SMALL RNAs

Leaner, meaner siRNAs

Single-stranded small interfering RNA offers a potent and simple way to silence gene expression in the body.

Scientists at Isis Pharmaceuticals are cutting the fat from small interfering RNAs (siRNAs). These molecules trigger the RNA interference pathway to silence gene expression with remarkable specificity, making them a promising tool for gene therapy. But getting them into cells is not straightforward—the double-stranded RNA needs to be coated with complex lipid formulations that can trigger the body's immune response.

To shed the fatty layer, Walt Lima and his team at Isis exploited insights into the RNA interference machinery (Lima *et al.*, 2012). siRNAs contain a guide strand and a passenger strand and are loaded into an RNA-induced silencing complex, where they direct Ago2 protein to destroy target transcripts. Only the guide strand interacts with Ago2. “It led us to believe that we probably didn't need the passenger strand,” says Lima. Work on single-stranded siRNA has shown that uncoated single strands do not face the same problems with cellular uptake as double-stranded molecules.

Past attempts to use single-stranded siRNAs, however, ended with feeble performance in animals, in which they would rapidly fall apart. “We needed an approach to modify the molecule so that it would be stable in the animal and ... figure out where to put these modifications and still bind Ago2 efficiently,” says Lima.

The researchers raided the chemical cabinet to come up with modifications to stabilize the RNA molecule in blood serum and in cells. As a functional test, they measured how well purified Ago2 could use each synthetic RNA to degrade a target transcript in cell extracts.

A key innovation was the use of a phosphonate analog in place of the first phosphate in the RNA. The team discovered that this phosphate is critical for Ago2 interactions but is removed in cells. It took extensive searching to find a metabolically stable molecule with the right stereochemistry for activity.

Phosphorothioate-modified bases are known to bind serum protein and prevent

clearance into the urine. But these had to be alternated with another modified base to achieve the highest potency. The team also found that purines at the tail end of the message are tolerated by Ago2 and protect against 3' exonuclease, the most common RNA predator in the cell and in serum. A modified 5' terminal nucleotide attached to the phosphonate prevents degradation by 5' exonucleases.

The heavily optimized single-stranded molecule hardly resembles natural RNA—every base is modified—but the researchers showed that it can reduce the levels of three different target transcripts in liver and other tissues over days. The agents were delivered simply by injecting them via saline solution into the bloodstream or under the skin of living mice. One advantage is that the modifications can be applied to any RNA sequence. As a bonus, off-target effects of passenger strands are eliminated in the single-stranded approach.

In an impressive application, David Corey at the University of Texas Southwestern Medical Center and his colleagues collaborated with the Isis scientists to design and administer single-strand siRNAs to a mouse model of Huntington's disease (Yu *et al.*, 2012). The siRNAs selectively reduced transcript levels of a disease-inducing version of the huntingtin gene in brain tissue.

It is expected that further optimization should improve the potency of single-stranded siRNAs. The molecules are much simpler to manufacture than lipid-coated siRNAs. Lead Isis chemist Thazha Prakash points out that complex lipid formulation strategies are still not very efficient and, after all, “making one molecule is much cheaper than making two.”

Prakash makes another compelling argument for the leaner siRNAs: “So far we do not see any cellular toxicity for any of these molecules.”

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RESEARCH PAPERS

Lima, W.F. *et al.* Single-stranded siRNAs activate RNAi in animals. *Cell* **150**, 883–894 (2012).

Yu, D. *et al.* Single-stranded RNAs use RNAi to potently and allele-selectively inhibit mutant huntingtin expression. *Cell* **150**, 895–908 (2012).