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Morphogens, Compartments, and Pattern: Lessons from Drosophila?

Review

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Over the last 20 years, the essential mechanisms of development have become clearer, mainly because modern molecular genetics has transformed traditional embryology. The longest dispute in embryology has been resolved: we now know that the adult is not preformed in the fertilized egg and that animals arise by step-by-step elaboration from simple beginnings, that is, by epigenesis. Much of this knowledge has come from ingenious experiments on invertebrates such as Drosophila and the nematode, Caenorhabditis elegans. Nevertheless, because so much of the genetic machinery is shared, many of the principles uncovered may apply, with variations, to all multicellular animals.

Here, we describe a simple and beautiful mechanism that is used to build pattern in the development of flies. Like the Central Dogma in molecular biology, the heart of the matter is not so much the individual molecules involved, but more the flow of information and the logic of the system they participate in. With this proviso, it becomes reasonable to ask whether pattern formation in other animals uses all or some of the same logical steps; and does it use similar molecules? Although this review is mainly about flies, we do approach these questions, briefly, with respect to vertebrates. Our aim is not to provide a specialized review of the fly work—this has already been done well (for example, see Cohen, 1993; Blair, 1995)—but to emphasize mechanisms and principles, to simplify, even to oversimplify, and to introduce some new hypotheses.

Epigenesis, at least in Drosophila, depends on three steps which interlock and overlap; we simplify and refer to these as the "central dogma." **First,** positional information in the form of morphogen gradients allocates cells into nonoverlapping sets, each set founding a compartment (Garcia-Bellido et al., 1973; Lawrence, 1973). **Second,** each of these compartments acquires a genetic address (Garcia-Bellido et al., 1979), the combination of active and inactive "selector" genes (Garcia-Bellido, 1975) that tells the founding cells and their descendents not only which part of the body to make, but also how to interact with cells in neighboring compartments. **Third,** interaction between cells in adjacent compartments initiates new morphogen gradients, gradients that organize the pattern. These gradients are initiated in a logically simple way: initially one compartment makes a **short-range inducer** (Basler and Struhl, 1994; Capdevila and Guerrero, 1994; Tabata and Kornberg, 1994), a signal to which cells of a neighboring compartment are sensitive. Those cells in range are then stimulated and become a source of a **long-range morphogen** that carries positional information to the cells of both neighboring compartments (Zecca et al., 1995; Nellen et al., 1996). We summarize each of the first two steps and then discuss the third step in more detail, taking the Drosophila wing disc as an example. We consider properties of morphogen gradients in general, as they are central to the model.

The Three Steps

(1) The Definition of Sets of Cells

At gastrulation, the Drosophila embryo consists of about 6000 cells that are assigned to a series of precisely defined primordia. By "precise," we do not mean that the number of cells in each primordium is fixed—it is the number and arrangement of the primordia and, also, the regions of the larva and adult that they generate that is invariant and precise. The cells are allocated according to their positions with respect to both the dorsoventral and the anteroposterior axes and not because they descend from particular ancestors. In both axes, this is achieved by morphogen gradients, each

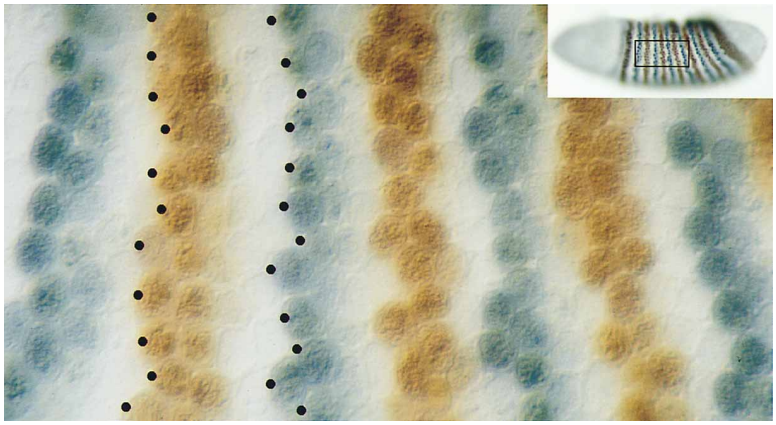

Figure 1. The Origins of Parasegment 4

At early gastrulation, the Drosophila embryo consists of a single layer of cells. As a result of the step-by-step interpretation of the Bicoid gradient, the embryo expresses two genes, *fushi tarazu* (stained in brown) and *even-skipped* (grey), in accurately spaced and positioned stripes. These asymmetric stripes have sharp anterior boundaries that delineate the boundaries of the parasegments (reviewed by Lawrence, 1992). Parasegment 4, which is the set of cells that will generate the larval and adult epidermis of the anterior part of the wing segment (T2) and the posterior part of T1, is outlined in black dots.
Photograph of dissected embryo, stage 7.

being initiated maternally by localized signals or determinants (reviewed by Lawrence, 1992; St Johnston and Nüsslein-Volhard, 1992). Allocated sets of cells in the dorsoventral axis constitute the germ layers, such as mesoderm or neurectoderm, while the subdivisions in the anteroposterior axis are into segmental units, known as parasegments (Martinez-Arias and Lawrence, 1985). The result is an embryo that is divided up like a checkerboard into "squares": an example of such a "square" would be a subset of the founders of parasegment 4 (Figure 1), those cells in the ectoderm that will generate a precise region of the larval and adult epidermis including the front halves of the wing and second leg.

(2) Selector Genes, Genetic Addresses, and Compartments

There is a special class of selector genes (Garcia-Bellido, 1975) responsible for giving the founder cells of compartments and their descendents unique instructions; the classical examples are *engrailed* (Morata and Lawrence, 1975) and elements of the bithorax complex (Lewis, 1978; Sánchez-Herrero et al., 1985). The model is a simple one: concomitant with the topographic allocation of sets of founder cells, selector genes are activated in different combinations so that each set becomes genetically "addressed" in a binary code. During subsequent development, a typical group of cells and its descendents may make successive binary decisions, each coinciding with the activation of a selector gene in a subset of the cells and its inactivation in the remainder.

Once these selector genes are turned on, or off, they become fixed in that state, so that the genetic address of the founder cells and their descendents becomes locked or "determined." For example, the genes of the bithorax complex must remain off in all cells in which they were initially repressed and this state must be maintained through subsequent DNA replication and cell division (Lewis, 1978; Struhl, 1981; Struhl and Brower, 1982). It is because all the founder cells are so inflexibly committed that their descendents together generate the whole compartment, with none straying away to make something else, and no cells from outside contributing. What is not fixed is the contribution any particular founder cell will make to the compartment, for example, which region the cells it generates will construct or how many divisions it will undergo. This flexibility makes the whole system more robust and able to compensate for cell loss.

Because we use the development of the Drosophila wing disc as an example, we digress briefly to describe how the cells within this disc are initially allocated and genetically addressed. The wing primordium arises from a small cluster of founder cells that straddle the border between parasegments 4 and 5; it forms just there because the selector genes of the Antennapedia and bithorax complexes give those parasegments specific addresses, and because these cells are already specified as lateral ectoderm in response to an early dorsoventral gradient of Dorsal protein in nuclei (reviewed by St Johnston and Nüsslein-Volhard, 1992; Jiang and Levine, 1993). The cells of the wing primordium are also specified by cell interactions across the parasegment 4/5 boundary, interactions that appear to depend on the intercellular signal Wingless and lead to expression of the homeodomain protein Distal-less (Cohen, 1993).

When the wing primordium is allocated, it is already subdivided into two sets of founder cells destined to form the anterior and posterior compartments of the adult wing (Garcia-Bellido et al., 1973, 1979). The posterior cells and their descendents express the *engrailed* selector gene (Morata and Lawrence, 1975; DiNardo et al., 1985; Fjose et al., 1985; Kornberg et al., 1985; Vincent and O'Farrell, 1992). Later still, both anterior and posterior compartments within the wing disc are subdivided once more by the *apterous* selector gene, which is activated in dorsal and repressed in ventral cells (Blair, 1993; Diaz-Benjumea and Cohen, 1993; Williams et al., 1993). The result of these subdivisions is a disc divided into four compartments.

The selector genes do more than specify the pattern and the structures that the compartments will eventually make—they also specify, indirectly, a surface property. This property has been termed cell affinity (Garcia-Bellido, 1975), meaning that cells that share the same affinity, owing to the same binary code of selector genes, will intermingle during growth. There are a number of different experiments that lead to this conclusion, but perhaps the simplest is the observation that when the selector gene *engrailed* is removed, in vivo, from a posterior clone of cells in the wing, those cells gain anterior affinity: they now sort out from posterior cells and, if they are in contact with anterior cells, will sort into and mingle with them (Morata and Lawrence, 1975; Lawrence and Struhl, 1982). Cells from neighboring compartments will have different affinities and tend to minimize their mutual contact, so that where the two compartments abut, there is a relatively straight line across which the cells do not stray.

As we have seen, the proliferation of cells is delimited by compartment boundaries, and this suggests that compartments act as units to control growth. One aspect of this control is cellular competition, a little understood but important mechanism that eliminates the **relatively** weaker cells in a population (Simpson and Morata, 1981). Simpson and Morata looked at the Drosophila wing and at the survival of clones of cells that grow more slowly than wild-type cells because they are weakened by a *Minute* mutation. *Minute* cells are perfectly competent when not mixed with wild-type cells—if the whole embryo consists of *Minute* cells, it gives rise to a normal fly, though development takes a few days longer than usual. However, if *Minute* cells are generated in an otherwise wild-type wing disc, they are actively eliminated. This important discovery deserves more attention, because it implies that cell death and, probably, cell division are regulated by a competitive process that can, in effect, compare cells within a population and weed out the weaker ones. Cell competition does not occur across compartment boundaries, another sign that the control of growth is autonomous to compartments.

(3) Morphogen Gradients

Initially, all the cells within a compartment are equipotential, but they must become diversified as they proliferate to make the final shape and differentiate to make the pattern. Here, we argue that pattern formation largely depends on gradients of morphogens, gradients that are initiated along compartment boundaries.

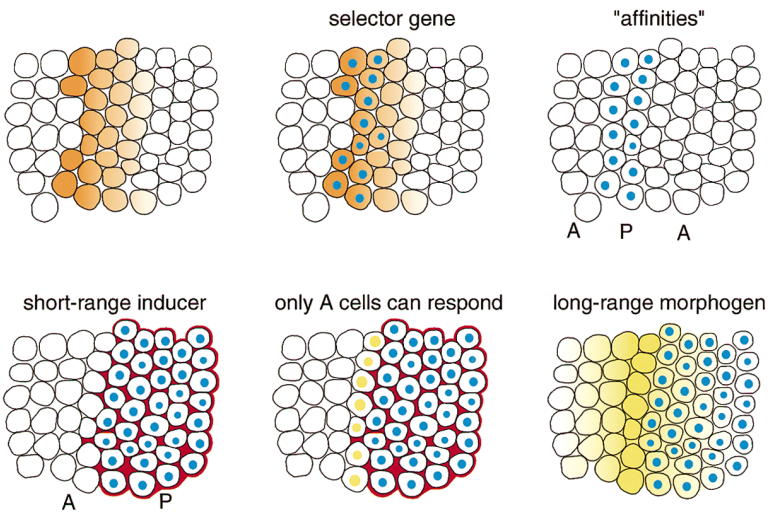

Figure 2. How Pattern Is Specified in the Drosophila Wing: A Summary

While the embryo is still a monolayer of cells, stripes of pair-rule genes allocate the parasegments (see Figure 1), and later *engrailed* (blue dots) is activated in a subset of cells (P) in each parasegment. After some refinement, these become the founders of the posterior compartment. The activation of *engrailed*, a selector gene, gives the now-posterior cells different affinities, and the P and the anterior (A) cells minimize their region of contact by lining up. During subsequent development, *engrailed* directs synthesis of Hedgehog, a short-range inducer (red), in the P cells. A cells that are in range respond and express Dpp, a long-range morphogen (yellow) that diffuses away from the line source and sets up two gradients, one in the A compartment, and one in the P. The final shapes and patterns of the two compartments are distinct, because A and P cells interpret their own gradient differently owing to their different states of *engrailed* gene expression ("off" versus "on").

It is important to distinguish a morphogen from other types of organizing molecules: as originally defined, a morphogen is a "form generating" substance that diffuses through a tissue, its distribution dictating the development of cells in the tissue (Turing, 1952). In gradient models of pattern formation (see Wolpert, 1969; Lawrence, 1992), a morphogen emanates from a localized source and diffuses away to make a concentration gradient. This gradient is interpreted as pattern—for example, downstream genes are activated in particular places; elements such as bristles are arranged and oriented with precision. The complete morphogen does more than just turn genes "on" or "off" at different concentrations; it orchestrates cellular behavior coherently so that its distribution **prefigures** the pattern. Hence, if the distribution changes, even details of the pattern change in a predictable and coordinated way. There are two aspects to the logic of this: first, how are morphogen gradients established, and second, how do they work?

How Are Gradients Established?

Just recently, there has been a big advance in understanding the wing disc, enough to outline a simple logic for establishing gradients within tissues and to question whether it might apply more generally. The principle (Figure 2) is as follows: two adjacent compartments have different genetic addresses; at its simplest, this difference is specified by a selector gene that is active in one but not in the other. In all the cells of the compartment in which a selector gene is active, it directs the expression of a short-range signal. The selector gene also makes these same cells refractory to the signal. However, in the adjacent compartment, the selector gene is inactive and this ensures that the cells there are sensitive to the signal. Thus, when the signal crosses over the compartment border and into the adjacent and responsive compartment, all the cells within range are affected. This range is probably only a few rows of cells. The cells respond by becoming a line source of a long-range morphogen, and the morphogen diffuses away from this source in all directions. The result is a mirror-image gradient landscape with a peak at, or just one side of, the compartment border, and this patterns the two compartments (differently, because they have different genetic addresses). This model has come mainly from studies on the two axes of the wing disc, which we now discuss one by one.

(1) The Drosophila Wing Disc, Anteroposterior

As we have seen, *engrailed* is the selector gene responsible for the posterior compartment, its absence specifying anterior. *engrailed*, like most selector genes so far identified, carries a homeobox and regulates other genes, some of which are known (for example, see Sanicola et al., 1995; Tabata et al., 1995). It has been suggested that the selector gene function of *engrailed* depends also on a sister gene *invected* (Hidalgo, 1994; Guillén et al., 1995; Simmonds et al., 1995); however, *invected* cannot be doing anything important or unique, because *invected*$^-$ embryos are reported to develop into normal flies (Tabata et al., 1995).

Experiments best illustrate how the paradigm (selector gene, short-range inducer, long-range morphogen) works in the wing: removal of the *engrailed* gene from a clone of posterior cells transforms them into anterior, and generates an ectopic compartment border where the *engrailed*$^-$ (now anterior) cells meet the surrounding posterior cells. At the ectopic border, these now-anterior cells receive the short-range inducer from the surrounding posterior cells and establish a new gradient peak that reorganizes the pattern. The patch of *engrailed*$^-$ cells also "sort out," meaning a circular boundary forms between them and the posterior cells—they have acquired the affinities appropriate to anterior cells and try to minimize contact with posterior cells (Lawrence and Struhl, 1982; Sanicola et al., 1995; Tabata et al., 1995; Zecca et al., 1995). Conversely, artificially expressing *engrailed* in a clone of anterior cells transforms them into posterior cells and causes them to sort out from adjacent anterior cells (which do not express

engrailed). It also creates an ectopic compartment border where the now-posterior cells send the short-range inducer into surrounding anterior cells and this establishes a new gradient peak that again reorganizes the pattern (Zecca et al., 1995). Both the sorting-out and reorganizing behavior of these clones is due to new interfaces beween cells with different states of *engrailed* gene activity ("on" or "off"): cells lacking or artificially expressing *engrailed* develop normally only when their state of *engrailed* gene activity matches that of surrounding tissue.

The short-range inducer specified by *engrailed* is the secreted protein Hedgehog; it is regulated by *engrailed*, being expressed in all posterior cells, and being absent from all anterior ones (Lee et al., 1992; Mohler and Vani, 1992; Tabata et al., 1992). It is secreted and processed to give a product (Tabata and Kornberg, 1994; Porter et al., 1995) that crosses over the border to the anterior side, where it activates *decapentaplegic* (*dpp*), a member of the transforming growth factor β (TGFβ) family of genes (Padgett et al., 1987; Posakony et al., 1991; Basler and Struhl, 1994; Tabata and Kornberg, 1994). If *hedgehog* is artificially activated in a clone of anterior cells, even if they are far from the compartment border, *dpp* becomes turned on in the clone and in neighboring cells (Basler and Struhl, 1994; Zecca et al., 1995). *hedgehog*-expressing clones in the posterior compartment have no effect. As would be expected, *hedgehog*$^-$ clones are normal in the anterior compartment and usually in the posterior compartment as well. However, if posterior cells mutant for *hedgehog* are adjacent to the compartment border, they fail to activate *dpp* on the anterior side of that border, and this has a global effect on pattern (Mohler, 1988; Basler and Struhl, 1994).

There is evidence that Dpp is the long-range morphogen. Patches and clones of cells that express *dpp* ectopically change the pattern of the wing, both in the anterior and posterior compartments, the effects varying with location (Capdevila and Guerrero, 1994; Zecca et al., 1995). In the model for the wing, the pattern derives from a line source of Dpp, located immediately anterior to the anteroposterior border—it follows that the concentration of Dpp should be highest there, and grade away anteriorly and posteriorly. If a *dpp*-expressing clone is of medium activity, it should only be able to alter the concentration landscape where the background level is low, that is, far from the endogenous Dpp source in the middle of the wing. This is exactly what is observed (Zecca et al., 1995): Figure 3 shows two examples of marked *dpp*-expressing clones; such clones can produce duplications of veins I, II, or V, but have little effect on the central region containing veins III and IV. Note that the effects on pattern extend far outside the clone; it is as if the concentration landscape of Dpp prefigures and determines the finest of pattern details, such as the type, sequence, and spacing of the veins. Note also that ectopic "peaks" in the gradient landscape (identified by those pattern elements found nearest to the anteroposterior compartment borders in the normal wing) always appear to be in the center of the *dpp*-expressing clones (Figure 3).

It is possible to make a wing primordium that lacks Dpp; this wing can form only a little stump (Spencer et al., 1982). *dpp*-expressing clones can be made in these flies, and when they occur in the wing primordium, they rescue pattern and growth of the wing. When the clone has posterior provenance (cells expressing *engrailed*), it organizes a duplicated winglet of posterior type, and when anterior, it forms an anterior winglet (Zecca et al., 1995). In this experiment, the clone acts in a wing primordium that has no endogenous Dpp; therefore, the total concentration of Dpp that can be reached is less than when such clones are made in a normal wing (where there is some Dpp already present). As a consequence, the pattern elements formed are those normally found nearer to the extreme anterior and posterior margins of the wing—more evidence that the level of Dpp is what counts. The above evidence does not prove that Dpp is the long-range morphogen; however, it must be closer to it than Hedgehog—for it appears that, while Hedgehog may act chiefly as a switch to activate Dpp expression, Dpp does much more. Indeed, the experiments suggest it is the amount of Dpp that determines which part of the wing pattern is formed. Note also that, in all these experiments, net growth is coordinated with the reorganization of pattern so that, finally, the extra pieces of wing have all the normal elements, such as veins, and are complete in size and proportion.

As we have seen, Dpp has long-range effects; it could achieve these directly by diffusing over as many as 30 cell diameters. Another possibility is that it could be the first of a "bucket brigade" of short-range inducers that together build a long chain of indirect effects. The above experiments do not favor bucket brigades; they indicate

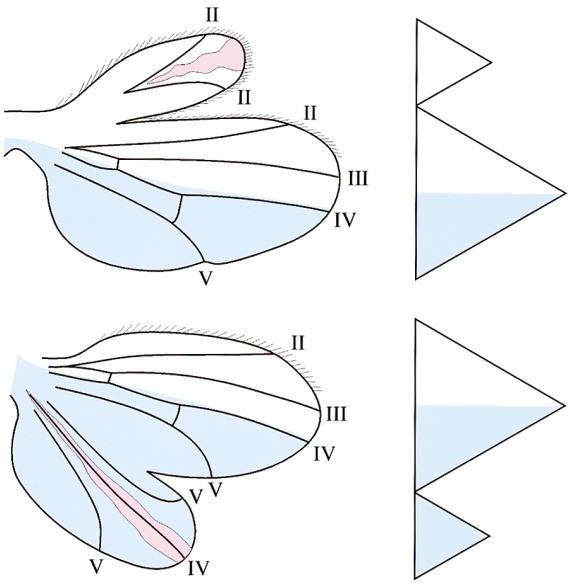

Figure 3. Pattern Reorganization in the Wing; Clones Expressing *dpp*

When clones that express *dpp* (pink) are produced in either the anterior (upper figure) or posterior compartment, they produce an amount of Dpp protein that induces a pattern normally found near to either edge of the wing, such as veins II or V. The gradient interpretation is shown on the right, the height of the peaks depending on the amount of Dpp produced. Posterior compartments are shown in blue. For data, see Zecca et al. (1995).

that cells far from the source can respond directly to low concentrations of Dpp, belying the need for intermediate inducers (Tickle et al., 1975; Struhl and Basler, 1993). Also, there is new evidence that favors direct action at a distance: Basler's group has used two genes, *spalt* and *optomotor blind* (*omb*), which are activated in response to Dpp; *spalt* is activated near the Dpp source and *omb* overlaps with it but extends further away (Nellen et al., 1996). Clones of cells that express activated Dpp receptors cause both the *omb* and *spalt* genes to be ectopically expressed, but only in those cells within the clone and no others, even when these clones are far from the source of Dpp. Had Dpp been the first link in a chain, the effect of activating the receptor should have triggered the second link and, as a consequence, expression of *spalt* and *omb* would have spread outside the clone.

If there were a chain, Dpp receptors should be required only near to the source of Dpp, so that a different receptor would be needed for each link. Nellen et al. (1996) looked at clones of cells with incapacitated receptor; these eliminate the Dpp response from all cells in the clone, even when the clone is far away from the source of Dpp. They also made clones of cells that express *dpp*; these trigger *spalt* and *omb* expression in the cells surrounding the clone in nested circles, with Spalt seen in the inner circle (where the Dpp concentration is higher) and Omb further out. Taken together, these experiments build a strong case that Dpp acts as a long-range morphogen.

(2) The Drosophila Wing Disc, Dorsoventral

Recent work has suggested that the same paradigm—selector gene, short-range inducer, long-range morphogen—can be applied to the dorsoventral axis in the wing. The wing consists of two apposed surfaces, which are lineage compartments, with the border between them running around the perimeter of the wing (Bryant, 1970; Garcia-Bellido et al., 1973). *apterous* is the selector gene that makes the dorsal distinct from the ventral surface; it specifies the genetic address "dorsal." Experiments give results exactly comparable with *engrailed* in the anteroposterior axis; if *apterous* is removed from dorsal cells, they transform into ventral ones and a new border with long-range reorganizing properties forms where the now-ventral cells and the surrounding dorsal cells meet (Diaz-Benjumea and Cohen, 1993; Williams et al., 1993; Blair et al., 1994). The border is not wiggly, because dorsal and ventral cells have different affinities. It also has been shown that, if *apterous* is removed from dorsal cells that are located near the ventral compartment, these now-ventral cells assort with the ventral compartment and become completely subsumed into it—they can only be traced if they are genetically marked (Blair et al., 1994).

The way *apterous* works appears to be more complex than *engrailed*. *apterous* has at least two distinct outputs: first, it is responsible for making the dorsal cell type distinct from ventral, a property that may be due to its activating the gene *Dorsal wing* (Tiong et al., 1995); second, it directs the expression of *fringe* and *Serrate* in the dorsal compartment. Fringe is remarkable because a boundary forms wherever *fringe*-expressing and nonexpressing cells meet, a boundary that can organize long-range pattern (Irvine and Wieschaus, 1994). Serrate, a

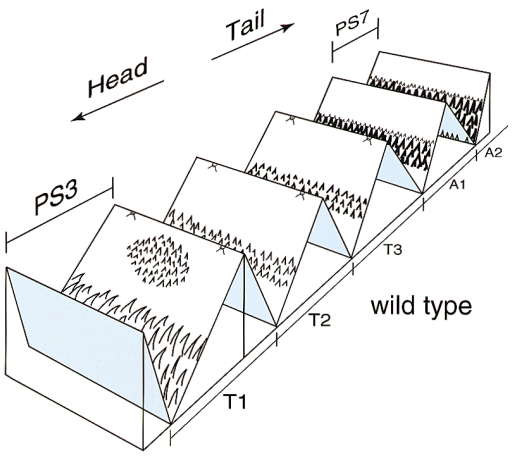

Figure 4. The Postulated Gradient Landscape in the Embryo

The peaks are drawn at the parasegment boundaries (cf Sampedro et al., 1993). The cuticle pattern of the different thoracic segments (T1–T3), the first abdominal segment (A1) and a more typical abdominal segment (A2) are indicated. Note that the pattern elements occur at particular heights in the morphogen gradient and depend on whether the compartment is of anterior or posterior (blue) identity. For example, in the abdominal segments A2–A7, all posterior compartments are naked except for a single row of hairs at the lowest level of morphogen. Anterior compartments are naked at high values in the gradient, except in T1, where a beard forms.

transmembrane protein, appears to signal from dorsal to ventral cells to elicit the production of a long-range morphogen (Diaz-Benjumea and Cohen, 1995; Kim et al., 1995; de Celis et al., 1996). Reciprocally, Delta, another transmembrane protein, may signal from ventral to dorsal cells and trigger the production of the same morphogen (de Celis et al., 1996; Doherty et al., 1996). Both Serrate and Delta may activate the receptor Notch (Diaz-Benjumea and Cohen, 1995; de Celis et al., 1996; Doherty et al., 1996).

wingless is transcribed along the dorsoventral compartment boundary, apparently in response to Notch activation. Wingless has some important function in genesis of that boundary—so much so that ectopic expression of *wingless* alone is sufficient to induce adventitious border (Diaz-Benjumea and Cohen, 1995). It could be that the long-range morphogen induced by Serrate and Delta is Wingless itself, as there is circumstantial evidence that Wingless acts as a gradient morphogen elsewhere, for example, in the embryonic epidermis (Bejsovec and Martinez-Arias, 1991), the gut (Hoppler and Bienz, 1994), and the adult leg (Struhl and Basler, 1993).

(3) Segment Patterning in Embryos

It is a little unconventional to go back to the embryo after looking at the wing disc, but the discoveries in the disc may have general implications. It is known that the anteroposterior compartment boundary within the disc derives directly from the parasegment boundary in the embryo (Struhl, 1984), and, hence, the same rules may apply. Specifically, in the embryo as in the disc, it is possible that Hedgehog may serve principally as a short-range inducer to elicit a long-range morphogen that controls patterning on both sides of the parasegment boundary. In Figure 4, we show the distribution of such

a putative morphogen as it applies to the ventral pattern of the first instar larva. It should peak at, or just in front of, the parasegment boundary and form gradients of opposite slope extending anteriorly and posteriorly. As we describe for the wing, anterior cells would interpret this gradient to form different cuticular patterns than posterior cells, because they have an anterior (*engrailed* off) as opposed to a posterior (*engrailed* on) genetic address.

The identity of the putative long-range morphogen induced in the embryo by Hedgehog is unlikely to be Dpp, as it is not present just anterior to the border in the ventral ectoderm. One candidate would be Wingless, which is expressed as a line source just anterior to the parasegment boundary (van den Heuvel et al., 1989), apparently in response to Hedgehog protein secreted by posterior cells just across the boundary (Ingham, 1993).

The patterning of insect segments was studied long ago, using transplantation experiments, and the results led to the hypothesis that growth and patterning in the epidermis is organized by gradients of positional information (reviewed by Lawrence, 1992). Such gradients are usually depicted as having a high point at one edge of the segment and declining to a low point at the other edge, creating a sawtooth pattern with a precipice at each segment boundary. However, these experiments were concerned with what we now know to be just the anterior compartment, and hence left open the possibility that the gradient distribution shown in Figure 4 may be an equally, if not more valid, representation.

How Do Morphogen Gradients Work?

(1)What Do They Do?

According to what we have called the "central dogma," developing cell populations are progressively subdivided into compartments and programmed so that the interface between a pair of compartments becomes the line source of a morphogen (cf. Meinhardt, 1983). The experimental results suggest that the morphogen diffuses through the pair of neighboring compartments and sets up concentration gradients that control patterning, polarity, and proliferation.

In principle, a concentration landscape contains three types of information (reviewed by Lawrence, 1992). First, given that the peak is at a fixed height, the scalar concentration provides positional information about how far an individual cell is from the peak. Second, the vector, that is, the direction of maximal change at any point in the gradient landscape, provides information about orientation with respect to a source; it could polarize cells. Third, the slope of the gradient relates to the size of the field; for example, if both upper and lower limits of the gradient are at fixed levels, the steepness becomes some measure of the length of the compartment in one axis. This measure could affect the probabilities of each individual cell dying or dividing, probabilities that determine the overall growth rate. The attraction of this last hypothesis is that it offers the means for a single cell (the unit that must wait, divide, or die) to make a decision based on local information—yet, this local information, in effect, could tell the cell the length of the compartment in the axis in which the gradient is operating.

There is circumstantial evidence that all these three types of positional information are used to organize patterning and growth within insect compartments (Locke, 1959; Lawrence, 1966; Stumpf, 1966; Bohn, 1974; reviewed by Lawrence, 1992). The scalar values of the gradient do appear to be read out as the type of cuticle; perhaps the most eloquent experiment is one by Stumpf (1968) (reviewed by Lawrence, 1992). Similarly, the vectors do appear to determine the cell polarity; this is revealed in some insects by ripples in the cuticle that run parallel to contours in the presumed landscape of concentration (Stumpf, 1966) and, in other insects, by the orientation of bristles that appear to point down, or up, the local steepest slope in the same landscape (Lawrence, 1966). Finally, there is suggestive evidence that relates growth to the slope of the gradient. In particular, the experiments of Bohn (1974) indicate that, in the segments of the limb, the amount of growth stimulated by juxtapositions between host and donor cells is determined by the disparity between their original positions prior to grafting.

In the developing Drosophila wing, the gradient of Dpp, like that of the putative segmental gradient, also appears to organize distinct scalar responses as well as growth. For example, the genes *spalt* and *omb* are activated in broad domains that extend different distances from the Dpp-secreting cells, as if reflecting different contour lines of the Dpp gradient landscape. Moreover, in experiments in which ectopic peaks of Dpp are generated by forcing clones of cells to express *dpp* constitutively, large extra portions of wing are produced and these are properly patterned and made to scale. In these cases, it is certain that most of the growth is induced outside the *dpp*-expressing clone (Zecca et al., 1995) (Figure 4) and must be a reaction to that clone; we suggest that net growth is stimulated by the new and sharp slopes and this continues until gradient landscapes of normal steepness are restored.

(2) How Are Morphogen Gradients Read?

Little is known about interpretation of gradients, except in the case of the Bicoid protein gradient, the primary determinant of anterior body pattern in the Drosophila embryo. Bicoid is a very different kind of protein from Dpp; it has a homeodomain and functions largely as a DNA binding transcription factor. It also operates under different circumstances: unlike cellular primordia such as the wing disc, the early embryo is a syncytium in which transcription factors can diffuse through a common cytoplasm from one nucleus to the next. Nevertheless, we believe that the way the Bicoid gradient is read could help in understanding how extracellular morphogens such as Dpp might organize growth and patterning in epithelia.

Bicoid protein arises from mRNAs that are localized at the anterior pole of the egg (reviewed by St Johnston and Nüsslein-Volhard, 1992). Following fertilization, these RNAs are translated and the protein diffuses posteriorly to form a gradient extending about half-way down the body, a gradient that organizes the segmentation of the head and thoracic primordia (Nüsslein-Volhard and Frohnhofer, 1986; Driever and Nüsslein-Volhard, 1988a, 1988b). Under some conditions, Bicoid can even specify the pattern of most of the abdominal segments (Hülskamp et al., 1990; Struhl et al., 1992). In

principle, the Bicoid gradient could act directly to organize the segment pattern: the scalar values of concentration could set thresholds that define the boundaries between parasegments. Also, the direction of slope of the gradient at each point, that is, the vector, could polarize cells throughout. Indeed, it is found that when the Bicoid gradient landscape is altered, the pattern changes and it **appears** as if every nucleus measures the exact concentration of Bicoid protein, and directly reads the slope of the gradient. However, appearances can deceive; it has been shown that Bicoid works more indirectly.

In the anterior part of the embryo, Bicoid drives high levels of *hunchback* transcription and then binds cooperatively with Hunchback protein to several target genes, such as *orthodentical*, *giant*, and *hunchback* itself; as a result, the target genes are activated in specific zones (Driever et al., 1989; Struhl et al., 1989; Small et al., 1991; Simpson-Brose et al., 1994). Hunchback also diffuses posteriorly, beyond the apparent range of direct Bicoid action, to form a morphogen gradient of its own. This gradient helps dictate the striped expression of other target genes in the posterior half of the body, particularly the gap genes *Krüppel*, *knirps,* and *giant* (Hülskamp et al., 1990; Struhl et al., 1992).

The protein products of all of these regulatory genes form local concentration gradients, gradients that control the transcription of genes further down the hierarchy, such as the homeotic selector genes of the bithorax complex and the pair-rule genes *hairy* and *even-skipped* (Lewis, 1978; Nüsslein-Volhard and Wieschaus, 1980). Some of the pair-rule genes govern the expression of the segment polarity genes, including *hedgehog* and *wingless*. It is only the genes at the bottom of the hierarchy, such as *even-skipped*, *Ultrabithorax,* and *wingless* that allocate cells to parasegments, determine the type of parasegments formed, and polarize the cells. Thus, Bicoid acts to orchestrate body patterning through diverse and largely indirect mechanisms of information processing.

The indirect nature of the way Bicoid works is underlined by experiments that alter the distribution of products of the gap, pair-rule, homeotic, or segment polarity genes (for example, see Lewis, 1978; Nüsslein-Volhard and Wieschaus, 1980). These experiments change the number, size, and sequence of segments or the polarity of the cells within them—yet they do this in the context of a normal Bicoid gradient. Nevertheless, Bicoid clearly sits at the top of the hierarchy, as there seems to be no way to organize a coherent body plan without it.

(3) The Dpp Gradient

Here, we consider the thorny question of how Dpp might act to organize growth and patterning within the wing disc. As we have seen, two types of experiments establish that Dpp is not the first member of a bucket brigade of signals but itself acts as a long-range morphogen, being distributed as a gradient. So how are concentration gradients read? At one extreme, one might envisage a simple model in which Dpp acts directly. At each locale within the field of cells, its concentration would impart a scalar value, and the slope of concentration across one cell or the difference in concentration from one cell to the next, or both, would impart a vector. Such a model works in principle. However, it is demanding: cells would have to be able to discriminate between relatively small differences in concentration across the entire gradient. For example, in the Drosophila wing, longitudinal veins form at precise distances from the compartment boundary and each would have to form in response to a precise threshold concentration. Similarly, in many, maybe all, tissues, cells are polarized in the plane of the epithelium (they may form bristles and cell hairs that point posteriorly), and these cells would have to be able to detect shallow slopes of concentration across themselves or to read small differences in concentration from one cell to the next.

An alternative view, which we favor, is that Dpp acts in an indirect way, formally reminiscent of Bicoid. Even though Dpp forms an extracellular gradient, it could be transduced via receptors and drive regional and possibly graded distributions of intracellular transcription factors. Then, just as with the Bicoid system, these factors could dictate different cellular behaviors, and also initiate the synthesis of subordinate tiers of regulatory proteins. The main difference between the early embryo and the wing disc is that signals in the disc must pass between cells, rather than diffuse between nuclei within a common cytoplasm. Hence, transcription factors in the disc would act in part by driving the expression of new intercellular signals. These signals would then be received by receptors on neighboring cells in order to influence the expression of genes further down the regulatory hierarchy, ultimately building a comprehensive system of positional information similar to that which arises under Bicoid control in the early embryo.

There are two indications of such a mechanism. First, *spalt* and *omb* are downstream genes of *dpp*; they both encode DNA-binding proteins and, in that respect, resemble the proteins immediately downstream of Bicoid, for example, Hunchback. A graded distribution of Dpp outside the cells organizes the domains of *spalt* and *omb* transcription inside, and even produces a graded distribution of both these proteins near their boundaries of expression (Nellen et al., 1996). Second, there is evidence that subordinate genetic systems help interpret the Dpp gradient in the wing. For example, even though the pattern of wing veins is dictated by the Dpp gradient landscape, the veins are not initiated in one step: they are first defined as broad pre-vein territories that are later refined by lateral inhibition involving the *Notch* gene (Garcia-Bellido and de Celis, 1992; Sturtevant and Bier, 1995).

The Three Steps in Vertebrates?

What is the evidence in vertebrates for the three steps we have outlined? The relevant results in vertebrates are more biochemical and cellular than genetic, but there are some suggestive parallels. For example, the hind brain of vertebrate embryos is divided into rhombomeres, units of development that share key features with compartments: like compartments, their boundaries act to confine cells of a common lineage (reviewed by Lumsden, 1990). This is an example of the first step of the "central dogma" (the allocation of cells). The rhombomeres are the units of expression of vertebrate Hox genes, genes which are homologous to homeotic

selector genes in flies (reviewed by Krumlauf, 1994). This is an example of the second step (the genetic address). As we describe above, the genetic address in flies is heritable: once the correct combinations of active selector genes are established, they are maintained subsequently by a silencing mechanism that keeps the inactivated genes off in all cells descending from the founder group. This mechanism is the responsibility of the *Polycomb* family of genes (Duncan, 1982; Paro, 1990; Bienz and Müller, 1995), a family that is functionally conserved in mammals (Alkema et al., 1995; Müller et al., 1995)—there may be, therefore, a universal mechanism used to maintain the genetic address.

At present, there is no clear evidence that the boundaries between rhombomeres act as cellular interfaces that build morphogen gradients (but see Graham and Lumsden, 1996). However, in the vertebrate limb, Sonic-hedgehog, a homolog of Drosophila Hedgehog, acts as a secreted signaling molecule that organizes the anteroposterior pattern (Riddle et al., 1993). Moreover, it may induce the expression of bone morphogenetic proteins, homologs of Dpp (reviewed by Roelink, 1996). Finally, in flies Hedgehog protein activates high levels of expression of the *patched* gene in anterior, but not posterior, cells; this results in thin stripes of *patched* expression in those anterior cells that receive Hedgehog (for example, see Capdevila et al., 1994; Tabata and Kornberg, 1994). Recently, vertebrate homologs of *patched* have been identified, and these show a remarkably similar pattern of expression in the limb: they are silent in *Sonic-hedgehog*-expressing cells, but are transcribed at high levels in adjacent cells (Goodrich et al., 1996). While the lineage of these sets of cells is unknown, the conserved relationships between Sonic-hedgehog, Patched, and the bone morphogenetic proteins provide a hint that the third step (induction of morphogens across compartment boundaries) might also apply to vertebrates.

There are also good arguments for gradients of morphogens in vertebrates, especially in the embryonic mesoderm. In the dorsoventral axis of the frog mesoderm, there are at least five different cell states. Smith and colleagues have shown that the concentration of a secreted protein called activin can determine the cell state in mesodermal cells (Green and Smith, 1990; Green et al., 1992). Moreover, as the concentration is increased, the sequence of cell types induced corresponds with the order in which these cell types are arranged in vivo. Gurdon and colleagues have studied the molecular responses of aggregates of mesoderm cells to a concentration gradient of activin. Activin triggers two genes, *Xgoosecoid* and *Xbrachyury*, one at high and one at low concentration; when a localized source of activin is made, these two genes are activated in nested circles, just like *spalt* and *omb* in Drosophila. Again, it appears that the cells respond differently depending on the local concentration of inducer; they can even change their response if the concentration of activin increases with time (Gurdon et al., 1994, 1995). It may not be just coincidence that the activin molecules, like Dpp from insects, belong to the TGFβ family.

Of course, these examples do not prove the universality of the three steps and many questions remain. For example, in vertebrates, Hox genes are deployed in a specific sequence not only along the anteroposterior axis of the body, but also along the proximodistal axis of the limbs and even in the genitalia (Dollé et al., 1991). In the limbs at least, these Hox genes help specify pattern (Dollé et al., 1993), yet it is unknown whether or not expression of Hox genes correlates with lineage, that is, whether there are compartments in the limb that might carry a heritable genetic address.

Another uncertainty is whether the mechanism we have outlined for generating positional information within the Drosophila limb—short-range induction of long-range morphogens—might be an oversimplification. Even for the case of Hedgehog, there is evidence both in flies and in vertebrates that Hedgehog itself may serve as a gradient morphogen (Heemskirk and Dinardo, 1994; Roelink et al., 1995; Goodrich et al., 1996). In the Drosophila wing, Hedgehog signaling appears to elicit several different responses in anterior cells close to the compartment boundary (for example, see Capdevila et al., 1994; Tabata and Kornberg, 1994), responses that may contribute to pattern. This situation is reminiscent of Bicoid in the early embryo: Hedgehog may not only act indirectly and at long range by inducing Dpp (as Bicoid controls thoracic and abdominal determining genes via Hunchback), but also directly and at short range as a morphogen itself (as Bicoid directs expression of head determining genes).

Important questions also remain about how morphogens work. It is clear that remarkably small differences in concentration can be interpreted to give patterned transcription of genes, although the mechanism is only partially understood (but see Small et al., 1991; Jiang and Levine, 1993; Simpson-Brose, et al., 1994; Ma et al., 1996). Also, in the case of Dpp, the normal distribution of the protein is not known; it is not clear whether it spreads by simple diffusion, or whether translocation of the protein involves processing or binding to other extracellular components. There is even evidence that the movement of putative morphogens in insects might be linked to cell proliferation (Lawrence et al., 1972; Nellen et al., 1996). Finally, it remains uncertain how gradients are used to link the scalar and the vector, or how they regulate growth. While we think the model of Bicoid provides a general guide as to the logic of how morphogens work, there will be special molecular mechanisms that apply to intercellular gradients such as Dpp.

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Note Added in Proof

After completion of this manuscript, three papers were published on the morphogen action of Dpp in the Drosophila wing (de Celis et al., 1996; Grimm and Pflugfelder, 1996; Lecuit et al., 1996). The paper by Lecuit et al. (1996) presents similar but not identical results to those of Nellen et al. (1996) and reaches somewhat different conclusions about the mode of action of Dpp.

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