RNA INTERFERENCE

# A slice of the action

The RNA-induced silencing complex (RISC) mediates sequence-specific gene silencing by the cleavage of mRNA targets, to which it is directed by complementary, short interfering (si)RNAs. In addition to siRNAs, RISC contains proteins from the Argonaute family. Two papers in *Science* — resulting from a collaborative effort by the groups of Hannon and Joshua-Tor — now provide convincing evidence that Argonaute is the enzyme that mediates mRNA cleavage during RNA interference (RNAi). And, of the four Argonaute proteins present in mammals, Argonaute2 (Ago2) is the 'slicer' protein of mammalian RISC.

Hannon, Joshua-Tor and colleagues affinity-purified mammalian Argonaute-containing RISC complexes, and showed that only when RISC contained Ago2 was it able to cleave mRNAs that were complementary to the siRNA used. They also showed that the knockout of mouse *Ago2* produced an embryonic-lethal phenotype with severe developmental defects. In addition, mouse cells that lacked Ago2 — but did contain other Argonaute proteins — were unable to repress gene expression in response to an siRNA. This defect could be rescued by adding back Ago2.

Using relatively pure Ago2, the authors could reconstitute RISC activity *in vitro* in the presence of several different siRNAs and complementary RNA substrates. Although it is possible that a putative slicer protein co-purifies with Ago2, these findings strongly implicate Ago2 as the catalytic engine of RISC.

In parallel to the genetic and biochemical studies, the authors determined the crystal structure of an Argonaute protein from the archaebacterium *Pyrococcus furiosus*. The overall structure reveals a crescent-shaped base that comprises the amino-terminal, middle and PIWI domains, with a PAZ domain held above the base by a stalk-like region.

The recently characterized PAZ domain is thought to interact with the 3′ ends of siRNAs. The current study shows surprising insights into the PIWI domain, which has a fold that is characteristic of enzymes with nuclease activity, and closely resembles RNase H. RNase-H enzymes cleave single-stranded RNA, as guided by the DNA strand in a DNA–RNA hybrid, and produce RNAs with 3′-OH and 5′-phosphate groups that are similar to the cleavage products of RISC. Moreover, both catalytic reactions are $Mg^{2+}$ dependent. These parallels make a strong case for Argonaute being the elusive slicer enzyme.

The PIWI domain of Argonaute contains three highly conserved catalytic carboxylate groups that are contributed by one glutamic acid and two aspartate residues. And indeed, mutant Argonaute proteins in which either conserved aspartate residue was mutated lost their nuclease activity while still binding siRNA. Interestingly though, the mutation of other residues outside the 'catalytic triad' also affected the enzymatic activity of Argonaute. So, there are likely to be additional determinants, including possible interacting factors, that are needed for full slicer activity.

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## References and links

**ORIGINAL RESEARCH PAPERS** Liu, J. *et al.* Argonaute2 is the catalytic engine of mammalian RNAi. *Science* 29 July 2004 (doi:10.1126/science.1102513) | Song, J.-J. *et al.* Crystal structure of Argonaute and its implications for RISC slicer activity. *Science* 29 July 2004 (doi:10.1126/science.1102514)