Wnt/Frizzled pathway at work in vertebrate embryos. Early studies of the South African clawed frog, *Xenopus laevis*, established that the overexpression of some Wnt proteins stabilizes β-catenin and results in twinned embryos, complete with two heads. Yet overexpression of other Wnt proteins perturbs cell movements during gastrulation[3] — a crucial stage of development that leads to formation of the three main tissue layers: endoderm, mesoderm and ectoderm. These different effects hinted that there is more than one Wnt pathway in vertebrates.

The idea was substantiated by further work in zebrafish and *Xenopus* embryos, which suggested that the Wnts that perturb cell movements activate a non-canonical Wnt/Frizzled pathway[3] involving increases in intracellular Ca^{2+} levels and the consequent activation of the enzymes protein kinase C (PKC) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)[5]. But questions remain, including the relationship between the non-canonical pathways in fruitflies compared with vertebrates, whether the Wnt/Ca^{2+} pathway works in any context other than early embryos, and whether it modulates gene expression or only gastrulation movements.

Saneyoshi *et al.*[2] enter this arena of uncertainty by proposing that if signalling from certain Wnt proteins leads to rises in intracellular Ca^{2+} levels, it may also activate calcineurin, a Ca^{2+}/calmodulin-dependent protein phosphatase, leading to the removal of phosphate groups from NFAT and hence its accumulation in the nucleus. And indeed, they find that Wnt5A and Frizzled-2 — the same Wnt–receptor combination reported to lead to intracellular Ca^{2+} increases in zebrafish and to activate CaMKII and PKC in *Xenopus* — strongly induces the movement of NFAT into the nucleus in cells of *Xenopus* embryos.

The authors also observe that overexpression of a constitutively active NFAT perturbs the cell movements that occur during gastrulation in *Xenopus*, and encourages cells to take on characteristics typical of the belly (ventral) rather than the dorsal part of the embryo. Finally, this hyperactive NFAT antagonizes the duplication of the dorsal axis that can be induced by the canonical Wnt pathway. Similar effects have been seen[3] with overexpression of Wnt5A. So Saneyoshi *et al.*'s findings support the existence of the Wnt/Ca^{2+} pathway in *Xenopus*, and identify NFAT as a likely downstream target of this pathway.

What, then, is the most plausible role of normal Wnt/Ca^{2+} signalling and NFAT in early frog embryos? Saneyoshi *et al.*'s data lend much weight to the previous suggestion[5] that this non-canonical pathway helps to determine a ventral fate for cells and opposes the canonical pathway, which promotes dorsal cell fates in early embryos (Fig. 1, previous page). In support of this, Saneyoshi *et al.* show that expressing a presumed inhibitor of NFAT in frog embryos results in a new dorsal axis, as would be predicted if the NFAT had been promoting ventral cell fates.

So the new data are a tantalizing hint that Wnt/Ca^{2+} signalling promotes ventral cell fates by activating NFAT. But there are questions to be answered before we can be sure. First, does NFAT actually activate gene transcription in response to a Wnt/Ca^{2+} signal? Saneyoshi *et al.* show that, in the presence of one of its cooperative partners — the transcription factor AP-1 — a constitutively active *Xenopus* NFAT activates transcription of a 'reporter' gene inserted into *Xenopus* embryos. But it is not yet certain whether normal NFAT would do so in response to Wnt/Ca^{2+} signalling, or whether Wnt5A leads to the transcription of NFAT-responsive genes, as would be predicted. There is evidence that Wnt5A regulates AP-1-dependent transcription as well as Ca^{2+}-mediated signalling[6]. So the Wnt/Ca^{2+} gene targets may encompass those regulated by AP-1 alone, or those regulated by complexes of AP-1 and NFAT.

Second, Saneyoshi *et al.*'s data predict that more NFAT proteins will be found in cell nuclei on the future ventral side of *Xenopus* embryos than on the future dorsal side. If that is the case, does this nuclear accumulation depend on Wnt signals? The activity of CaMKII is increased on the future ventral side in a Wnt-dependent manner[5], but it remains to be seen whether the nuclear accumulation of NFAT is likewise increased. Third, we need to test the proposed role of NFAT by knocking out its function, using genetic or molecular means.

Finally, it will be interesting to see whether the Wnt5A-driven movement of NFAT to the nucleus is unique to early vertebrate development, where all characterization of the Wnt/Ca^{2+} pathway has been conducted to date. Unpublished work from Chris Hughes (Univ. California, Irvine) suggests the contrary: that this Wnt protein also induces NFAT to accumulate in the nuclei of the immune system's T cells. Whatever the answers, it is clear that it takes many Wnt pathways to make a frog — and, by extension, any vertebrate. ■

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Daedalus

Augmented eggs

The size of an egg is one of evolution's battlegrounds. The chick wants the biggest possible egg, to aid its development; the mother wants the smallest possible egg, to help her to lay it. Neither party gets its ideal. Daedalus now has a compromise. He wants the egg to expand after being laid.

Sadly, all birds' eggs have a rigid carbonate shell. So DREADCO biologists are breaking fertilized eggs with great care. They are opening them into larger glass or plastic cavities, filled with distilled water or nutritive solution and sealed to the unbroken section of shell with silicone resin. The chick within will find plenty of room to develop further, before having to break its way out. Mother birds may not want to sit on and hatch augmented eggs, although some females appreciate outsize ones. Design problems will loom large. It will probably be easier to hatch augmented eggs in an incubator.

In normal conditions, most birds hatch fairly simple-minded offspring — Daedalus recalls Konrad Lorenz's goslings, which followed him because he pretended to be their mother. But a chick that develops in an expanded egg would take longer in development, and could be far shrewder than average. Even more cunning, imagine two eggs sealed together by a tube the diameter of one of them, the extra space filled with nutrient solution. Would one chick be aware of the other? Would they develop as a clever pair, tackling problems neither could work out alone? Might they even have some subtle avian empathy? A whole new type of bird might result from this research. It could emerge from the augmented egg with much enhanced mental or physical powers.

Daedalus is not sure whether to try the idea on domestic fowl. They are selected for stupid tractability; bright ones could be very troublesome. But ducks seem to have a lot of enterprise already. They could be ideal. The new improved chicks might swim or fly better, or be stronger and more decisive. They could come to dominate their fellows. Even so, an augmented duck that came to dominate a flock of them would be unable to hand on its abilities to the next generation. So Daedalus's egg augmenters are concentrating on birds that have recently lost the power of flight, such as penguins, kakapos and certain rheas. Is the ability to fly still inherent in a chick? If so, could it be recovered by egg augmentation?

David Jones