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The modern RNA world

When one hears the phrase 'RNA world', it is common to think of the prebiotic era and the origin of life. However, a recent conference* entitled "The RNA World" focused on current times rather than ancient history. This meeting, which was organized by Jennifer Doudna (Yale University) and Olke Uhlenbeck (University of Colorado at Boulder), featured some of the diverse scientific approaches that are underway to investigate problems ranging across a broad spectrum of RNA biology. Research challenges in this RNA world include: understanding RNA structure and catalysis, determining how RNAs are transported from the nucleus to the cytoplasm, identifying the *in vivo* functions of natural RNA modifications, and developing RNAs as therapeutic agents. Space precludes a complete account of all of the excellent presentations here, but the flavor of this dynamic RNA meeting can be seen from some of the highlights that follow.

Toward a high-resolution ribosome structure

The ribosome, which contains three ribosomal RNAs (with more than 4,500 nucleotides) and over 50 different proteins, is clearly on the frontier of RNA science. The structures of many individual ribosomal components are known in isolation, but it is not at all clear how these molecules interact to perform their functions. The most exciting presentations suggested that near-atomic resolution structures of the entire ribosome are on the horizon.

Tom Steitz (Yale University) described X-ray crystallographic studies of the 50S ribosomal subunit of *Haloarcula marismortui*. He presented a 5 Å electron density map clearly showing density for duplex RNA; so far, ~500–1000 base pairs of duplex RNA have been tentatively placed. Importantly, electron density of proteins can be distinguished from that of RNA in many cases. However, the exact identities of only a few proteins have been identified since many of the ribosomal proteins with known structures have significant β-sheet content, and at 5 Å resolution it is difficult to recognize β-sheets. However, even without allowing individual identifications, this map permits a striking observation — many of the proteins are interacting with two or three regions of duplex RNA, suggesting that the proteins are serving as 'cement' to hold together various parts of RNAs, perhaps placing them in appropriate conformations or positions. Harry Noller (University of California, Santa Cruz) also offered exciting structural results: a 7 Å electron density map of the entire ribosome. It is difficult to distinguish the 30S and 50S subunits in many areas of this map, but it is possible to model aminoacyl tRNA in the appropriate region of the 30S subunit. This exercise reveals interactions between the anticodon stem-loop of the tRNA and the 30S subunit, but the limited resolution of the map prevents ascertaining whether the major interactions are likely to be with protein or RNA components of the ribosome.

Ribozymes

While progress on ribosomal RNA structure and function is just now accelerating, work on smaller RNAs, such as ribozymes, has been moving along rapidly for many years. Topics in the session on ribozymes included: the packing and metal ion requirements of the Tetrahymena group I ribozyme active site (Scott Strobel, Yale University; Joseph Piccirilli, University of Chicago)[1–3], efforts to improve the catalytic rates of hammerhead ribozymes (Olke

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Uhlenbeck), and the structure, catalysis, and folding of the hepatitis delta virus ribozyme (Jennifer Doudna; Philip Bevilacqua, Pennsylvania State University).

The story of the hepatitis delta virus ribozyme presents some twists that have not been commonly observed in the RNA world thus far. Hepatitis delta virus replicates using the host Pol II enzyme. Replicated circular RNA intermediates have an ~85 nucleotide sequence that folds into a ribozyme and subsequently cleaves the RNA, thereby allowing further processing. Bevilacqua's group has shown that RNA sequences 5' of the minimal sequence required for ribozyme activity can inhibit folding of the RNA into its active state and thus could be involved in regulating the cleavage event. Once folded, the active site is buried, as seen in the structure of a self-cleaved hepatitis delta virus ribozyme[4]. There is no evidence of metal binding in this structure, as expected since this ribozyme, unlike most others, is not dependent on high concentrations of metals for activity. How does the cleavage reaction of the properly folded ribozyme occur without metals? Doudna's group proposes that a C nucleotide found at a sharp kink in a strand near the active site acts as a general base in the reaction. Nucleotide analog substitutions and measurements of the pK_a of this nucleotide are underway to test this likelihood of this hypothesis.

RNA transport in eukaryotes

In a eukaryotic cell, RNAs are made in the nucleus and must be exported to the cytoplasm. Elsebet Lund (University of Wisconsin, Madison) presented work showing that tRNAs are proofread for proper folding and maturation of their 5' and 3' ends: they must be aminoacylated in the nucleus before they can be transported efficiently to the cytoplasm. This finding was surprising — aminoacyl tRNA synthetases were once thought to be present only in the cytoplasm[5]. The transport of mRNAs to the cytoplasm also appears to involve quality-control mechanisms. Christine Guthrie (University of California, San Francisco) presented results from a screen of yeast cold-sensitive mutants for those deficient in mRNA export. One mRNA transport-deficient mutant, *brr3-1* (for bad response to refrigeration), also exhibits splicing and poly-A adenylation defects of mRNAs. The *BRR3* gene has also been identified (and named *GLE1*) by another group in a search for mutations that are lethal in the background of a nuceloporin mutation[6–8]. Together, these results support the idea that there are quality-control links between the pathways for mRNA processing and transport.

Medical applications of RNAs

Although much RNA research is aimed at a basic understanding of biological processes, this RNA meeting also focused on applied RNA science. Bruce Sullenger (Duke University Medical Center) presented progress on developing RNAs for potential use as therapeutics to prevent unwanted blood clotting. His group has performed *in vitro* selections to identify RNAs that bind specifically to coagulation factor VIIa, a key initiator of clotting. This strategy has let to development of an RNA that binds factor VIIa with a K_d of ~250 nM at 37 °C. In an *in vitro* coagulation assay, addition of this RNA results in a two-fold increase in coagulation time. To put this number into perspective, the anticoagulants, such as heparin, that are most commonly used in humans give only a ~50% increase in coagulation time *in vitro*. Optimization of the factor VIIa-binding RNA, with an eye toward therapeutic uses, is underway.

Pamela Pavco (Ribozyme Pharmaceuticals Inc.) described Angiozyme™, a ribozyme targeted to cleave the mRNA of a vascular endothelial growth factor (VEGF) receptor, which is involved in blood vessel formation. Angiogenesis can play a role in cancer growth, and therefore drugs that block blood vessel proliferation may be useful anti-cancer therapies. Angiozyme™ is effective in two animal models: it diminishes VEGF-induced angiogenesis in rat cornea and reduces growth of a highly metastatic line of Lewis lung carcinoma in mouse. The enzyme is currently in phase I clinical trial, in which an assessment of safety is the goal.

Overview

This was an excellent meeting for anyone wanting to learn about the variety of research in the RNA field. The only shortcoming was the small size of the poster session. While some exciting studies were presented in poster form (including results published recently in the pages of *Nature Structural Biology*[9,10]), it is surprising that in such an active and growing field, more people did not contribute. However, the plenary talks compensated well for limited number of posters, and together they gave not only exciting snapshots of the landscape of current RNA research, but also glimpses of possible future RNA worlds.

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