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The making of a maggot: patterning the *Drosophila* embryonic epidermis

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Abstract

Cell fates are instructed by signals emitted from specialized cell populations called organizers. The study of epidermal patterning in *Drosophila* is contributing novel insights concerning the establishment and action of such organizers. Juxtaposed rows of cells express either the *wingless* or *hedgehog* signaling molecules and thereby act as organizers of segment pattern. These signals mediate a mutually re-enforcing interaction between the two rows of cells to sustain organizer function. In a distinct and subsequent phase, wingless and hedgehog act to specify the fates of cells.

Introduction

One of the early successes of experimental embryology was the demonstration of the importance of induction to embryonic development. In this process, organizing centers emit signals that direct the choice of fate in surrounding cells [1]; for example, a signaling center in the posterior portion of the limb bud, the zone of polarizing activity (ZPA), organizes the pattern of digits across the entire limb [2]. Such organizers also operate within the insect epidermis, in which signaling centers near the borders of each segment specify cell fate [3,4]. Although the organisms favored by early experimental embryologists were well suited to the transplantation experiments that defined organizers, genetically tractable organisms are more suited to investigating the mechanisms by which organizers act.

In *Drosophila*, genetic screens have identified mutations in many of the genes involved in patterning the epidermis [5,6]. Genetic and molecular analyses of these segment polarity genes have revealed that two signaling molecules, encoded by *wingless* (*wg*) and *hedgehog* (*hh*), specify most of the epidermal cell fates. The *wg* product is a member of the evolutionarily conserved Wnt family of signaling proteins [7,8], and the *hh* product defines a novel class of conserved developmental signaling molecules [9–12,13•–16•]. Recent experiments have demonstrated that not only are the molecules conserved across species, but their function is

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also conserved. This observation has stimulated broad interest in the action of these two proteins, as well as other segment polarity genes. In this review, we outline a new framework for thinking about the action of these patterning genes. We focus on the establishment and action of the *wg*- and *hh*-expressing cells as organizers of pattern. The other segment polarity genes fit into this framework as components of signal transduction pathways, or as factors required to maintain and accurately position the signaling centers.

Steps in segmental patterning

Early in development, a cascade of regulatory genes generates stripes of localized transcription factors that define repeating units along the body axis, called parasegments [17]. Within each parasegment, the transcription factors initiate the expression of *wg* in one row of cells, and the expression of both the secreted protein Hh [9–12] and the transcription factor Engrailed (En) in the adjacent, posterior row [18–21] (Fig. 1). These two rows of cells flank the boundary between adjacent parasegments and have been identified as sources of signaling. Each row of cells signals at two different times during segmentation, with distinct outcomes [22–26,27•, 28••].

The early phase: stabilization of the signaling centers

The *wg*- and *hh*-expressing cells signal to each other, reinforcing gene expression in each cell (Fig. 1; for a comprehensive review, see [29]). It has been demonstrated that the secreted glycoprotein Wg is the ligand required for continued expression of *en* and *hh* in the neighboring cells [22–24,30••]. Reciprocally, it has been proposed that the secreted protein Hh is the signal that maintains *wg* expression [31,32].

Several segment polarity genes act in the signal transduction pathways that operate during the stabilization phase. Although no Wg receptor has yet been identified, the genes *porcupine*, *dishevelled*, *zeste-white3* and *armadillo* have been implicated in the sending or transduction of the *wg* signal (Fig. 1; [33,34•,35•,36••,37,38•–40•]; review in preparation, J Klingensmith, R Nusse, personal communication). One target of this transduction cascade may be *en* autoactivation, and *en*, in turn, positively regulates *hh* expression [25,41].

Less is known about transduction of the putative *hh* signal, although some genetic evidence [42,43••] implicates the *gooseberry* and *cubitus interruptus*^{D} transcription factors [44,45] and the *fused* serine/threonine kinase [46] in this pathway (Fig. 1).

At this stage in patterning, both *wg* and *hh* signaling appear to act over only short distances [30••,32]. Locally restricted signaling ensures that the domain of cells expressing either *wg* or *hh* remains narrow during development, even though the width of the parasegment grows threefold; for instance, as cell division and movements occur, some *en*/*hh*-expressing cells are displaced from the interface with *wg*-expressing cells and the expression of *en* is shut off in cells furthest away from the sustaining *wg* influence [47••]. Such refinement in the domains of *en* and *wg* expression is crucial for patterning, as several studies have demonstrated that widened domains of either *wg* or *en*/*hh* expression cause severe mis-specification of cell fates [22,23,32,48,49].

The late phase: signaling centers specify fates

The cell signaling events executed during the stabilization phase do not specify the final fate of cells, as alterations in the expression of either signal at later times dramatically affect cell fates [24,26,28••,32,48,50,51]. After the positions of the signaling centers have stabilized, however, the two signals then act to specify the distinct cell types across the parasegment [24,26,28••,50,51] (Fig. 1).

Roughly ten diverse epidermal cell types are generated within each parasegment. The final fate adopted by a cell is visualized at differentiation when the cytoskeleton distorts cells into distinct shapes (Fig. 2) [52]. Each cell then secretes a cuticular covering that indelibly reflects its shape change; therefore, cellular identity is easily visualized in the stereotyped pattern of segmentally repeated cuticular features [53].

The *wg* input is necessary for several rows of cells anterior to the *wg*-expressing cells to adopt their normal smooth cell fate [24,50]. The *wg* product also signals in the posterior direction, but in this case its effect is local. Two rows of cells posterior to the *wg*-expressing cells express *en*/*hh*. The most posterior row of *en*/*hh*-expressing cells adopts a denticle fate. However, *wg* input to the more anterior row, instructs those cells to adopt the smooth fate (Fig. 2) [26].

The *hh* gene appears to signal many of the remaining cell fates across the parasegment [28••]. In the dorsal epidermis, Hh acts as a morphogen in executing this role, whereas ventrally, Hh may cooperate with an unidentified signal from the *en*-expressing cells ([54••]; S DiNardo, unpublished data).

Uncoupling stabilization from fate specification

The mutual dependence of *wg* and *hh* expression during the stabilization phase initially masked their later, separate roles in fate specification. The standard genetic approach by which to uncover a role for a gene is to remove gene function and analyze the consequences on cell fate specification. If this gene is required for the expression of another signaling molecule, however, it is difficult to determine which ligand is responsible for which fate changes; for example, any of the changes in cell fate observed in a *wg* mutant could be attributed to loss of direct action of Wg, to subsequent loss of Hh activity, or to combined loss of both Hh and Wg activity. Two kinds of experiment have enabled researchers to distinguish between these possibilities and have led to the above proposal that *wg* and *hh* signaling centers operate as the two organizers of segmental pattern.

First, a temperature-sensitive allele of *wg* made it possible to inactivate *wg* at various times during development [24,28••,50,55]. Loss of Wg activity during the later, fate specification phase no longer affects the continued expression of *en* or of the *hh* signal [24,25]. This has provided a way in which to analyze the contribution of *wg* to fate specification without affecting the fates specified by *hh* signaling. Second, a key role for *hh* has been uncovered through experiments that bypass the stabilization phase, maintaining the expression of *hh* in the absence of any *wg* input. In this manner, we have discovered that *hh* can organize substantial pattern in the dorsal epidermis independently of *wg* signaling [28••].

The difficulty in identifying the separate early and late roles for *wg* and *hh* illustrates a general problem concerning all segment polarity genes. Does a given gene act early, during stabilization, or does it act both early and later, during fate specification?

Components of the *wg* signal transduction pathway act both early and late. These genes were first identified through their action during the stabilization phase, in which the target is *en* gene expression. However, the same signal transduction cassette mediates *wg* signaling during limb and wing patterning [35•,37,38•–40•], even though the target is not *en* expression. Thus, it is likely that *porcupine*, *dishevelled*, *zeste-white3*, and *armadillo* mediate *wg* function during fate specification also (Fig. 1).

In contrast to this, the segment polarity genes implicated in transduction of the *hh* signal act only early. In embryos lacking *gooseberry*, *cubitus interruptus*^{D} or *fused*, *wg* expression is lost, but *hh*-dependent cell types are still specified ([42,56]; J Heemskerk, S DiNardo, PH O'Farrell, unpublished data). Therefore, these three genes act only during early *hh* signaling, when *hh* is

needed for the maintenance of *wg* expression, and are not required in the *hh* pathway for signaling cell fates.

Particular segment polarity genes position the signaling centers

Segment polarity mutants that result in mis-specification of some cell fates were first thought to define genes involved directly in the specification of the affected cell types. Genes in this class include *naked*, *patched*, and *costal-2*. We argue that mutations in these genes affect cell fates only indirectly and do so because they change the distribution of the important signaling molecules Wg and Hh. The changes in Wg and Hh expression precede cell fate specification [22,23], and the ultimate changes in cell fate can be explained by the altered positions of the two signaling centers, or the distance over which they now act ([26]; J Heemskerk, S DiNardo, PH O'Farrell, unpublished data). One test of this hypothesis is to determine whether the cell fates missing in a mutant background can be restored by manipulating organizer function without restoring the missing gene product; for example, *naked* mutants mis-express *wg* and lack several cell types [22,26], but if *wg* function is inactivated after its mis-expression, but prior to final fate specification, the missing cell types are restored [26]. Therefore, *naked* activity is not required for the fates of these cells, but, rather, for the control of where *wg* is expressed. Analogous experiments have not yet been carried out for *patched* and *costal-2*. Nevertheless, as mutations in these genes also change the position of the organizers, we postulate that these genes do not act directly in establishing cell fate, but, rather, constitute a genetic circuit that assures the accurate positioning of the signaling centers during the stabilization phase (Fig. 2). The *patched* and *naked* genes may execute this role by modulating the transduction of either the *wg* or *hh* signal during stabilization, perhaps by encoding components of the signal transduction apparatus itself [31,32,54••]. It is presently less clear how *costal-2* acts to modify patterns of *wg* and *hh* expression. The view that these genes do not specify cell fate directly contrasts with a recent proposal by Bejsovec and Wieschaus [54••].

Conclusions

The *wg* and *hh* genes act as organizers of epidermal pattern. Most other segment polarity genes fit into this framework as components of the signal transduction apparatus, or as factors required to maintain or accurately position these signaling centers. Thus, few if any of the other segment polarity genes act specifically in signaling final cell fate; instead, most act in the feedback between the adjacent signaling centers.

Reinforcement is a general property of organizers

The mutual reinforcing signals that occur during the stabilization phase may be a general feature of organizers. In vertebrates, cell signaling interactions appear to sustain organizers; for instance, in the limb bud, feedback from the apical ectodermal ridge is required for the maintenance of the ZPA [57]. Recent analysis strongly suggests that the signaling molecule in the apical ectodermal ridge is fibroblast growth factor 4 [57,58], whereas the vertebrate *hh* homolog (*vhh*) encodes a ZPA signal [13•]. Although such feedback will maintain an organizer, it may also serve a larger purpose. Neighboring organizers that rely on mutually reinforcing signals would remain highly localized during growth and proliferation. This would constrain each organizer from inappropriately extending its influence and thereby disrupting overall pattern.

Distinct responses to the same signal

Early *wg* input stabilizes *en* and *hh* expression, whereas the later input specifies the smooth cell fate. It appears that the same components transduce the *wg* signal at both times. At present,

we do not understand how the same transduction pathway leads to different read-outs from the responding cell. The same issue is unresolved in other inductive cell signaling processes used during development. For instance, the activation of most receptor tyrosine kinases leads to the same intracellular cascade of Ras→Raf→mitogen-activated protein (MAP) kinase interactions, yet the response of the cell differs depending on the tissue type being patterned (reviewed in [59]). In the fly epidermis, either there are novel components in the *wg* transduction pathway yet to be identified, or the available targets in the responding *en*/*hh* cell must be different at the two times. Perhaps a solution will be found by focusing on the fate specification phase, in which more components the *wg* pathway need to be identified.

Abbreviations

en	*engrailed*
hh	*hedgehog*
wg	*wingless*
ZPA	zone of polarizing activity

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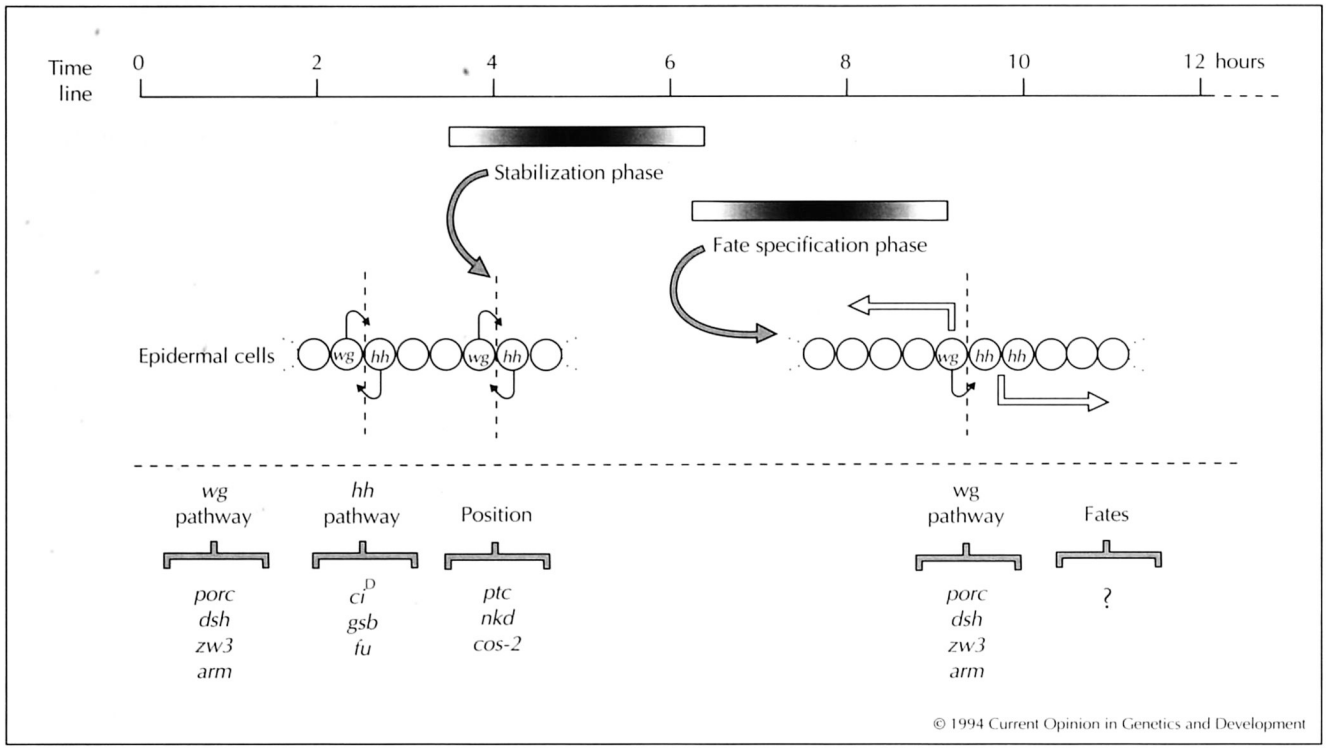

Fig. 1.

The stabilization and fate specification phases of epidermal patterning. Cells first form at two hours after fertilization, and the epidermal cells differentiate at twelve hours (time line). Shaded bars indicate the approximate period during which signals stabilize *wingless* (*wg*) and *hedgehog* (*hh*) expression, or specify cell fates (shading reflects uncertainty in timing). Line of circles represent a short antero-posterior strip of epidermal cells. During the early period, *wg*- and *hh*-expressing cells signal to one another (short arrows) across the parasegment boundary (vertical dashed line). During the late period, *wg* specifies fates anteriorly (leftward open arrow) and the fate of the adjacent *hh*-expressing cell [which co-expresses *engrailed* (*en*)]. *hh* function specifies cell fates posteriorly (rightward open arrow). The other segment polarity genes are grouped below according to their postulated roles in either the *wg* or *hh* signaling pathways, or in restricting the position of the signaling cells. *porc—porcupine*, *dsh—dishevelled*, *zw3—zeste-white3*, *arm—armadillo*, Ci^D*—cubitus interruptus*^{D}, *gsb—gooseberry*, *fu—fused*, *ptc—patched*, *nkd—naked*, and *cos-2—costal-2*. The mechanism by which pair-rule segmentation genes first establish *wg* and *en*/*hh* expression is reviewed in [60].

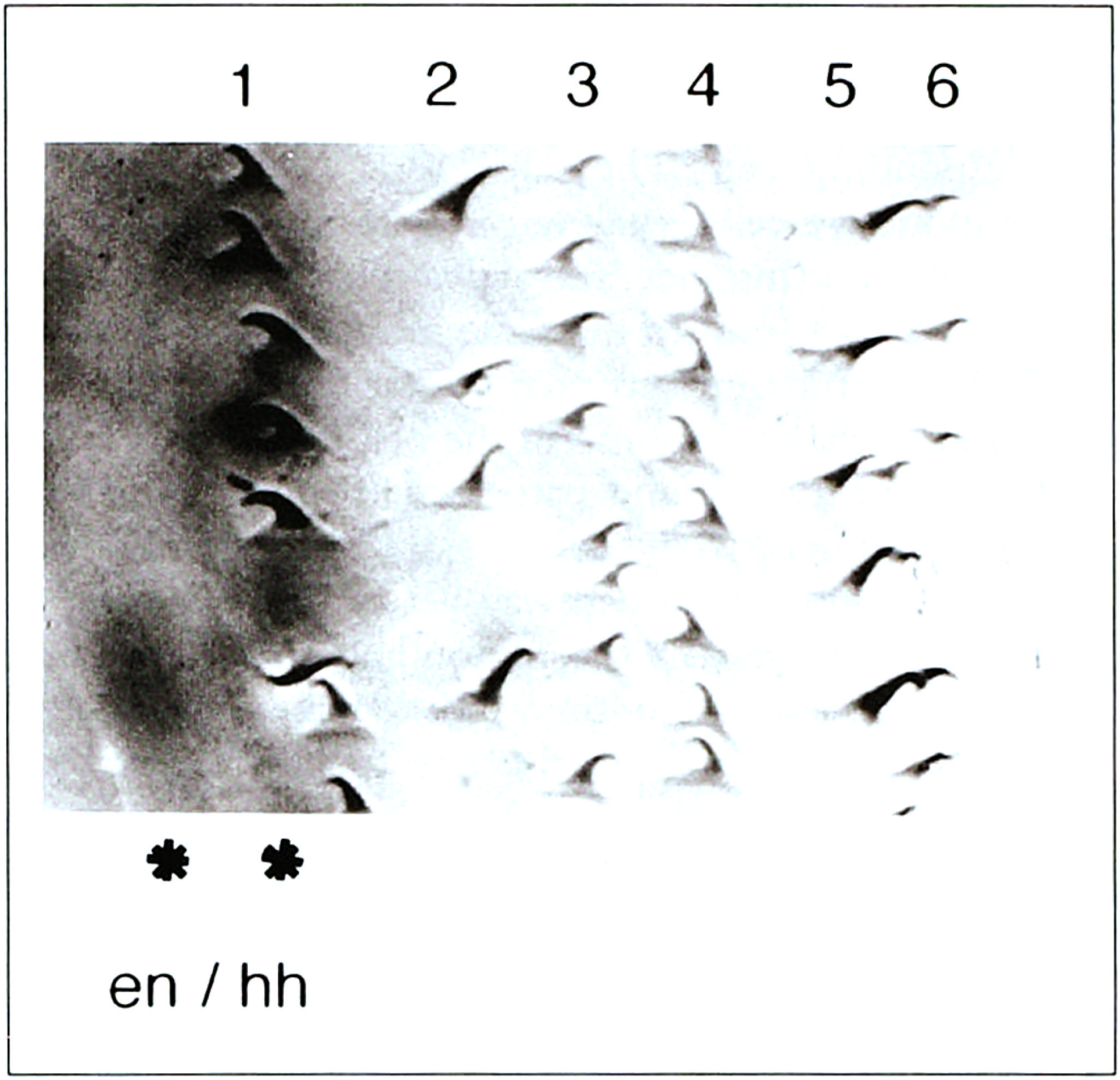

Fig. 2.

Epidermal cell fate. The photograph shows a portion of the ventral epidermis within a parasegment (anterior to the left, posterior to the right). Cell bodies are below the plane of focus. Six rows of cuticular protrusions, called denticles, are indicated, and each row exhibits unique characteristics of size and orientation that reflect the distinct positional identity of the row of underlying epidermal cells. Two rows of cells at the left express *en*/*hh* as revealed by an *en*–lacZ reporter gene (*; dark stain). Note that the posterior row adopts a denticle row #1 fate, whereas the anterior row adopts a smooth cell fate. This smooth fate is instructed by late *wg* input [26], as are other smooth cell fates further to the anterior in the segment [24] (not shown).