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Report:

RNA interference (RNAi), an endogenous mechanism of gene silencing in eukaryotes, is based on the sequence-specific recognition of the target mRNA. Argonaute, the primary enzymatic component of the RNA-induced silencing complex (RISC), requires the sequence-dependent recognition of the target mRNA by an Argonaute / guide strand siRNA complex to cleave target mRNA. We report the crystal structure of *Aquifex aeolicus* Argonaute (AaAgo) at 3.1 Å resolution. Relative to previous Argonaute structures, our structure reveals a reorientation of the PAZ domain that opens a basic cleft between the N-terminal and PAZ domains, and exposes the guide strand 3'- binding pocket of PAZ. This leads to a branched, Y-shaped system of grooves that extends through the molecule and merges in a central channel containing the catalytic residues. Activation of the Argonaute catalytic cycle may therefore require conformational rearrangement of the PAZ domain. Based on our structure and recent biochemical studies on RISC, we suggest separate pathways for loading of Dicer-processed siRNA and the guide strand / mRNA duplex, which occupy distinct branches of the Y-shaped groove. We propose a model in which the groove between the PAZ and N-terminal domains recognizes one end of the mRNA / guide strand duplex. The groove

between the N-terminal and PIWI domains serves to accept the Dicer-processed siRNA for passenger strand cleavage and subsequent guide strand loading.