DEVELOPMENTAL BIOLOGY

Limbs make progress

According to the well-established progress zone (PZ) model of vertebrate limb development, undifferentiated cells acquire positional information in the PZ — a region at the distal end of the developing limb. The fate of these cells is set by how long they spend in this zone: cells that leave early contribute to proximal limb structures and cells that leave late, to distal ones. This theory neatly explains why removing the apical ectodermal ridge (AER) — which overlies the PZ and is a likely source of signalling molecules, such as the FGFs (fibroblast growth factors), that are involved in distal limb patterning — from developing limbs causes the truncation of distal structures. Now, two *Nature* papers question the validity of this model and shed new light on the cellular events that occur during limb development.

Gail Martin's group took a genetic approach to investigating limb development by conditionally inactivating both *Fgf4* and *Fgf8* in the mouse limb. Although the hindlimbs of this mutant do not form, their forelimbs do, owing to the early transient expression of *Fgf8* and *Fgf4*. These limbs have normal proximal structures, as predicted by the PZ model, and more distal elements. However, they often lack middle limb structures — a phenotype that the PZ model, which requires that distal elements are specified only after more proximal ones, cannot easily explain. Sun *et al.* suggest that the poor development of mutant forelimb skeletal structures is due, not to a lack of progressive specification, but to the loss of skeletal precursor cell populations, as assayed by *Sox9* expression, caused by abnormal cell death in the most-proximal part of mutant limb buds.

Cell death also features in the study by Cliff Tabin's group. Dudley *et al.* found that, on AER removal, a region of cell death would extend 200 μm from the bud's distal tip. Could this cell death alone account for the distal limb truncations that occur on AER ablation? If this cell-death zone remained constant in size over several developmental stages, as indicated by the authors' studies, then a proportionally larger part of the distal limb would be lost at each stage.

According to the PZ model, on AER removal, distal cells should incorporate into proximal populations as their distal fate should not yet be fully specified. Instead, Dudley *et al.* found that distal cells die, while proximal cells expand and develop. Together these data indicate that patterning after AER loss might reflect the proportion of cells that lie outside the AER's influence — and that therefore remain unaffected by the changes in cell death and proliferation caused by its removal — rather than the level of patterning achieved by a particular developmental stage, as predicted by the PZ model.

In subsequent cell-fate mapping and embryological studies, the authors found evidence to indicate that positional specification and fate committment might be two distinct events in limb development. By labelling proximal, middle and distal cell populations, they found that cells in these regions do not intermix, indicating that they might be positionally specified at an early stage. Grafting experiments supported this conclusion, and additional cell re-aggregation studies indicated that the fate of distal cells is not fixed until later in limb development.

The new picture of limb development proposed by Tabin's group is one in which cell fates are established early, rather than progressively, and are then fixed in a proximal–distal wave of precursor-cell expansion and differentiation. Martin's group report limb phenotypes that cannot readily be explained by the PZ model, and provide new evidence on the involvement of FGFs in preventing cell death, in influencing the initial size of the limb bud, and in maintaining cell proliferation and gene-expression programmes during limb development. Although dispensing with the PZ model might be premature, further progress in the field might yet lead to its downfall.

Jane Alfred, Editor,
Nature Reviews Genetics

References and links

ORIGINAL RESEARCH PAPERS Sun, X. *et al.* Functions of FGF signalling from the apical ectodermal ridge in limb development. *Nature* **418**, 501–508 (2002) | Dudley, A. T. *et al.* A re-examination of proximodistal patterning during vertebrate limb development. *Nature* **418**, 539–544 (2002)

IN THE NEWS

Why I get a kick out of coffee

Have you ever wondered why some people (like me) need several cups of rocket-fuel-strength espresso just to get out of bed, whereas others can go all day on the strength of just one instant coffee?

According to a paper in *Nature*, this is due to the phosphorylation and dephosphorylation of a protein called DARPP-32.

"When a mouse drinks coffee or when a scientist injects it with caffeine … the caffeine stimulates the mouse's nerves. The mouse gets jittery, its heart rate increases, and it runs around. Once the effects from caffeine get started, DARPP-32 keeps them going", said *CNN* (14 August 2002).

"When this protein is combined with small doses of caffeine, it helps reduce the amount of other brain chemicals which would inhibit excess nerve activity", explained *BBC News* (14 August 2002). "The caffeine/protein combination also subdues a protein called kinase A, whose job it is to stop DARPP-32 working. The net effect is a circular one, with the combination working to effectively knock down various mechanisms designed to end the caffeine buzz".

"DARPP-32 keeps us going until the next coffee break by extending the effects of the last cup", wrote Dr Jean Marie Vaugeois in the accompanying News and Views article in *Nature*. "This knowledge should provide an even better understanding of caffeine's effects. So, wake up, smell the fresh coffee and enjoy its effects for a long time, thanks to your dependable DARPP-32!"

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