can be paraphrased by the words of Dylan Thomas[10]: "The force that through the green fuse drives the flower". Here, the force is the solar energy that drives photosynthesis. The flower, in this case the organic carbon of the phytoplankton, sinks from the surface waters drawing down carbon dioxide from the atmosphere and, if buried in sufficient quantities in the sediment, creating oil-rich rocks. For the past 100 and possibly 200 million years, diatom algae have been the primary 'green fuse' in this process. If we are to adequately understand the workings of biogeochemical cycles in earlier periods, then identifying the 'green fuse' of the past is essential. ■

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Developmental biology

# Giving limbs a hand

Martin J. Cohn

Vertebrate limbs are complicated structures. The genetic programme that directs their development is also proving to be complex, and trying to understand how it works is keeping developmental biologists busy. Three papers in *Development*[1–3] now bring the molecular basis of limb development closer to hand, or rather to *dHAND*, a gene with a key role very early on in the process.

As three-dimensional structures, limbs develop along three axes. In the arm these are: shoulder to fingertips, thumb to small finger, and palm to back of the hand. All limbs start out as limb buds in an embryo. Each bud is an undifferentiated swelling made up of only two kinds of cells — a core of mesenchymal cells, and a surrounding jacket of epithelial cells. The challenge for the embryo is to transform the limb bud into the elaborate system of different tissues found in the mature limb. This patterning process is orchestrated by specialized groups of cells that act as signalling regions and tell other cells where they are and what to do. For example, patterning along the anterior to posterior (thumb to small finger) axis is controlled by mesenchymal cells at the posterior edge of the limb in an area known as the polarizing region. Here, cells secrete the signalling molecule Sonic hedgehog (Shh), a protein that dictates the identities of skeletal elements and is needed for the limb to develop (Fig. 1). But what is signalling to the signaller? In other words, how is the polarizing region established?

The *dHAND* gene (also called *HAND2*) encodes a transcription factor, the dHAND protein, best known for its involvement in heart and face development[4,5]. It now turns out that this protein is also required for the development of paired appendages (fins, as well as limbs; Box 1, overleaf), where it either aids or induces expression of the *Shh* gene in the posterior part of the bud[1–3]. The three groups reporting in *Development* found that *dHAND* is first expressed throughout the part of the embryo from which limb and fin buds develop (the lateral plate mesoderm; Fig. 1), before limb budding or *Shh* expression begins. As limb buds emerge, *dHAND* expression becomes restricted to the posterior regions of forelimb and hindlimb buds, and to the non-limb-forming region between forelimb and hindlimb buds known as the flank. Only a subset of these *dHAND*-expressing cells will go on to express *Shh*.

If *Shh*-expressing cells from the polarizing region are transplanted to the anterior margin of another limb bud, they can induce mirror-image digit duplications by instructing anterior cells to form posterior structures. This is known as polarizing activity. The polarizing potential (the ability to express *Shh* and polarize the limb) is initially encoded over a much larger area of the lateral plate mesoderm than will eventually express *Shh* and have polarizing activity[6].

To understand how the polarizing region is established, we need to go back a step and identify which molecule(s) encodes polarizing potential. Several years ago, a strong contender emerged in the form of the *Hoxb8* gene (Fig. 1), which is normally expressed in posterior forelimb and flank cells. But if this gene is overexpressed in the anterior part of mouse forelimb buds, *Shh* is expressed there and forelimb digits are duplicated in the anterior region[7].

Now, two of the research groups[1,2] report that overexpression of *dHAND* in chick and mouse limbs can also lead to anterior *Shh* expression, or activation of the *Shh* pathway (sometimes with no detectable *Shh* expression), causing duplications of structures in the forelimbs and hindlimbs. Retinoic acid, long suspected of being involved in the polarizing-region pathway, can induce overexpression of *Hoxb8* (directly), which in turn is followed by expression of *dHAND* and then *Shh*, when applied to the anterior part of the chick limb bud. Expression of *dHAND* not only precedes that of *Shh*, but is required for it. Loss of dHAND function in knockout mice[1] and in zebrafish *Hands off* mutants[3]

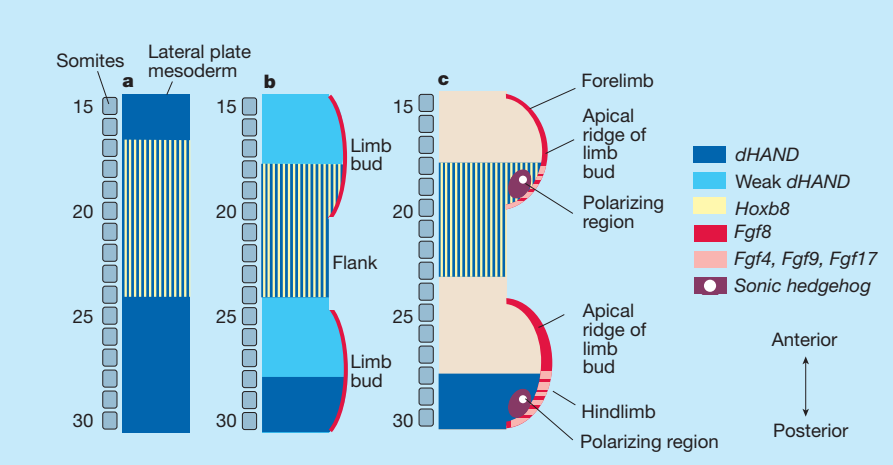


Figure 1 **Development of the polarizing region in paired appendages, as suggested by the new work[1–3].** a, **Before limb and fin buds begin to form, *dHAND* is expressed throughout the lateral plate mesoderm. Within the *dHAND* territory is an area of *Hoxb8* expression extending from the posterior region of the prospective forelimb bud down to the posterior part of the flank, which corresponds to a region opposite somites (segmented blocks of mesoderm) 17 to 24 in the chick embryo.** b, **As the limb buds start to form, high levels of *dHAND* expression are maintained posteriorly in limb buds and in the flank, but levels decrease in anterior forelimb and hindlimb.** c, ***Sonic hedgehog* expression is induced in limb bud mesenchyme where the domain of *dHAND* expression (and of *Hox8* expression in the forelimb) intersects with the posterior edge of the apical ridge (along the leading edge of the limb), which expresses fibroblast growth factors *Fgf4*, *Fgf8*, *Fgf9* and *Fgf17*. This defines the polarizing region.**


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## 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 landmines 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**


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