



## 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 dupli-

cated *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 Smoothed (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

**ORIGINAL RESEARCH PAPER** Cooper, M. *et al.* A defective response to Hedgehog signaling in disorders of cholesterol biosynthesis. *Nature Genet.* **33**, 508–513 (2003)

**FURTHER READING** Tabata, T. Genetics of morphogen gradients. *Nature Rev. Genet.* **2**, 620–630 (2001)