TECHNOLOGY

Down a hairpin

Short hairpin RNAs (shRNAs) — synthetic molecules that are modelled on small, non-coding microRNA molecules with a 'hairpin' secondary structure — can silence gene expression by RNA interference (RNAi), much as small interfering RNAs (siRNAs) do. As reported in *Nature Structural Biology*, Thomas Rosenquist's group, in collaboration with Greg Hannon's group, explored whether germline transmission of shRNA constructs was feasible in mammals, as this would enable stable, long-term silencing of gene expression.

The initial attempt of Rosenquist and colleagues to achieve germline transmission using standard transgenics methods, in which linearized constructs were injected into pronuclei to create transgenic founder animals, was unsuccessful. Choosing *Neil1* (a DNA *N*-glycosylase that initiates base-excision repair) as the target gene, they turned to a different approach — based on the use of embryonic stem (ES) cells. The authors created a single shRNA expression construct against the *Neil1* target gene, which was introduced into mouse ES cells by electroporation. Stable ES cell lines showed ~80% reduction of Neil1 protein, which correlated with a similar reduction in mRNA levels, and the cells were approximately twofold more sensitive to ionizing radiation — consistent with Neil1's role in DNA repair.

To obtain transgenic animals, the authors injected cells from two independent ES cell lines into blastocysts. The chimeric mice that contained a high percentage of ES-derived cells were outcrossed, and germline transmission of the shRNA expression construct was detected in several F1 offspring. Neil1 protein and mRNA levels were reduced in several tissues of F1 mice, as was observed in the ES cells. In addition, siRNA was detected, by northern blotting, in animals that carried the shRNA expression vector, but not in those that lacked the vector.

The authors conclude, therefore, that shRNAs can be used to create germline transgenic mice in which a target gene is silenced by RNAi. These findings open the door for tissue-specific, inducible and reversible suppression of gene expression in mice.

Arianne Heinrichs, Senior Editor,
Nature Reviews Molecular Cell Biology

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GENE EXPRESSION

The ESSENCE of correction

As many as half of the disease-associated single-nucleotide mutations in the coding regions of genes affect RNA splicing. Often, such mutations result in the exclusion of exons from mRNA, a process known as exon skipping. So, finding a way to reinstate these exons into the transcript could be an effective route to treating the underlying cause of a wide range of diseases.

Adrian Krainer and Luca Cartegni now describe the development of ESSENCE (exon-specific silencing enhancement by small chimeric effectors), which can correct these genetic typos by emulating the function of essential splicing factors called serine/arginine-rich (SR) proteins. SR proteins work by binding to exonic splicing enhancers (ESEs) and recruiting the cutting-and-pasting components of the splicing machinery through protein–protein interactions mediated by an RS domain (a domain characterized by several Arg–Ser dipeptides).

To restore normal splicing in exon-skipping models, Krainer and Cartegni fused a synthetic RS domain to an antisense fragment that binds to specific exons. They tested the ESSENCE concept first in the breast cancer 1, early onset gene *(BRCA1)*, in which a natural mutation in an ESE in exon 18 causes exon skipping. The addition of an ESSENCE compound with the exon-18-specific antisense fragment restored accurate splicing *in vitro*, and required both the antisense-targeting fragment and the synthesized RS-protein-recruitment domain.

The authors then looked at one of the best-characterized examples of an ESE associated with disease: spinal muscular atrophy (SMA), a paediatric neurodegenerative disorder caused by the loss of both functional copies of the survival of motor neuron 1 *(SMN1)* gene. SMN1 could be compensated by SMN2— a related gene product — but a single-nucleotide mutation in exon 7 of *SMN2* produces a defective isoform that lacks this exon. The authors showed that creating an ESSENCE compound that targets this mutation restored the inclusion

of exon 7 in the transcript *in vitro*. The technique is being developed to optimize *in vivo* delivery and activity, with the hope that the next generation of ESSENCE compounds will represent a viable approach for the treatment of SMA and many other genetic diseases.

Simon Frantz, Associate Editor,
Nature Reviews Drug Discovery

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