TECHNIQUE

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 now 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* (which encodes 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 *Neil1*, 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 the role of Neil1 in DNA repair.

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

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POLARITY

Two become one

Take two pathways — Cdc42–Par6–protein kinase Cξ (PKCξ), and glycogen synthase kinase-3β (GSK-3β)–β-catenin–adenomatous polyposis coli (Apc) — that have previously, but independently, been implicated in the control of cell polarity and what have you got? A single mechanism to spatially control cell polarity, report Alan Hall and Sandrine Etienne-Manneville in *Nature*.

What lay downstream of Par6–PKCξ in cell-polarity control was unknown, so Hall and Etienne-Manneville studied GSK-3β phosphorylation. Using a scratch-induced cell migration assay, they noticed an increase in the levels of GSK-3 phosphorylated at serine 9 soon after scratching. Anti-phospho-GSK-3 (Ser9) staining occurred predominantly at the leading edge of migrating cells, colocalizing with Cdc42, where Par6 and PKCξ have previously been observed. They also showed that GSK-3β and PKCξ are in a complex, which dissociates during scratch-induced migration, and that the same complex might also contain Par6. As phosphorylated GSK-3β couldn't be detected in PKCξ precipitates, phosphorylation probably causes GSK-3β to dissociate from the complex.

Inhibiting Cdc42 or PKCξ prevented GSK-3β phosphorylation, whereas transfection of Par6 or PKCξ induced GSK-3β phosphorylation. Phosphorylation of GSK-3β on Ser9 inhibits its catalytic activity, and when the authors microinjected a non-phosphorylatable, constitutively active mutant of GSK-3β (GSK-3 S9A) into leading-edge cells, they saw that it blocked centrosome reorientation, which is a measure of polarity.

As in the Wnt pathway, inactivation of GSK-3β results in the accumulation of β-catenin, but during astrocyte migration, β-catenin accumulated at the leading edge and not in the nucleus. Although β-catenin is not required for polarity, Apc, another target of GSK-3β in the Wnt pathway, is. Two to four hours after scratch-induced migration, in a Cdc42-, PKCξ- and phospho-Ser9-GSK-3β-dependent manner, Apc associated with the plus ends of microtubules at the leading edge. Apc binds to microtubules directly or indirectly through the microtubule-binding protein EB1, but EB1 localization at microtubules was independent of Cdc42, PKCξ and GSK-3β. Deletion of the carboxy-terminal domain of Apc, which contains the microtubule- and EB1-binding sites, inhibited centrosome reorientation.

So the spatially restricted association of Apc with the plus ends of microtubules — crucial for establishing polarity — arises from the action of Cdc42 on Par6–PKCξ, which, in turn, results in GSK-3β phosphorylation and its inactivation. GSK-3β might affect several other microtubule-associated proteins, too.

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