

Exploring the possibility of an RNA world

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The RNA world, second edition, Edited by Raymond F. Gesteland, Thomas R. Cech and John F. Atkins. Published by Cold Spring Harbor Press, Cold Spring Harbor, New York, USA; 1999. 709 pages, US \$129. ISBN 0-87969-561-7.

RNA molecules are very adept, capable of storing genetic information, adopting complex shapes and catalyzing chemical reactions. The title of this book refers to a time before the evolution of protein synthesis when life forms may have depended exclusively on RNA for information storage and catalysis. In this second edition of *The RNA world*, chemists, biologists and geologists investigate the plausibility of an RNA world. Nine new chapters have been added and many of the chapters from the first edition have been entirely reworked. Each chapter begins with a general introduction to a topic and proceeds on to detail recent advances in the field. Most authors also point out unanswered questions and evaluate prospects for the future — features that I found especially worthwhile.

The enterprise of probing a world that may once have existed but is now largely gone is necessarily an indirect pursuit. Attempts are made to address three broadly defined questions. The question of 'what is' examines life in our present-day RNA-protein (RNP) world while that of 'what was' asks about life in the beginning, ~4 billion years ago. The answers to both of these questions must obey the basic laws of nature and thus, must lie within the realm of 'what is possible'. The central issue is whether 'what was' happened to be an RNA world, either from the start, or as a transient stage between a pre-RNA world and the modern RNP world.

The new edition is divided into three parts. The first, "The origins of RNA and RNA at the origin", contains nine chapters that consider the likelihood of molecular evolution of RNA polymers under prebiotic conditions, and, in the event that RNA could form, what kind of chemistry could it have performed. Two of the new chapters are particularly engaging. Mojzsis (UCLA), Krishnamurthy (Scripps), and Arrhenius (Scripps) apply geophysical constraints based on conditions presumed to exist in the early atmosphere and hydrosphere and make a case to limit the vastness of 'what is possible' to those reactions that could take place under prebiotic conditions. Gilbert and de Souza (Harvard University) contribute a lucid and compelling chapter sup-

porting an RNA world. Based on the contemporary RNP world, the authors make biochemical arguments that RNA most likely preceded DNA. Credit is given to the enzyme-like properties of RNA — its ability to perform synthetic chemistry, form complex binding pockets for substrates, and bind multiple catalytic metal ions. The authors invoke an RNA world that was dependent on an intron-exon structure for efficient recombination events. Such a structure may have allowed for the assembly of large RNAs out of small functional or structural modules, made the folding of a catalytic RNA thermodynamically less favorable and therefore more accessible as a template, and enhanced the number of recombination events by allowing alternative splicing. There are many other intriguing chapters in this section that tackle issues of how ancient RNA chemistry arose, how it differed from present-day chemistry, and what evolutionary events may have led to the RNP World.

Some of the most significant advances in RNA research since the first edition (1993) have occurred in the realm of RNA structural biology. In "How to build a functional RNA", six chapters are devoted to characterizing RNA structures, metal ion interactions, and the folding properties of RNA. These chapters firmly establish that 'what is possible' for RNA includes a number of diverse chemical reactions and an almost limitless variety of complex shapes. McKay and Wedekind (Stanford University) discuss the nature of small ribozymes and their structures and functions. The authors point out that several crystal structures of the hammerhead ribozyme have been solved, either using different constructs or under different conditions. These structures have led to new insights and principles for RNA tertiary folding and metal binding. Unfortunately, none of these structures appears to be in a catalytically active conformation. To address this problem, trapping techniques and modified nucleotides have been used in more recent crystal structures, leading to insights into intermediates that resemble the expected transition states. Other chapters highlight functional and structural roles for metal ions, the energetics and structures of RNA

building blocks, crystal structures of large portions of a Group I intron, and methods for predicting and determining RNA folding pathways and structures.

In "Transition to the RNP world," nine chapters are devoted to the process by which life evolved into its current RNP form. These chapters are not as tightly connected to one another as those in the first two sections, due in part to the enormous complexity of the topic. One particularly interesting chapter by Simpson (UCLA) directly addresses the issue of what transitions are likely to have taken place en route to the present day RNP world. He provides an evolutionary perspective on RNA editing, outlining recent advances in editing mechanisms and the need for strong selective pressures to maintain it. From this perspective, editing may simply be a patch that can be added later to alter an organism's genetic make-up, allowing, for example, multiple proteins to be produced from a single gene.

Several areas of research may impact future editions of *The RNA world*. An explosion in genetic information may allow insights into biochemical interactions and RNA evolution to be made using genomic and bioinformatic approaches. Growth in the number of identified snRNPs, in particular those of low abundance, should bolster an ever-growing understanding of RNP function and assembly. Structural biology of RNA and RNP complexes, especially of the ribosome (as evidenced by the recently reported structures of the small and large ribosome subunits), will continue to reshape RNA research. In the wake of genomic and structural advances, fundamental questions of RNA biophysics will remain. What are the energetic and kinetic principles that lead to a functional RNA structure? What are the fundamental catalytic strategies of ribozymes, and how do proteins facilitate the process? How could RNA catalysis have evolved? Results from these areas should allow the plausibility of an RNA world to be more clearly defined.

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An updated guide to enzyme and protein folding mechanisms

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Structure and mechanism in protein science: A guide to enzyme catalysis and protein folding by Alan Fersht. Published by W.H. Freeman Company, 41 Madison Avenue, New York, New York 10010, USA; 1999. 614 pages, US \$67.95. ISBN 0-7167-3268-8.

Protein structure and dynamics are intimately linked with biological activity. Understanding the physical basis for this activity requires knowledge of the equilibrium and kinetic properties of the protein in solution, in various free and ligand bound states. For example, conformational changes in enzymes may be crucial for optimal function and may result from subtle movements, such as the rotation of a peptide bond; from large scale fluctuations, such as the folding of regions of a protein around a prosthetic group, or from changes in the relative orientation of domains (proteins) with respect to one another in a multidomain (multiprotein) complex. Thus, understanding the interplay between structure, dynamics and function of enzymes in solution is an important goal in modern biochemistry.

Generations of students have been introduced to this subject with the first and second editions of *Enzyme structure and mechanism* by Alan Fersht. The detailed introductory descriptions of stopped-flow, rapid quench, steady and pre-steady state kinetics included in this text have made it a classic in graduate level courses in mechanistic enzymology. Rapid advances in protein engineering and structure elucidation over the past decade in addition to the rebirth of interest in the protein folding question require a modernized text that includes strategies and analyses of protein engineering and its effects on folding and activity. In an admirable effort, Fersht has expanded his original text to include discussions on the impact of advances in protein engineer-

ing combined with efforts directed towards understanding protein folding and catalysis. The foundation of the text is grounded on solid principles encompassing chemical reactivity, kinetics and thermodynamics in the context of the three-dimensional structure of the system under investigation.

While the scope of the book is broad, the unifying reliance on simple chemical principles in discussing enzyme mechanism and protein folding makes it a coherent work from start to finish. Thus it remains an excellent text for modern graduate courses in biochemistry and biophysics. Much of the subject matter from the original work has been retained while some chapters have been extensively updated and new sections that focus on protein stability, folding pathways and energy landscapes have been added. A particularly appealing aspect of this text is the author's writing style, which is engaging and rigorous not only in discussing the key topics of interest in protein engineering today, but also in his insistence on the importance of using correct terminology when discussing important concepts in enzymology and protein chemistry.

The systems that Fersht has chosen for a detailed examination of the benefits and limitations of applying a protein engineering approach to elucidating an enzyme reaction or folding mechanism generally come from detailed studies in his own laboratory. The discussion of studies on tyrosyl-tRNA synthetase highlights the principles of overcoming very unfavorable equilibrium by the use of

binding energy in the enzyme-product complex; facilitating a reaction by lowering the transition state energy by virtue of increased favorable interactions in the enzyme-transition state species and the process of stopping unwanted side reactions during catalysis by the favorable interactions between the enzyme and highly reactive intermediates in a process known as enzyme-intermediate complementarity. The new focus on the protein folding problem, which includes a discussion on the kinetic consequences of rapid pre-equilibria and the relevance of transition state theory in light of advances in theoretical methods for the analysis of folding mechanisms, is an especially noteworthy addition to this text. While the discussions of key concepts in protein folding and stability focus mostly on barnase and chymotrypsin inhibitor 2 and are technically sound, a more inclusive overview of contributions from leaders in the field would have been appropriate. Nonetheless, his discussions on energetics of structure formation, propensity for structure in the denatured state and thermodynamics/kinetics of protein folding provide a wonderful transition for the beginning student from introductory biochemistry to a more advanced understanding of structure formation and stabilization.

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