Ribozymes: The First 20 Years

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Twenty years have passed since the first reports that certain RNAs mediate self-splicing and precursor tRNA processing reactions in the absence of proteins. An entire field emerged to learn how RNAs that lack the chemical versatility of amino acids nonetheless assemble into enzymes that accelerate chemical reactions with efficiencies that rival those of their protein counterparts.

RNA enzymologists assembled at a recent Biochemical Society Focused Meeting/EMBO workshop held in Dundee, Scotland, in August 2002 entitled “Ribozymes and RNA Catalysis” (organized by David Lilley and Fritz Eckstein) to evaluate how far the field has come and to anticipate the work that lies ahead. The meeting covered a broad array of mechanistic and structural perspectives on RNA catalysis ranging from detailed views of active site biochemistry in self-splicing of the Tetrahymena group I intron, the original ribozyme, to the largest ribozyme, the ribosome, with glimpses into the spliceosome where new evidence points to the possibility that it might also be an RNA enzyme at its core (Saba Valadkhan from James Manley’s group at Columbia Univ.) and a novel ribozyme identified through an in vitro selection strategy that catalyzes carbon-carbon bond formation (Andres Jäschke, Ruprecht-Karls-Universität). We viewed structures of RNA enzymes at resolutions ranging from atomic level structures of potential intermediates along the hammerhead ribozyme reaction pathway (Bill Scott, UC Santa Cruz) to lower-resolution, yet striking, cryo EM images of spliceosomess (Reinhard Lühmann, Max-Planck-Institute of Biophys. Chem., Göttingen) and models of global folding of the VS ribozyme based on constraints obtained through fluorescence resonance energy transfer experiments (David Lilley, Univ. Dundee).

The highlight of the opening session on catalytic mechanisms of the small nucleolytic ribozymes was Adrian Ferré-D’Amare’s (Fred Hutchinson Cancer Research Center) presentation of the crystal structure of a hairpin ribozyme in complex with a vanadate transition state mimic (Rupert et al., 2002). Comparison of this complex with structures of ribozyme-substrate and ribozyme-product complexes revealed potential hydrogen bonding interactions that seem to be specific to the transition state, supporting a transition state stabilization mechanism. In the Nucleic Acids Award Lecture, Olke Uhlenbeck (Univ. Colorado) announced with characteristic glee that most of what we thought we knew about the role of metal cations in hammerhead catalysis is wrong. While metal cations once were viewed as essential cofactors responsible for electrostatic stabilization of the transition state and for mediating proton transfer through bound water, recent studies suggest that metal cations contribute no more than 50-fold to catalytic rate enhancement by the hammerhead ribozyme. Other talks on mechanisms of RNA catalysis focused on the ways that RNA nucleobases might participate directly in catalytic chemistry and the use of positioning and orientation of functional groups to achieve catalytic rate enhancement. No consensus emerged regarding catalytic mechanisms of RNA self-cleavage, but the tremendous impact that the availability of active site structures is having on the direction of biochemical studies was inescapable.

Tom Cech himself opened the second session with a retrospective of “the first 20 years” of investigations of group I intron self-splicing. This plenary lecture began with the “It needs protein - It doesn’t” Cech lab debate of the early 1980s and ended with current efforts to exploit in vitro selection strategies to facilitate RNA crystallization (Guo and Cech, 2002). Joe Piccirilli (Univ. Chicago) presented results, based on the metal specificity switch pioneered by Fritz Eckstein, of a long-standing collaboration between the Piccirilli and Herschlag (Stanford Univ.) labs to develop a detailed thermodynamic description of three metal cations essential for Tetrahymena ribozyme catalysis, and Scott Strobel (Yale Univ.) described an interference suppression strategy used to identify the first ribozyme ligand for one of the three active site metal cations. Characterizations of metal ions important for RNase P folding and catalysis were the subject of platform (Leif Kirsebom, Uppsala Univ.) and poster (E. Christian and M. Harris, Case Western) presentations (Kaye et al., 2002). As Michael Gait (MRC) observed, the active sites of the large ribozymes seem to be gaining metal cations even as the small ribozymes lose them.

A number of investigators reported the results of recent studies of ribozyme folding, taking advantage of the fact that catalysis provides a clear signal that an RNA enzyme has adopted its functional structure. Early studies focused mainly on metal-cation-induced folding pathways that are characterized by divalent cation-stabilized kinetic traps that might not reflect normal RNA assembly pathways during transcription in vivo. Renée Schroeder (Univ. Vienna) presented the results of her group’s efforts to investigate folding of the td group I intron directly in E. coli and the influence of proteins on folding, particularly the opposing effects of two proteins,
StpA and Cyt-18, on tertiary structure stability (Waldsich et al., 2002a, 2002b).

One striking theme that transcended boundaries between the large and small ribozyme families was the dramatic effect of peripheral RNA sequences on RNA assembly and reaction pathways. Sarah Woodson (Johns Hopkins) reported that circular permutants of the Tetrahymena ribozyme fold through distinct pathways with different intermediates depending on the location of 5' and 3' termini within the ribozyme sequence. Hammerhead ribozymes are among the most thoroughly investigated ribozymes, being the subject of over 1000 research articles published since 1989. Virtually all of these studies focused on minimal hammerhead ribozymes that consist of three short base-paired helices and 14 conserved nucleotides that comprise the catalytic core. In an astonishing development, Anastasia Khvorova and Suahmeda Jayasena (Amgen, Inc.) reported that extending hammerheads to include sequences that naturally flank the minimal hammerhead motif in plant satellite RNAs transforms the reaction entirely. Enhanced cleavage and ligation activity was attributed to stabilization of the folded tertiary structure through interactions between the extended helices. Although the structural basis of a similar VS ribozyme transformation is less apparent, Rick Collins (Univ. Toronto) found that altering the connection between the stem-loop that contains the reactive phosphate and the catalytic core accelerates VS ribozyme cleavage by over two orders of magnitude and reduces divalent cation requirements dramatically. These results with hammerhead and VS ribozyme variants evoke similar changes in structural and catalytic properties observed when hairpin ribozymes were designed to assemble in the natural context of a 4-way helical junction compared to minimal ribozyme constructs with 2-way junctions.

Ironically, deletion of peripheral sequences was motivated many years ago by the desire to avoid “alternative conformer hell,” that is, the propensity of RNA sequences to adopt stable misfolded structures that interfere with assembly of functional ribozymes (Olke Uhlenbeck). It now seems that peripheral sequences that were deleted from minimal ribozyme constructs can direct ribozyme folding and stability. Some investigators have taken this strategy one step further, by appending sequences to minimal ribozymes that confer allosteric regulation through sensitive and specific binding to small molecules, oligonucleotides, or proteins (Ron Breaker, Yale, and Michael Famulok, Univ. Bonn). Clearly, the same propensity to adopt alternative conformations that can give rise to “conformational hell” also can be exploited as an opportunity for ribozyme engineering for potential applications in biological sensing or in drug discovery.

The meeting concluded with updates on the most recent addition to the ribozyme family, the ribosome. As if the stunning 30S, 50S, and 70S ribosome crystal structures of 2001 didn’t give us enough to absorb, Harry Noller (UC Santa Cruz), Peter Moore (Yale) and Ada Yonath (Weizmann and Berlin) brought out an expansive array of crystal structures of ribosome complexes with tRNAs, translation factors, and antibiotics. These snapshots of ribosomal complexes representing various functional states can now be strung together to create movies portraying conformational transitions (“undulations,” as Harry Noller put it) that occur throughout the translation cycle. New insights into the mechanism of action of antibiotics provided by the three groups gave an apt reminder of the practical as well as aesthetic implications of studies of RNA enzymes.

References


