have shaped the genomes of the flowering plant lineage. A similar method has recently been used to determine when an important duplication event in *Saccharomyces cerevisiae* occurred, so this approach might become a useful tool for mapping out important evolutionary events in a wide variety of species.

Louisa Flintoft,
Nature Publishing Group

References and links

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WEBSITE

Plant Genome Mapping Laboratory:
<http://www.plantgenome.uga.edu>

GENE EXPRESSION

Emerging from hibernation

In keeping with its namesake, Hedgehog (Hh) — or, at least, components of the Hh pathway — has come out of hibernation. Thanks to work by Phil Beachy and colleagues, reported in *Science*, Dally-like protein (Dlp) and casein kinase 1 α (CK1 α) have newly assigned roles in Hh signalling.

Because classical genetic approaches to studying Hh signalling have their limitations — not least because the pathway functions during embryogenesis — the authors turned to RNA interference (RNAi) in *Drosophila* cultured cells. They transfected cells derived from the wing imaginal disc (cl-8 cells) with a Hh pathway-responsive luciferase reporter and double-stranded RNA (dsRNA) for RNAi. Their use of exogenous Hh ligand rules out components that are involved in Hh synthesis or distribution, and makes the assay quantitative. Also, because several dsRNA species can be used at a time, the assay can be used to study gene interactions and epistasis.

To test the system, the authors used a library containing dsRNAs that corresponded to all the kinases and phosphatases predicted from the *Drosophila* genome sequence. Several dsRNA pools that affected reporter activity were identified and, when re-screened, three dsRNAs emerged. Of these, CK1 α had not previously been implicated in Hh signalling.

Extending the screen to a dsRNA library based on the *Drosophila* Gene Collection Release 1 — which contains cDNAs corresponding to ~43% of the predicted genes in *Drosophila* — identified four gene targets that had not been previously

implicated in Hh signalling. CK1 α was one of them; another was Dlp, a glycoprotein heparin sulphate proteoglycan. Both are thought to function in the Wingless (Wg) signalling pathway.

Immunostaining showed that Dlp localized to the surface of cl-8 cells. RNAi of Ptc suppressed the requirement for Dlp in the Hh response, indicating that Dlp could function upstream or at the level of Ptc. As Ptc is a membrane protein, Dlp might help deliver Hh to Ptc by concentrating Hh on the cell surface and, indeed, preliminary evidence showed that Dlp associates with Hh. Consistent with such a role, when Hh was expressed in a membrane-anchored form, RNAi of Dlp did not block the signal response, which indicates that Dlp might function normally to concentrate the Hh signal.

Indications from the library screens were that CK1 α might control basal pathway activity, as dsRNA increased basal reporter activity. Ci was required for this increase, whereas Smo and Fu were not, which implied that CK1 α is upstream or at the level of Ci, but downstream of Smo and Fu.

As a regulator of the basal activity of Hh — and Wg — signals, CK1 α could function as a tumour suppressor in a number of cancers that are associated with overactivation of one or the other pathway. Similarly, deletion of a human chromosomal region that contains GPC6, a mammalian glycoprotein member that is closely related to Dlp, is associated with many human malformations. So, too, are mutations in one of the two remaining genes identified in the screen that had not previously been implicated in Hh signalling. Hopefully, discovering the functions of these genes in the Hh pathway will not be too prickly a problem!

Katrin Bussell, Associate Editor,
Nature Reviews Molecular Cell Biology

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