Editorial

RNA Takes Center Stage

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In the last several years RNA has attracted so much scientific attention that it seems hard to believe (especially to those of us who learned about catalytic RNAs from textbooks) that there was a time when RNA was the stepchild of biochemists—not stable enough to store genetic information and not structurally and functionally diverse enough to catalyze reactions. From today's perspective, it is therefore impossible to fully appreciate the intellectual merit of the breathtaking proposal, put forward in parallel by Orgel, Woese, and Crick in 1968,[1–3] that an RNA world could provide a simple solution to the old chicken-and-egg problem of evolution—which came first, the DNA to encode proteins or the proteins required to synthesize DNA?—as RNA might be able to perform both functions.

One way to appreciate the forward-thinking nature of this proposal is to consider the fact that it took another 15 years of scientific research before the first evidence for catalytic RNA was documented. One early finding that suggested RNA might be able to exert catalytic action was Aaron Klug's determination of the crystal structure of tRNA in 1974.[4] This structure exemplified the intricate and compact tertiary structures that RNA is capable of adopting, a key feature of proteins that are required for catalytic activity. Another 8 years later Cech and coworkers, as well as Altman and coworkers, finally stumbled upon catalytic RNAs involved in self-splicing and tRNA maturation, respectively.[5,6]

The unveiling of catalytic RNA has transformed the scientific community's perception of RNA and has led to the birth of an entire new field devoted to the study of the structure and function of RNA. Numerous examples of catalytic RNAs have since been found to function in processes such as viral replication (HDV, hammerhead, hairpin, and other small ribozymes[7–9]), protein translation,[10] and the maturation of tRNAs (RNase P[6]), rRNAs (RNase MRP[11]), and mRNAs (self-splicing introns[5,12,13] and possibly the spliceosome,[14,15] itself a Nobel-winning discovery by Phil Sharp and Richard Roberts). As a result we now have a fairly sophisticated understanding of RNA's capabilities and limitations. The mechanism of some RNA enzymes is understood as well as or some may say in even greater detail than that of many protein enzymes.[16–19] In addition, we now have atomic resolution structures of many RNA molecules,[20–28] including the ribosome.[29–32] Such information has allowed us to make significant progress in determining the details of the cellular role of many RNA molecules, including the cell's largest RNA machine, the ribosome.

This progress by itself is impressive and would have been unthinkable 25 years ago, when the Cech and Altman labs were preparing their seminal publications. However, more recently there has been yet another RNA revolution: In 1998, Fire and Mello reported that double-stranded RNA could strongly and specifically repress gene expression in worms.[33] They had uncovered the RNAi pathway, for which they were awarded the most recent Nobel price in Medicine. Their groundbreaking discovery has proven to be one of the most exiting new frontiers in RNA research, as well as an invaluable "genetic" tool for the study of higher eukaryotic organisms. This discovery was also the first insight into how RNA is used in biology to regulate gene expression in a spatial and temporal manner via targeting of the mRNA through complementary base-pair interactions. A related regulatory role was uncovered when Kadner's and Soberon's labs reported the existence of metabolite-sensitive structures in the 5′-regulatory regions of individual mRNAs in bacteria.[34,35] These types of RNA sequences, now dubbed "riboswitches," have been extensively characterized by the Breaker lab at Yale (e.g., Refs. 36–39). Breaker and coworkers have shown that metabolite binding induces conformational rearrangements in the RNA, which can regulate transcription or translation of mRNAs. In addition, it is now clear that some riboswitches are evolutionarily conserved and coregulate entire metabolic pathways in response to intermediates in that pathway.

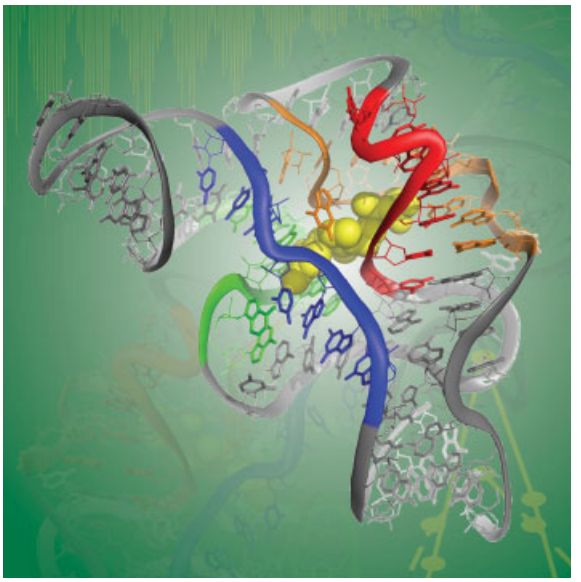

FIGURE 1 Shown is an artistic rendering of the 2.05 Å crystal structure of the thiamine pyrophosphate (TPP) riboswitch bound to TPP.[44] The structure represents a striking example of an intricately folded RNA element that precisely binds a specific ligand through the exact organization of multiple binding pockets that each recognize and coordinate with a particular defined functional moiety of the ligand. Figure courtesy of Maximilian Bailor.

It was thus fitting that Ron Breaker gave the keynote address at the Ninth Annual Michigan RNA Society meeting held in April 2007 at the University of Michigan campus in Ann Arbor. In his talk he gave a beautiful summary of this field, which has been almost single-handedly unraveled by his lab. He and his coworkers have now uncovered 16 classes of riboswitches,[40] explored the folding of the RNA in the presence and absence of the metabolite (e.g., Ref. 41, see Figure 1), and mapped the biosynthetic pathways regulated via these riboswitches.[38,40] Bioinformatics tools have been invaluable for these discoveries and have been aided by the simplicity of RNA secondary structure prediction, given that phylogenetic information is available from genome sequencing. Thus, the beauty of Breaker's work is that experiments and computation go hand in hand. In an exciting new frontier, Breaker also reported the discovery of metabolite-sensitive regulatory elements that affect alternative splicing in fungi.[42] In addition to this tour de force talk, three posters from the Walter lab at the University of Michigan, as well as one from the Walton lab at Michigan State explored conformational dynamics of riboswitches and their use as chemical sensors.

This meeting, however, was by no means devoted solely to riboswitches. Instead, it covered a broad range of RNA biochemistry, biophysics, and biology and included talks and posters on RNA structure and dynamics, the function of ribozymes, analysis of the translation machinery, and ribosome assembly. Highlights from oral presentations include the characterization of the dynamics of the transactivation response element (TAR) from HIV, using the recently pioneered NMR technique of residual dipolar coupling. In his work, which was recognized with an award for the best talk, Max Bailor from the Al-Hashimi lab at the University of Michigan characterized the binding of four closely related aminoglycoside antibiotics to TAR. He was able to show a surprising degree of promiscuity in the recognition of these antibiotics, demonstrating the structural flexibility that is characteristic (for better or for worse) of RNA molecules. Interestingly, the changes in the RNA molecule depend on the chemical modifications of these antibiotics in a modular manner. Additional posters from the Hoogstraten (Michigan State University) and Al-Hashimi labs explored further the use of NMR techniques to analyze RNA's structural dynamics.

In related work, Tuhina Banerjee, a postdoctoral researcher from Andrew Feig's lab at Wayne State University, presented an analysis of conformational changes in RNAs as modeled by kissing interactions, which can be found throughout biology, including in HIV viral maturation and in the regulation of gene expression via noncoding RNAs in bacteria. Banerjee used isothermal titration calorimetry and single molecule fluorescence spectroscopy to show that her model hairpins form kissing duplexes unproductively, many times before they eventually melt into long duplexes. This provides insight into the function of proteins that catalyze duplex formation, such as Hfq in bacteria. These proteins are thus predicted to work by lowering the barrier to duplex formation and not by stabilizing the kissing complexes or by lowering the barrier to kissing loop formation. A review by Feig on "Applications of Isothermal Titration Calorimetry in RNA Biochemistry and Biophysics" is included in this issue of Biopolymers.

Single molecule experiments are used routinely by the Walter and Rueda (Wayne State) labs to study RNA structure. These labs presented posters discussing conformational heterogeneity in the hairpin ribozyme, loop–loop interactions in the hammerhead ribozyme, and RNA folding using U2 and U6 RNA, which form the active site of the spliceosome. In addition, Miguel Pereira from the Walter lab presented a single-molecule fluorescence resonance energy transfer-based analysis of how kissing loops and the central junction cooperate in a Mg^{2+}-dependent manner to ensure folding of the Varkud satellite(VS) ribozyme from the mitochondrial VS RNA of *Neurospora crassa* to its catalytically active structure. Pereira's data provide information on the interactions stabilizing the catalytically active fold and will allow further dissection of the role of specifically bound

Mg^{2+}-ions as well as structural components in stabilizing this fold. The use of single molecule experiments to unravel catalytic mechanisms of RNA enzymes is reviewed by Walter and coworkers in "Focus on Function: Single Molecule RNA Enzymology", which is also in this issue of Biopolymers. Ribozymes were also the focus of additional talks and posters from the Hoogstraten, Fierke (University of Michigan) and Engelke labs (University of Michigan). Kristin Smith from the Fierke lab discussed her work on bacterial RNase P holoenzyme, which cleaves the 5′-leader sequence from pre-tRNA molecules. She has dissected the contribution of a conserved charged protein motif, the RNR (arginine–asparagine–arginine) motif, found in the single protein component from bacterial RNase P, on binding of both RNase P RNA as well as pre-tRNA substrate using catalytic activity as a readout. Her work comparing the activities of wild type and mutant holoenzymes indicates that the RNR motif is located at the interface between RNaseP and pre-tRNA and helps to correctly assemble a catalytically active holoenzyme. Mutations in this region weaken binding to RNase P RNA and pre-tRNA substrate and, furthermore, reduce catalytic activity of the assembled RNP.

In contrast, work from Scott Walker in Dave Engelke's lab focuses on dissecting the topology of the much more complex yeast RNase P enzyme, which, in addition to the RNA component, contains nine proteins. Walker's work uses a novel crosslinking approach and tagged yeast strains to dissect RNA-RNA and RNA-protein interactions and gain a first handle on this biochemically challenging complex. "RNA-protein interactions in RNase P" are also reviewed by Fierke and colleagues in this issue. Given the importance of ribozymes to the RNA field, this issue of Biopolymers contains two related reviews on catalytic nucleic acids. Charles Hoogstraten gives a broad overview on "Functional strategies of catalytic RNAs and RNPs." "Recent Advances in DNA Catalysis," which are taking simplification of the chemical repertoire of catalysts a step further, are reviewed by Scott Silverman (University of Illinois, Urbana Champagne).

Toward more complex biological systems, the Feig lab presented work indicating that Hfq, the bacterial Sm-like protein, binds tRNAs with strong affinity, and also interacts with proteins involved in tRNA metabolism. This supports the idea that Hfq might play a hitherto unrecognized role in tRNA metabolism. An outstanding poster on this work was presented by Taewoo Lee and was recognized as the best of over 40 posters.

Given the exciting, recent, high-resolution structures of ribosomes, it may not be very surprising that there were many talks aiming to understand the role of modifications as well as dynamics for ribosome function. Work presented from the Chow, Santa Lucia, and Cunningham labs (all at Wayne State) used NMR, chemical biology tools, genetic, and biochemical experiments to address these exciting questions. A talk presented by Tek Lamichhane, from the Cunningham lab, described the use of mutational analysis to tease out the function of base modifications in a conserved loop of the mature 16S rRNA that resides near the ribosomal P-site. Lamichhane's data indicate that a specific modifications improve fidelity by limiting stop-codon read-through and misincorporation of incorrect amino acids into the nascent polypeptide. By combining these functional results with the available structural information, this group has begun making hypotheses as to how the wild-type structure ensures proper protein translation.

The recent ribosome structures as well as large-scale mass-spectrometry approaches have provided a strong background and renewed interest in studies on ribosome assembly. Work in this area, presented by the Britton (Michigan State), Maddock and Karbstein labs (both at the University of Michigan), covered the function of GTPases and other assembly factors in bacteria and yeast, respectively. This work has revealed that GTPases are the largest class of essential ribosome assembly factors in bacteria and has started to provide insight into the role of GTP hydrolysis by the yeast GTPase Bms1 in promoting binding of a putative RNA endonuclease to nascent ribosomes. The role of GTPases in ribosome assembly was also reviewed in a recent issue of Biopolymers.[43]

In summary, the meeting showcased work on every aspect on RNA's function, a repertoire, which is likely to further expand in the future. It is clear that RNA-centered research will remain at the forefront of biological research in Michigan and beyond, and we look forward to new discoveries in the years to come.

For more information on the RNA community and RNA meetings refer to http://www.rnasociety.org and http://www.umich.edu/~superrna.

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