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Suck it up

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Molecular sponges bind up microRNA, preventing the downregulation of microRNA target genes.

A net designed to catch tuna may let a minnow go free. Similarly, techniques like RNA interference (RNAi) that knock down the expression of mature genes may not be appropriate to block small RNAs. How can researchers block small RNAs, including microRNA (miRNA)? Ebert *et al*. report 'sponges' that competitively bind miRNA in a recent article in *Nature Methods*.

Mature miRNAs are 19-24-nucleotide small RNAs that suppress gene expression by inhibiting translation or degrading mRNA. Approximately 30% of protein-coding genes are regulated by miRNAs, which are involved in a variety of physiological processes, from development to disease. Knocking down miRNA expression would provide insight into miRNA function; however, the size, localization and secondary structure of miRNA and miRNA precursors make them difficult targets for traditional RNAi.

Mature miRNA is integrated into a multiprotein complex that includes RNA-induced silencing complex (RISC) and Argonaute 2. This complex seeks out complementary mRNA and degrades it if it is perfectly complementary to the miRNA. However, if the mRNA and miRNA are imperfectly complementary, the activated complex blocks mRNA translation. The authors designed sponges that bind up endogenous miRNA. They made a plasmid with tandem repeats of specific miRNA-binding sites. To prevent sponge degradation, they included physical 'bulges' in the miRNA-binding sites that prevented perfect miRNA binding.

Sponges with miR-20-binding sites increased the expression of an miR-20 target reporter in cells that endogenously express miR-20, suggesting that the sponges blocked miRNA-induced downregulation of the target gene. Sponges with bulged miRNA-binding sites increased target gene expression more than sponges with perfectly matched miRNA-binding sites, consistent with RISC blockade. For all miRNAs tested, sponges increased target gene expression more than antagomirs, the modified antisense oligonucleotides that have been most successful at blocking miRNA.

The miRNA 'seed' region (nucleotides 2-7) is important for target binding. Families of related miRNAs have the same seed region, and related miRNAs cross-regulate gene targets. To eliminate miRNA regulation of a given gene, researchers previously had to design antagomirs to each miRNA family member. Sponges with miRNA-binding sites complementary to the seed region of miR-30e also derepressed expression of an miR-30c target, suggesting that one sponge can block multiple related miRNAs.

Cells transfected with sponge plasmids showed increased expression of endogenous target proteins. Perhaps sponges could be used in transgenic animals to determine the function of the miRNAs that are enriched in the brain, including miR-9, miR-124, miR-128a and miR-128b.

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1. Ebert, M. S., Neilson, J. R. & Sharp, P. A. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nature Methods* **4**, 721–726 (2007). | Article |