EVOLUTION

Double dating

Duplication — be it of a single gene, a chromosomal region or a whole genome — is a powerful tool for evolution. Being able to tell when duplication events took place is important for analysing the relationships between species. But the numerous rounds of copying and subsequent loss of genes, especially in plants, make carrying out comparative genomics studies a daunting task. A recent study by Bowers *et al.* describes how these difficulties might be circumvented to understand how duplication events have shaped the evolution of plant genomes.

Most studies date duplication events using a 'molecular clock' method, in which the number of nucleotide differences between a pair of duplicated genes is counted. The larger the number of differences, the longer the period since the duplication took place. This measurement is then calibrated against the number of changes that occur in a known period of time, for example, by using the fossil record.

Because different molecular clocks can run at different speeds, Bowers *et al.* chose a different approach. Instead of trying to put a precise date on duplication events, they concentrated on mapping them in relation to the divergence of different species — an all-important factor in comparative genomics. They compared duplicated *Arabidopsis* genes to those from other plants, and determined the amount of difference between pairs of genes from the different species. This allowed them to work out whether the duplication happened before or after the divergence of the various species.

In this way, the authors identified several important duplication events in *Arabidopsis* evolution. The first of these is extremely ancient, dating back to before the evolution of the flowering plants. A second event took place after the monocotyledons and dicotyledons went their separate ways. Relatively recently, a third duplication occurred in a common ancestor of *Arabidopsis* and cabbage.

So, without calculating actual time periods, Bowers *et al.* have pinpointed three major events that

DEVELOPMENTAL GENETICS

Picking up the signals

Faulty autoprocessing of Hedgehog (Hh) proteins was thought to be the link between mutations in genes involved in cholesterol biosynthesis and the developmental abnormalities that they cause. However, Michael Cooper and colleagues now show that it is more likely that an impaired ability to respond to the Hh signal is the culprit.

Cholesterol biosynthesis disorders cause birth defects in structures and organs patterned by Hh signalling. Cholesterol also has an integral role in the Hh signalling pathway, helping to cleave and ultimately replace the autoprocessing domain of the Hh precursor. So, impaired autoprocessing of the precursor has been the prime suspect in investigations of the molecular basis of these defects.

Cooper and colleagues administered cyclodextrin — which depletes cholesterol — to chicken embryos and studied their development. They found that the facial abnormalities caused by cholesterol depletion mimic those that are caused by alkaloids that inhibit Hh signalling and, in more severe cases, phenocopy Sonic hedgehog (*Shh*) mouse knockouts.

They then showed that decreasing cholesterol levels reduced the sensitivity to Hh signalling, because the high-threshold response in neural-plate explants, induced by recombinant Shh protein, could be converted to an intermediate-level response by administering high levels of cyclodextrin. Lower levels of cyclodextrin were less inhibitory, which indicates that the level of cholesterol affects the response to Hh signalling.

So, cholesterol affects both Hh signal production and response — but which has a bigger role in the abnormalities that are caused by cholesterol biosynthesis disorders? The authors neatly address this question by looking at the effects of signal production and response in cell lines from mouse models of cholesterol biosynthesis disorders. They show that these cells retain their ability to autoprocess Shh protein — even when cholesterol depleted — probably because cholesterol levels are only reduced by ~50%. This is a reasonable model for the situation in individuals with cholesterol biosynthesis disorders in whom cholesterol is reduced but never absent. By contrast, the responses of these cell lines to exogenously supplied Shh signal were clearly impaired.

Cooper and colleagues go on to show that sterol depletion could block pathway activation by Smoothened (Smo), a membrane-bound protein that is a key activator in Hh responding cells. This indicates that Smo is probably the link through which cholesterol influences Hh signal response.

Taken together, these studies indicate that mutations of cholesterol biosynthesis probably cause developmental abnormalities by decreasing Hh pathway activation through their effects on Smo. What remains to be discovered is exactly how this occurs.

Perhaps the most important lesson from this work is that the most obvious answer is not always the right one. In future studies of developmental abnormalities we would do well to keep an eye on all the possible influences that a defect might have on the signalling pathway involved.

Nick Campbell

References and links

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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 cereviseae* occurred, so this approach might become a useful tool for mapping out important evolutionary events in a wide variety of species.

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Nature Publishing Group

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WEBSITE

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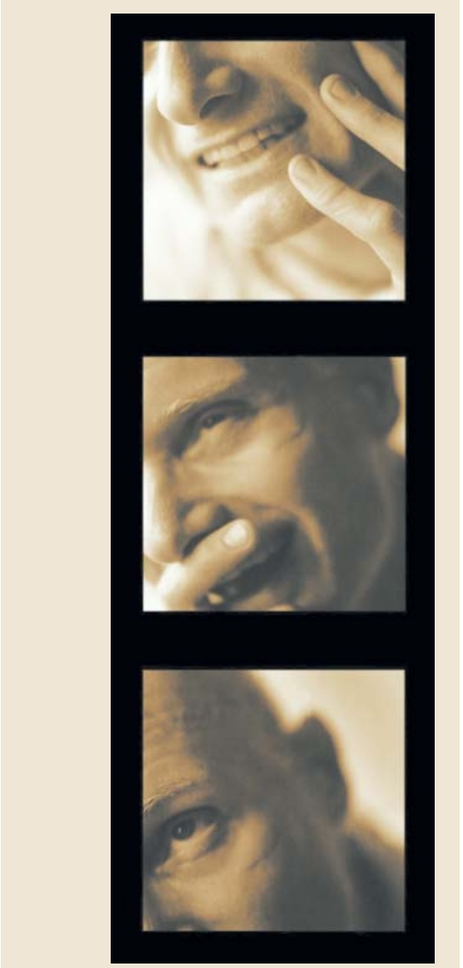

GENE EXPRESSION

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 1α (CK1a) 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, CK1α 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. CK1α was one of them; another was Dlp, a glycipan 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 CK1α 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 CK1α 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, CK1a 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 glycipan 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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