Plant development

The flowers that bloom in the spring

Deciding when to flower is of crucial importance to plants; every season has advantages and disadvantages, and different plant species adopt different strategies. Elsewhere in this issue, Sibum Sung and Richard M. Amasino (*Nature* **427,** 159–164; 2004) and Caroline Dean and colleagues (*Nature* **427,** 164–167; 2004) investigate how such decisions are made at the molecular level. They uncover a mechanism that prevents the model plant *Arabidopsis thaliana* (pictured) from blooming until the coming of spring.

Plants take a variety of environmental factors into account when choosing when to flower, such as the length of the day, the plant's age and the requirement for an extended cold period (a process called vernalization). All of these factors work in part through the gene *FLOWERING LOCUS C* (*FLC*), whose protein product blocks flowering by repressing numerous genes required for flower development. During a prolonged cold spell, for example, the normally high levels of expression of *FLC* are lowered, remaining low even after warm weather returns.

Several genes are needed for vernalization: Dean and colleagues studied two of these, *VRN1* and *VRN2*, whereas Sung and Amasino identified another, *VIN3*. All three encode proteins with counterparts in animals that either bind DNA directly, or change the structure of the chromatin into which DNA is packaged.

Following this lead, the two groups found that vernalization induces changes in histone proteins (components of chromatin) in the vicinity of the *FLC* gene — and that *VRN1*, *VRN2* and *VIN3* mediate these

changes. Specifically, cold causes the loss of acetyl groups from particular lysine amino acids in histone H3. Such patterns of deacetylation mark genes that are permanently inactivated or silenced. The researchers found that whereas *VIN3* is needed to deacetylate H3 during a cold snap, *VRN1* and *VRN2* are required afterwards, to maintain the silenced state.

Interestingly, these changes in histone acetylation are confined to a region of the *FLC* gene that was recently shown to contain a binding site for the FLOWERING LOCUS D (FLD) protein (Y. He *et al. Science* **302,** 1751–1754; 2003). FLD is related to a component of the human histone deacetylase complex, and is also involved in promoting flowering by silencing *FLC*. Plants lacking FLD show both high levels of histone acetylation and a considerable reluctance to flower.

Silencing is an effective means of controlling long-term gene expression, as it persists even after cells divide. In animals, switching silencing on or off is a well-known way to control development. It seems that plants share this system, using it to preserve the memory of winter's passing. **Christopher Surridge**

support of this, the authors discover that ligand-dependent activation of Notch in cultured cells is sensitive to Ca^{2+} concentrations in the range observed around the chick node.

These findings provide a convincing picture of how Notch can trip the Nodal switch asymmetrically. The Nodal gene is, however, expressed only in a restricted region immediately neighbouring the node (Fig. 1), whereas the Ca^{2+} concentration increases in a much broader domain. Raya *et al.* show that this spatial restriction depends on a second input to the Notch pathway. The Notch ligands Dll1 and Srr1 are expressed on both the left and right of the node, in regions that abut at an interface that lies roughly perpendicular to the embryo's head-to-tail axis. It is around this interface on the left of the node — where Ca^{2+} levels are high — that Nodal is expressed (Fig. 1). This is not a coincidence: Raya *et al.* find that experimentally disrupting this interface results in loss of left-sided Nodal expression.

A third input is required to determine the time at which the Notch pathway turns on Nodal expression. Raya *et al.* show that the Lunatic fringe (Lfng) protein is an essential component of this input. The expression of this protein is highly dynamic — several short pulses of Lfng expression sweep up the embryo from tail to head[7]. Raya and colleagues' findings suggest that, as these pulses cross the Dll1–Srr1 interface, they enhance Notch activation. On the left of the node, where Notch activity is already higher than on the right because of the asymmetry in Ca^{2+} levels, the fifth wave of Lfng expression raises Notch activity to a high enough level to allow Nodal to be expressed (Fig. 1).

This work represents a significant advance in our understanding of how left–right asymmetry is established. It shows for the first time how transient non-genetic biases can become fixed in stable asymmetric patterns of gene expression. It also provides a concrete example of a patterning mechanism that is driven by the spatial modulation of a kinetic parameter (the affinity of Notch for its ligands)[8]. A central role is played by the Notch pathway, which acts as a robust signal integrator and amplifier, using three disparate inputs to ensure that Nodal is expressed at the correct time and place. Raya and colleagues' approach illustrates the benefits that can be gained by exploiting the complementarity of theoretical and experimental approaches, especially in systems as complex as vertebrate embryos.

There are, of course, a few gaps yet to fill. Most obviously, how is left–right symmetry broken in the first place? In mice, an attractive candidate for the symmetry-breaking event is the right-to-left flow of extracellular fluid seen around the node[9]. The motile cilia that generate this flow have been observed in several different vertebrates before left-sided Nodal expression is established, prompting speculation that fluid flow has an evolutionarily conserved role in generating left–right asymmetry[10,11]. But expression of Notch around the node and fluid flow (or its consequences) appear to be largely independent of each other[4,5]. It is intriguing that fluid flow also generates a brief increase in Ca^{2+} levels to the left of the node — although this rise is intracellular rather than extracellular[12]. Perhaps these seemingly parallel mechanisms are somehow integrated at the level of Nodal expression.

There are further issues. How does the juxtaposition of Dll1 and Srr1 expression enhance Notch activity? How is this potentiated by Lfng? And are there parallels with the activation of Notch at Fringe-demarcated boundaries in fruitflies? The dramatic progress made in recent studies has opened up many new fronts on which to explore these fascinating questions. ■

Nick Monk is at the Centre for Bioinformatics and Computational Biology and in the Department of Computer Science, University of Sheffield, Regent Court, 211 Portobello Street, Sheffield S1 4DP, UK.
e-mail: n.monk@shef.ac.uk

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