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Silencing by stealth

Genetic manipulation can allow one to determine gene function in tractable organisms, but other systems seemed unusable for these studies, until the discovery of RNA interference (RNAi), a naturally occurring process in eukaryotes.

Studies in the mid-1980s found that introducing antisense RNA into a cell could shut down mRNA expression, but the process by which this happened was not understood. A breakthrough occurred in 1998, when Fire, Mello and colleagues examined the specific requirements for antisense RNA activity in a *Caenorhabditis elegans* system. They found that both mRNA and protein levels were reduced, and that double-stranded RNA (dsRNA) was much more effective than single-stranded RNA (ssRNA), suggesting that a model involving simple base-pairing with the mRNA was not sufficient. Unexpectedly, the effect also persisted into the progeny of the injected animals. The authors proposed that dsRNA could be used as a genetic tool to investigate the function of any coding region.

A year later, Hamilton and Baulcombe addressed the basis of a putative antiviral protection phenomenon, known as post-transcriptional gene silencing (PTGS), in plants and fungi. They set out to find the endogenous RNAs involved in this process and discovered that plant PTGS was tightly associated with the presence of ~25 nucleotide (nt) RNAs antisense to the transcripts investigated. This study confirmed that antisense RNA mediated an endogenous process.

The term RNAi became commonly used after publication of a paper by Tuschl and colleagues in 2001. By this time, it was known that longer dsRNAs were processed to 21–23 nt pieces. In this work, Tuschl and colleagues showed that 21–22 nt RNAs with short 3′ overhangs, which they called small-interfering RNAs (siRNAs), were effective in promoting mRNA degradation. They also found that the mRNA was cleaved 9–10 nt from the 5′ end of the siRNA, and that the end from which processing occurred dictated whether the active strand was sense or antisense.

From these studies, the mechanism of RNAi was sufficiently understood that researchers could begin to make specific siRNAs that would cleave mRNAs at a desired location. This technique has since revolutionized our views of the value of non-coding RNAs, the regulation of gene expression in development and the potential for specific gene silencing in a therapeutic setting.

Angela K. Eggleston,
Senior Editor, Nature

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