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RNA folding: A clear path to RNA catalysis

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A unique feature of Ribonucleic Acid (RNA) is that it can both carry genetic information and perform catalysis in the cell¹. Until the discovery of a self-splicing group I intron in *Tetrahymena*, only proteins were known to fold into complex three-dimensional enzymes capable of catalyzing the biochemical reactions of life². The process by which a single stranded RNA biopolymer achieves a catalytically active conformation is often a key regulator of biochemical processes in the cell. Folding of globular proteins is a relatively well-understood process, generally driven by the formation of a hydrophobic core³. Since RNA biopolymers are only comprised of four bases (A, C, G and U), there is no hydrophobic collapse driving folding. Instead, the RNA must follow a carefully planned path to its catalytically active conformation⁴.

In this issue, Zhao *et al.* reveal unprecedented atomic details of RNA folding dynamics through a new high-resolution crystal structure of an *Oceanobacillus iheyensis* group II intron ribozyme intermediate⁵. The group II intron assembles through an on-pathway intermediate, similar to the stepwise folding of proteins like myoglobin. Therefore, although some RNAs adopt stable misfolded states during folding⁶, others follow stepwise folding pathways sampling only native-like intermediates. By comparing this intermediate structure with the previously determined catalytically active conformation⁷, the authors reveal the molecular details of the conformational rearrangements necessary to properly choreograph a path to catalytic activity. The structural snapshot enables atomic resolution visualization of a pathway to catalysis.

RNA remains stubbornly difficult to study structurally, whether by crystallography or Nuclear Magnetic Resonance. The protein data bank (PDB) is unfortunately (for RNA at least) still aptly named; only a small fraction of three-dimensional structures deposited in the database contain RNA⁸. Catalytic RNA is one of the more amazing discoveries of modern molecular biology⁹. Self-splicing is a profoundly complex reaction that requires equivalent specificity and sensitivity to ribosomal protein synthesis¹⁰. Based on the findings of Zhao *et al.* a model for group II intron self-splicing can be envisioned⁵ (Figure 1). Fundamentally, the group II intron must excise itself from the transcript (Figure 1A) by ligating the two exons. This reaction must be carried out with single nucleotide precision. Indeed, any errors in splicing will alter the reading frame and lead to the completely wrong sequence being translated into protein.

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The ability to carry out this reaction efficiently and specifically is governed by the process of RNA folding. Secondary structure formation (specific Watson-Crick base-pairs, G/C and A/U) is very fast, and is the first step of the folding reaction (Figure 1B). The key finding reported by Zhao *et al.* is that D1 isolated from the rest of the intron adopts a significantly different conformation, which is incompatible with the final catalytically active conformation in the folded ribozyme⁷. Thus, to proceed to the fully active ribozyme, where domains D2-D6 are also folded and a fully formed catalytic site is formed, D1 must sample conformations; creating a kinetic folding intermediate. By comparing the D1 conformation isolated to the D1 conformation in the presence of D2-D6, the Pyle lab is providing the first atomic resolution structure of a large RNA folding intermediate.

One of the surprising aspects of the D1 structure is the use of rigid helical junctions and loop hinges that allow formation of a paradoxically stable and flexible scaffold in D1; rigid helical junctions are central to organizing and stabilizing the RNA. However, D1 adopts two structures: a closed structure that blocks the active site and an open structure that can accommodate the other domains and the exons. Transition between the closed and open state is governed by RNA hinges in loop regions that allow the RNA to sample different states with little energetic penalty. As the rest of the intron folds (Figure 1D), it is likely that D2 and then D3 stabilize D1 in the open state. Finally, the remaining domains can fold onto the scaffold; in fact, D5, the catalytic domain, should be able to quickly dock into place without further rearrangements, as all of its binding partners in the intron are open and available. The two steps of splicing (Figure 1E) and release of product (Figure 1F) can then occur efficiently.

This new structure by Zhao *et al.* is of particular interest given that it reveals how specific structural elements of RNA are able to confer emergent dynamic properties to the molecule⁵. Furthermore the crystal packing includes a palindromic surprise that will interest labs attempting to harness RNA/DNA self-assembly in nanotechnology. The key crystal packing interactions are mediated by symmetric tetraloop/receptor interactions (commonly occurring in structured RNAs) with helical segments containing sticky ends that are remnants of the transcription reaction. This results in a surprisingly elegant symmetric module, which will inspire novel self-assembling scaffolds with high specificity and affinity. In the end, this is what RNA folding is all about, synchronizing a series of hierarchical steps ultimately leading to a new genetic message, Now we have a better understanding of how this occurs at the atomic level.

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Stand: A new high-resolution crystal structure of the subdomain from a catalytically active group II intron reveals important conformational rearrangements necessary to achieve the fully formed catalyst. This structure provides the first atomic-resolution structure of and RNA folding intermediate.

Figure 1.

Folding to the correct three-dimensional structure is essential for the *Oceanobacillus ihayensis* group II intron RNA to carry out self-splicing. A.) the mRNA containing two exons (E1 and E2), as well as the group I intron, begins as a single stranded, unfolded molecule. B.) The RNA secondary structure then rapidly forms creating six domains (labeled D1 to D6). C.) In a critical first step of the folding reaction, D1 folds to a distinct and unique three-dimensional structure, effectively creating the core of the ribozyme. The structure must then rearrange to allow folding to proceed further with D2-D6 now folding as well. D.) When this happens, the intron is correctly folded and can carry out catalysis. E.) The first step of splicing which removes the first exon can occur through a branching pathway in which the branch point A, analogous to the spliceosome, acts as the nucleophile, or the hydrolytic pathway in which water acts as the nucleophile. The second step of splicing yields the F.) final spliced mRNA product and the lariat intron (branching) or linear intron (hydrolysis). In some cases, the intron can go on to act as a mobile genetic element. Until now, we had no atomic resolution insight into this fundamental physical process, and a new high-resolution structure by Zhao *et al.* of the D1 intermediate reveals a significant D1

conformational rearrangement, providing unprecedented molecular details into three-dimensional RNA folding intermediates.

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