RNA INTERFERENCE

Fine-tuning RNAi *in vivo*

An inducible microRNA-based short hairpin RNA (shRNA) construct is the basis for the tissue-specific control of endogenous gene expression in transgenic mice.

In a nutshell, the goal is total control. Scientists are no longer satisfied with deleting a gene; they want to regulate its endogenous expression in a time- and tissue-dependent manner. Scott Lowe at Cold Spring Harbor Laboratories found a way to show how it could be done.

For years the Lowe team has been working on the regulation of endogenous gene expression by RNA interference (RNAi). An important finding by the Lowe group, together with Gregory Hannon, the team of Bryan Cullen and others, was that sh RNAs embedded in a microRNA scaffold are transcribed by polymerase II and can thus be handled like a protein-encoding cDNA and, for example, be driven by a tetracycline (tet)-inducible promoter.

Lowe wanted to go beyond what he calls a chimeric setting, where cells are manipulated *in vitro* and then put back in the mouse, to a system in which the mouse expresses an shRNA that is stably integrated into its genome and can be induced to target an endogenous gene (Dickins *et al.*, 2007).

The proof-of-principle experiment was simple as far as transgenic mice go: the researchers introduced a tet-inducible shRNA construct into a fertilized oocyte via pronuclear injection and crossed the founder mice with strains expressing the tet-transactivator (tTA)—a protein needed for expression from the tet promoter—either from a universal or a tissue-specific promoter. The doxycylcin, required to kick off expression, they supplied with the 'drinking water'.

The Lowe team showed that they could target an endogenous tumor suppressor gene in specific tissues. If its expression was switched off, tumors would form; if expression came back on, tumors receded.

Lowe is convinced that the system is ready for use with any gene of interest but also cautions that one might encounter some difficulties. Not every tTA strain may drive enough shRNA to efficiently knock down the target gene. If too much shRNA is expressed, it may interfere with the processing of endogenous microRNAs.

To gain more control over the number of shRNA copies that integrate into the genome, Lowe is now working on a targeted knock-in strategy that allows single-copy integration at a known location.

The quest for total control over RNAi is progressing.

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RESEARCH PAPERS

Dickins, R.A. *et al.* Tissue-specific and reversible RNA interference in transgenic mice. *Nat. Genet.*; published online June 17, 2007.