Published in final edited form as:

Nature. 1994 March 17; 368(6468): 188–189. doi:10.1038/368188a0.

Developmental Biology. Unanimity waits in the wings

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Embryological development of intricate biological pattern is a multistep process. Beginning with initial asymmetries in the egg, each step incrementally adds detail to a molecular code of positional information — a code that directs the development of structures in their appropriate places. Hedgehog is a signalling molecule that acts at several steps in this process, and two new papers — one in *Cell*¹ and one on page 208 of this issue² — tell us a great deal about this protein's activity in *Drosophila*. Not only does the work reveal remarkable parallels with the action of Hedgehog homologues in vertebrates^{3–5}, but it also points to a link that would unify two schools of thought, each emphasizing different views of the code of positional information.

According to one school, supported by molecular genetic studies in *Drosophila*, positional information is encoded by developmental regulators expressed in localized patterns. Like a paint-by-number kit, where contours delimit areas whose colour is indicated by a number code, domains of gene expression delimit territories of the embryo in which cell fate is specified by a code of regulatory molecules.

In contrast to this mosaic of regulators, experimental embryology suggests that pattern in a variety of organisms is encoded by graded signals. Organizing centres, which are identifiable as transplanted bits of tissue that direct surrounding cells to adopt particular fates according to their distance from the transplant, are thought to emit signalling molecules called morphogens. The idea here is that the fates of cells are dictated by morphogen concentration, which is graded with distance from the organizer.

The phenotype of *Drosophila* mutant in the *engrailed* gene, which is expressed in segmentally repeated territories in the early embryo, stimulated the original proposal that pattern is specified by a mosaic of territories expressing different developmental regulators^{6,7}. Heemskerk and DiNardo¹ now show that territories of *engrailed* expression act as organizers to specify the fates of surrounding cells, and that Hedgehog, which is produced by *engrailed*-expressing cells^{8–10}, is the morphogen mediating this patterning activity. Hedgehog acts at least twice during the cascade of interactions producing the segmental subdivisions. Mutations in *hedgehog* reveal that Hedgehog signalling is required early for the maintenance of Wingless expression in cells anterior to the *engrailed* territory. To identify the second role, Heemskerk and DiNardo had to distinguish the direct effects of Hedgehog from those due to the lack of Wingless, an important patterning molecule in its own right.

In an elegant piece of work, they show that the production of several distinct types of epidermal cells is independent of Wingless, but dependent on Hedgehog. Using a temperature-sensitive allele of *hedgehog*, they show that the time at which Hedgehog activity is required to specify segmental cell fate (6–9 hours) is distinct from the time at which Hedgehog signals to *wingless*-expressing cells (3–6 hours). Importantly, by genetically manipulating levels of Hedgehog, they provide convincing evidence that Hedgehog acts as a morphogen whose concentration specifies which cell type will form at different positions posterior to the *engrailed* stripe. Thus, Hedgehog signalling by the *engrailed* territory suggests a link between the gradient concept of positional information and the code of localized gene expression.

This relation between Hedgehog production and the territory of *engrailed* expression is maintained when the limbs are patterned during the larval-to-pupal transition. As in the embryo, Hedgehog is produced by *engrailed*-expressing cells, which comprise the posterior compartment of the wing and leg disk primordia. In an impressive series of experiments, Basler and Struhl² dissected the role of Hedgehog in limb patterning. They exploited an ingenious technique in which the *flp* recombinase of yeast is used to induce clones that express Hedgehog constitutively (see Fig. 1 on page 208). When these clones occur in the posterior compartment, where the cells naturally produce Hedgehog anyway, they have no effect on the subsequent morphological pattern. But when they occur in the anterior compartment, *hedgehog*-expressing clones can promote dramatic pattern duplications. The most extreme of them — a new wing blade composed of two anterior compartments symmetrically displayed around the clone of *hedgehog*-expressing cells — bears striking similarities to the pattern duplications seen in chick wing upon ectopic expression of the vertebrate homologue, *Sonic hedgehog*⁵.

The pattern duplications demonstrate that Hedgehog, which ordinarily emanates from the posterior compartment, can organize the pattern of the anterior wing blade. However, this patterning action of Hedgehog appears to be indirect. Another signalling molecule (Dpp), the product of the *decapentaplegic* gene (*dpp*), is required for wing formation. Dpp is ordinarily expressed in a stripe of cells just anterior to the posterior compartment. Basler and Struhl show that Hedgehog is required for this expression of Dpp, and that clones that ectopically produce Hedgehog induce Dpp. Indeed, they suggest that the only important function of Hedgehog is to turn on Dpp, an inference which is based on the finding that loss of *dpp* results in the same wing phenotype as loss of *hedgehog*. Thus, Dpp is downstream of Hedgehog in a signalling cascade.

The situation in the leg is similar, but somewhat more complex. As in the wing, ectopic Hedgehog can induce duplications, but the limits of many of these duplications suggested that the dorsal half of the leg differs from the ventral half. Indeed, although Hedgehog induces Dpp in a stripe, this stripe is intense dorsally and faint in the ventral half of the leg disk, where Hedgehog induces another signalling molecule, Wingless. Like Dpp, Wingless is essential for limb patterning. Furthermore, clones that ectopically express *wingless* support a proposal that Wingless is the intermediary of Hedgehog action in the ventral part of the leg¹¹. Presumably, responses of dorsal and ventral cells to Hedgehog differ because other genes distinguish these two territories.

As emphasized, both embryonic and larval Hedgehog signalling emanate from an *engrailed*-expressing territory. What is the importance of this linkage between a territory of localized gene expression and production of a morphogenetic signal? Neither of the original concepts — patterning by a mosaic of localized regulators, and patterning by the action of graded morphogens — fully describes the progressive refinement of pattern that characterizes embryogenesis. By itself, the mosaic of regulators can represent pattern but it is static; and although interpretations of a graded signal can give a step in pattern formation, the model cannot mimic the progressive nature of the process unless one adds a mechanism to specify new organizers. But combining the models overcomes the limitations of either alone (see figure), and the validity of such a combination is supported by the finding that *engrailed* territories of gene expression act as the source of a morphogen.

The power of a combination of mosaic and graded codes has been documented for the patterning of the early syncytial *Drosophila* embryo. Imprints of maternal pattern present in the egg promote accumulation of regulators (Bicoid, for example) in concentration gradients that specify the embryonic axes. These long-range gradients provide coarse positional information that guides expression of the gap gene RNAs in sharply defined, striped territories. The proteins produced by these RNAs diffuse to create local gradients, each steeper and more

localized than the primary gradients that define the body axes. This second round of gradients provides local information that guides new gene expression in more finely divided stripes, the pair-rule stripes (territories). And so the process continues, each step refining pattern in the developing embryo¹². In each step, territories of localized gene expression serve as organizers — sources of morphogens — and the gradients of morphogens define new territories. I refer to this alternation between mosaic and graded codes of pattern as a sequential subdivision model; all such models require communication between territories, but in some formulations signalling does not use graded morphogens^{13,14}.

Clearly, the molecular mechanisms involved in communication between different parts of the embryo must change when the embryo makes the transition from syncytial to cellular development. For this reason, there has been little attempt to generalize the lessons learned from early stages of *Drosophila* development. However, the new results^{1,2} suggest that sequential subdivision continues into later stages.

In Heemskerk and DiNardo's work¹ we see that a territory of gene expression created by the early syncytial cascade now acts as an organizer secreting a Hedgehog morphogen. Furthermore, Basler and Struhl's observations² resemble the steps of sequential subdivision. A territory of localized gene expression, the posterior compartment, serves as the organizer producing a signalling molecule, Hedgehog. In the wing, Hedgehog induces a new territory composed of cells transcribing *dpp*. This new territory is the organizer for another patterning signal, Dpp itself. But this may not be the end of the story. Because it is unlikely that the extraordinary detail of final wing pattern is directly specified by graded Dpp concentrations, further rounds of sequential subdivision may well precede specification of individual cell fates.

Finally, the beautiful analyses of the expression of *Sonic hedgehog* in the chick wing-bud⁵ invite comparisons with the results in *Drosophila*. In fly and chick, Hedgehog signals are produced in the posterior of the developing wing field, and in both ectopic expression induces duplications of more anterior patterns. It seems likely that Sonic hedgehog is itself a step in a cascade of sequential subdivision. There are proposals that the limb organizer (the zone of polarizing activity), and perhaps *Sonic hedgehog* expression, depend on expression of FGF-4 by the overlying apical ectodermal ridge¹⁵. The zone of polarizing activity and Sonic hedgehog are thought to induce Hox gene expression, but it is not clear whether the Hox genes respond directly to Sonic hedgehog concentrations⁵. More likely there are intermediaries in this action and, by analogy with Dpp and Wingless in *Drosophila*, other members of the TGF- β and Wnt families are attractive candidates for intermediate signals. Furthermore the different territories of Hox gene expression are not likely to be the end of the patterning cascade: these domains are too coarse to be responsible for the complete patterning of a limb.

As well as its role in patterning of the limb, *Sonic hedgehog* is expressed in several different regions that have organizer activity, including the notochord, the floorplate of the neural tube and the zone of polarizing activity^{3–10}. It is possible that Sonic hedgehog activates different downstream cascades in each of these cases. But whatever the molecular details, the insights gained from the *Drosophila* studies are likely to throw new light on what may prove to be a general mechanism of embryonic patterning.

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The sequential subdivision view of pattern formation brings together two modes of encoding positional information, gradients of morphogenic molecules and mosaics of localized gene expression, in an alternating sequence that creates new pattern. As illustrated, a primary gradient of a morphogen across an egg can specify three different territories of gene expression (red, blue and green) as a result of concentration-dependent effects. These three territories can direct formation of new gradients, each of which can direct formation of new territories of gene expression. This sequence can be repeated to specify pattern with increasing accuracy.