protein synthesis, and the inhibition of DNA replication following stress-induced release of the protein nucleolin[8].

There has been a remarkable convergence of recent evidence — including the Rubbi and Milner paper[1] — suggesting that nucleoli are important in monitoring cellular stress. The health of the nucleolus is an excellent surrogate for the health of the cell, and conditions that lead to nucleolar disruption are unlikely to be safe for continued cell proliferation. The notion that intact nucleoli are necessary to hold the p53 response in check provides an attractive model in which a default pathway of p53 induction and inhibition of cell growth is overcome only by the maintenance of nucleolar well-being. These ideas reinforce the growing realization that the nucleolus — long regarded as a mere factory for assembling ribosomal subunits — is a vital command unit in monitoring and responding to stress. ■

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Developmental biology

Asymmetric fixation

Nick Monk

Computer simulations and laboratory experiments have shed light on how an asymmetric pattern of gene expression is fixed in vertebrate embryos — an early step towards asymmetric development of the internal organs.

As judged by external appearances, the left and right sides of vertebrate bodies are (more or less) identical. There are, however, consistent left–right differences in the structure and placement of the internal organs. The heart, for instance, usually forms on the left, the liver on the right. In recent years, researchers have uncovered several different molecular events that are involved in establishing this left–right asymmetry as embryos develop[1]. But the picture that has emerged from these studies contains significant gaps. The paper by Raya *et al.*[2] on page 121 of this issue goes some way towards completing this picture, revealing an explicit link between an early, temporary asymmetry and later, stable patterns of asymmetric gene expression.

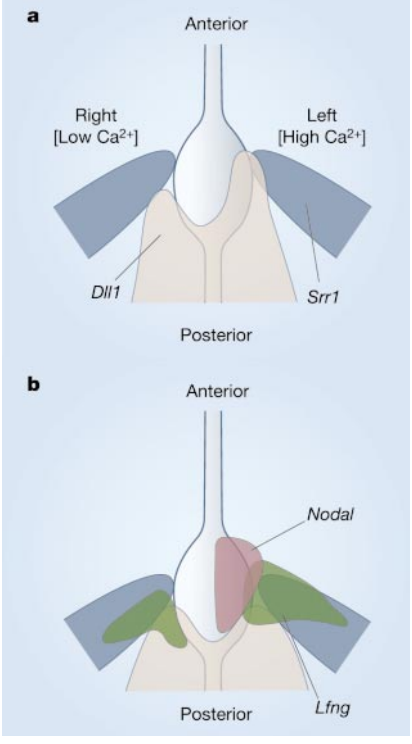

Figure 1 **Fixing asymmetry in vertebrates. According to convention, embryos are viewed from the 'front' — so the left-hand side of the embryo appears on the right of this diagram. An early manifestation of asymmetry in chick embryos is the expression of the *Nodal* gene on the left of the 'node' (oval). Raya *et al.*[2] put forward a model for how this occurs. It was known from studies in mice that *Nodal* expression depends on the Notch pathway, which is in turn activated by Dll1 and Srr1.** a**, At stage 5 of development (19–22 hours after fertilization), *Dll1* expression extends further towards the head (the anterior) on the left than on the right. This is the earliest indication that Notch activity is higher on the left (as *Dll1* is a target of Notch activity).** b**, During stage 6 (23–25 hours after fertilization), the *Dll1* and *Srr1* expression domains are symmetrical. But, as the fifth pulse of expression of the *Lfng* gene sweeps up the embryo, it moves further to the anterior on the left. *Nodal* expression is then induced around the boundary between *Dll1* and *Srr1* expression. This occurs only on the left, where the Ca^{2+} concentration is high; this might enhance the affinity of Notch for its ligands. Note that the node 'regresses' posteriorly between stages 5 and 6.**

The events that lead to the initial breaking of left–right symmetry in vertebrate embryos are not fully understood, but they are believed to provide only weak transient biases[3]. So additional mechanisms must exist to amplify these biases, converting them into stable and heritable asymmetric patterns of gene expression[1]. The earliest detected feature of left–right asymmetry that is common to all vertebrates studied is the expression of the secreted growth-factor protein Nodal on the left side of the 'node'. This region, located on the midline of the embryo, acts as an organizing centre during development. In mice, Nodal expression has been shown to depend on a second signalling pathway, centred on the cell-surface-located receptor Notch[4,5]. But how the Notch pathway becomes activated to a sufficient degree to trigger Nodal expression only on the left side of the node remains an open question.

Raya *et al.*[2] use a combination of modelling and experimentation to address this problem in chick embryos. Having determined the patterns of expression of various key genes around the node, the authors capitalize on this information to construct a mathematical model of the network of molecular interactions underlying Notch activation and Nodal expression. As Nodal enhances its own production, it can act as an on–off switch: only a transient increase in activity of the Notch pathway is required to induce stable Nodal expression. Raya *et al.* find that the simplest way to achieve this in their model is to enhance the affinity of Notch for its activating partners (ligands) — the Delta-like 1 (Dll1) and Serrate 1 (Srr1) proteins. So the model suggests that a transient lateral bias in this affinity should be enough to convert the initially symmetric pattern of gene expression into one that is manifestly asymmetric.

The authors carry out a range of experiments that show that this is indeed the case. In doing so, they uncover a chain of events that lead from a left–right asymmetry in the electrochemical potential across the membranes of cells around the node, to the left-specific expression of Nodal. The first step in this cascade is a previously described left-sided reduction in the activity of a membrane-spanning ion pump (the H^+/K^+-ATPase); this reduction results in membrane depolarization[6]. Raya *et al.* find that this depolarization leads to a transient increase in the extracellular concentration of Ca^{2+} ions on the left of the node. And this in turn is necessary for left-sided Nodal expression — suggesting that it could be Ca^{2+} that modulates the affinity of Notch for its ligands. In

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. ■

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