Structural Biology of Riboswitch-mediated Gene Regulation and Argonaute-mediated Gene Silencing

Dinshaw J. Patel
Memorial Sloan-Kettering Cancer Center, New York, NY, 10021

Riboswitch-mediated Gene Regulation

The 5′-untranslated regions of bacterial mRNAs can act as molecular sensors, responding to fluctuating metabolite concentrations by appropriate modulation of the expression of genes governing metabolite synthesis, transport and degradation. Such riboswitches are composed of metabolite-sensing and expression platform domains, with the sensing domain composed of highly conserved sequence and structural elements required for generation of binding pockets associated with specific metabolite recognition and discrimination against closely related analogs.

Alexander Serganov and Lily Huang in my group have solved the crystal structures of the bound state of the metabolite-sensing domains of two new large riboswitches. The secondary structure of each sensing domain contains multi-helical junctions involving large internal bubbles that form compact scaffolds on complex formation. The structures of the complexes address the following questions: What is the pattern of coaxial alignment of helical stems on complex formation? What is the pattern of loop-loop and loop-receptor interactions stabilizing the overall architecture? Are the binding pockets located at the multi-stem junctional site, and if so, how many junctional nucleotides are involved in forming the binding pocket and do they originate from different segments of the internal bubble? Do the ligands bind in an extended or fold-back conformation in the complex and does a pathway exist within the RNA scaffold for their entry and release? How are the charged ligands recognized by their respective RNA scaffolds and what is the role of ions in mediating recognition? To what extent can one design and test analogs of the ligands for their ability to modulate interaction specificity?

Argonaute-mediated Gene Silencing

Previous crystallographic studies have reported on the structures of archael Pyrococcus furiosus and eubacterial Aquifex aeolicus Argonautes (agos) in the free state. An unexpected feature of such thermophilic prokaryotic Ago’s is their preferential propensity to bind guide DNA strands and their requirement for DNA guide strand-mediated mRNA cleavage.

Yanli Wang in my group has solved the crystal structure of binary complex of Thermus thermophilus Ago bound to 5′-phosphorylated 21-mer DNA guide strand. The structures of the complexes address the following questions: Can one trace the bound guide strand in its entirety and does it interact with all domains of the Ago scaffold? In such a complex, are both ends of the DNA guide strand anchored in their respective pockets – Mid (for 5′-phosphate end) and PAZ (for 2-at 3′-end)? What about the seed nucleotides of the guide strand – to what extent are they stacked and are their Watson-Crick edges positioned for pairing with the message? What is the structure of the bound DNA guide strand at the 10-11 step, which is positioned opposite the cleavage site on the RNA?

Yanli Wang has also solved the crystal structures of a ternary complex of Thermus thermophilus Ago bound to 5′-phosphorylated 21-mer DNA guide strand and complementary message. In one such complex, the Ago protein is wild-type and the DNA-RNA duplex contains two mismatches at the 10-11 step to prevent cleavage of the message strand. In the second complex, the Ago protein contains an Asp to Ala catalytic mutant to prevent cleavage and the DNA-RNA duplex, which lacks mismatch pairs. The structures of the complexes address the following questions: Are both ends of the DNA guide strand still anchored on ternary complex formation? Do the seed nucleotides of the DNA guide strand pair with the RNA message, and how regular is the resulting DNA-RNA duplex? Does the DNA-RNA duplex at the 10-11 catalytic cleavage step adopt an A-form helical conformation, thereby adopting a backbone conformation competent for catalytic cleavage? What is the arrangement of divalent Mg cations at the cleavage site and what is the nature of the coordination to the three catalytic Asp residues? Are there conformational changes within the Ago scaffold on proceeding from the binary to ternary complexes, and do they reflect translational and/or rotational transitions?