Box 1 Evolution of fins and limbs

Four-legged (tetrapod) vertebrates are a subdivision of the lobe-finned fish. As such, forelimbs and hindlimbs are actually modified pectoral and pelvic fins. Most vertebrates have two sets of paired limbs or fins, but none have more than two. These appendages are built along a similar plan and have to solve many of the same problems during development, such as initiation of budding, outgrowth, and patterning the skeleton along three axes.

Although fin development has not been studied in lobe-finned fish, analyses of fin development in zebrafish (a more distantly related, ray-finned fish) have uncovered remarkable similarities with limb development at the cellular and molecular levels. Some of these characteristics are global features of all animal outgrowths, but others specifically highlight the homology of fins and limbs.

Fin and limb buds are patterned by a posterior polarizing region, which expresses the *Sonic hedgehog* gene, and bud outgrowth is sustained by fibroblast growth factors secreted by epithelial cells at the distal tip (the apical ridge; Fig. 1). These features reflect the common origin of fins and limbs.

Studies of the transcription factor dHAND in a variety of vertebrates[1–3] indicate that its role in establishing the polarizing region is also common to fins and limbs. This role of dHAND may be as ancient as paired fins themselves — the earliest known fin skeletons have anterior–posterior asymmetry. **M.J.C.**

results in a failure to activate *Shh*, and in the fish causes an absence of paired fins (the mice die shortly after limb budding). At face value, these data suggest a linear genetic cascade of induction, although — as ever — the reality is more complex.

Molecular feedback loops are an integral part of limb development, and several of the new results point to a regulatory feedback loop between dHAND and Shh. Overexpression of either *dHAND* or *Shh* can induce the expression of the other gene. Moreover, mice in which dHAND is knocked out do not express *Shh*, and in Shh-knockout mice *dHAND* expression is severely reduced[1,2]. Shh also participates in a positive feedback loop with signalling molecules known as fibroblast growth factors (FGFs), and these also seem to be needed for dHAND expression[2]. Further experiments should determine whether FGFs regulate Shh and dHAND independently.

Understanding the mechanism by which dHAND functions in the vertebrate limb should be helped by analyses of its roles in heart, paired-fin and face development. In zebrafish *Hands off* mutants — which have severe defects of the heart, pectoral fins and jaws — precursors of the heart and pectoral fins are specified but then fail to differentiate. It is not yet known what happens to these cells, but in *dHAND*-knockout mice, underdevelopment of the branchial arches (from which structures such as jaws and gills form) and the right ventricle of the heart is a result of extensive cell death through apoptosis[4,5].

In any developing system that requires a large increase in the number of cells (as in heart, face, fin and limb development), the benefits of proliferation are realized only if the cells are kept alive[8]. If limb budding requires not only cell proliferation, but also active inhibition of apoptosis, then one interpretation of the limb and fin defects in dHAND mutants is that cells in the limb bud suffer the same apoptotic fate as cells in the face and heart of dHAND-knockout mice.

The results of *dHAND* overexpression may also be consistent with this interpretation. Mesenchymal cells at the anterior margin of limb buds undergo apoptosis during normal limb development, but cell death is attenuated or absent in several multidigited mutants[9]. Moreover, anterior limb-bud cells, which normally lack polarizing activity, can develop it under experimental conditions[10]. So if the normal function of dHAND is to keep cells with polarizing potential alive in the posterior part of the limb, then overexpression of dHAND might prevent apoptosis in anterior cells, which in turn could induce digit duplications. The ability of Shh, retinoic acid and polarizing-region grafts to induce dHAND expression suggests a mechanism by which the polarizing region could ensure its own survival. The situation might be similar in the face, where Shh is needed for neural crest cells to survive[11]. Whether dHAND really does exert its effect on the limb by modulating cell death remains to be tested experimentally. But if true, we may have a new tool for studying the cell biology of embryonic development. ■

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Daedalus

Power in the dust

Last week Daedalus devised his 'Stressed Powder', whose tiny ring-particles were needle crystals bent into a highly strained circular form. A suitable shock, rupturing the tense rings, releases their enormous energy density. DREADCO's chemists are now crystallizing many different substances as Stressed Powders, and exploring their properties.

Hard materials such as metal oxides should, if undisturbed, hold their stress indefinitely. Daedalus hopes to exploit such Stressed Powders as long-lasting, non-chemical insecticides. He points out that insects, being so small, are greatly at risk of dehydration. Even a minute crack in their exoskeletal armour allows their vital fluids to evaporate. If the Stressed Powder were scattered around their living space, they would inevitably set off a few grains of it in their wanderings. Each grain would explode like a tiny landmine, firing its crystal fragments at enormous velocity and lethally puncturing the insect's exoskeleton. No nasty chemical residues would remain. To human hands, the powder would be annoyingly prickly, but would pose no serious threat. Like land-mines themselves, the powder would remain lethal for years.

Softer materials, such as salt and sugar, would as Stressed Powders slowly creep and relax their tension. But if used promptly they would dissolve in soup or tea with a vigorous release of energy. The resulting drink would be heated almost to boiling point, creating a new 'instant' product. In the same way, many industrial chemicals might be crystallized in stressed form. Like 'nascent' hydrogen, they should be extremely reactive. They would speed up many reactions, and perhaps undergo new ones. Indeed, even an inert Stressed Powder could be chemically useful. In a solvent that crazed or weakened its crystals, it would break up with intense local heat and vigour. It might catalyse many otherwise tricky reactions, both by its energy, as with ultrasound, and by the sudden release of a vast amount of new surface as its particles disintegrate.

For the ultimate in energy density, Daedalus recommends stressed carbon nanotubes. A nanotube bent into a tiny toroid, and thickened by deposition of sucessive sleeves of carbon, could store far more energy than dynamite. And its explosive disintegration would release a vast amount of active carbon surface. Sadly, even DREADCO's redoubtable chemists can see no way of making Stressed Nanotube Powder. **David Jones**