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Complete pairing not needed

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Abstract

Structures of microRNA-mRNA-human Argonaute reveal where and how intermolecular interactions are specified

Gene expression can be silenced in eukaryotic cells by small noncoding RNAs that target mRNAs and prevent their translation into protein. These microRNAs (miRNAs) recruit a ribonucleoprotein complex called the RNA-induced silencing complex (RISC) and guide it to target mRNA, where miRNA-mRNA annealing allows RISC to execute translational repression (see the first figure, panel A). Argonaute (Ago) protein, a key component of RISC, is highly conserved from prokaryotes to eukaryotes and is essential for gene silencing (1). The mechanistic basis underlying the silencing initially emerged from x-ray crystallographic studies of prokaryotic Ago proteins bound as binary complexes to guide strands (DNA) and as ternary complexes with added target strands (RNA or DNA). These structural studies focused on small interfering RNA (siRNA)-mediated cleavage of target mRNA, whereas more recent studies of the same complexes in eukaryotes have focused on miRNA-mediated translational repression (2–4). On page 608 of this issue, major mechanistic insights are gleaned from structural studies by Schirle *et al.* (5) on binary and ternary complexes of human Argonaute 2 (hAgo2).

The Ago protein adopts a bilobal scaffold composed of N-terminal (N and PAZ domain) and C-terminal (MID and PIWI domain) lobes (6). The PIWI domain, which adopts a ribonuclease H (RNase H) fold, contains the catalytic acidic residues involved in siRNA-mediated target cleavage (6). Structural studies on the binary complex of *Thermus thermophilus* Ago (*TtAgo*) with a bound guide strand (in this case, DNA) identified the nucleic acid binding channel between the two lobes, with the 5′-phosphorylated end of the guide anchored within the MID domain of Ago and the 3′-end anchored within the PAZ domain (7). Notably, nucleotides 2 to 6 of the bound guide were in a helical conformation with their base edges directed outward, available for pairing with target RNA. This configuration thus constitutes the nucleation step of target recognition.

Follow-up structural studies examined ternary complexes of *TtAgo* bound to a DNA guide strand with an added target RNA (or DNA). These studies defined the pivot-like conformational changes associated with nucleation, propagation, and cleavage steps of the catalytic cycle (8). These structural studies used target strands of varying length complementary to the guide's seed segment (nucleotides 2 to 8), target-cleavage site (nucleotides 10 and 11), and the supplementary segment (nucleotides 12 to 19). The studies also defined the role of a pair of Mg²⁺ ions and acidic residues positioned within the PIWI

domain of Ago in mediating the cleavage chemistry. From a functional viewpoint, both DNA- and RNA-guided prokaryotic Agos were shown to be involved in defense against mobile genetic elements (9, 10).

Unlike siRNA-mediated cleavage of target mRNAs, which require perfect complementarity between guide and target, miRNAs require pairing of guide and target to primarily span the seed segment (nucleotides 2 to 8) of the target mRNA for translational repression and degradation (4, 11). Two enzymes, Dicer and Ago, mediate miRNA biogenesis, converting precursor miRNA to mature miRNA that is then loaded onto Ago (4). To understand the principles underlying miRNA-mediated translational repression, structural studies on prokaryotic Ago (pAgo) complexes have been extended to their eukaryotic Ago (eAgo) counterparts. These include studies of binary complexes of hAgo2 (12, 13) and budding yeast *Kluyveromyces polysporus* Ago (*KpAgo*) (14) with bound guide RNA strands. The seed segments of guide RNAs—either mixed-sequence (12, 14) or defined-sequence (13)—were anchored at both ends in the eAgo complexes, with nucleotides 2 to 5 directed outward and available for pairing with target RNA, similar to their prokaryotic counterparts. In addition, structural studies on *KpAgo* binary complexes with guide RNA identified a key glutamic acid that inserted into the catalytic pocket of eAgo to complete a catalytic tetrad (composed of four acidic residues) associated with formation of a cleavage-competent conformation (14).

Extension of these studies to ternary complexes required Schirle *et al.* to generate and purify milligram amounts of hAgo2 bound to a defined-sequence guide RNA (15), which could then be used to generate ternary complexes in amounts sufficient for crystallization and structure determination. The structural analysis was aided by x-ray data sets (at 2.9 Å resolution) of hAgo2 binary complexes with 5'-phosphorylated guide RNA (21 nucleotides in length) (see the first figure, panel B) and ternary complexes with added target RNAs (11 nucleotides in length) that were complementary to nucleotides 2 to 7, 2 to 8, and 2 to 9 of the guide (at 1.8 to 2.5 Å resolution) (see the first figure, panel C).

Schirle *et al.* show that ternary complex formation involving hAgo2 is facilitated by shape complementarity between the minor groove of the guide-target duplex that spans seed nucleotides 2 to 7, and hydrophobic residues of the interacting Ago scaffold. This explains why mismatches between guide and target are not tolerated within this region of the seed segment. By contrast, there are no such intermolecular contacts with hAgo2 for the duplex spanning nucleotides 8 and 9, explaining why mismatches are tolerated at these positions. Earlier structural studies of binary eAgo complexes had identified a kink between nucleotides 6 and 7 of the guide as a result of insertion of an isoleucine residue from helix $\alpha 7$ of the protein (12–14). Strikingly, Schirle *et al.* show that upon ternary complex formation, nucleotides 6 and 7 adopt a stacked helical conformation, with $\alpha 7$ undergoing a 4 Å shift. This change is necessary for accommodating the guide-target duplex within the hAgo2 scaffold.

Another notable feature of the binary complex of hAgo2 with defined-sequence guide RNA is that in addition to the expected anchoring of both ends of the guide and the helical alignment adopted by the seed segment, the guide strand spanning nucleotides 14 to 18

could be traced for the first time. This segment is anchored within a narrow channel between the N and PAZ domains, with their base edges directed inward and unavailable for pairing. By contrast, upon formation of the ternary complex with target RNA, nucleotides 14 to 18 underwent a profound conformational change from an unstacked to a stacked state, whereby their base edges were directed outward and available for pairing.

These distinct conformational transitions within the guide strand upon proceeding from the hAgo2 binary to ternary complex formation (see the second figure) led Schirle *et al.* to propose a stepwise model for miRNA targeting, whereby the miRNA guide strand initially interrogates mRNAs to identify candidate target sites through pairing involving seed nucleotides 2 to 5. This, in turn, triggers a concerted conformational transition in hAgo2, involving helix $\alpha 7$ and the PAZ domain, which exposes seed nucleotides 6 to 8 and supplementary segment nucleotides 13 to 16 in a helical conformation with base edges available for additional target recognition. In this model, there is no pairing involving nucleotides 9 to 12, showing that annealing within this segment is not important for translational repression. Hence, guide-target recognition spanning nucleotides 2 to 8 and 13 to 16 by Ago2 avoids the topological constraints associated with complete pairing within its nucleic acid-binding channel.

Future research should examine longer target RNAs, so as to observe guide-target pairing in miRNA-mediated hAgo2 ternary complexes spanning not only nucleotides 2 to 8, but also 13 to 16. Also, the mechanistic basis underlying siRNA-mediated cleavage of perfectly complementary target mRNAs by hAgo2 (but not by the related hAgo1, hAgo3, and hAgo4) has yet to be addressed. Such cleavage by the RNase H fold of the PIWI domain requires pairing of the guide and target spanning nucleotides 10 and 11, as well as precise positioning of a pair of Mg^{2+} cations and a catalytic tetrad of histidine and three acidic residues (8) at the hAgo2 cleavage site. Additional structural studies will also be required to elucidate the contribution of the N domain of hAgo2 in siRNA-mediated slicing of mRNA.

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Superposition. The guide (g) RNA in binary (blue) (PDB: 4W5N) and ternary (purple) (PDB: 4W5O) complexes compares nucleotides 1, 5 to 7, and 13 to 16.