RNA INTERFERENCE

siRNAs meet their match

A new study addresses how to design highly specific small interfering RNAs (siRNAs) capable of distinguishing between similar variants of the same gene.

The use of siRNAs, which are small RNAs that can reduce the expression of target mRNAs, has emerged recently as an important tool for biologists. siRNAs also have therapeutic potential as agents that could reduce the expression of mutant disease-causing genes. To unlock the full potential of siRNAs, however, it is important to understand the rules governing targeting specificity. One major challenge has been to develop siRNAs that can distinguish between two similar variants of a gene, such that one of the gene variants is knocked down but the other remains unaffected.

Recently, a team of scientists lead by Phillip Zamore, Zuoshang Xu and Neil Aronin at The University of Massachusetts Medical School in Worcester, Massachusetts, USA, tackled this problem. Zamore recalls, "when we started, not only was I certain that it would be easy to find rules for designing siRNAs that could distinguish between gene variants, I thought I pretty much knew the rules already. Needless to say, I was wrong. The task proved harder and more interesting than I had anticipated."

For much of their study, Zamore and colleagues focused on the *SOD1* gene. A single base pair mutation in *SOD1* can lead to the familial form of the neurological disease amyotrophic lateral sclerosis (ALS). Individuals with familial ALS often carry one mutated version of the gene and one normal version. Therefore, it would be desirable to find a way of reducing expression of the mutant *SOD1* while at the same time preserving the function and expression of the normal gene. As a first step toward accomplishing this, Zamore and colleagues generated a series of siRNAs to target *SOD1*. Each of these siRNAs contained, in a different position, a 1-base-pair mismatch to wild-type *SOD1*, but all of these siRNAs perfectly matched the mutant *SOD1*. The researchers hoped that some of the siRNAs in this panel would knock down the mutant version of *SOD1* without affecting the normal gene.

By systematically testing this panel of siRNAs, Zamore and colleagues found that the position of the 1-base-pair mismatch within the siRNA was extremely important for achieving selectivity; if the mismatch was located in certain positions, the siRNA was not able to effectively discriminate between the normal and mutant versions of *SOD1*, and it reduced expression of both versions. Generally, they achieved better discrimination when the mismatch was not located in the 5′ seed region of the siRNA, and the most effective location for the mismatch was at nucleotide position 16 in the siRNA. Although the reason why these mismatches are more effective at allowing discrimination than others is not completely clear, Zamore believes they may affect the mechanism through which siRNAs cause target degradation: "I suspect that these mismatches block mRNA cleavage by [the protein] Argonaute, without blocking target-RNA binding. In a sense, they cause the [RNA-induced silencing complex] RISC to bind nonproductively. Intriguingly, there are hints that nucleotide position 16 is important for plant microRNAs, which like mammalian siRNAs act by directing Argonaute to cleave the target mRNA at a single phosphodiester bond. So there must be something special about nucleotide position 16 once the siRNA guide is bound to Argonaute in RISC." In another set of experiments, the authors found that mismatches between two purines were more effective at discriminating between two similar gene variants than a mismatch between a purine and a pyrimidine.

In addition to establishing important guidelines for siRNA design, this research also resulted in the generation of highly specific siRNAs that could be useful for ALS therapy. Zamore cautions, however, that "for ALS, delivery remains a huge hurdle, so I can't predict how long it will take before we could use siRNAs as therapy for this devastating disease."

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RESEARCH PAPERS
Schwarz, D.S. *et al.* Designing siRNA that distinguish between genes that differ by a single nucleotide. *PloS Genet.* **2**, 1307–1318 (2006).