

## Editorial: Mechanism of Catalytic RNA

Since the discovery over 20 years ago that RNA can be catalytic, there has been great interest in understanding how an ostensibly simple polymer can have such complex properties as rate acceleration and specificity. The articles in this issue provide a perspective on progress in this area. The cornerstone of mechanistic insight into enzyme function has always been the relationship between structure and function. Unfortunately, one of the defining features of RNA structure is conformational heterogeneity including the formation of multiple alternative pairings, or misfolds, at the secondary and tertiary structure levels, which can be exceptionally stable. These misfolds arise because the limited functionality of RNA leads to a degeneracy in base pairing and the possibility for a large number of kinetically stable short helices. Moreover, the large number of dihedral angles in the RNA backbone leads to a considerable conformational entropy penalty for rigidifying the backbone. Another source of heterogeneity is population of non-functional ionizations of critical nucleobases such as general acids and bases, and putative oxyanion holes. Lastly, RNA might “resist” the functional fold if it requires an energetically unfavorable conformation; especially the close positioning of negative charges. An emerging picture of RNA enzymes thus appears to be one of a large ensemble of states, the population of which is dictated by *physical* processes, only one of which is highly reactive or functional, a *chemical* process. Clearly, understanding RNA folding is key to understanding RNA catalysis. This leads to several questions. How do features of RNA enzymes select the functional state? What roles do higher order tertiary structure and positive patches—metal ions, protons, and basic proteins—play in this function? Can the exceptionally stable energetics of RNA folding be parlayed into additional functional diversity of the bases? These themes are common to half of the articles in this issue.

Ferré-D’Amaré provides an overview of the structure and mechanism of one of the best characterized catalytic RNAs, the hairpin ribozyme. His research group has solved several crystal structures of this small ribozyme, including a recent structure with the transition state mimic vanadate bound at the active site. He discusses implications of these structures on the mechanism of the ribozyme. One of the interesting features pointed out is that while the major conformation of stem B from NMR studies differs from that found in the crystal structure, a minor conformation appears to be the same. Thus, one might think of docking of domains as a process in which a collection of nonfunctional RNA conformations can be winnowed to a single functional one. A corollary of this observation is that hierarchical folding of secondary and tertiary structure is subject to exceptions.

Fierke and co-workers provide an overview of the role of proteins in facilitating the function of catalytic RNAs. They point out that “*protein subunits appear to be necessary for naturally occurring ribozymes to carry out multiple turnover reactions under in vivo conditions.*” Fierke and co-workers also discuss important RNA–protein (RNP) systems and the known roles of proteins in these complexes. One of the challenges of this field is defining the relative contributions of the protein and RNA subunits to catalysis. One function of the protein subunits of certain RNPs is to aid the folding of RNA. An early idea was that proteins facilitate the close approach of negative charge since they could be substituted for by very high concentrations of monovalent salts. “Capture” of a functional structure from an assortment of nonactive structures is another key concept in facilitation of RNA folding by proteins. However, not all proteins in RNPs function by facilitating RNA folding. For example, RNase P protein appears to increase the affinity of RNase P RNA for both pre-tRNA substrate and metal ions. This property might arise from “preorganization” of the metal sites by the protein.

Bevilacqua and co-workers discuss ways in which the energetics of RNA folding might be used to drive the four nucleobases into unusual forms: protonated, tautomeric, or both. Coupling of protonation or tautomerization events to the formation of new secondary and tertiary structure provides an energetically feasible means of populating such species. Adenine and cytosine protonations are divided into two classes: those in which the proton is sequestered and those in which it is not. The former class is suggested to be a possible source of oxyanion holes for electrostatic catalysis, while the latter might also serve as general acids and bases. Comparisons to arginine/lysine- and histidine-like functions are made. Quantitative contributions of general acid–base catalysis and electrostatic catalysis to rate acceleration are also discussed, which leads to a second theme in these articles: What features of the functional form of the ribozyme, once properly enriched, lead to rate acceleration?

Harris and co-workers provide an introduction to the transition states of phosphodiester bond cleavage reactions through studying the effects of heavy atom isotopes. The authors provide a comprehensive overview of the problem, focusing primarily on the transition state of uncatalyzed reactions. Differences between intra- and intermolecular attack reactions are provided, as are the associative and dissociative nature of the resulting transition states. Interestingly, divalent metal ions and general acids and bases affect the transition state and mechanism of nonenzymatic bond cleavage. Since RNA-catalyzed reactions also employ these species, a future challenge will be to compare transition states of enzymes to those of model systems.

An important tool for discovery of new chemistries has been *in vitro* selection, or SELEX. Hodgson and Suga report on their mechanistic studies of acyl-transferase ribozymes. One of the elegant features of their work has been coupling of one functionally selected domain to another. In particular, they combine an acyl-transferase ribozyme domain with an amino acid-selective domain and select the proper amino acid for acylation, much like a tRNA synthetase. In a related study, they combine acyl-transferase activity with a tRNA-selective domain, and select and acylate the proper tRNA substrate. Continuing with the mechanistic theme of the articles, the authors describe their

extensive characterization of the kinetic mechanisms of these ribozymes, with a special emphasis on the contributions of approximation and divalent metal ions to folding and catalysis. Design of RNAs that fold effectively, with a large fraction folded, is another theme of this article.

Turner and co-workers provide an introduction to how mechanistic understanding of RNA folding can be used to purposely misdirect ribozymes into adopting inactive conformations. The authors discuss three approaches to inactivating RNA function: (1) enhancing binding interactions by tertiary interactions (BETI), (2) suicide inhibition, and (3) oligonucleotide directed misfolding of RNA (ODMiR). In the first approach, the extra tertiary interactions employed by the substrate are co-opted to increase specificity for the target RNA over competing RNAs in the cell. Interestingly, the usage of excess binding energy to advantage is a common theme between this approach and the population of rare tautomeric and protonated states of RNA. In the second approach, oligonucleotides that mimic the natural substrate are used to tie up the RNA in an inactive conformation. In the third approach, one of the aforementioned misfolds is enriched by binding of an oligonucleotide, effectively hitting RNA in its Achilles heel.

These articles leave one with the impression of RNA as a molecule that has the intrinsic ability to “do it all”. With apparent examples of many of the chief catalytic strategies of protein enzymes in hand, including metal ion catalysis, general acid–base catalysis, and electrostatic catalysis, there appear to be few reactions that RNA should not be able to catalyze, especially with the aid of a cofactor and the application of clever *in vitro* selection schemes. This view lends credence to the “RNA-world” hypothesis. Curiously, the limiting factor in RNA’s ability to catalyze reactions may have lain not so much in what catalytic features it lacks, but in how to rein in the large number of nonfunctional ionizations and misfolded states. Perhaps it was the conformational heterogeneity of RNA that was the greatest limitation to the RNA world.

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