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The Genetic Basis of Patterned Baldness in Drosophila

Minireview

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Studies of segment polarity genes, which specify cellular identities within the Drosophila segmented embryonic epidermis, have identified two secreted proteins, wingless (wg) and hedgehog (hh), that play key roles in instructing cells about their fates within the segments. These molecules appear to function as signals in two temporally distinct pathways. First, through short-range interactions, cells expressing the wg and hh signals stabilize their mutual expression. Second, these signals pattern cell types at a distance, acting either as morphogens or by the triggering of a cascade of local signaling responses and ultimately a determination of whether epidermal cells differentiate various kinds of hairs or naked cuticle. The functions of many other segment polarity genes can be explained either as regulators or transducers within the signaling pathways of these two primary signals. Wg and hh are also implicated in long-range signaling that establishes cellular patterning within the imaginal discs. The components that mediate the wg and hh effects in both the embryo and imaginal discs are similar, indicating that these signals activate biochemical cascades that have been conserved between cell types. Identification of these molecules in vertebrates also suggests that these pathways have been conserved during evolution.

Intrasegmental Patterning

In the Drosophila embryo, the first morphological sign of segmentation begins at mid–stage 10 with the formation of metameric units or parasegments (Figure 1). The anterior boundaries of the pair rule genes *fushi-tarazu* and *even-skipped*, which are initially expressed in overlapping domains of seven stripes, define the parasegmental borders, as well as initiate the 14 striped expression patterns of some of the segment polarity genes, in particular *wg* and *engrailed* (*en*). The secreted glycoprotein wg and the homeodomain-containing transcription factor en are expressed on either side of the parasegmental border. A few years ago, it was noticed (see review by Martinez-Arias, 1993) that continued *en* expression (after stage 9) requires *wg* activity and vice versa. This interdependence of *wg* and *en*, which has been reproduced with Drosophila tissue culture cells (Cumberledge and Krasnow, 1993), led to the model that wg is the signal that maintains *en* expression. Initially, wg promotes *en* autoregulation, which subsequently evolves to a wg-independent state by stage 11 (Figure 1). The reciprocal maintenance of *wg* expression by en suggests a requirement for a signal from the *en*-expressing cells to the *wg*-expressing cells. Since *wg* transcription decays after stage 9 in *hh* mutant embryos and since *hh* expression is not only restricted to the *en*-expressing cells but also regulated by en (Tabata et al., 1992), it was proposed that *hh* encodes the signal sent by the *en*-expressing cells to the *wg*-expressing cells (Ingham et al., 1991). The molecular characterization of *hh* is consistent with this model since it encodes a secreted protein (Lee et al., 1992; Mohler and Vani, 1992; Tabata et al., 1992, 1994; Taylor et al., 1993). Later in embryogenesis, *wg* expression evolves into an en/hh-independent autoregulatory phase (Figure 1) in which *gooseberry*, a transcription factor with both homeodomain and paired-domain motifs, has been implicated (Li et al., 1993).

Morphogens and Intrasegmental Patterning

It has been postulated that morphogens (diffusible molecules organized in molecular gradients that specify cell-type diversity in a concentration-dependent manner) define positional information across each segment. How-

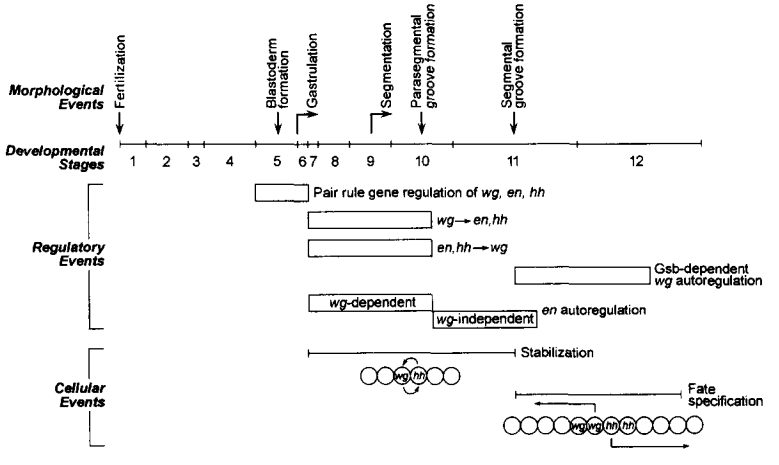

Figure 1. Patterning Events in the Embryonic Epidermis

The position of the parasegmental borders along the antero-posterior axis is established by the anterior boundaries of the pair-rule genes *fushi-tarazu* and *even-skipped*. A function of these pair-rule genes is to initiate the 14 stripes of expression of some of the segment polarity genes, beginning at stage 5. After stage 9, stripes of expression of the pair-rule genes decays, while expression of the segment polarity genes is maintained. It is through short-range interactions between the *wg*- and the *en/hh*-expressing cells that the wg- and hh-organizing signals ultimately organize epidermal cell types. At stage 9 the wg protein is initially detected in a gradient from the cells in which it is synthesized. Subsequently, at stage 10 the gradient of wg protein becomes restricted in an anteriorly directed gradient. Hh protein is detected at stage 8, peaks at stage 10, and disappears by stage 12. Hh is observed symetrically around its domain of expression, indicating that the parasegmental border does not affect protein movement (Taylor et al., 1993; Tabata and Kornberg, 1994). Epidermal cells begin to differentiate during stage 16. This figure is adapted from Martinez-Arias (1993) and Heemskerk and DiNardo (1994).

ever, it is also possible that positional information within the epidermis is established through a cascade of local cell-signaling interactions (for references see Heemskerk and DiNardo, 1994). One candidate for a morphogen in segmental patterning is wg, which regulates the differentiation of individual cells that secrete naked cuticle in the ventral epidermis. High levels of wg have been proposed to be required for maintenance of *en* expression and naked cuticle secretion, suggesting that it may act as a morphogen. However, since so few cell states are instructed in this patterning process, it has not been possible to distinguish whether wg acts as a morphogen or initiates a cascade of local signaling responses (Dougan and DiNardo, 1992). Unlike wg, hh has recently been shown to fulfill the properties of a morphogen. Temperature-shift experiments using a *hhts* allele, as well as overexpression of *hh*, allowed Heemskerk and DiNardo (1994) to demonstrate that various cell fates in the embryonic dorsal epidermis, visualized by the type of denticles they secrete, can be instructed depending on the amount of hh provided.

The Wg Signaling Pathway

Analyses of mutations associated with segment polarity phenotypes similar to *wg* have identified three genes that are required for wg function. One gene, *porcupine*, may encode a function necessary for wg secretion or transport since in *porcupine* mutant wg is not secreted, but accumulates within the cells that produce it (van den Heuvel et al., 1993; Siegfried et al., 1994). Two genes, *armadillo* (*arm*) and *dishevelled* (*dsh*), are required in the *en*-expressing cells for the interpretation of the incoming wg signal. Following reception of the wg signal, both the level and subcellular distribution of arm, which is similar to the cytoskeletal proteins β-catenin and plakoglobin, is post-transcriptionally regulated (Peifer et al., 1994). The biochemical function of the novel protein dsh is, as yet, unknown; however, it contains a domain of homology found in several junction-associated proteins (Klingensmith et al., 1994).

Generalized expression of *wg*, under the control of a heat shock promoter, any time between embryonic stages 8–12, results in expansion of the domain of *en*-expressing cells to approximately one half of the cells of the parasegment and subsequently to generation of naked cuticle (the so-called hs–wg phenotype; Noordermeer et al., 1992). Since this phenotype is the reciprocal of the loss of function phenotypes of *wg*, *dsh*, and *arm* mutants (i.e., a solid lawn of bristles), it was used to determine the genetic epistatic relationships among these genes (Noordermeer et al., 1994). These analyses have shown that both arm and dsh are required for the interpretation of the wg signal. This is consistent with the analysis of the level and intracellular localization of arm since in response to wg signal, cells accumulate high levels of cytoplasmic arm (Peifer et al., 1994).

Two segment polarity genes, *zeste-white*^{3} (*zw*^{3}, also known as *shaggy*) and *naked*, have a segment polarity phenotype reminiscent of the hs–wg phenotype. The activity of *zw*^{3}, which encodes multiple related serine/threonine protein kinases, is most likely inactivated by reception of the wg signal since in both *wg* and *zw*^{3} double mutant and

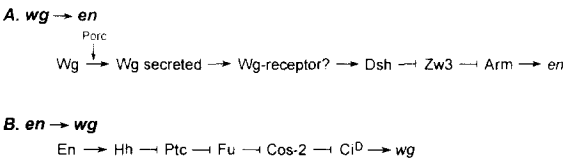

Figure 2. Epistatic Relationships between Segment Polarity Genes

(A) Genes involved in wg to *en* signaling. For details see Siegfried et al. (1992, 1994) and Noordermeer et al. (1994). (B) Genes involved in en to *wg* signaling. For details see Forbes et al. (1993).

zw^3 single mutant animals, *en* expression is expanded (Siegfried et al., 1992). Epistatic experiments between *zw*^{3} and *dsh*, as well as *zw*^{3} and *arm*, further establish that dsh acts upstream and arm downstream of zw^3 (Siegfried et al., 1994). The functional relationship between zw^3 and arm is supported by the observation that in *zw*^{3} mutant embryos, high levels of arm protein are found throughout the embryo (Peifer et al., 1994; Siegfried et al., 1994).

Combined, these results have led to the working model presented in Figure 2A. Wg, which requires porcupine activity to be properly secreted, activates, through its interaction with a receptor as yet unidentified, a signal transduction pathway that is mediated by dsh, zw^3, and arm. This signaling pathway ultimately regulates *en* autoregulation and the subsequent generation of naked cuticle. The position of arm in this pathway, downstream of the *zw*^{3} kinase and upstream of *en*, may indicate that wg signaling regulates cellular junctions, possibly regulating the reception of other signaling molecules (Siegfried et al., 1994). Alternatively, an unknown biochemical activity associated with arm may still be uncovered.

The Hh Signaling Pathway

The observation that *wg* expression disappears in *hh* mutant embryos, beginning at embryonic stage 9, identified the hh signal as a positive regulator of *wg* transcription. Patched (ptc), a transmembrane protein, was also identified as a player in this regulatory pathway. However, unlike *hh*, *ptc* acts as a negative regulator of *wg* since in *ptc* mutant embryos, *wg* expression is broadened. *ptc* expression during segmentation is complex and dynamic; however, this spatial regulation appears irrelevant to patterning events since ubiquitous *ptc* activity under the control of a heat shock promoter is able to rescue *ptc* mutant animals (Ingham et al., 1991). This result demonstrates that the repression of *wg* by ptc is spatially regulated in the wild-type embryo. Two lines of evidence suggests that ptc activity is negatively regulated by hh. First, in *hh ptc* double mutants, *wg* transcripts do not decay but behave as in *ptc* mutant embryos (Ingham et al., 1991). Second, generalized expression of *hh* under the control of a heat shock promoter generates phenocopies of *ptc*, the hs–hh phenotype, which consists of ectopic activation of *wg* to approximately one half of the cells of the parasegment (Ingham, 1993; Tabata and Kornberg, 1994).

The observation that the transmembrane ptc protein acts as a repressor of *wg* and that only cells receiving the hh signal can overcome this repression suggests that ptc

may encode the hh receptor (Ingham et al., 1991). Although biochemical analyses will be necessary to demonstrate whether ptc is the hh receptor, the observation that the hh protein stripes are more intense and broader in *ptc* mutants than in wild type (Taylor et al., 1993; Tabata and Kornberg, 1994) is consistent with this model.

Reasoning that mutations in genes acting downstream of hh should abolish *wg* transcription without eliminating *en* expression, Forbes et al. (1993) identified *fused* (*fu*) and *Cubitus interruptus Dominant* (Ci^D) as components of the hh signaling pathway. The role of the serine/threonine kinase *fu* in this pathway was further supported by the finding that *fu* activity is required for the generation of the hs–hh phenotype (Ingham, 1993). Finally, gene expression and double mutant analyses indicate that the segment polarity gene *Costal²* (Cos^2), which has yet to be molecularly characterized, is required downstream of fu and upstream of the transcription factor Ci^D. In this pathway (Figure 2B), ptc may repress the fu kinase, which is required for Ci^D activity, and Cos^2 may act as a negative regulator acting upstream of Ci^D.

Functions of Wg and Hh during Imaginal Disc Development

Both *wg* and *hh*, as well as several other segment polarity genes, are expressed at multiple times during development. In fact, genetic analyses have revealed that wg and hh are required for establishment of the polar coordinates within the imaginal discs during the first and second larval instar stages (Couso et al., 1993). In the leg imaginal discs, *wg*, which is expressed in the region from which ventral cells arise, is associated with a ventralizing activity. Ectopic expression of *wg* in dorsal disc regions reorganizes the pattern and results in the differentiation of ventrolateral patterns (Struhl and Basler, 1993). In addition, wg, together with *decapentaplegic* (*dpp*), which encodes a transforming growth factor β (TGFβ) homolog expressed in a stripe of cells in the anterior compartment along the anteroposterior boundary, is involved in proximodistal organization of the limb. The intersection between *dpp*-expressing cells and the *wg*-expressing cells has been postulated to define the domain of expression of the homeobox gene *aristaless*, which specifies the most distal structures of the appendage (Campbell et al., 1993). A new proximodistal axis that results in the production of duplicated appendages is generated when a second intersection of *wg*- and *dpp*-expressing cells is created (Campbell et al., 1993).

hh, like *en*, is expressed in the posterior compartment of imaginal discs. Ectopic expression of *hh* in the anterior compartment of the wing causes reorganization of the pattern (Tabata and Kornberg, 1994; Basler and Struhl, 1994). Characterization of these pattern rearrangments indicate that the normal activity of *hh* in the posterior compartment is to organize the pattern of the anterior compartment. Cells that ectopically express *hh* also induce local ectopic expression of *dpp*. Combined, these results suggest that hh may control pattern indirectly by activating expression of dpp in cells located in a stripe of cells along the anteroposterior compartment boundary. Subsequently, dpp would control growth and cell patterning. This model is consistent with the observation that loss of *hh* activity at the compartment boundary can block *dpp* expression and perturb cellular proliferation and patterning (Basler and Struhl, 1994).

One question is whether components involved in wg and hh signaling in the embryo also mediate wg and hh functions in the imaginal discs. Although little information is available to address this question, the answer appears to be yes. Both *dsh* and *arm* mutant animals exhibit polar patterning defects in their imaginal discs that are reminiscent of defects in *wg* mutants (Peifer et al., 1991; Klingensmith et al., 1994). In addition, induction of zw^3 clones in dorsal domains of wing discs leads to ventralization, a phenotype similar to ectopic induction of *wg* in the wing discs (E. Wilder and N. P.). In the case of hh signaling, it has been proposed that ectopic expression of hh in the anterior compartment of wing discs leads to repression of *ptc* activity. This in turn would allow genes such as *dpp*, which are normally repressed by *ptc*, to be expressed (Tabata and Kornberg, 1994; Capdevila et al., 1994; Basler and Struhl, 1994).

Patterning of the Wing Margin by Wg

In the larval third instar disc, *wg* is expressed along the presumptive margin of the wing (Couso et al., 1993), a region that gives rise anteriorly to an array of innervated bristles and posteriorly to noninnervated bristles. Wg activity is required in this patterning process since animals that lack *wg* gene activity at the margin lack bristles. At the margin, *wg* is expressed in a region three cells wide and its protein can be detected at low levels up to three cells on either side of the *wg*-expressing cells (Couso et al., 1994).

Components involved in wg signaling in the embryo also mediate wg patterning at the wing margin. Clones of either *dsh* or *arm* mutant cells at the margin exhibit a phenotype reminiscent of lack of *wg*, i.e., loss of bristles (Couso et al., 1994; Klingensmith et al., 1994). In addition, zw^3 exerts the opposite phenotype since clones of zw^3 mutant cells differentiate ectopic bristles. *dsh* activity is not required for the cellular transformation associated with loss of zw^3 activity since ectopic bristles differentiate from clones of *dsh* zw^3 double mutant cells (Couso et al., 1994). Altogether, the functional relationships described at the margin among wg, arm, dsh, and zw^3 are consistent with the epistatic relationships between these gene products during embryonic segmentation.

Perspectives

In this minireview, I have described our current understanding of the cellular functions and relationships among segment polarity genes. A striking observation is that the requirements for these genes as well as their functional relationships have been conserved in different developmental processes. For example, dsh, zw^3, and arm regulate wg effects on *en* autoregulation, formation of naked cuticle, establishment of polar coordinates, and margin formation. Further, the epistatic relationships among these genes are similar in various cellular contexts. In addition, the functions and relationships of components involved in hh signaling have been conserved. Taken together, it appears that wg and hh activate biochemical pathways that are conserved between cell types, though

Table 1. Molecules Encoded by Segment Polarity Genes

Drosophila Gene	Product or Motif	Vertebrate homologs
wg	Secreted protein	Wnt-1
dsh	Novel protein	MDsh1, MDsh2
zw^3	Serine/threonine kinase	GSK3
naked	Not determined	
arm	Cytoskeletal protein	β-Catenin, plakoglobin
porcupine	Not determined	
en	Homeodomain	En1, En2
Ci^D	Zinc finger	GLI
hh	Secreted protein	M-Dhh, M-Ihh, M-Shh, C-Shh, Z-Shh
ptc	Transmembrane protein	
fu	Serine/threonine kinase	
Su(fu)	Not determined	
gooseberry	Homeodomain, paired box	
Cos^2	Not determined	

this model will have to await biochemical tests to be substantiated.

To complete our understanding of the basic mechanisms underlying positional information, it will be critical to continue the ongoing genetic dissections of the pathways controlled by the segment polarity genes. The genetic screens conducted to date have not identified all segment polarity genes since many of those associated with maternal-effect phenotypes remain to be characterized (Perrimon et al., 1989). Interactive screens for second site modifiers, which have been successful in dissecting other signaling pathways, should also help in identifying additional segment polarity genes. This last approach, which has not been extensively applied to the study of segment polarity genes, has the promise of revealing possible redundancy or intricate relationships among the genes. For example, a dominant suppressor of *fu* null mutations, *Suppressor of fused* (*Su(fu)*), was isolated in such a screen (Preat, 1992). Interestingly, null *Su(fu)* mutations have no phenotypes by themselves, indicating that fu and Su(fu) either inhibit each other, respectively, or compete in an antagonistic fashion for a common substrate.

Further genetic analyses of segment polarity genes will certainly lead to the identification of additional components involved in the wg and hh signaling pathways and possibly to the identification of additional signals that, together with wg and hh, organize patterning. As indicated in Table 1, many of the segment polarity genes have vertebrate counterparts, and in some instances the roles of some of these molecules are comparable. The most striking example of homology is the recent finding that both the fly and vertebrate hh proteins function as morphogens during patterning (Heemskerk and DiNardo, 1994; see review by Smith, 1994). It is also important to realize that most, if not all, of the molecules implicated as transducers of both the wg and hh signals have been conserved during evolution, thus raising the possibility that wg and hh identify basic signaling pathways used throughout the animal kingdom to establish positional information.

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