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STRUCTURAL BIOLOGY RNA exerts self-control

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Abstract

A crystal structure of two bound RNA molecules not only provides insights into how regulatory riboswitch sequences affect messenger RNA regulation, but also expands understanding of RNA structure and architecture¹. See Letter p.xxx

Over the past three decades, our knowledge of the role of RNA in cellular processes has expanded enormously². For example, structures of different regulatory RNA sequences called riboswitches have uncovered how they affect transcription or translation of their downstream messenger RNA sequences by specifically recognizing and binding to ligand molecules³. Now, in a paper published on *Nature*'s website, Zhang and Ferre D'Amare¹ provide another structural insight — this time, into a bacterial riboswitch called T-box in complex with a transfer RNA molecule. Their data elucidate how an RNA molecule recognizes another RNA molecule of similar size to regulate its own transcription through a simple switching mechanism.

To support the process of protein synthesis, cells must regulate the pool of tRNAs that covalently bind to (become charged with) specific amino acid residues and deliver them to the growing protein chain. Distinct enzymes known as amino-acyl tRNA synthetases carry out tRNA charging. In Gram-positive bacteria, the T-box riboswitch upstream of each amino-acyl tRNA synthetase mRNA sequence negatively regulates its synthesis⁴.

The T-box consists of at least two independently folded domains: a sensory 'aptamer' domain that forms a long stem called Stem I and which binds to specific tRNAs; and a second domain, which can switch between two alternate conformations depending on whether the bound tRNA is charged or uncharged⁵. Whereas binding to a charged tRNA leads to termination of transcription (Fig. 1a), an uncharged tRNA stabilizes an antiterminator conformation of the T box, leading to transcription and subsequent protein synthesis (Fig. 1b).

Stem I is a roughly 100-nucleotide long RNA domain studded with several phylogenetically conserved structural motifs along its length⁴. Previous work has implicated most of these motifs in tRNA recognition and binding⁴, yet atomic-level details of this process have remained largely unknown. Zhang and Ferré-D'Amare provide the first glimpse into this mechanism by describing the crystal structure of a complex between the Stem I of the T-box of the tRNA synthetase for the amino acid glycine and an uncharged glycine-tRNA. The authors' cocrystal structure also explains the precise role of the Stem I motifs, delivers intriguing information about their synergy and offers a structural rationale for their sequence conservation.

Specific recognition of a tRNA by Stem I seems to involve two main tRNA regions: the anticodon loop and the T- and D-loops. Specifically, Stem I bends to follow closely the

tRNA and reach the anticodon loop and the T- and D-loops with its proximal and distal ends, respectively.

The interaction between the tRNA T- and D-loops and the T-box Stem I was anticipated, based on recent bioinformatics, biochemical and structural data^{6, 7}. For example, the crystal structure of a distal segment of Stem I showed⁷ two interacting loops forming a similar arrangement to the one observed in two other large and unrelated RNAs — RNase P and an RNA component of the ribosomes^{8, 9}. (Ribosomes are RNA-protein complexes that mediate protein synthesis). In these large RNAs, the interacting loops participate in tRNA recognition and binding by piling or stacking their planar nucleobases over conserved nucleobases sticking out of the tRNA.

The present structure of Stem I and tRNA corroborates the predictions and demonstrates conclusively that the interacting loops recognize the conserved tRNA ‘elbow’ in a similar fashion as in the previously observed cases^{8, 9}. The structure, however, also reveals an unexpected surprise — interactions between the three-nucleotide anticodon sequence of tRNA and Stem I are almost identical to the ones previously noted^{9, 10} between the tRNA anticodon and mRNAs associated with ribosomes.

The present paper also shows an elegant ‘mutually induced fit’ mechanism for T-box riboswitches, by which both binding partners change conformation to attain shape complementarity. Finally, whereas the inherent flexibility of a tRNA is well established¹¹, the cocrystal structure shows how Stem I turns sharply around two hinge regions to embrace the tRNA using a conserved dinucleotide bulge and K-turn motif and explains why mutations in these hinges lead to impaired tRNA binding and regulation^{1, 12}.

Zhang and Ferré-D’Amaré’s work, therefore is a major step forward towards understanding the mechanism of action of T-box riboswitches at the atomic level. It explains how a relatively small RNA molecule can recognize specifically a tRNA by maximizing interactions. Details of the other important part of the switching mechanism — namely, recognition of the charged state of the tRNA and the conformational changes that lead to regulation — remain unknown, but this study brings us closer to understanding this fascinating riboswitch in even greater detail.

An added bonus is that the authors’ structure provides truly interesting information on RNA–RNA recognition and RNA structure and architecture. Despite the paucity of structures of large RNA molecules, some commonalities are starting to emerge. In all known cases, tRNA recognition involves at least two distinct and distant areas. RNA flexibility plays a notable part in recognition and serves to maximize interactions and achieve shape complementarity. In addition, the recurrent use of a few structural motifs seems to have a large role in RNA architecture, as illustrated by the presence of several previously known structural motifs along Stem I.

The structure of the T-box Stem I–tRNA complex shows one more example of the conformational versatility of RNA that allows it to perform various functions. As we learn more about RNA structure and function, it frequently emerges that RNA molecules can execute many of the functions that are normally ascribed to proteins. One can, thus, imagine a time when many of the functions that are now performed by proteins or by proteins in complex with nucleic acids were performed solely by versatile RNA molecules. We look forward to more examples of RNA molecules performing unexpected roles in biology.

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Figure 1. RNA meets RNA

Zhang and Ferré-D'Amaré¹ present the structure of the Stem I domain of a T-box riboswitch in complex with an uncharged tRNA. Stem I bends at the dinucleotide bulge and the K-turn to recognize tRNA by binding to its anticodon loop and the T- and D-loops. a, The amino acid present in a charged tRNA is postulated to prevent interaction between the T-box sequence downstream of Stem I and the tRNA acceptor end, favouring formation of a terminator loop that stops transcription of the mRNA downstream of the T box. b, By contrast, the free acceptor end of uncharged tRNA interacts directly with the T-box sequence and leads to the formation of an antitermination loop, allowing transcription of the mRNA and its subsequent translation into a protein.