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Regeneration in insects

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In insect limbs, networks of gene regulation mediated by secreted morphogens play key roles in controlling development, repair and regeneration. Two models for limb patterning and regeneration were proposed in the 1970s: The Polar Coordinate Model and The Boundary Model. Here we describe the molecular networks driving limb development and regeneration and how they support a hybrid PCM-Boundary Model, where circumferential positional values emerge from the interplay of two morphogens with two compartments and distal outgrowth is initiated by the combinatorial signaling of two morphogens, Wingless (Wg) and Decapentaplegic (Dpp). Autoactivating loops and lateral inhibition are prominent mechanisms in these networks.

Key words: regeneration / pattern formation / gene regulation / morphogen / distalization

Introduction

TISSUES EXHIBIT AN impressive ability to respond to a myriad of insults by repairing and regenerating complex structures. The elegant and orderly process of regeneration provides clues to the mechanisms of pattern formation but also offers the hope that the process might one day be manipulated to replace damaged body parts. To manipulate the process, it will be necessary to understand the genetic basis of the process. In the case of the insect leg, we are coming close to such a level of understanding and many of the lessons learned are relevant to vertebrate systems. A dynamic web of gene regulatory networks appears to create a robust self-organizing system that is at once extremely intricate but also perhaps simple in its reliance on a few key signaling pathways and a few simple processes, e.g. autoactivation and lateral inhibition. Here we will summarize what has been learned about the networks of gene regulation present in the *Drosophila* leg discs and then we will explore how the regenerative responses to different insults can be understood as predictable responses to these networks. Each of the regulatory networks could themselves serve as the subject of a detailed review and that is beyond the scope of this discussion. Here we will focus on the interplay between the regulatory networks in patterning the tissue.

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The tissue: imaginal discs

The appendages of *Drosophila* arise from imaginal discs. Topologically, discs are bags of cells comprised of a single epithelial sheet (reviewed in refs 1,2). The disc is asymmetric with one side comprised of folded columnar epithelial cells that will give rise to the adult structures and the other side comprised of a few very squamous cells that make up the peripodial membrane (Figure 1). During metamorphosis, the columnar side telescopes out to form an appendage.

Imaginal discs arise from approximately 10 to 30 cells at the intersection of *wingless (wg), decapentaplegic (dpp)* and *engrailed (en)* expression on the flank of the embryo and hence initial asymmetries in the disc are defined by embryonic gene networks.[2] The disc field invaginates from the embryonic ectoderm to become a physically independent bag of cells connected to the larval hypoderm by a thin stalk. A leg disc will increase from 10–30 cells to 10–30 000 cells before ceasing growth and undergoing metamorphosis.[1,2] Cell division occurs throughout the disc without any obvious growth zones[1,2] until very late. Cell lineage's apart from the A/P compartment lineage's, appear for the most part indeterminate,[1,2] suggesting that cell interactions play an important role in evolving towards the final pattern.

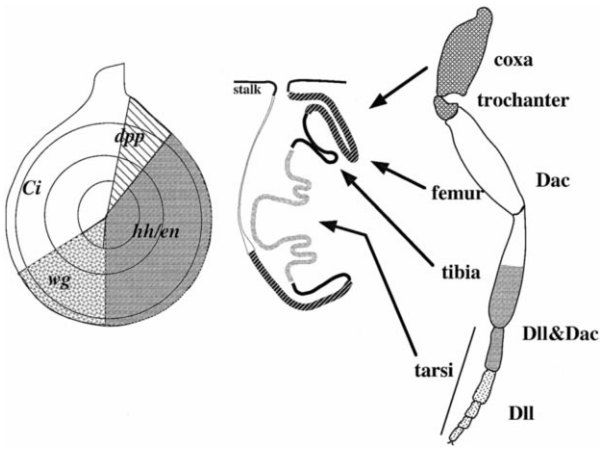

Figure 1. Anatomy of a leg disc and leg. (A) The frontal view of the leg disc shows a series of rings demarcated by folds that will telescope out of the plane to form the leg during metamorphosis. Dorsal is up and anterior is to the left. The domains of *ci, dpp, hh, en* and *wg* expression are indicated. (B) The side view shows the approximate sources of the adult structures with the tarsi arising from the central fold(s), the tibia from the outermost folds and the femur, coxa, trochanter arising from the outmost ring of the epithelium. The peripodial membrane covers the apical surface of the epithelium. The domains of *dac* and *Dll* expression relative to the adult structures are indicated.

Requirements for normal patterning in *Drosophila* leg discs

The minimum requirement for a chiral structure such as a limb is three axes of asymmetry. In discs (and other epithelia), one axis is the apical basal axis of the cells that is fixed. Disruption of this axis leads to a complete breakdown of tissue architecture and no pattern at all (e.g. ref 3). The bulk of the patterning stems from the interaction of the other two axes of asymmetry, namely the Anterior/Posterior (A/P) and Dorsal/Ventral (DV) axes. Briefly, asymmetry in the A/P axis is provided by a lineage restriction between the anterior and posterior compartments (reviewed in ref 4), while asymmetry in the D/V axis is generated by the expression of *dpp* dorsally[5,6] and *wg* ventrally.[7,8] For this discussion, we postulate that localized expression of just two morphogen organizers, *wg* and *dpp*, is sufficient to produce a symmetrical outgrowth (limb) and that the added feature of differential response of cells in the anterior and posterior compartments is sufficient to account for production of a chiral limb. This assumes the following:

- Two morphogens, Wg and Dpp establish ventral and dorsal territories, respectively, by a process of autoactivation and lateral inhibition.
- Combinatorial signaling at the intersection of the two morphogen territories initiates distal outgrowth.
- A short-range inducer from the posterior compartment, i.e. Hedgehog (Hh), maintains localized expression of *wg* and *dpp*.
- Different responses of Anterior and Posterior compartment cells can generate asymmetry in the A/P axis thus establishing chirality.
- The properties of the autoactivating loops and lateral inhibitory networks are sufficient to account for regeneration of stable pattern after insult.
- The continuous input of these morphogens is essential throughout the developmental process up until the last hours before differentiation thus providing a dynamic system able to respond at many times in development to injury.

Wg and Dpp are the key morphogens

Many experiments have demonstrated that the localized expression of *wg* and *dpp* are essential for normal pattern formation. In leg discs, both *wg* and *dpp* are expressed in narrow wedges of anterior cells abutting the A/P compartment border[5–8] (Figure 1). Loss of *wg* causes loss of ventral structures and symmetrical duplication of dorsal structures, while ectopic expression of *wg* in the dorsal region causes production of ectopic ventrolateral structures and organization of an ectopic leg.[7–12] The reverse is true for *dpp*. Loss of *dpp* causes loss of distal and dorsal pattern elements, sometimes accompanied by duplication of ventral tissue,[13,14] while ectopic activation of *dpp* in a ventral region produces a new leg axis.[15,16] Thus, localized expression of *wg* and *dpp* is both essential for patterning and sufficient to initiate new (regenerative) patterning.

Dorsal and ventral territories of *dpp* and *wg* expression are achieved by autoactivation and lateral inhibition

With the initial discovery of compartments in the A/P axis, it was predicted that compartments would provide a general mechanism for patterning along other axes, like the D/V axis. D/V compartments would provide a convenient mechanism to maintain

wg expression ventrally and *dpp* expression dorsally. In fact, no evidence for a D/V compartment has been found in leg discs.[17] Rather, localized expression of *wg* and *dpp* is maintained by a territory system that involves autoactivation and lateral inhibition[18] (Figure 2). As we will see, such territories are much more dynamic and robust in their ability to regenerate coherent pattern after a myriad of insults than fixed lineage systems.

Several studies reveal that Wg and Dpp inhibit each other's expression.[16,18–23] For example, eliminating Wg signaling in the entire disc by using temperature sensitive alleles of *wg* leads to ectopic *dpp* expression in the *wg* domain over a time span that is sufficient for only a few cell divisions.[18] In addition, clones of cells that lack Wg response (e.g. *dsh*) activate *dpp*.[11,23] Conversely, ectopic expression of *wg* suppresses *dpp* expression dorsally.[18,19,21] Manipulations of Dpp signaling affect *wg* transcription in a similar fashion. Loss of Dpp signaling allows ectopic *wg* expression along the A/P border, while ectopic expression of *dpp* suppresses *wg* expression ventrally.[16,18–20,22]

In addition to inhibiting each other's expression, Wg and Dpp each autoactivate their own expression as suggested by manipulations of either pathway downstream of the ligand. For example, Wg signaling inhibits Shaggy activity (Sgg, ZW-3 or mammalian Glycogen Synthase Kinase) and the regenerative responses to engineered loss of Sgg activity resemble the responses to ectopic *wg* expression.[12,23,24] Sgg clones also activate *wg* expression.[20,23] On the other hand, Dishevelled (Dsh) activity is required to transmit the Wg signal and loss of *dsh* causes loss of *wg* expression.[23,25,26]

Autoactivation of *dpp* in leg discs is indicated by the observation that ectopic expression of *dpp* can trigger ectopic expression of a *dpp > LacZ* reporter gene.[16] Interestingly, the regulatory networks can be quite tissue-specific. For example, Wg repression of *dpp* that is clear in the leg discs, does not occur in the wing disc[23] and in the developing gut, Wg and Dpp signaling act synergistically[27] rather than antagonistically as in the leg.

In summary, a system of autoactivation operates throughout development to maintain both *wg* and *dpp* expression in the leg disc and a system of cross-inhibition ensures that the initial D/V bias of *dpp* and *wg* expression will be perpetuated (Figure 2). This regulatory circuit helps us to understand how forced confrontation of inappropriate cells in response to genetic or surgical manipulations can be resolved into coherent and organized patterns with respect to Wg and Dpp.

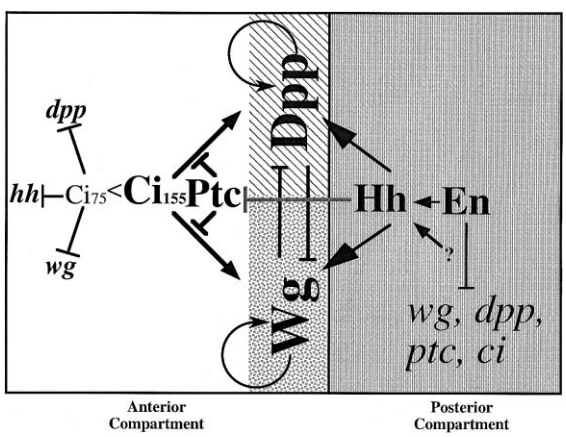

Figure 2. Local sources of *dpp* and *wg* expression are restricted to the region along the A/P boundary. Ci_{155} is required for *dpp* and *wg* expression but is continuously inhibited by the action of Ptc. Hh diffusing from the posterior compartment, relieves this inhibition; thus, maintaining a localized source of *dpp* and *wg* expression. The different dorsal and ventral territories of *dpp* and *wg* expression, respectively are maintained by a network of autoactivation of, e.g. Wg activating itself, and lateral inhibition in which Wg inhibits *dpp* expression and visa versa.

The A/P axis is characterized by compartments of lineage restriction and an inductive circuit at the boundary between two compartments involving Hh

Restriction of *wg* and *dpp* expression to defined wedges requires the interplay of the A and P compartments. Unlike the dynamic network that maintains D/V territories of *dpp* and *wg* expression, asymmetry in the A/P axis is achieved by lineage restriction.[4,28] The posterior compartment is defined by expression of the homeotic selector gene *engrailed* (*en*) that inhibits expression of anterior genes, e.g. *wg*, *dpp*, *patched* (*ptc*) and *cubitus interuptus* (*ci*) while promoting expression of *hh*.[29,30] By defining the posterior compartment, En also modulates the response of posterior cells to the secreted morphogens Wg and Dpp.

Localized expression of *wg* and *dpp* is achieved by an inductive circuit at the boundary between two compartments involving Hh. Hh is expressed only in the posterior compartment[31–33] and diffuses a short distance into the anterior compartment where it

counteracts the inhibition of Smoothened (Smo) signaling by the Patched protein (Ptc) thereby allowing *wg* and *dpp* to be expressed (Figures 2 and 3). Smo encodes a seven-pass transmembrane protein[34,35] which acts constitutively to promote *wg* and *dpp* expression.[36] However, the transmembrane protein Ptc[37,38] constitutively inhibits Smo activity, thereby inhibiting *wg* and *dpp* expression in the anterior compartment. Hedgehog can bind the Ptc receptor and thereby relieve the inhibition of Smo activity.[36,39–41] In addition to its role in transducing the Hh signal, Ptc also impedes the movement of Hh thereby limiting the region of *wg* and *dpp* activation to a narrow stripe abutting the A/P compartment boundary[36,41–43] (Figure 2).

The anterior compartment is defined by expression of Zn finger transcription factor Cubitus interruptus (Ci) which is related to the Gli proteins of vertebrates.[44,45] Ci can be proteolytically processed to produce two different forms, Ci75 a repressor and Ci155 an activator (Figures 2 and 3). At the A/P boundary, Hh signaling causes Ci to be converted to an activator of *wg*, *dpp* and *ptc* expression.[46–49] In A cells that do not receive the Hh signal, i.e. distant from the A/P border, Ci75 predominates and represses *hh*, *wg* and *dpp* but not *en* expression.[45,46,48] The fine tuning of this regulatory circuit is known to involve interactions with other factors such as Fused Suppressor of fused, PKA, Costal-2, Smo, Ptc, Groucho, Polyhomeotic (Figure 3; refs 43,47,50–56).

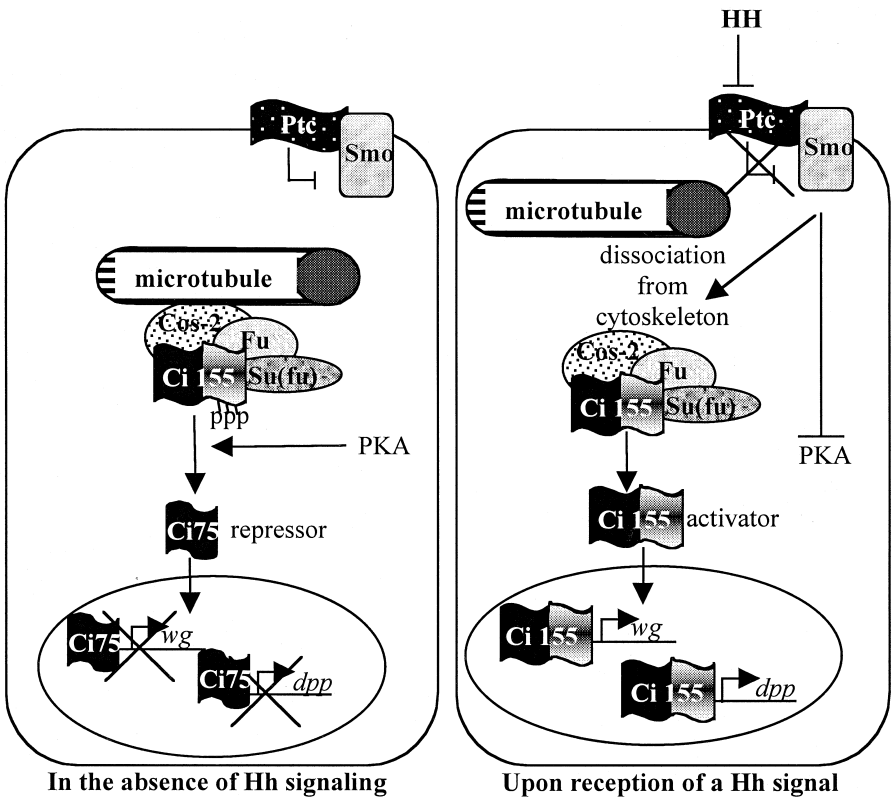

Figure 3. Ptc and Smo have opposite effects on Ci activity. In the absence of Hh signaling, Ptc inhibits Smo activity. When Smo activity is inhibited, full length Ci (Ci 155) is held in a cytoplasmic multiprotein complex that is associated with microtubules and includes Cos2, Fu and Su(fu). PKA phosphorylates Ci and promotes the cleavage of Ci to form Ci75, which acts as a transcriptional repressor and inhibits *wg* and *dpp* transcription. Upon reception of a Hh signal, Pte is inactivated and Smo activity is no longer inhibited. Smo inhibits PKA, thus inhibiting phosphorylation and proteolysis of Ci155. Smo also promotes the dissociation of the Ci155 containing multiprotein complex freeing the transcriptional activator, Ci155, to activate *wg* and *dpp* expression. Thus, in cells that receive a Hh signal, the Ci75 repressor is not produced and the Ci155 transcriptional activator is released from a multiprotein complex.

The ability to maintain two distinct populations of cells, A and P, that do not mix appears to require Hh signaling to establish boundaries of affinity that distinguish the two compartments.[53,57] An important issue is how the regulatory network between the A and P compartments can account for the apparent regeneration of posterior compartment cells in disc fragments that regenerate. We will consider this in the discussion of responses to surgical manipulations below.

Wg plus Dpp are sufficient to distalize

Establishment of the proximal distal axis has long been perplexing.[58] Meinhardt[59] proposed that a unique morphogen whose expression required input from Wg, Dpp and Hh, would be expressed at the center of the disc and would pattern the P/D axis. The disc appears to have adopted a more conservative mechanism to accomplish the same objective (Figure 4). A regulatory network exists in which combinatorial input from Wg and Dpp directs gene expression in discrete domains along the proximodistal axis. High levels of Wg and Dpp activate *Distalless (Dll)* expression in the distal leg.[60–63] Dll in turn inhibits *homothorax (hth)* expression[62,64] which is required for targeting Extradenticle (Exd) to the nucleus.[65–67] These interactions set up a proximal domain of active nuclear Exd and a distal domain of inactive cytoplasmic Exd. The nuclear Exd in the proximal domain of the leg prevents cells from responding to Wg or DPP signaling thus dividing the leg into Wg/Dpp responsive and Wg/Dpp non-responsive domains along the P/D axis.[62–64] The Wg/Dpp, responsive domain is further divided into *Dll* and *dachshund (dac)* expressing regions by the differential response of cells to levels of Wg and Dpp signaling and repression of Dll by Dac.[61,64] These discrete domains of gene expression are required for normal P/D patterning. Dll is required for the more distal regions of the leg,[68] while Dac specifies intermediate pattern elements[69] and Exd and Hth are required for the formation proximal structures.[70,71]

The shape of a new outgrowth is influenced by the spatial regulation of the combinatorial inducers, Wg and Dpp. If not inhibited by other mechanisms (e.g. Ptc), Wg and Dpp will autoactivate and laterally inhibit to produce two territories of expression with a long border. If *hh* is expressed uniformly,[60,72] a paddle shaped limb will develop.[60] If expression is limited to wedges, a pointy leg (natural) will develop. Wherever *Dll* is activated, a P/D axis will develop as Dll activity inhibits *hth* expression leaving a proximal ring of Hth which then forms an autoregulatory loop with Exd. Since Hth blocks the effect of both Wg and Dpp on their target genes, the range of action of these cytokines is restricted in the P/D axis.[60–64] Thus, the juxtaposition of high levels of Wg and Dpp is sufficient to initiate a cascade of gene regulation that defines a P/D axis and organizes a new limb.

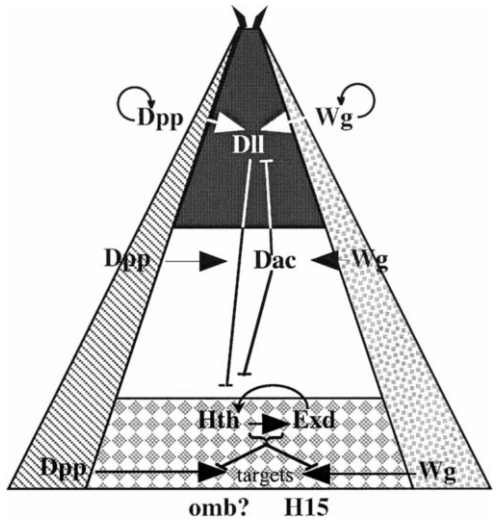

Figure 4. Wg and Dpp trigger a cascade of regulatory networks in the P/D axis. High levels of Wg and Dpp induce *Dll* expression and low levels induce *dac*. Where levels of Dll and Dac are low, *hth* is expressed. Hth causes ubiquitously expressed Exd to enter the nucleus. Nuclear Exd in turn stabilizes Hth; thus, forming an autoactivating loop and providing a source of regulatory proteins that inhibit the expression of targets of Wg and Dpp regulation, e.g. H15 for Wg and *omb* in the wing for Dpp. Whether the action of Dll on *hth* is direct or indirect is not resolved. These interactions divide the P/D axis into three regions: a distal region defined by Dll, an intermediate region, where Dac is required to specify cell fates; and a proximal region defined by Hth and Exd.

Understanding regeneration in terms of regulatory networks

A number of experimental manipulations will induce ectopic patterning including surgical manipulations, misexpression experiments and loss of heterozygosity. All of these manipulations can (but do not always) produce supernumerary truncated limbs.

Misexpression experiments

Expression of genes in ectopic locations can be accomplished by several strategies including the flipout technique[11] and the Gal4/UAS system.[73] In these experiments, the gene is constitutively active since it has been removed from the feedback regulation that may characterize the normal gene. For example, ectopic activation of *wg* in a dorsal region causes bifurcated limbs with the ectopic limb arising completely from the A compartment with no P tissue.[60,72] Based on the networks described above, these double anterior regenerates can be explained by the establishment of an ectopic focus of Wg and Dpp juxtaposition that activates Dll expression and a new P/D axis. The lack of posterior tissue in these regenerates reflects the inability to regulate *en* expression in Anterior cells in order to reassign compartment identity.

In another example, misexpression of *hh* in just the dorsal or just the ventral anterior regions affects patterning but does not produce distal outgrowth.[60,72] Only when a *hh* expressing clone is present in both the dorsal and ventral anterior regions, are both *wg* and *dpp* activated and consequently Dll and a new P/D axis established. These outgrowths contain only anterior tissue suggesting that Hh cannot induce *en* that is required for posterior cell fates.

Morimura *et al*[16] used the dpp^{blk} > GAL4:UAS to ectopically express *dpp*. When low levels of *dpp* were expressed ventrally, leg duplications emerged suggesting that the Dpp/Wg combination activated *Dll* expression and produced an outgrowth. On the other hand, if high levels of *dpp* were expressed, ventral structures were lost entirely and duplications of dorsal structures were observed. One imagines that the higher levels of Dpp were sufficient to extinguish *wg* expression[16,18–20,22,23] and the artificial expression of *dpp* would drive cells toward a dorsal fate.

Loss of heterozygosity experiments

Induction of mitotic clones can be used to engineer the loss of heterozygosity in a particular cell and all its descendants. The patterning responses to loss of heterozygosity appear to be due to disruption of autoactivating or inhibiting loops that result in misexpression of one morphogen or another. For example, Shaggy activity antagonizes Wg signaling[74] and loss of Sgg function leads to activation of *wg* expression.[20,23]. If *sgg* clones occur in the *dpp* expressing regions of a disc, two new foci of Dpp/Wg confrontation are created along the P/D axis. This causes two new distalization centers to be established and a pattern triplication to emerge with the ectopic limbs comprised solely of anterior tissue[23,60] implying the absence of a mechanism to activate *en* and posterior compartment genes. Dsh activity antagonizes Sgg, and *dsh* clones have the opposite effect namely a ventral clone of *dsh* loses *wg* expression and activates *dpp* expression.[20,23]. The resulting Dpp/Wg confrontation activates *Dll* and organizes a new center of distalization with opposite polarity to that produced by *sgg* clones.

Similarly, clones of cells that have lost the Dpp receptors, Punt, Thickveins or Saxophone activate *wg*.[22] Such activation of *wg* would create a new focus of Dpp/Wg confrontation and consequent activation of Dll and outgrowth, e.g. see Figure 1C in ref 22. Thus, the responses to loss of genes downstream of ligands in signaling pathways can best be understood by their affects on the autoactivating and laterally inhibiting regulatory networks affecting expression of morphogen genes.

Transcriptional regulation can account for the patterning responses to manipulations of Hh signaling. Loss of Hh function results in the loss of distal leg elements[33,72] while ectopic Hh can lead to the formation of double anterior supernumerary limbs.[72] Since Hh signaling is required to maintain *wg* and *dpp* expression[72] loss of Hh indirectly leads to loss of Dll and truncated legs.[60–63] On the other hand, clones of ectopic Hh that fall along the D/V midline of the anterior compartment activate *dpp* in the dorsal region and *wg* in the ventral region of the clone. These clones produce double anterior outgrowths suggesting that activated *wg* and *dpp* are sufficient to specify P/D growth that is symmetrical if there is no input from the posterior compartment, but input from the posterior compartment does confer a chirality to a fully patterned leg.[72]

Surgical manipulations

Central to the insect regeneration field have been the responses to surgical manipulations of *Drosophila* imaginal discs.[75–81] One of the key observations has been that the anterior dorsal quadrant of a first leg disc (A1/4) will regenerate while the remaining P3/4 fragment will duplicate (Figure 5). One perplexing aspect of these observations has been how a fragment completely lacking a posterior compartment (A1/4) could regenerate and why a fragment with at least some *wg*, *dpp* and *en* regions (P3/4) would

duplicate.[28] In a series of elegant experiments, Gibson and Schubiger[79] provide an explanation that forces us to reinterpret many of the older experiments. They find that first leg discs are unique in having a small patch of *en* and *hh* expressing cells in the peripodial membrane that appears to serve as a source of posterior compartment material in these fragments (Figure 5). Notably, second and third leg discs do NOT have this patch of *en* and *hh* and they do NOT exhibit the same regenerative capacity. Almost all the literature describing manipulations of *Drosophila* leg discs is based on manipulations of the first leg disc exclusively [i.e. the prothoracic (L1) disc]. In the process of wound closing, the unique domain of *en/hh* expression in L1 discs provides a basis for reestablishing a posterior compartment that other discs (L2; L3) do not possess.[79] From the regulatory networks described above, one would expect that posterior 3/4 fragments of leg discs (roughly 12 to 9 o'clock) would regenerate as the cut edges expressing *wg* and *dpp* come into contact and begin to regulate. In fact, P3/4 fragments from L2 and L3 discs do just that. The exception is the P3/4 fragment from L1 discs which duplicates posterior structures because the wound closing brings a novel patch of *en* expressing cells to the anterior. If Hh is eliminated from the P3/4 fragments of L1 discs (e.g. by hh^{ts}), then the Anterior compartment regenerates via the action of *wg* and *dpp* as the other discs do. Similarly, the unique patch of peripodial cells that express *en/hh* in first leg discs provides a starting source of posterior cells that allows regeneration in the A1/4 fragments from L1 discs. Notably, A1/4 fragments from L2 and L3 discs fail to thrive when cultured as does the A1/4 fragment from L1 discs if Hh activity is blocked, e.g. by a temperature sensitive allele of *hh*.[79] It is clear that the small patch of *hh/en* expressing cells is able to direct the growth of a new posterior compartment. Although the exact mechanism whereby this occurs is unclear, one immediate response to wound healing is activation of patterning genes such as *dpp* and H15.[82] In some experiments, Hh does not seem able to reprogram A cells to P fates[30] although in other experiments ectopic Hh activity can activate *en* expression in anterior wing cells[51,83] and leg discs[79]. Perhaps the many experiments with L1 discs are the exception that proves the rule, i.e. *wg* and *dpp* are capable of regenerating the disc IF they have a mechanism to support their expression which in *Drosophila* leg discs requires sustained Hh to relieve the repression by Ptc.

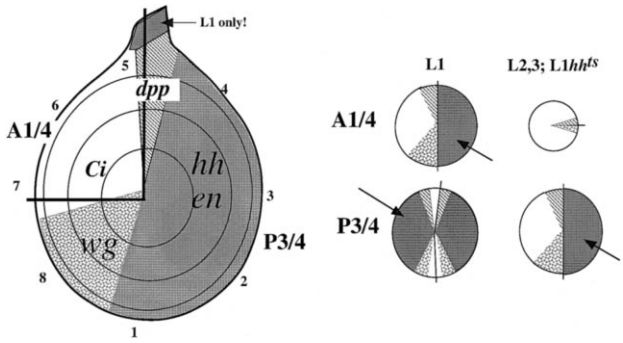

Figure 5. A source of Hh and En are required to establish a new posterior compartment. (A) *Expression patterns of genes relative to cuts (dark black lines).* Dorsal is up, anterior is to the left. Expression of *en* and *hh* are restricted to the posterior compartment in all leg discs. The first leg disc is unique in having *en, hh* expressing cells in the dorsal peripodial cells (arrow). (B) *Patterning responses to surgical cuts described in (A)* in *L1, L2* and *L3* discs. The A1/4 fragment from L1 has a small bit of *en, hh* expression (arrow in A) which provides the starting point to regenerate a posterior compartment (arrow in B). Because the L2 and L3 discs do not have these *en, hh* expressing peripodial cells, they cannot create a posterior compartment and the A1/4 fragments from L2 and L3 do not regenerate. In P3/4 L1 disc fragments, the small patch of *en, hh* expression closes on the wounded edge and provides a second source of posterior compartment (arrow), and hence creates a mirror image duplication. The P3/4 fragments from L2 and L3 have an intact posterior compartment and thus, regeneration is dominated by Wg and Dpp regenerating the A compartment. L1 discs that have lost Hh function, L1 hh^{ts}, behave like L2 and L3 discs emphasizing the importance of a source of Hh and En in the outcome of a regenerate.

A second set of surgical manipulations addressing the processes of regeneration involved grafting experiments with cockroach legs and other insects (reviewed in refs 60,80,84. The results of those experiments can be understood if one assumes spatial patterns of gene expression and regulatory circuits similar to those observed in the *Drosophila* discs. Contralateral grafts of legs to stumps would bring *wg* and *dpp* expressing regions together which would be expected to induce Dll and produce outgrowths which they do. Rotation during grafting brings A and P cells in apposition thus creating a new A/P boundary with attendant induction of *wg* and *dpp* and distalization. For an excellent discussion of these experiments, see ref 60. Notably, 90° rotations do not produce supernumerary limbs perhaps reflecting the fact that only modest changes in *wg/dpp* or A/P confrontations would be created which would likely be resolved with minimal alterations of pattern.

In summary, the resolution of the paradoxically different behavior of first leg discs versus the other discs in *Drosophila* coupled with the regulatory networks that have emerged from the contributions of many authors provides a framework to understand a molecular basis for regeneration in insect legs in response to surgical manipulations.

Models and molecules

There has been much discussion about compartments, boundaries, morphogens and positional identity. Bateson[85] articulated rules to account for spontaneous triplications in insect legs such as the fact that the three legs lie in a plane. The spatial locations of *wg / dpp / hh* account for that observation. Wolpert[86,87] defined the property of positional information to account for the behavior of surgically manipulated tissues. The polar coordinate model[58,80] postulated a set of positional identities in two dimensions, i.e. radial and circumferential. The interplay between *wg* and *dpp* in promoting *Dll* expression and radial P/D differentiation and the interplay between these two morphogens and the A and P compartment in assigning circumferential identities fit this model well.[88] A notable point was the suggestion that positional values would be continuous without boundaries of discontinuity. Meinhardt[59] proposed a hybrid model that called for three compartment boundaries to act as organizing centers with the intersection of the three compartments to induce a proximal morphogen.[89] Indeed, the networks described here fall somewhere between these two models. The distinction between D and V is not defined by a compartment but rather by a dynamic continuum of Wg and Dpp signaling. The continuous need for this input to maintain patterns of expression can account for the robust ability of tissues to reorganize and regenerate after a myriad of insults. The combinatorial action of Wg and Dpp is sufficient to define the P/D axis without the need for new morphogen, but the addition of Hh from the posterior compartment relieving a repression keeps that interaction focused to a point rather than a line.

A working hypothesis

What are the key mechanistic interactions that appear to make the system work? One is the involvement of territories of morphogen activity that are maintained by a system of autoactivation and lateral inhibition that requires continuous input. Such a system is dynamic allowing confrontations of cells to be resolved in a coherent manner as morphogen gene expression is activated or repressed depending on the confrontation. A second key feature is the extensive use of negative regulation such that loss of one component leads to activation of another. Thus, loss of heterozygosity for a negative element in an autoactivating pathway or in a cross-inhibiting pathway can lead to activation of a growth factor and thus, initiation of growth and patterning. Some hyperplasias may originate by such a mechanism. The use of combinatorial input from two morphogens to define a unique point in a tissue replaces the need for a distal morphogen.

Compartments play two roles. One is to serve as a spatially restricted source of Ptc inhibition; thus establishing the boundary as an organizer and restricting confrontation of Wg and Dpp to a point resulting in a circle of Dll expression and a tube-like leg. Compartments also affect the response of cells to these two morphogens resulting in chirality. Formally, it seems possible that a second pair of morphogens orthogonal to the first could be used to pattern the A/P axis resulting in four wedges of morphogen expression without the need for compartments. However, we must wait to see if such a mechanism exists in other systems or if the flexibility of such a system might be its own undoing. It seems likely that different morphogens could be used to pattern limbs in other systems by employing similar regulatory circuits.

Although the discussion above can serve as a reasonable working hypothesis, there is much to learn. For example, the mechanism of how a few posterior cells can serve to regenerate a compartment needs further study. How these thoughts apply to the topologically more complex wing disc that nevertheless follows many of the same rules is not fully understood. Perhaps most notable, very few of the formal interactions discussed here have been documented at the level of understanding the direct physical and functional interactions of the participating gene products.

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