.

How could the incredibly complicated translation apparatus, with its hundreds of components, ever have evolved? H. F. Noller reviews biochemical, genetic, and structural studies indicating that the RNA components of the ribosomal subunits play a central role in the ribosome-mediated processes of decoding RNA, tRNA selection, and the catalysis of peptide bond formation. Surprisingly, the regions of tRNA and rRNA that interact with each other are relatively compact. Codon recognition involves only about 15 nucleotides of the tRNA structure and a small subdomain of 16*S* rRNA, and the peptidyl transferase function requires only the CCA terminus of tRNA and a limited number of features of one domain of 23*S* rRNA. It is therefore possible that the two primary functional domains and their tRNA subfragment substrates may be simple enough to have arisen from preexisting RNAs. J. F. Atkins *et al.* consider the dynamics of the genetic code and present scenarios for the stepwise evolution of the modern translation apparatus. They speculate that the catalytic center for peptide bond formation and mRNA (the informational sequence) could once have been parts of one and the same RNA molecule. In this molecule, a proto-anticodon branch (having a tRNA-like sequence) could fold back and pair with "codons" to direct the synthesis of short oligopeptide chains. Separate binding pockets for different amino acids in the RNA could help deliver the amino acids to the active site for peptide bond formation. Later in evolution, the mRNA would be separated from the primitive ribosome; eventually the codon recognition and amino acid delivery functions would also be removed and would evolve into individual tRNAs, each specific for a different amino acid.

W. Gilbert and S. J. de Souza review the evidence that RNA was an antecedent to DNA. They explain why an intron-exon organization could have been crucial for the evolution of RNA-based proto-cells and for

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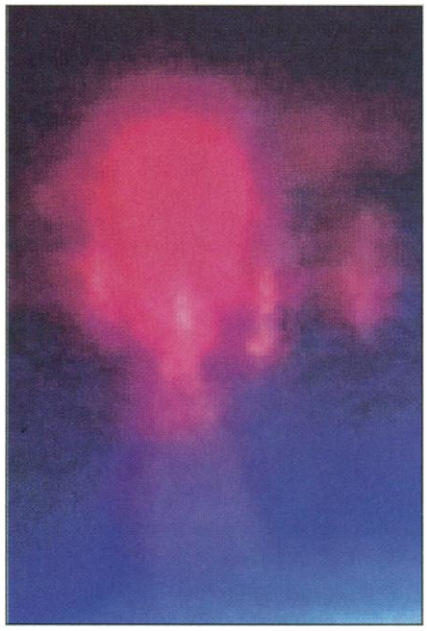
the subsequent transitions, first to RNA-protein forms and then to the present DNA-RNA-protein forms. Gilbert and de Souza propose that in the RNA world the genetic material consisted of RNA exons held together by self-splicing introns. This organization gave the RNA an extended structure adapted for copying; after copying, the introns could splice themselves out, leaving a set of exons tied together. These molecules could fold up and become functional ribozymes, which could catalyze the synthesis of protein building-blocks and membrane components of these primitive cells. The intron-exon organization exhibits another important feature, the possibility of creating RNA molecules with novel functions through shuffling exons by RNA transposition. At this stage the gradual development of a protein synthesis machinery could have set in, eventually leading to the emergence of proteins with full enzymatic functions and the replacement of ribozymes in most biochemical processes. Introduction of DNA and its highly accurate replication mechanism facilitated the formation of very large genomes. The maintenance of the intron-exon organization of DNA genes allowed an enhanced recombination rate, which could lead to novel combinations of protein modules and to the generation of entirely new structures by illegitimate recombination.

M. E. Burkard, D. H. Turner, and I. Tinoco Jr. detail the rich repertoire of noncovalent interactions that shape RNA structure. Besides base pairing to form intramolecular helices, these include interactions within simple helices generated by base stacking, hydrogen bonding, counterion condensation, and metal ion coordination. Tertiary interactions between secondary structure elements (A-form double helices, coaxial stacks, internal loops, and junctions) are responsible for the three-dimensional folding of RNA molecules into structural domains. The many possible contacts involved in such interactions include several of the interactions listed above as well as nucleotide triples and base pairing to form pseudoknots. In a separate chapter, A. L. Feig and O. C. Uhlenbeck review the many important roles played by metal ions in RNA folding and RNA catalysis.

The problems encountered in attempting to predict RNA structures from known sequences are reviewed by P. B. Moore. Fortunately, nuclear magnetic resonance and x-ray crystallographic methods for RNA structure determination are becoming increasingly tractable, and several structures have been solved. An example is the hammerhead ribozyme, a member of a family of small, self-cleaving RNAs whose reaction mechanism and ground-state structure are described by D. B. McKay and J. E. Wedekind. In their summary of the general features of RNA catalyzed reactions, T. R. Cech and B. L. Golden emphasize the large group I and group II self-splicing introns. Perhaps the most exciting development they discuss is the determination of the structure of the 160-nucleotide–long P4-P6 domain, which forms about half the active site of the *Tetrahymena* group I intron. The 2.6 Å resolution of this structure revealed both the expected nucleic acid features and features that appear protein-like. The molecule has a sharp bend at the top, allowing two punctuated double-helical regions to be aligned side by side. The protein-like feature is the tightly packed core produced by sandwiching together the two halves of the molecule. A striking role of divalent cations is seen in the A-rich bulge of P4-P6 where two magnesium ions turn a portion of the RNA "inside out," placing the backbone on the inside and displaying the adenosines for tertiary interactions.



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Prebiotic reaction sites? Sprites, luminous transient plasma discharges that reach altitudes of 90 to 100 km in the ionosphere above thunderstorms, offer a possible mechanism for the synthesis and transport of organic molecules from cold plasma in a nonoxidizing atmosphere.

T. A. Steitz examines the mechanisms by which proteins target particular RNA molecules. The many surface features displayed by folded RNA molecules are utilized to make specific protein contacts. Among the illustrative examples Steitz discusses are complexes between tRNA synthetases and their cognate tRNA substrates and RNA complexes with the recognition motifs found in many proteins that bind specific RNA molecules.

As A. M. Lambowitz *et al.* note, "group I and group II introns are not only catalytic RNAs but also mobile genetic elements." Both types of introns have become dependent on proteins to increase their folding and splicing efficiency (intron-encoded splicing factors and recruited host enzymes, such as tRNA synthetases) and to aid in their capacity to act as transposons and retrotransposons (intron-encoded endonucleases, reverse transcriptases). This has important implications for the evolution of RNP-based systems like eukaryotic spliceosomes and the RNP enzyme telomerase, aspects that E. H. Blackburn also discusses.

Ribonucleoprotein particles play central roles in many RNA processing reactions. Y.-T. Yu *et al.* summarize the recent progress in understanding small nuclear ribonucleoprotein particles (snRNPs), tight complexes of multiple proteins with a short RNA molecule, most of which are involved in the splicing of nuclear mRNA precursors. Highlights are the dynamic interactions seen in the assembly and disassembly of spliceosomes, the rearrangements before and during catalysis, and the discovery of new snRNPs as components of a separate class of low-abundance spliceosomes. In addition, a myriad of new small nucleolar RNP particles (snoRNPs) have been discovered. These particles orchestrate the processing and modification of ribosomal RNA precursors (pre-rRNAs), specifying the numerous cleavage and modification reactions by forming short intermolecular base-pairing interactions between their RNA moieties and target sites in the pre-rRNA substrates. C. B. Burge, T. Tuschl, and P. A. Sharp review the pre-mRNA splicing carried out in spliceosomes. Although the heart of the spliceosome's catalytic center contains the RNA moieties of U2 and U6 snRNPs, strongly implicating these molecules in the two catalytic steps of splicing, the individual steps in the spliceosome cycle are assisted by a surprisingly large number of protein factors. (The authors tabulate the features and mammalian homologues of 78 protein components involved in yeast splicing, and the list continues to grow.)

The contents of this volume overlap with material in another recent monograph from the same publisher, *RNA Structure and Function*, edited by Robert W. Simons and Marianne Grunberg-Manago (Cold Spring Harbor Laboratory Press, 1998). Both volumes are rich sources of information and indispensable reading for researchers investigating the biology of nucleic acids. Because of its wider scope, *The RNA World* will also attract nonspecialist readers interested in how life on Earth may have originated and evolved.