

### Resolving the Pivotal Role of Argonaut Proteins in RNAi

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The discovery of RNA interference (RNAi)<sup>1</sup> in 1998 quickly revolutionized the tool sets of both basic biological research and applied biomedical drug development. Almost weekly new groundbreaking details of the complex set of RNAi pathways involved in the regulation of exogenous and endogenous genes in all higher eukaryotes are being unearthed. No wonder then that biophysicists are struggling to keep up with the rapidly expanding list of key players to elucidate their structure and dynamic interactions, thought critical to tapping RNAi's full biological and biomedical potential.

Argonaut (Ago) proteins were previously known to be important for plant development and *Drosophila* stem-cell division, but stepped into the limelight when their pervasive function in RNAi became apparent.<sup>2</sup> Argonauts bind small interfering RNAs (siRNAs) and other non-protein coding RNAs as guide strands to form an RNA-induced silencing complex (RISC), the effector of RNAi. Ago proteins contain four domains, the N-terminal, PAZ, Mid, and PIWI domains. The PAZ domain binds single-stranded RNA (ssRNA), while the RNase H-like PIWI domain carries the cleavage activity of RISC that destroys a target complementary to the bound guide strand.

Attesting to their ancient evolutionary origin, Ago proteins are found in all three kingdoms of life, and previous work has characterized the crystal structures of both archaeal and eubacterial Ago proteins in free and siRNA bound form. In a recent article in *Nature*, Patel, Tuschl, and co-workers describe the structure of a eubacterial *Thermus thermophilus* argonaute with all four protein domains in complex with either a 21-nucleotide or a 10-nucleotide single-stranded guide DNA analog.<sup>3</sup> The 21-mer ligand complex shows the DNA 5'- and 3'-ends anchored by the Mid and PAZ domains, respectively, and maps out the nucleic-acid-binding channel between the PAZ and PIWI domains of Ago. A comparison

with the 10-mer ligand complex shows that the 5'-anchor is lost so that both the Mid and PAZ domains significantly pivot (by 22° and 25°, respectively) around the PIWI domain. The authors suggest that a similar conformational change may accompany closing of Ago around the RNA guide strand. In addition, two arginines of Ago pivot nucleotide 11 of the 21-mer guide DNA around nucleotide 10, directly juxtaposed to the expected target cleavage site, highlighting how RISC may predispose a particular segment of the guide-target RNA duplex for subsequent catalysis. In small catalytic RNAs such kinking commonly positions a specific bond for self-cleavage,<sup>4</sup> but it remains to be seen whether the kink or its removal upon target binding plays any role in RNA cleavage by Ago. At any rate, the new crystal structures provide another snapshot on the road to fully deciphering the pivotal role of Ago proteins in RNAi.

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### References

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