Drosophila Wingless: A Paradigm for the Function and Mechanism of Wnt Signaling

Esther Siegfried and Norbert Perrimon

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Summary

The link between oncogenesis and normal development is well illustrated by the study of the Wnt family of proteins. The first *Wnt* gene (*int-1*) was identified over a decade ago as a proto-oncogene, activated in response to proviral insertion of a mouse mammary tumor virus. Subsequently, the discovery that *Drosophila wingless*, a developmentally important gene, is homologous to *int-1* supported the notion that *int-1* may have a role in normal development. In the last few years it has been recognized that int-1 and Wingless belong to a large family of related glyco-proteins found in vertebrates and invertebrates. In recognition of this, members of this family have been renamed Wnts, an amalgam of int and Wingless. Investigation of *Wnt* genes in *Xenopus* and mouse indicates that Wnts have a role in cell proliferation, differentiation and body axis formation. Further analysis in *Drosophila* has revealed that Wingless function is required in several developmental processes in the embryo and imaginal discs. In addition, a genetic approach has identified some of the molecules required for the transmission and reception of the Wingless signal. We will review recent data which have contributed to our growing understanding of the function and mechanism of *Drosophila* Wingless signaling in cell fate determination, growth and specification of pattern.

Introduction

The *Wnt* genes encode a large family of putative signaling molecules implicated in the regulation of several developmental processes. The first member of this family to be identified was *Wnt-1* (*int-1*), which was isolated as a mammalian proto-oncogene capable of regulating cell proliferation[1]. Subsequently, related proteins have been identified in many species from *Drosophila* to humans, and multiple members of this family have been found within each species[2,3]. Examination of *Wnt* expression patterns has revealed that individual *Wnt*s have distinct spatial distributions in embryonic and adult tissues, suggesting that they have specific developmental functions[2,3]. Furthermore, ectopic expression of Wnts in *Xenopus*[4,5], and loss-of-function studies in the mouse[6,7], have demonstrated that alterations of *Wnt* gene expression have profound effects on the development of the organism.

The greatest understanding of the role of *Wnts* in development has come from genetic studies of the *Drosophila Wnt-1* gene, *wingless* (*wg*). *wg* was first identified by a mutation that caused the loss of adult wings, hence the name[8]. Subsequently, it was recognized that Wg signaling functions in embryonic segmentation, cell fate determination, cell proliferation and axis specification. Additional *Wnt* genes have been identified in *Drosophila*: *DWnt-2* and *DWnt-3*, which most closely resemble mouse *Wnt-7* and *Wnt-5* respectively[9,10]. Their expression patterns are distinct; *DWnt-2* is expressed in the ectoderm overlapping *wg* expression and *DWnt-3* is expressed primarily in the mesoderm. Mutations in these genes have not yet been isolated and the properties of the encoded proteins are unknown, therefore we will not discuss these genes further.

The realization that *wg* encodes the homologue of a putative secreted glyco-protein was very exciting, in the light of the proposed role for Wg in the inter-cellular communication process which regulates segmental patterning[11-13]. In the embryonic epidermis, *wg* expression is restricted to a small number of cells within each segment, whereas almost the entire segment is affected in *wg* mutant embryos[11,14]. In addition, earlier genetic mosaic analyses had shown that *wg* is not cell autonomous: patches of *wg* cells can be rescued by surrounding wild-type cells[15]. The similarity of Wg and Wnt-1 further supports a role for Wg in inter-cellular communication, and implicates Wg as the signal.

Drosophila Wg has the greatest similarity to the vertebrate Wnt-1 (54% amino acid identity), which it more closely resembles than *Drosophila* DWnt-2 or DWnt-3. Although the sequence predicts a secreted glycoprotein, no Wnt-1 protein has been detected in the media of cells expressing *Wnt-1* and no active protein has been isolated[16-18]. Wnt-1 protein has been shown to adhere to the cell surface[19] as well as to the extracellular matrix[20]. In addition, it has been demonstrated that Wnt-1 can transform cells *via* a paracrine mechanism: non-responsive cells transfected with Wnt-1 can cause morphological and proliferative changes in adjacent mammary epithelial cells that do not express Wnt-1[21]. The similarity between Wg and Wnt-1 is consistent with Wg functioning in a paracrine fashion, and there is direct evidence that Wg is a secreted factor. Wg protein has been detected in neighboring non-expressing cells[22,23], and in recent experiments Wg protein has been shown to be associated with the cell surface of transfected cultured cells[24].

As mentioned above, Wg has a role in embryonic segmental patterning. This is not the sole function of Wg but rather, reflects the earliest developmental process in which it is involved. Studies of Wg function using conditional mutations, mosaic analyses and ectopic expression have indicated that *wg* is required for several different developmental pathways in the embryo and imaginal discs. Wg activity is required at different stages of development, as well as being involved in diverse processes regulating cell fate determination, patterning and growth. In this review we will describe the different developmental processes in which Wg participates, and address the function and mechanism of Wg signaling.

The role of Wingless in embryonic segmentation

The overall anterior-posterior axis of the *Drosophila* embryo is determined by a hierarchy of maternal and zygotic genes

that subdivide the embryo into segmental units[25]. Segmentation is clearly visible in the cuticle of the hatched larva as an alternating pattern of naked cuticle and cuticle covered with small hairs, called denticles (Fig. 1). This pattern reflects the establishment of segmental borders, polarity and specific cell fates within each segmental unit. This is achieved through a mechanism of cell-cell communication, mediated in part by Wg[26,27]. Although Wg is expressed in a subset of the cells of each segmental unit, Wg activity is required for correct patterning of the entire segmental unit. In *wg* mutant embryos, the larval cuticle pattern is severely disrupted: the naked cuticle is lost and replaced by a mirror image duplication of rows of denticles (Fig. 1)[28].

In the early embryo, Wg is expressed in stripes of epidermal cells that are immediately adjacent, and anterior, to cells expressing the homeo-domain protein Engrailed (En)[11,14,29]. The juxtaposition of En- and Wg-expressing cells is crucial for the establishment of borders and polarity within each segmental unit (see Fig. 1; Wg expression is shown in blue and En expression is shown in brown). The first morphological manifestation of embryonic segmentation is the appearance of the shallow parasegmental grooves precisely at the interface of the En and Wg stripes (Fig. 1)[11,14]. The parasegmental borders are transient and out of register with the later segmental borders, but they do correspond to the domains of homeotic gene expression, at least in the thorax and abdomen[30]. These borders disappear and are replaced by the segmental borders which occur at the posterior edge of the En cells. It has been proposed that En expression not only demarcates posterior cells within each segment, but also is required for establishing a lineage restricted to the posterior fate[31,32]. This lineage restriction would divide each segmental unit into two distinct compartments, anterior and posterior.

The expression of both En and Wg are initiated by combinations of pair-rule genes[26]; however, the maintenance of their expression is mutually dependent. In *wg* mutant embryos, En expression fades from the epidermis[14,29,33], and in *en* mutant embryos, epidermal Wg expression disappears[14,34]. Wg signaling to adjacent En cells is critical for the maintenance of cell fate. Clonal analyses in embryos using a photo-activatable lineage marker have demonstrated that En expression does not define a lineage, as once thought[35]. Instead, the maintenance of En expression within individual cells determines which cells assume the posterior cell fate, and the stability of En expression is regulated by the proximity of cells to the Wg-expressing cells. Thus, one early function of Wg signaling during embryonic segmentation is to stabilize En expression, which in turn, determines the position of the segmental borders and specifies segmental polarity. Two lines of evidence strongly support the suggestion that the signaling from Wg- to En-expressing cells is mediated by Wg protein itself. First, Wg protein has been detected as far as two to three cells away from the cells where it is expressed, including the adjacent En-expressing cells[22,23]. Second, *in vitro* experiments have demonstrated that isolated En-expressing cells will maintain En expression when cocultured with heterologous cells transfected with *wg*, but will not if cultured alone[36].

Role of Wingless in tissue induction

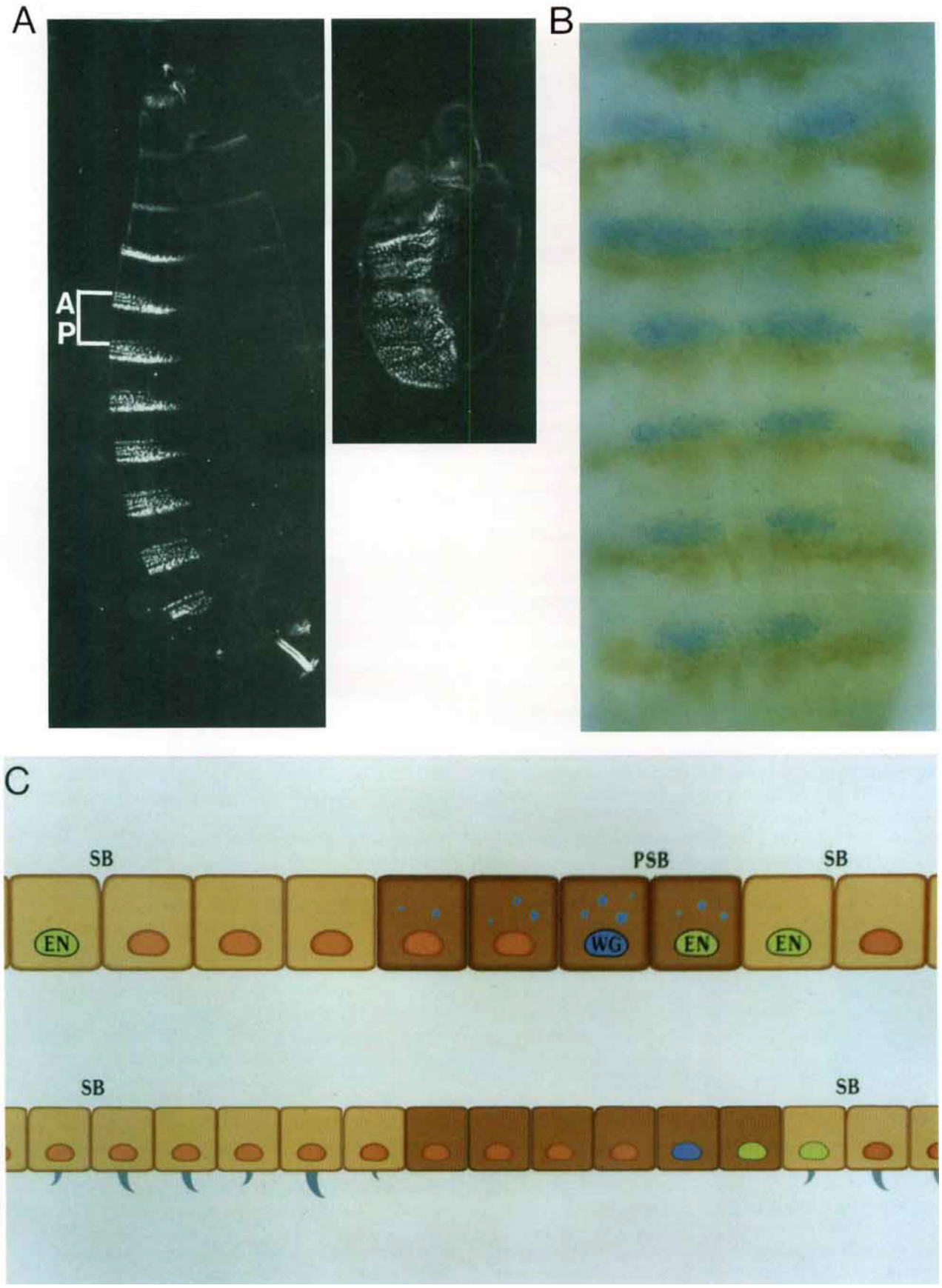
As described above, Wg is involved in local cell-cell signaling to establish segmental pattern within a sheet of embryonic epidermal cells. Another interesting function of Wg is its role in segmental patterning of the midgut epithelium[37,38]. Correct patterning of the unsegmented endoderm, which gives rise to the midgut epithelium, requires signaling from the overlying visceral mesoderm. The extracellular signals in this induction are encoded by *wg* and *decapentaplegic* (*dpp*), which are both expressed in spatially restricted domains in the visceral mesoderm. Wg and Dpp signal to the underlying endoderm to initiate the correct expression of the homeotic gene *labial* in the midgut epithelium, and to regulate the formation of the second midgut constriction (see Fig. 2). This is a mechanism for using positional information within one germlayer to determine pattern in an adjacent unsegmented germlayer.

Additional roles for Wingless during embryogenesis revealed by the use of a temperature-sensitive mutation

wg mutant embryos are extremely abnormal in development and morphology, suggesting that Wg is required in several developmental pathways during embryogenesis. A temperature-sensitive *wg* allele (wg^{ts}) has facilitated the analyses of Wg function throughout embryonic development and has revealed three distinct roles for *wg*: first, in the generation of diversity of cell types in the embryonic epidermis; second, in neuroblast determination and differentiation; and third, in the control of cellular proliferation during Malpighian tubule formation.

Inactivation of Wg activity at 6-9.5 h after egg laying (AEL) does not affect normal En expression, but does disrupt the differentiation of naked cuticle[34,39]. This is consistent with

Fig. 1. Wg signaling in the embryonic epidermis. (A) Cuticle of a wild-type (left) and a *wg* mutant (right) embryo. On the ventral surface of a wild-type embryo, each segment (indicated by the bracket) is comprises an anterior half (A) decorated with small projections called denticles, and a posterior half (P) covered with smooth cuticle. The *wg* mutant embryo is devoid of naked cuticle. (B) En protein (brown) and *wg* expression (blue) in a stage 11 wild-type embryo; this a ventral view with anterior to the top. En is detected by immunostaining with a monoclonal antibody directed against En and Wg expression is detected with the enhancer trap line Cyo,wg^{en11}, which expresses β-galactosidase in the Wg pattern[61]. In the early embryo, Wg is expressed in a circumferential stripe of epidermal cells that is immediately adjacent, and anterior, to cells expressing En. At a later embryonic stage, *wg* expression in the lateral epidermis is lost. (C) (Top) Schematic representation of En (yellow) and Wg (blue) expression in the embryonic epidermis (approximately stage 10). The position of the parasegmental borders (PS) is determined by the juxtaposition of En- and Wg-expressing cells; Wg stripes at the anterior and En stripes at the posterior edge of the borders[26]. The segmental borders (SB) form at the posterior edge of the En cells. Shaded cells represent cells with higher accumulation of Armadillo protein and blue dots represent Wg protein observed in surrounding cells. (Bottom) Schematic representation of the ventral cuticle of a single segment of a wild-type mature embryo. The different denticle types are illustrated.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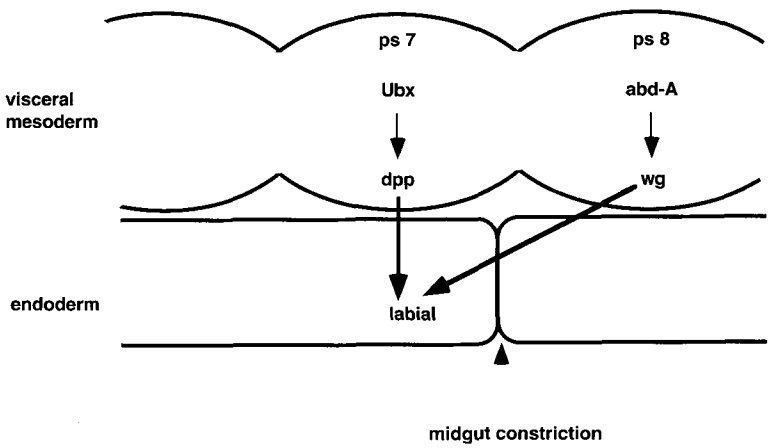

Fig. 2. Wg signaling and segmentation in the endoderm. The endoderm initially appears unsegmented; it is derived from two populations of cells located at either end of the embryo. These primordia migrate towards one another and eventually fuse to form the midgut epithelium beneath the segmented visceral mesoderm[37,38]. Later in embryonic development, three constrictions can be seen in the midgut epithelium, positioned along the anterior-posterior axis. In parasegments (PS) 7 and 8, the homeotic genes *Ubx* and *abd-A* turn on the expression of *dpp* and *wg*, respectively. *dpp* encodes a *Drosophila* member of the TGF-β family. These secreted proteins signal to the endoderm and regulate the expression of *labial*, and the formation of the second midgut constriction (indicated by the dashed arrow). The heavy arrows indicate intercellular signaling.

the observation that ubiquitous expression of Wg throughout the embryo results in complete loss of denticles on the ventral surface[40]. In addition to naked cuticle differentiation, Wg is required for the generation of normal denticle diversity[41]. The complete loss of *wg* activity results in a lawn of denticles, all of a single type (see Fig. 1). Shifting wg^{ts} embryos to the non-permissive temperature at 7 h AEL results in loss of naked cuticle, but a restoration of denticle diversity[34].

Inactivation of wg^{ts} transiently after gastrulation does not affect epidermal patterning, but has revealed a requirement for Wg in normal neuroblast determination and differentiation[42]. Wg is expressed in a subset of cells in the neuroectoderm that give rise to specific neuroblasts of the embryonic central nervous system. Loss of Wg activity does not affect the fate of Wg-expressing neuroblasts; however, the determination of adjacent cells in the neuroectoderm is altered, resulting in the loss and duplication of identified neurons. Furthermore, transient inactivation of wg^{ts} prior to neuroblast delamination from the neuroectoderm is sufficient to cause loss of identified neuroblasts, suggesting that neuroblast identity is specified by Wg signaling in the neuroectoderm.

Finally, Wg plays a role in the control of cellular proliferation during the development of the embryonic Malpighian tubules, an excretory epithelium associated with the hindgut. Loss of Wg activity results in aberrant development of the Malpighian tubules, due in part to a failure of cell proliferation of the tubule anlage[43]. This requirement for Wg in cell proliferation is consistent with experiments examining ectopic expression of Wg, in which over-proliferation of the Malpighian tubule cells has been correlated with ubiquitous expression of Wg.

The role of Wingless in imaginal disc development

In addition to the role of Wg in developmental processes in the embryo, Wg is expressed in the imaginal discs, where it is required for the proper differentiation of adult structures. The role of Wg in adult patterning was recognized by the phenotype of wg^{1}, the first *wg* mutation characterized, which causes the transformation of wing into notum[8]. Further analysis of *wg* loss-of-function and gain-of-function in imaginal discs has revealed three distinct roles for Wg in axis specification, cellular proliferation and cell-type specification.

The imaginal discs are derived from ectodermal cells set aside during embryogenesis. During larval development they proliferate, and finally differentiate during pupal development. As they differentiate, the two-dimensional discs evert to form a three-dimensional adult structure that has anterior-posterior (A-P), dorsal-ventral (D-V) and proximal-distal (P-D) axes. Wg is required for the development of imaginal discs. No imaginal tissue can be cultured from *wg* mutant embryos[44], and several adult structures are affected in individuals that are homozygous for pupal-lethal alleles of *wg*[45]. The expression of Distal-less, a homeo-domain protein which is required for limb formation, is dependent on Wg, as is the position of the leg imaginal discs along the A-P axis of the embryo[46,47]. Expression of *wg* in leg and wing discs can be detected in first and second instar, respectively[47-49]. The pattern of *wg* expression in these discs, and its relationship to the fate map, is illustrated in Fig. 3.

Examination of Wg function in the growth and development of leg discs has revealed a role for Wg in the specification of ventral fates. This conclusion comes from two different approaches; firstly, loss-of-function studies, using temperature-sensitive heteroallelic combinations of *wg* alleles to induce loss of Wg function[48], and secondly, gain-of-function studies, where random clones of Wg-expressing cells are induced in ectopic regions of the imaginal discs[50]. Loss of Wg function in leg imaginal discs results in loss of ventral structures and mirror image duplication of dorsal structures. In addition, deletions and duplications of the most distal portions of the affected legs are frequently observed. These mutant phenotypes have also been observed with pupal-lethal alleles of *wg*[45]. Alternatively, the gain-of-function studies indicate that ectopic expression of Wg in the presumptive dorsal or lateral regions of the leg discs results in expansion of ventral-lateral structures, not the most ventral structures[50]. Interestingly, supernumerary limbs which include a large number of wild-type cells are occasionally observed in these ectopic expression experiments, suggesting an inductive effect of ectopic Wg.

The role of Wg in leg disc growth and patterning is consistent with two alternative models that have been proposed to explain the growth and patterning of the imaginal discs. The polar coordinate model proposes that patterning in the disc derives from circumferential positional values (i.e. a clock face of positional values), and that proximal distal values are determined by short range cellular interactions[51]. Recently, a molecular basis for this model has been proposed, suggesting that expression of *wg*, as well as of other genes, mark the circumferential coordinates in the developing leg disc[48,52]. This proposal is consistent with both the expression of *wg* in leg discs and with the phenotype which results from loss of Wg activity during imaginal development. An alternative model proposes that there are distinct anterior-posterior and dorsal-ventral sectors in the disc, and that the juxtaposition of these sectors results in long-range signaling that determines pattern[53]. The expression of *wg* in the leg disc and the ability of ectopic Wg to induce ventral-lateral structures is consistent with a role for Wg in sector determination. Furthermore, it has been suggested that Dpp may also act to demarcate a sector and, in concert with Wg, define a focus for radial positional information[54]. Dpp is a member of the secreted type β transforming growth factor family and is expressed along the A-P border in discs[55,56]. It has been demonstrated that ectopic expression of *aristaless*, a marker of distal cell fate, occurs when cells expressing ectopic Wg are in close contact with cells expressing Dpp[57]. The proposed interaction between these two secreted factors is reminiscent of the patterning of the midgut epithelium, where Wg and Dpp also act together to specify positional information.

Finally, Wg appears to have an additional function in the wing imaginal disc: the specification of the wing margin[58]. The late pattern of Wg expression in the wing disc includes the presumptive wing margin, where the dorsal and ventral surfaces of the wing are juxtaposed (see Fig. 3). The adult wing margin is normally decorated by bristles, but when *wg* is inactivated in late larval development, all the marginal bristles are lost. The marginal bristle precursors are located in a zone of non-proliferating cells which is coincident with Wg expression in third instar larval discs[58,59]. In discs that are deficient for Wg activity, cell proliferation is enhanced along the presumptive wing margin, indicating that Wg is required for the normal decrease in cell proliferation that is associated with marginal bristle differentiation[58]. Interestingly, this is in contrast to the role of Wg in Malpighian tubule differentiation, where Wg is required for normal cell proliferation [43].

Does Wingless act as a morphogen?

The ability of small numbers of Wg-expressing cells to govern the spatial pattern of surrounding cells within the embryonic epidermis and the imaginal leg disc has led to the speculation that Wg acts as a morphogen[27,50]. For Wg to be called a morphogen it must meet several criteria: Wg should be diffusible, protein distribution should be graded and Wg should influence the fate of non-expressing neighboring cells. Finally, different concentrations of Wg should be capable of eliciting distinct responses in surrounding cells. The homology of Wg to Wnt-1, the observation of Wg protein in surrounding non-expressing cells and the non-autonomous nature of *wg* mutations, are all consistent with Wg being a secreted factor. Furthermore, Wg protein has been observed in a graded distribution in the embryonic epidermis[23], and Wg can influence the fate of neighboring non-expressing cells in a number of different developmental processes, which we have described in this review.

What remains controversial is the ability of Wg-expressing cells to act as a source of a graded signal that regulates pattern in a concentration-dependent fashion. Some data indicate that different levels of Wg protein can elicit different developmental outcomes; an example is Wg signaling to maintain En expression in adjacent cells. The cells closest to the Wg-expressing cell receive high levels of protein and maintain En expression, whereas more posterior cells do not receive sufficient levels of Wg protein to sustain En expression[35]. Furthermore, among the cells receiving sufficient levels of Wg to maintain En expression, only those exposed to the highest levels of Wg will give rise to naked cuticle[23,34,39]. Consistent with these conclusions is the observation that expression of high levels of Wg throughout the embryo results in expanded En expression and the suppression of denticle differentiation, resulting in completely naked cuticle[40]. However, an alternative conclusion to Wg acting as a morphogen, is the possibility that Wg regulates a switch in the choice between En expression or not, and the choice between differentiation of naked cuticle or denticles. In this case Wg would be an instructive, or perhaps a permissive, signal rather than a graded signal establishing different values of positional information. The possibility of Wg acting as a switch is supported by the observation that uniform expression of Wg in *wg* mutant embryos is sufficient to maintain epidermal En expression and restore some segmental pattern. Therefore, graded Wg may not be necessary to specify embryonic pattern, but Wg may be required in sufficient levels to act as a switch[60].

The strongest evidence for Wg functioning as a morphogen is the patterning of the D-V axis in the leg imaginal disc. It has been proposed that this arises through a graded distribution of Wg protein; high levels of Wg determine ventral fate, low levels define ventrolateral fate, and no Wg defines the dorsal fate[50]. This model derives from the observation that ectopic Wg-expressing cells in the dorsal regions of the disc have a ventralizing effect on neighboring non-Wg-expressing cells. Interestingly, these studies result in the induction of ventrolateral, but not ventral, cell fates. This may be accounted for by the relatively low levels of ectopic Wg protein achieved to date. However, in recent experiments higher levels of ectopic Wg have been expressed but the ventral-most structures are still not induced (E. Wilder, personal communication). When supernumerary limbs are generated by ectopic Wg expression, many wild-type cells are recruited into the new structure, which is consistent with Wg-expressing cells acting as a source of positional information. The ability of ectopic Wg to organize pattern in imaginal discs appears to extend to cells beyond the range of Wg diffusion; therefore, it has been suggested that long range patterning of the adult limb may arise through sequential local inductive events initiated by Wg signaling[50].

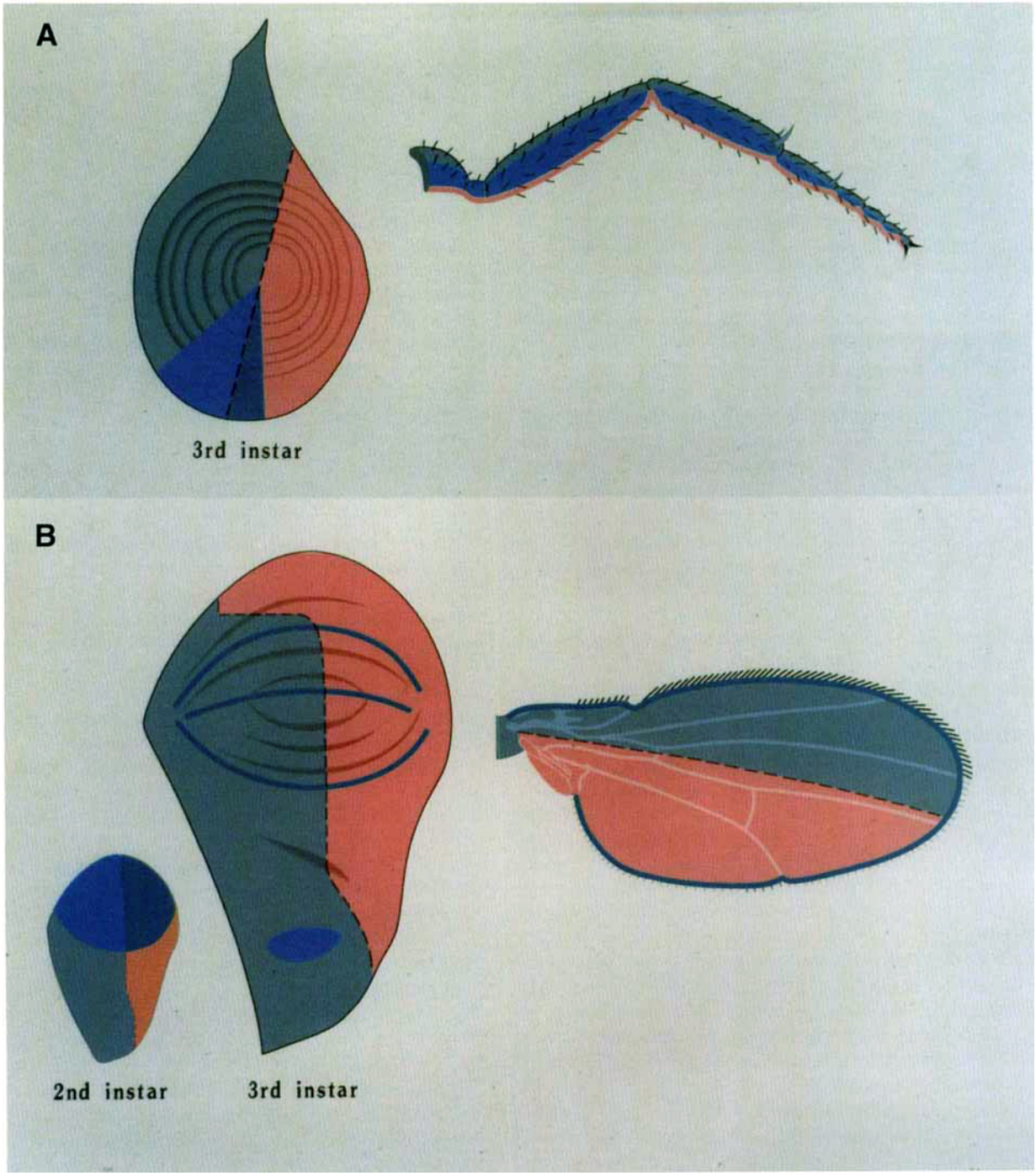

Fig. 3. Wg expression and the specification of pattern in the adult structures. (A) Schematic representation of En (pink) and Wg (blue) expression in third instar leg disc (shown on the left), and the second leg of an adult male (shown on the right). The regions of the adult structure derived from the En domain of the discs are shaded in pink, and those derived from the Wg domains are shaded in blue. The anterior-posterior boundary in the leg imaginal discs is illustrated by the dotted line. Just to the right of this dotted line are Wg-expressing cells that also express En (shaded in darker blue). The precursors of the leg imaginal discs are positioned over the parasegmental border, and express both En and Wg. En is expressed in the posterior half of the disc and Wg in the ventral-anterior half[47,48]. As the leg imaginal disc develops, Wg expression is maintained in an anterior-ventral quadrant[45,48]. (B) Schematic representation of En and Wg expression in second instar wing disc (right), third instar wing disc (middle) and the adult wing (left). The margin of the adult wing, derived from a Wg-expressing domain in the disc, is shaded in blue. The posterior half derived from the En-expressing domain is shaded in pink. In the embryonic wing imaginal precursors there is no Wg expression; by second instar larval stage, Wg (blue) is detected in the ventral region of the disc and En is expressed in the posterior half of the disc (pink)[48,49]. The dark blue area indicates the region of the disc which expresses both Wg and En. During third instar, this pattern changes and resolves into rings around the presumptive wing blade, an arc across the presumptive wing margin and a spot in the presumptive notum[45,48,49,58].

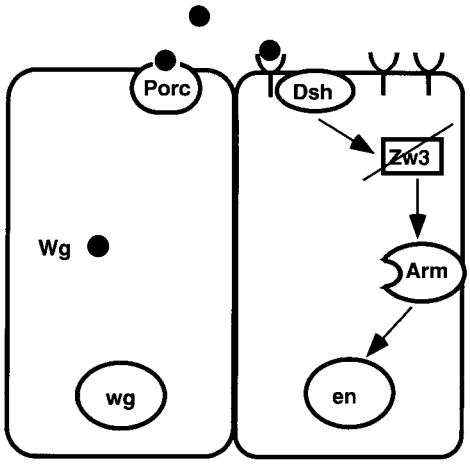

Fig. 4. A model for Wg signaling. Stable En expression in the embryonic epidermis requires Wg signaling from adjacent Wg-expressing cells. Secretion or distribution of Wg protein (shown as a black dot) is regulated by Porc. Wg may bind an unidentified receptor in adjacent cells and initiate a signal transduction cascade. The Wg signal is transduced through Dsh, which inactivates or antagonizes Zw3 kinase activity. This results in accumulation of high levels of Arm and a shift in the sub-cellular distribution of the protein, which is required for correct En expression and cell fate determination.

Components of Wingless signaling

We have so far described the multiple roles of Wg in development, and stressed its importance in patterning both the embryo and the adult. However, what these analyses have not revealed is the mechanism of Wg signaling. To this end, analysis of mutants that have segmentation phenotypes similar to *wg* mutants constitutes a promising approach. Recently, through the analyses of genetic interactions, some potential components of Wg signaling in embryonic patterning have been identified. One is Zeste-white 3 (Zw3), the *Drosophila* homologue of Glycogen Synthase Kinase 3β, which functions to mediate the Wg signal[61]. In *zw3* mutant embryos, En expression is expanded and only naked cuticle is secreted, the opposite phenotype to *wg* mutant embryos[61]. The phenotype of *zw3* mutant embryos is consistent with a role for Zw3 in the repression of both En expression and naked cuticle differentiation. In the absence of Zw3 activity, Wg is no longer required for En expression or naked cuticle differentiation. This was demonstrated by the analysis of embryos doubly mutant for *zw3* and *wg*, which resemble *zw3* mutant embryos[61]. These results suggest that Wg signaling functions by inactivation of the Zw3 repression, to maintain correct En expression and cell fate determination.

Several other genes may encode additional components of Wg signaling; these include the genes *dishevelled* (*dsh*)[62-64], *armadillo* (*arm*)[65,66] and *porcupine* (*porc*)[62]. Embryos mutant in any one of these genes exhibit similar embryonic phenotypes to *wg* mutant embryos: loss of epidermal En expression and a failure to differentiate naked cuticle. *dsh* encodes a novel protein of unknown function[63,64]; Arm is the *Drosophila* homologue of β-catenin and plakoglobin[67], proteins involved in cell adhesion, and it is not known what *porc* encodes.

Genetic interaction studies have suggested that *dsh, arm* and *porc* are in the Wg signaling pathway. Examination of the phenotypes of embryos doubly mutant for *zw3* and each of these three mutations has been used to order these gene products in the Wg signaling pathway[68]. This analysis takes advantage of the opposite phenotypes of *zw3* embryos and the *wg*-like mutant embryos to determine the epistasis of the genes. The epistatic relationships between genes are determined by examining double mutants of two single mutations with distinct phenotypes. If the double mutant phenotype resembles one single mutation, then that mutation is epistatic to the other mutation. For example, *zw3 porc* double mutant embryos resemble *zw3* mutant embryos, therefore *zw3* is epistatic to *porc*[68]. Genetic epistasis can be used to determine gene order, which gene is upstream or downstream, depending on whether the genes function in a positive or negative fashion in the pathway (for a review see ref. 69). Double mutant analyses have indicated that both *porc* and *dsh* function upstream of *zw3* in Wg signaling[68]. In contrast, *arm* is likely to act downstream of *zw3* because it is epistatic to *zw3*[68,70]. A second approach used to determine the role of these genes in Wg signaling has made use of the dominant phenotype resulting from ubiquitous expression of Wg in embryos; En expression is expanded and only naked cuticle differentiates, similar to the phenotype of *zw3* mutant embryos[40]. These effects of ubiquitous Wg expression can be suppressed by two mutations, *dsh* and *arm*, indicating that they are both required for the transduction of the Wg signal[71].

Consistent with these genetic data, more direct evidence that both Arm and Porc function in Wg signaling has been obtained. An examination of *porc* mutant embryos has revealed that Wg protein distribution is altered in these embryos and appears to accumulate in the cells that synthesize it[24,68]. This suggests that Porc is required for the correct secretion of Wg protein. In wild-type embryos, Arm is distributed in alternating stripes of high accumulation of protein, and inter-stripes of low levels of Arm protein. The stripes of Arm arise in response to post-transcriptional regulation by Wg signaling[72], and reflect both changes in the level and sub-cellular distribution of Arm protein[70]. In *zw3* mutant embryos, high levels of Arm accumulate throughout the embryo[68,70], suggesting that Wg signaling causes Arm accumulation by inactivating Zw3. This observation, and the fact that *arm* is epistatic to *zw3*, indicate that Zw3 activity may be mediated through the modulation of Arm protein distribution. These observations, and the results of genetic epistasis, have led to a model for Wg signaling which is illustrated in Fig. 4. Wg signaling requires Porc for the secretion or transport of the protein. Once received by adjacent cells, Wg initiates a signal transduction cascade through Dsh. Zw3 kinase activity is inactivated, which results in the accumulation and possible relocalization of Arm protein, which is required for En expression and correct cell fate determination.

Wingless may activate the same pathway at multiple times during development

As described above, Wg is able to elicit numerous cellular effects during development. One important issue is whether

the biochemical pathways activated by Wg are the same in the various cell types where it functions. A number of results suggest that the components of Wg signaling identified in embryonic epidermal patterning are likely to act in other Wg-dependent pathways. For instance, loss of *dsh* results in defects of midgut differentiation similar to those that are observed in *wg* mutant embryos[63]. There is also evidence that Zw3, Dsh and Arm are required for specification of the same adult pattern elements as Wg. Loss of Arm activity in the wing imaginal disc results in loss of the wing blade and duplication of more proximal structures, similar to the phenotype of *wg* mutations[66]. Zw3[73,74] and Dsh[63,74] are both required for correct differentiation of the marginal bristles, a cell specification which is Wg-dependent. Finally, reduction of Dsh[63,64] or Arm[66] activity in leg discs results in transformation of ventral fates to dorsal fates, and in the induction of supernumerary limbs. This suggests that, similar to Wg, Dsh and Arm are required for ventral cell fate determination in the leg imaginal disc. Interestingly, loss of Zw3 activity in the dorsal region of the leg disc gives rise to ectopic ventral cells and duplication of the legs, similar to ectopic expression of Wg in the disc (E. Wilder, personal communication)[75]. These results strongly suggest that the Wg pathway proposed for embryonic patterning may be the same as that for Wg patterning of adult limbs.

Conservation of Wg signaling between species

Are any functions of Wg conserved in the roles of Wnts in vertebrate development? Both Wg and Wnt-1 are required for the normal development of the embryonic nervous system. Whereas loss of Wg in *Drosophila* results in a failure of neuroblast determination[42], null mutations of *Wnt-1* in the mouse result in a severe disruption of the central nervous system; both the midbrain and cerebellum are affected[6,7]. Furthermore, this defect apparently arises from a loss of Wnt-1-expressing cells in the mid-brain early in fetal development, in addition to the loss of En expression in the mid-brain[76]. This indicates that Wnt-1 plays an instructive role in mid-brain development and possibly regulates En expression, analogous to the role of Wg in *Drosophila* embryonic patterning. The ability of ectopic Wg in imaginal discs to induce a supernumerary limb is reminiscent of axis duplication resulting from over-expression of vertebrate Wnts in *Xenopus*[4,5]. Finally, the ability of over-expression of Wg to affect proliferation and differentiation in Malpighian tubule may be similar to ectopic expression of Wnt-1, which can promote cell division in mammary glands, resulting in the formation of mammary tumors[77].

In addition to the function of Wg being conserved, the mechanism of signaling is also likely to be shared between species. As mentioned above, many of the components of Wg signaling are conserved: *zw3* is the homologue of mammalian GSK-3β, and expression of GSK-3β in *zw3* mutant individuals can restore wild-type pattern and viability[61,78]. Two other components of the Wg signaling pathway, Arm[67] and Dsh (D. Sussman, personal communication), have also been shown to have vertebrate homologues. There is suggestive evidence that β-catenin and plakoglobin, like Arm, may be involved in Wnt signaling. Injection of antibodies directed against β-catenin into *Xenopus* embryos results in induction of a secondary body axis, similar to the phenotype due to ectopic expression of Wnts[79]. In addition, expression of Wnt-1 in PC12 cells results in increased accumulation of plakoglobin and increased cell adhesion[80].

Perspectives

The use of genetic analysis to study a signaling pathway has proved valuable for understanding both the function and possible mechanism of Wg signaling in *Drosophila* development. However, our understanding of the Wnt signal transduction pathway is still extremely limited. This is in contrast to the receptor tyrosine protein kinase (RTK) signaling pathway, which has been studied both genetically and biochemically, and for which many of the molecules in the pathway have been identified. These studies have revealed that RTK signaling is highly conserved in evolution, and that different RTKs activate a common signal transduction pathway[81]. The study of Wnt signaling in *Drosophila* is still at an early stage. The foremost challenge is the identification of the Wg receptor and other components of the pathway. Once these components have been identified genetically, the biochemistry of the pathway must be determined. It will also be important to test whether this pathway is used throughout development in the different Wg-mediated processes. Recent genetic data, described above, indicate that this will be likely. Finally, what are the roles and mode of action of the other two Wnts in *Drosophila*, DWnt-2 and DWnt-3? It will be interesting to see if Wnts resemble other growth factor families which have unique receptors but share a common signal transduction pathway, or whether each Wnt protein functions through a distinct pathway. We expect the Wnt signaling pathway to be conserved in evolution; however, this possibility remains to be tested, and will surely be an important part of the future investigations of Wnt signaling.

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Esther Siegfried* is at the Department of Genetics, and **Norbert Perrimon** is at the Howard Hughes Medical Institute, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA.

*Current address: Department of Biology, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, 208 Mueller Laboratory, University Park, PA 16802, USA.