Published in final edited form as:
Curr Opin Chem Biol. 2008 December ; 12(6): 601–603. doi:10.1016/j.cbpa.2008.10.006.

Editorial Overview: Exploring the vast dynamic range of RNA dynamics

Philip C. Bevilacqua and
The Pennsylvania State University, Department of Chemistry, University Park, PA 16802, Tel: 814-863-3812, Fax: 814-863-8403, e-mail: pcb@chem.psu.edu

Rick Russell
University of Texas at Austin, Department of Chemistry and Biochemistry, Austin, TX 78712, Tel: 512-471-1514, Fax: 512-232-3432, e-mail: rick_russell@mail.utexas.edu

This issue presents the field of RNA research from the perspective of dynamics. Over the past 20 years or so, our appreciation of the functions of RNA has grown dramatically. RNA has been shown to catalyze chemical reactions and to regulate gene expression, both by binding to small molecule and undergoing conformational changes as riboswitches, and by binding to specific messages as miRNAs. During this period of explosion in RNA biology, much has been learned about RNA physical chemistry and structural biology. For instance, the thermodynamics of RNA folding are better understood, allowing secondary structures to be predicted with increasing success, and the kinetics and folding pathways of complex RNA molecules are being elucidated. At the same time, many structures of functional RNAs have been reported, including multiple ribosome structures and ribozyme and riboswitch structures. These advances have been nothing short of astonishing. Yet at the same time that new RNA biology has been discovered and analyzed in terms of structure and chemistry, new questions have been framed. Central among these is the nature of the dynamics that allow RNA to switch between conformations, as is demanded by these systems. The issue of RNA dynamics comprises the theme of this special issue. We are very fortunate to present here 10 articles from leaders in this field. Topics range from the ultrafast dynamics of simpler model systems to the dynamics of systems of intermediate complexity including ribozymes, to motions in systems of extreme complexity including the ribosome and other RNA-protein (RNP) complexes.

Application of ultrafast spectroscopy to RNA dynamics is in its early stages but promises to provide new and unique insights into RNA function. RNA molecules are well-known for their ability to adopt multiple conformations. Tianbing Xia writes about the femtosecond dynamics of RNA conformational changes, with an eye toward elucidating this ‘rugged’ conformational landscape of RNA. His review describes applications of femtosecond time-resolved spectroscopy to simpler RNA systems such as GRNA tetraloops, base stacking, and RNA-peptide complexes. In this method, the number of exponential decays and their relative amplitudes give insight into the nature and populations of the various states in the ensemble, which are related to structure and thermodynamics.

How do we visualize the dynamic motions of RNA? Molecular dynamics (MD) simulation is emerging as a powerful tool for generating highly specific predictions about dynamic behavior. An important challenge for researchers on both the computational and experimental sides is to

build a region of overlap, where predictions from computational studies are directly evaluated by experiments. Dynamics on the time scale of picoseconds to nanoseconds can be evaluated by NMR relaxation data and can be observed directly in MD simulations, as discussed in the next article by Kathleen Hall. This review focuses on two systems of strikingly different complexity, both of which have computational and experimental data available. They are the UUCG tetraloop and the complex between the L11 protein and its RNA binding partner within the large ribosomal subunit. The review highlights some great successes of the community at combining these approaches to push the frontiers of our understanding, and it outlines future challenges. Chief among the challenges is to develop a quantitative understanding of the interactions and effects of monovalent and divalent ions, which of course are an integral and ubiquitous component of RNAs in solution but are not yet sufficiently understood to be captured accurately in MD simulations.

Important strides toward understanding these interactions are described in the next review by Dan Herschlag, Seb Doniach, and co-workers. Functional RNA molecules tend to be compact, which necessitates a clustering of negative charge. This clustering is made possible by the presence of positively charged ions, a few of which are precisely positioned, but most of which form a diffuse ‘ion atmosphere’ around the RNA. This review provides an brief overview of the experimental approaches and theoretical treatments that gave rise to an appreciation of this ion atmosphere, and it describes exciting new experimental approaches that are now generating quantitative understanding of the ion atmosphere around simple model RNAs (and DNAs) and the effects of the atmosphere on nucleic acid conformation and dynamics.

Mike Harris and Adam Cassano describe RNA dynamics of a different nature—those of bond cleavage, or ‘chemical dynamics’—and take the reader from simpler model systems to large biological RNAs. The authors focus on RNA enzymes, or ribozymes, and provide a description of the wide range of concerted and stepwise mechanisms possible for RNA bond cleavage, as well as the structures of the transition states possible for such chemical transformations. Issues of leaving group bond cleavage, nucleophile bond formation, and early-versus-late and loose-versus-tight transition states are discussed in a lucid and accessible manner. This field is in its early stages with respect to RNA. The authors therefore approach the review by laying the theoretical foundation for describing chemical reactions and transitions states, then reviewing literature on relevant RNA-cleaving model systems, and culminating with a discussion of advances on RNase P, a large RNA enzyme that processes the 5′-end of tRNAs. The main experimental approach used to probe the structure of the transition state is heavy atom (^{18}O) isotope effects, which give insights to the structure of the transition state. Because such isotope effects are by their nature small, instrumental advances play an important role, and the authors give this important subfield a treatment.

Over the last few years, single molecule approaches have made invaluable contributions to the field of RNA dynamics. Two articles describe recent advances in single molecule approaches to RNA dynamics. Michael Woodside, Cuauhtemoc Garcia-Garcia, and Steve Block discuss the folding of RNA under force, which they describe as a ‘mechanical denaturant’. Their review also ranges from simple model systems, such as RNA and DNA hairpins and 3-helix junctions, to more complex RNA systems, including the adenine riboswitch and a co-transcriptionally prepared RNA terminator hairpin. Insights into experimental and theoretical approaches are provided, which include fluctuation theorems and ultrastable instruments. One remarkable accomplishment is the ability to map and manipulate the position of the transition state during hairpin folding. Whereas Block and co-workers describe force-extension-curves, David Rueda and co-workers focus on key advances for systems where the RNA is not constrained by force. In particular, they describe single-molecule FRET studies of Mg^{2+} ‘jumps’ on RNA folding and unfolding, cooperativity of tertiary contacts in the P4-P6 domain of the group I intron, and hierarchical assembly of two proteins onto the RNA scaffold in telomerase. Together these

two reviews show that single-molecule studies offer the ability to gain insight into RNA dynamics that is unique and remarkably clear.

The single molecule reviews complete a segue from simpler model systems to complex RNAs and RNP assemblies. The last 4 reviews of this issue focus on systems of increasing complexity. While touching on folding of diverse RNAs, from relatively simple riboswitches to the highly complex group I and group II introns, Michael Brenowitz, Alain Laederach and co-workers focus on the folding dynamics of a single group I intron, the 400 nt self-splicing RNA from *Tetrahymena thermophila*, which has yielded a wealth of knowledge on RNA folding, structure, and function since its discovery as one of the first catalytic RNAs. This review highlights two important developments in experimental approaches and the new insights that have already arisen from them. First is the elegant combination of two existing approaches. Small angle X-ray scattering (SAXS) can generate global information on the size and shape of an RNA as it folds, and hydroxyl radical footprinting can generate richly detailed local information. By combining these methods to study the same RNA under the same or closely-related conditions, one can correlate the large-scale changes with local ones, truly seeing both the ‘forest’ and the ‘trees’ at the same time. Second is development of a computational modeling program, Kinfold, that allows the enormous amount of data from footprinting experiments to be condensed to a smaller number of folding transitions and intermediate states. Using these methods, the authors and their collaborators have achieved new understanding of the folding pathways of this large RNA and the structures of intermediates along the pathways.

Two articles focus on the dynamics of the ribosome. Over the last decade, high-resolution structural biology has provided unprecedented insight into ribosome function, thus revealing actions that might be possible in other RNPs as well. The ribosome is a massive RNA-protein complex with approximately 4500 nucleotides of RNA and more than 50 different proteins; thus, its structural biology and biophysics are rich with dynamics of RNA-protein assembly and the mechanics of RNA-protein movements. Sarah Woodson describes the amazing process by which the bacterial 16S ribosomal RNA folds and assembles with 20 proteins to form the 30S ribosomal subunit, focusing on two sets of exciting studies. Time-resolved footprinting has recently generated snapshots from the RNA perspective, and a novel mass spectrometry method has been used to follow the same process from the perspective of the proteins. Together these studies suggest a highly dynamic process in which RNA folding and protein binding steps are intimately and inextricably linked, with RNA folding creating protein binding sites and protein binding inducing further RNA folding. In the next review, Harry Noller and co-workers describe movements of a fully assembled ribosome during protein synthesis, with structural insight garnered from X-ray crystallography, cryo-EM, and FRET. One remarkable feature of ribosome is its flexibility, as reflected in the wide range of distances functionally-relevant motions span. These include intersubunit rotational movements of 40 Å and localized changes of just a few Å; these motions accompany tRNA-mRNA translocation and peptide bond formation, respectively. One commonality to ribosome conformational changes is that they seem to arise from changes in the conformation of rRNA rather than proteins.

The final article of this issue provides a unique and compelling perspective on the roles and structural dynamics of some non-coding RNAs (ncRNA) in the cell. Robert Hogg and Kathy Collins present the viewpoint that we are today in an evolutionary period that can be referred to as an ‘RNP renaissance’; a period in which ncRNAs have evolved and are continuing to evolve new functions in a protein-rich environment. Many of these functions take advantage of RNA’s ability to form locally stable modules of structure, which are arranged within a large RNA to scaffold the binding and organization of proteins into functional RNP assemblies. A good example is the telomerase RNA, which brings together several protein components, as well as serving as a template for synthesis of DNA. Further, as recently shown by the authors and highlighted here, the protein components in these assemblies can be dynamic, changing

in response to cellular conditions and thereby regulating the functions of the RNP assemblies. New methods of tagging and purifying RNP assemblies are sure to yield exciting insights, and almost certainly some surprises, about the structural and functional properties of cellular RNAs that have evolved in the RNP renaissance.

We have learned a tremendous amount in recent years about the structures and dynamics of RNA molecules in their functional contexts and about the theoretical underpinnings of these properties. It has been thrilling to see how the development and implementation of new experimental and computational approaches, as well as the marriages of existing ones, have allowed large advances into areas that once seemed all but impenetrable. It will be fascinating in the future for the community to confront new challenges, such as striving to understand which dynamic motions are necessary for the functions of RNA and which are simply a by-product of its physical composition and properties, and on the biological side, exploring how the dynamic properties of RNA are exploited and changed by specific and non-specific interactions within cellular environments. Last, we note that it has recently become clear that a staggering number of different non-coding RNAs are produced in cells, but that we know nothing about the functions of most of them. With this viewpoint, we expect that the explosion of knowledge of the biological processes mediated by structured RNAs will continue into the foreseeable future, underscoring the importance of understanding the dynamics of RNA in biology.

Biographies

Philip Bevilacqua is a Professor in the Department of Chemistry at the Pennsylvania State University. He received a BS from John Carroll University and a PhD from the University of Rochester, studying with Doug Turner. After conducting post-doctoral research with Tom Cech at the University of Colorado, he joined the faculty at Penn State University in 1997. His research interests include RNA folding, RNA catalysis, and roles of RNA in innate immunity.

Rick Russell received a BA in chemistry from Earlham College in 1991 and then performed graduate studies with Roger McMacken at Johns Hopkins University. After completing his PhD in 1997, he was an NIH postdoctoral fellow with Dan Herschlag at Stanford University. He joined the faculty at the University of Texas at Austin in 2002 and was promoted to Associate Professor in 2008. His current research interests include folding and stability of structured RNAs and the mechanisms of RNA chaperone proteins.