DOI:
10.1038/nrg1865

URLs
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RNA WORLD

Designer plant miRNAs meet their targets

RNAi is widely used to knock down gene expression in plants. However, although it is a tremendously useful genetic tool, it is not perfect. The agents of RNAi, small interfering (siRNAs), are able to move between plant tissues (so their effects are not cell-autonomous), and there have been suggestions that siRNAs can affect non-complementary 'off-target' mRNAs — effects that are hard to predict. Furthermore, siRNAs are limited in their ability to silence weakly expressed targets and in many cases lead to only a low frequency of silencing. Now, two research groups have shown that 'designer' microRNAs (miRNAs) that are produced *in vivo* overcome some of these problems, providing another powerful toolkit for gene manipulation in plants.

Detlef Weigel's group previously showed that endogenous plant miRNAs are much more specific than miRNAs or synthetic siRNAs in animals. To confirm that this is due to genuine differences between the RNAi machineries in plants and animals, rather than selection against miRNAs that have broader specificities in plants, they designed a range of miRNAs to target various endogenous genes in *Arabidopsis thaliana*. In cases for which the effects of mutating the endogenous target genes were already known, the phenotypes that resulted from the overexpression of the corresponding designer miRNAs were remarkably similar to the outcomes of mutation. Weigel and colleagues also used microarrays to show that, like endogenous miRNAs, designer miRNAs (known alternatively as artificial or synthetic miRNAs) are highly specific — they knock down their predicted targets, but little else. This confirms the intrinsic specificity of miRNA-mediated silencing in plants and highlights designer miRNAs as potentially useful tools.

In independent work, Yuval Eshed and colleagues found that designer miRNAs work not only in *A. thaliana*, but also in other species including tomato and tobacco — a finding that is perhaps unsurprising given the considerable conservation of some miRNAs. This highlights the potential for this new tool to be applied to plant species that are not normally amenable to genetic manipulation.

Functional redundancy is common in plant genomes, so knocking down a whole gene family simultaneously could be very useful. Sure enough, both groups found that designer miRNAs can simultaneously target whole gene families, producing the same effects as combinations of multiple mutants. Designer miRNAs can also function quantitatively — Eshed and colleagues show that shifting the degree of complementarity of the miRNA to its target genes strengthens or weakens the resulting phenotypes when the miRNA is expressed. Finally, miRNAs that are expressed under the control of a tissue-specific promoter have largely cell-autonomous effects, which is a potentially large advantage over siRNAs.

Both groups discuss this and several other possible merits of designer miRNAs as genetic tools. For example, the remarkable specificity of these molecules raises the possibility of making the equivalent of knockouts that would be specific for particular alleles or splice variants. So, along with more established methods using siRNAs, designer miRNAs promise to make important contributions to our ability to manipulate plant genes.

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ORIGINAL RESEARCH PAPERS
Alvarez, J. P. *et al.* Endogenous and synthetic miRNAs stimulate simultaneous, efficient and localized regulation of multiple targets in diverse species. *Plant Cell* 7 April 2006 (doi:10.1105/tpc.105.040725) | Schwab, R. *et al.* Artificial microRNAs silence target genes with high specificity. *Plant Cell* 10 March 2006 (doi:10.1105/tpc.105.039834)
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Image courtesy of John Alvarez, Weizmann institute.