GENE EXPRESSION

# A matter of trust

RNA interference (RNAi) has proved to be revolutionary for scientists studying gene function, as it allows them to silence the expression of a target gene without the difficulties of making knockouts or inducing mutations. An all-important assumption when using this technique is that the effects produced are a result of the specific inactivation of a particular gene. But just how specific is RNAi? In a recent paper, Jackson *et al.* used microarray analysis to investigate this question. The results are sobering — researchers might have to rethink the way in which they carry out RNAi experiments and interpret their results.

The authors made several small interfering RNAs (siRNAs) against *MAPK14* and *IGF1R* and looked at their effects on global gene-expression patterns. If RNAi were truly specific, it would be expected that each siRNA targeted against a particular gene would affect the expression of the same set of genes: the target gene and any genes that are regulated by it. But Jackson *et al.* found that each siRNA directed against *MAPK14* or *IGF1R* had effects on a different set of genes, indicating that RNAi is not target-specific, but siRNA-specific.

To investigate this further, the authors looked more closely at the effects of one of the siRNAs against *MAPK14*. They found that for many of the genes affected, the kinetics of downregulation were similar to those of *MAPK14*, which indicated that the effect might have occurred too quickly to be a secondary effect of target-gene inactivation. In support of this, none of these genes function in the same pathway as *MAPK14*. Importantly, the authors also found that the transcripts of these genes all contained regions with partial sequence identity to the siRNA, indicating that siRNAs also downregulate genes other than their intended targets that contain similar sequences.

This is in sharp contrast to reports that RNAi is highly specific, and that changing just a single nucleotide in an siRNA can abolish its ability to downregulate its target. Jackson *et al.* suggest that a stretch of as little as 11 nucleotides of similarity might be all that is required for an siRNA to affect the expression of a gene, which means that most are likely to downregulate genes other than their intended targets. So, until there is a reliable way of predicting which siRNAs will lead to such off-target expression, it seems that researchers should take extra care to ensure that the results of RNAi experiments are biologically meaningful.

*Louisa Flintoft,*
Nature Publishing Group

**References and links**

**ORIGINAL RESEARCH PAPER** Jackson, A. L. *et al.* Expression profiling reveals off-target gene regulation by RNAi. *Nature Biotechnol.* **21**, 635–637 (2003)

**FURTHER READING** McManus, M. T. & Sharp, P. A. Gene silencing in mammals by small interfering RNAs. *Nature Rev. Genet.* **3**, 737–747 (2002)