MILESTONE 7

Mechanism of RNA interference discovered

Following the landmark discovery that exogenously provided double-stranded RNA (dsRNA) reduces the expression of homologous mRNAs in *Caenorhabditis elegans*, and the suggestion that such RNA interference (RNAi) may have a catalytic component (MILESTONE 4), gene silencing triggered by dsRNA was observed in many species including *Drosophila melanogaster*, trypanosomes, fungi and plants. However, the mechanism of unwinding energetically-stable dsRNA to promote the search for complementary targets remained unknown.

In 2000, two papers reported the elucidation of the mechanisms underlying RNAi using biochemical approaches. These papers suggested that the key to RNAi is the conversion of dsRNA into small RNAs, which then guide specific cleavage of complementary targets.

Work from the Hannon laboratory showed that incubation of synthetic mRNAs with extracts of *D. melanogaster* S2 cells transfected with dsRNA recapitulates RNAi in vitro. The activity responsible for the sequence-specific mRNA degradation was termed RNA-induced silencing complex (RISC).

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Pre-treatment of extracts with micrococcal nuclease abolished RISC activity, whereas DNase I had no effect, suggesting that RISC contains RNA components. By purifying RISC activity using chromatography, the RNAi-active fraction was shown to contain ~25 nt-long small RNAs that were homologous to target mRNAs. This was in line with the presence of ~25 nt small RNAs during post-transcriptional gene silencing in plants (MILESTONE 6).

Zamore, Tuschl and colleagues provided more direct evidence of the active role of small RNAs in RNAi using *D. melanogaster* embryo lysates and radiolabelled RNAs. Both strands of long dsRNAs were processed into 21–23 nt small RNAs. Interestingly, the cleavage products of the target mRNAs were produced at 21–23 nt intervals, the same interval as during dsRNA processing. These results suggested that small RNAs guide the cleavage of target mRNAs.

Shortly thereafter, several laboratories were able to identify enzymes and cofactors in the RNAi pathway using genetic and biochemical methods. These efforts revealed that dsRNAs are cleaved by the RNase III enzyme Dicer to generate small RNAs, which were termed small interfering RNAs (siRNAs). RISC comprises an Argonaute protein loaded with siRNA, which cleaves complementary mRNAs.

The discovery of the mechanism of RNAi revolutionised experimental gene regulation. siRNAs targeting complementary mRNAs can be easily designed and rapidly synthesized. Of clinical relevance is the targeting of multiple mRNA molecules by the same siRNA molecule in consecutive rounds of base-pairing, compared with drugs that remain bound to their target molecule. Thus, the discovery of RNAi mechanisms has laid the groundwork for the development of RNAi-based therapeutics.

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MILESTONE STUDIES Hammond, S. et al. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* **404**, 293–296 (2000) | Zamore, P. D. et al. RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell* **101**, 25–33 (2000).