Wnt/Wingless Signaling in *Drosophila*

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The Wingless (Wg) pathway represents one of the best-characterized intercellular signaling networks. Studies performed in *Drosophila* over the last 30 years have contributed to our understanding of the role of Wg signaling in the regulation of tissue growth, polarity, and patterning. These studies have revealed mechanisms conserved in the vertebrate Wnt pathways and illustrate the elegance of using the *Drosophila* model to understand evolutionarily conserved modes of gene regulation. In this article, we describe the function of Wg signaling in patterning the *Drosophila* embryonic epidermis and wing imaginal disc. As well, we present an overview of the establishment of the Wg morphogen gradient and discuss the differential modes of Wg-regulated gene expression.

Evolutionarily conserved cell signaling pathways regulate the development of metazoans through their reiterative implementation, both spatially and temporally. Wnt signaling represents one such pathway that has multiple, essential roles during both embryogenesis and adult homeostasis to regulate cell proliferation, cell polarity, and the specification of cell fate (for review, see Wodarz and Nusse 1998). Wnt genes encode secreted glycoprotein ligands that can act both as short-range signaling molecules and long-range morphogens, depending on the developmental context. Members of the Wnt family are defined by sequence homology to Wnt-1 (Nusse and Varmus 1982; Nusse et al. 1984), the first identified Wnt protein, rather than by functional homology. As such, subsequent to the identification of Wnt-1, diverse Wnt-regulated processes have been identified that when aberrantly regulated result in myriad diseases, ranging from developmental disorders to cancers. Thus far, 19 vertebrate Wnt family members have been discovered, of which there are seven homologs in *Drosophila* (Table 1). Much of our understanding of the role of Wnt proteins during development has come as a result of genetic analyses of the *Drosophila* *wnt-1* (*Dwnt-1*) or *wingless* (*wg*) gene.

As the name suggests, the *wg* gene is required to pattern the *Drosophila* wings and other adult body structures. It was originally identified through a hypomorphic allele, *wg*¹, which harbors a deletion in a regulatory element of the gene and causes the variable transformation of the adult wing(s) to thoracic notum (Sharma and Chopra 1976; Babu 1977). Subsequent to characterization of the viable *wg*² allele, large-scale genetic screens performed by Eric Wieschaus, Christiane Nusslein-Volhard, and colleagues yielded embryonic lethal,
loss-of-function alleles of wg (Nusslein-Volhard and Wieschaus 1980; Nusslein-Volhard et al. 1984). In the years that followed, the wg gene was cloned (Baker 1987; Cabrera et al. 1987; Rijsewijk et al. 1987), and through the use of conditional mutants, mosaics analyses, and ectopic expression, it was shown to have important roles at several stages of development in multiple tissues, including the embryonic ectoderm (Baker 1988a; Bejsovec and Martinez Arias 1991; Dougan and DiNardo 1992; Bejsovec and Wieschaus 1993), head (Schmidt-Ott and Technau 1992), midgut (Immerglück et al. 1990; Reuter et al. 1990; Thuringer and Bienz 1993; Bienz 1994), wing disc (Simcox et al. 1989; Cohen 1990; Cohen et al. 1993; Phillips and Whittle 1993; Williams et al. 1993), and leg disc (Baker 1988b; Campbell et al. 1993; Couso et al. 1993). Moreover, through genetic and biochemical analyses performed predominantly in Drosophila over the years, the molecular mechanism of canonical Wnt or Wg signaling has emerged. In the absence of the Wnt/Wg ligand, cytoplasmic levels of β-catenin/Armadillo (Arm), the transcriptional effector of the pathway, are kept low through its constitutive degradation by a protein destruction complex composed of Axin, APC, GSK3/Zw3, and CK1. As a result, Wnt/Wg-regulated genes are kept off by the DNA-binding transcription factor T-cell factor (Tcf) with the aid of other transcriptional corepressors. Binding of the Wnt/Wg ligand to its coreceptors, Frizzled2 (Fz2) and LRP/Arrow (Arr), initiates a sequence of cytoplasmic events that leads to the Dishevelled (Dsh)—mediated inactivation of the protein destruction complex, thereby allowing stabilized β-catenin/Arm to translocate to the nucleus, where it binds Tcf to direct the activation of Wnt/Wg-target genes (for review, see Bejsovec 2006).

In this article, we discuss the role of the Wg molecule as an organizing center during embryonic segmentation and patterning of the wing disc, because these are now considered the classic systems for demonstrating different aspects of Wg signaling.

### FUNCTION OF WINGLESS SIGNALING IN THE EMBRYO

During Drosophila embryogenesis, a hierarchy of maternal and zygotic (gap, pair-rule, and segment polarity) genes progressively subdivides the embryonic syncytium into transverse regions that determine the anterior/posterior axis (for review, see Ingham and Martinez Arias 1992; St. Johnston and Nusslein-Volhard 1992). The cellular blastoderm is formed during stage 14 of embryogenesis and coincides with the division of the anterior/posterior axis into segmental units as directed by the segment polarity genes wg and hedgehog (hh) (for review, see Perrimon 1994). These segment polarity genes interact with one another to define the segment boundaries and intrasegmental pattern of the embryo (Fig. 1). At the end of embryogenesis, the outcome of the segmentation and patterning events is a larva characterized on the ventral epidermis by an alternating pattern of protrusions called denticles that are separated by regions of naked cuticle (for review, see Martinez-Arias 1993). We here describe the mechanism through which Wg signaling establishes and patterns each segment to generate this stereotypical arrangement of denticles and naked epidermal cuticle. This process can be divided into four successive events: establishment of the organizer, asymmetric signaling from the organizer, subdivision of each segment into...

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**Table 1. Comparison of Wnt genes between Drosophila and vertebrates**

<table>
<thead>
<tr>
<th>Drosophila genes</th>
<th>Dwnt-1 or wingless</th>
<th>Dwnt-2</th>
<th>Dwnt-5</th>
<th>Dwnt-4</th>
<th>Dwnt-6</th>
<th>Dwnt-8 or Dwnt-D</th>
<th>Dwnt-10</th>
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<tr>
<td>Vertebrate homologs</td>
<td>Wnt-1</td>
<td>Wnt-7</td>
<td>Wnt-5</td>
<td>Wnt-9</td>
<td>Wnt-6</td>
<td>Wnt-8</td>
<td>Wnt-10</td>
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signaling domains, and cell fate specification by the signaling domains (Fig. 1).

The expression of wg and hh is initiated by the pair-rule genes in adjacent, non-overlapping domains during stage 9–10 of embryogenesis, and subsequently, they reciprocally regulate each other to stabilize their expression (Fig. 1) (for review, see DiNardo et al. 1994). Wg protein that is transcribed and secreted from an anterior row of cells maintains the expression of a transcription factor, engrailed (en), in adjoining, posterior cells. The En-expressing

Figure 1. Wingless-regulated patterning of the Drosophila embryonic epidermis. The interplay between the Wg and Hh signaling pathways initially establishes the parasegment boundaries and subsequently directs the intrasegmental pattern to establish the stereotypical arrangement of denticles and naked cuticle at the end of embryogenesis (see text for details). The embryo is positioned with its anterior end to the left. (Top panel courtesy of L.R. Braid.)
cells, in turn, transcribe and secrete the Hh ligand, which reciprocally maintains Wg expression in the neighboring, anterior cells (DiNardo et al. 1988; Martinez Arias et al. 1988; Hidalgo and Ingham 1990; Bejsovec and Martinez Arias 1991). The interface between these two adjacent domains defines the parasegment boundary or organizer, with en/hh transcribed at the anterior and wg at the posterior end of each parasegment, respectively (Baker 1987; Lee et al. 1992; Mohler and Vani 1992).

Initially, after the parasegment boundary is established, the distribution of the Wg ligand is bidirectional and triggers a response through its signaling cascade at equivalent levels in both the anterior and posterior directions (Riggleman et al. 1990; Peifer et al. 1994). However, during stage 11, the distribution of extracellular Wg is only graded anterior to the Wg-expressing domain and is completely lost in the posterior direction (Fig. 1) (van den Heuvel et al. 1989; Gonzalez et al. 1991). This asymmetry in Wg distribution and correspondingly signaling activity is a result of its rapid endocytosis and degradation in cells posterior from which it is secreted, in a process that is promoted by Hh signaling (Sanson et al. 1999; Dubois et al. 2001). Interestingly, Wg signaling, in turn, attenuates Hh signaling anterior to the En/Hh-expressing cells, thereby allowing its activity in only the posterior direction (Gritzan et al. 1999). Thus, a pattern of polarized signaling of Wg and Hh at the parasegment boundary is formed that can direct cells on either side to follow distinct developmental programs.

The wg- and en/hh-expressing domains initially established successively specify two additional domains within each parasegment that are defined by the expression of Serrate (Ser) (a ligand for the Notch pathway) (Wiellette et al. 1999) and rhomboid (rho) (promotes EGFR signaling) (Golembo et al. 1996). Wg and Hh signaling antagonize the posterior and anterior boundaries, respectively, of Ser expression, thereby restricting its domain to the center of each parasegment (Alexandre et al. 1999; Gritzan et al. 1999; Sanson et al. 1999). Rho expression is repressed by Wg signaling and is positively reinforced through a combination of Hh and Ser signaling, thereby establishing its domain immediately posterior to the en/hh domain (Alexandre et al. 1999). During stage 12, each parasegment on the ventral epidermis is divided into four domains that express specific genes that are responsible for the intraparasegmental patterning of the embryo. This period also coincides with the formation of a segmental groove at the posterior edge of each en/hh domain and defines the segment boundary (Fig. 1).

The four signaling domains established within each segment control the binary decision between specification of naked cuticle or denticle cell fates. The outcome between these two choices is dependent on the expression of a transcription factor encoded by the shavenbaby (svb) gene, which is necessary and sufficient to direct denticle formation cell autonomously (Payre et al. 1999). The expression of svb is inhibited in cells that specify naked cuticle, whereas cells that make denticles express svb. Wg signaling specifies naked cuticle (Bejsovec and Martinez Arias 1991; Noordermeer et al. 1992; Lawrence et al. 1996) by repressing the expression of svb (Payre et al. 1999). Due to the asymmetric distribution of Wg, the repression of svb is asymmetric and results in one row of cells posterior to, and four rows of cells anterior to the Wg domain that produce naked cuticle (O’Keefe et al. 1997; Szuts et al. 1997), across the posterior half of each segment. The six rows toward the anterior half of each segment do not receive the Wg ligand but instead transduce the EGFR signal that promotes svb expression (Payre et al. 1999), thus resulting in the generation of denticles that vary in shape, size, and polarity. The outcome of the patterning events mediated by regulated cell signaling is a repeated mosaic of denticle belts and naked cuticle on the ventral epidermis of the embryo, composed of segments 11–12 cells wide along the anterior/posterior axis and 36–40 cells wide along the dorsal/ventral axis (Fig. 1). In wg loss-of-function mutants, svb expression is not repressed and naked cuticle is not specified, resulting in the excess specification of denticles. Conversely, when wg is ectopically expressed, excess naked cuticle is produced with
a reduction or loss of denticles (Fig. 2) (Payre et al. 1999).

Components of the Wg signal transduction cascade were initially identified in large-scale genetic screens designed by Eric Wieschaus and Christiane Nusslein-Volhard to isolate zygotic mutations that disrupt the *Drosophila* embryonic cuticle. These screens yielded numerous genes that displayed segment polarity phenotypes when mutated, including both positive and negative regulators of the Wg pathway (Nusslein-Volhard and Wieschaus 1980; Jürgens et al. 1984; Nusslein-Volhard et al. 1984; Wieschaus et al. 1984). Mutations in arm and arr resemble the wg loss-of-function phenotype to specify excess denticles, whereas naked, an inhibitor of the pathway, was also isolated and displays a wg gain-of-function phenotype with an excess specification of naked cuticle. Although the aforementioned genetic screens led to the characterization of several downstream components, they did not yield the entire set of genes involved in the Wg pathway. This is because some genes are maternally contributed and zygotic mutants thus retain sufficient maternal product to pattern a relatively normal embryo. This problem was circumvented through the induction of mitotic recombination in the germline, thereby eliminating maternally contributed gene product, and this technique was used to genetically screen the X-chromosome for maternal effect mutations that disrupt patterning of the embryonic cuticle (Perrimon et al. 1989). arm was reisolated in this screen along with additional new components of the pathway—dsh, porcupine, and zw3.

Traditional loss-of-function epistasis experiments rely on evaluating phenotypes in double-mutant combinations, thus allowing one to determine which of the two distinct phenotypes “overrides” or is epistatic to the other. This information allows the ordering of genes within a pathway, because the gene that acts most downstream will generally be epistatic to those acting upstream. However, the similar phenotypes of many of the first identified patterning genes precluded such analyses. To overcome this limitation, numerous studies were performed using combinations of gain- and loss-of-function fly strains, which provided distinct phenotypes (e.g., excess naked cuticle due to gain of wg compared with excess denticles in an arm mutant). These studies allowed the relationships between the early pathway components to be defined and have continued to serve as a template for characterizing and positioning novel pathway members. Remarkably, already in 1994, the minimal components of Wg signaling were known and ordered into a rudimentary pathway leading from *wg* to *dsh*, *sgg*, *arm*, and into the nucleus to regulate *en* expression (Noordermeer et al. 1994; Siegfried et al. 1994). Thus, through the implementation of genetic screens, several components of the Wg pathway were first defined based on their ability to disrupt embryonic patterning, and the order of these genes in the pathway was deduced through genetic epistasis. The genetic and molecular characterization of these components formed the
foundation of our current understanding of Wnt/Wg signaling.

FUNCTION OF WINGLESS SIGNALING IN THE WING IMAGINAL DISC

Like the embryonic ectoderm, the wing imaginal disc represents another tissue for which the function of Wg signaling has been well elucidated. The wing disc is an epithelial sac that is composed of 20 cells when it is formed during embryonic development and then proliferates during the larval stages to generate a disc of \(~75,000\) cells in the late third larval instar. At this stage, the cells comprising the major elements of the wing primordium can be identified through the use of molecular markers, including the notum, hinge, blade, and margin (Fig. 3). \(wg\) is expressed in two ring-like domains in the hinge region, along the dorsal/ventral compartment boundary dividing the wing blade, and in a broad band in the dorsal part of the disc. The inner ring-like domain frames the wing blade and gives rise to the hinge, whereas the dorsal/ventral boundary region forms the wing margin. The most dorsal part of the wing disc gives rise to the notum of the adult fly (Fig. 3).

As previously mentioned, the first \(wg^1\) mutant displayed a transformation of wing structures into thoracic notal structures (Sharma and Chopra 1976; Morata and Lawrence 1977). Conversely, misexpression of \(wg\) at a specific time leads to the ectopic induction of wing structures in the notum (Ng et al. 1996; Klein and Arias 1998a). These phenotypic analyses suggest that the \(wg\) gene has a crucial function during the development of the wing disc to specify the region that eventually gives rise to the adult wing. During the second larval instar, \(wg\) is expressed in the ventral region of the wing disc and specifies the wing field, while the EGFR ligand, \(vein\), is expressed in the dorsal region of the wing disc to specify the notum (Simcox et al. 1996; Wang et al. 2000). The loss of \(vein\) during the second larval instar results in the loss of all notal structures (Simcox et al. 1996; Wang et al. 2000), whereas the misexpression of \(vein\) in the wing field prevents wing formation and produces ectopic notum structures (Baonza et al. 2000; Wang et al. 2000), phenotypes that are complementary to those of \(wg\). The expression of \(vein\) is restricted to the dorsal region of the wing disc by the suppressive influence of Wg signaling in the ventral region (Baonza et al. 2000). Conversely, \(wg\) expression is antagonized in the dorsal region of the disc by EGFR signaling to limit its expression to only the ventral region (Baonza et al. 2000; Wang et al. 2000). This mutual antagonism between the Wg and EGFR pathways segregates the early wing disc into notum and wing regions and provides an explanation for the wing-to-notum transformation that occurs in the absence of \(wg\) function.

Multiple pathways contribute to the refinement of the \(wg\) expression pattern as larval
development proceeds. During the late second instar, the expression of the selector gene *apterous* (*ap*) overlaps ventrally with that of *wg*, to divide the developing wing field into two regions (Williams et al. 1993; Ng et al. 1996). The Notch pathway ligands, *Serrate* and *Delta*, are transcribed at the *ap*-expression boundary (dorsal/ventral boundary) and lead to the induction of downstream genes that are ultimately required for the establishment of the distal wing fates, including the wing blade and margin (Williams et al. 1994; Klein and Arias 1998b; Milan and Cohen 2000). Subdivision of the wing field is initiated by the Notch-induced expression of *vestigial* (*vg*) from its boundary enhancer (*vgBE*). Vg specifies the wing blade and collaborates with Notch signaling to induce the expression of *wg* along the dorsal/ventral boundary (Kim et al. 1996; Klein and Arias 1998a, 1999). The Wg ligand is secreted from cells at the dorsal/ventral boundary and subsequently patterns the wing margin through activation of its target genes in a concentration-dependent manner (Couso et al. 1994; Neumann and Cohen 1997). After the establishment of the expression of *wg* and *vg* in the wing primordium, the wing blade begins to grow during the third instar. The expression of *vg* is induced in cells of the wing blade that lie outside of the domain of Notch signaling through its quadrant enhancer (*vgQ5*) (Kim et al. 1996). This enhancer requires input from Vg itself, Wg (produced at the dorsal/ventral boundary), and Dpp (produced at the anterior/posterior boundary) for its activity.

Flies deficient for *wg* do not develop wings, and mutant patches of cells that cannot respond to the Wg ligand are eliminated from the wing field in the wing disc (Chen and Struhl 1999). This suggests that there is an absolute requirement of Wg for growth of the wing. However, until recently it had been unclear whether cells of the wing disc required Wg signaling as an instructive signal or a permissive signal in order to proliferate. Early studies suggested that although the misexpression of *wg* has mitogenic effects in the hinge region, it does not induce cell proliferation within the wing pouch but rather respecifies these cells to assume a wing margin fate (Neumann and Cohen 1996). It was subsequently shown that high levels of Wg at the presumptive wing margin, in fact, arrest the cell cycle (O’Brochta and Bryant 1985; Johnston and Edgar 1998). The ability of uniform expression of moderate levels of Wg to indeed promote growth throughout the presumptive wing region was shown only recently (Baena-Lopez et al. 2009). In this study, the investigators propose a model in which Vg-expressing cells expand their numbers by inducing their neighboring, non-wing cells to express Vg, provided these cells also receive the Wg signal, and thereby be recruited into the wing field through a feed-forward mechanism. In this scenario, Wg is proposed to act as a permissive signal to enable the recruitment of cells into the wing field to promote growth of the wing disc (Zecca and Struhl 2007a,b).

**DOES WINGLESS ALWAYS BEHAVE AS A MORPHOGEN?**

As a minimal definition, a “morphogen” is a molecule that diffuses away from a localized source to directly instruct cell identities in a concentration-dependent manner. For a secreted signaling ligand such as Wg to qualify as a morphogen, it must form a graded distribution away from its source, directly act on cells at a distance rather than indirectly through a relay mechanism, and induce the commitment of cells in the field, as a function of their distance from the morphogen source, to distinct developmental fates through the expression of different sets of genes.

Experiments performed in the wing disc suggest that Wg does indeed behave as a morphogen in this tissue. During the third larval instar, Wg, secreted from cells at the dorsal/ventral organizing boundary of the wing disc, diffuses away from its source on either side and acts directly on cells at long range to induce the nested expression of its target genes *achaete* (*ac*), *distal-less* (*dll*), and *vestigial* (*vg*) (Fig. 4) (Zecca et al. 1996; Neumann and Cohen 1997). The modulation of Wg signaling through clonal analyses or ectopic expression of *arm* and *dsh*, positive regulators of the pathway, shows
a cell-autonomous change in Wg-responsive target gene expression, regardless of the position of the cell from the source of the Wg signal (Zecca et al. 1996; Neumann and Cohen 1997). These data suggest that the Wg ligand acts directly on cells distant from its source, and the expression of target genes is not activated via a secondary or relay signal. In addition, in contrast to the wild type secreted Wg, a membrane-tethered form of the ligand that is unable to diffuse away from its source, activates expression of target genes only in its immediate neighbors (Zecca et al. 1996; Neumann and Cohen 1997). Furthermore, through the use of a temperature-sensitive allele, wgts, it has been shown that the level of Wg activity minimally required to activate expression of ac, a high-threshold target gene; dll, a medium-threshold target gene; and vg, a low-threshold target gene; is progressively lower. Because the activity of Wgts is decreased (by raising the temperature), the expression of ac is lost at a temperature at which dll expression is retained, and as the temperature is further increased, the expression of dll is lost with no effect on vg expression. In both cases, there is a concomitant reduction of the expression domains of target genes toward the source of the ligand as the activity level of Wg is reduced. Consistent with this result, low levels of ectopically expressed Wg are able to activate dll but not ac (Neumann and Cohen 1997). These data suggest that Wg activates different target genes in a concentration-dependent manner and defines their expression domains through different activation thresholds. This does not exclude the possibility that activation of target genes requires other permissive signals, but it does argue that the level of the Wg ligand is the instructive signal. Lastly, the extracellular concentration gradient of the Wg protein can be detected up to 10 cell diameters away from the secreting cells at the dorsal/ventral boundary.

Figure 4. Morphogen gradient of Wingless. In the third instar wing disc, Wg is secreted from cells at the dorsal/ventral boundary and forms a concentration gradient on either side to regulate the expression of its target genes, senseless (Sens, blue, high threshold), distalless (Dll, red, medium threshold), and vestigial (Vg, green, low threshold). The expression of distalless is graded throughout its domain, whereas vestigial expression is graded only at its edges. senseless, like achaete (discussed in the text), requires high levels of Wg signaling and is expressed only in cells abutting the dorsal/ventral boundary.
boundary and represents direct evidence that Wg acts as a morphogen in the wing disc (Strigini and Cohen 2000).

Wingless has been proposed to behave as a morphogen additionally in the embryonic epidermis and midgut, but whether it actually does so in these developmental contexts is unclear. In the embryonic ectoderm, Wg secreted from the parasegment boundary adopts a graded distribution in the anterior direction up to four cells away to specify naked cuticle (Bejsovec and Martinez Arias 1991). Although in this context Wg fulfills the criteria of having a localized source, forming a gradient, and acting at long range, there is no evidence for a concentration-dependent induction of target genes. In fact, the epidermal phenotype of wg mutant embryos can be rescued through the ubiquitous expression of a wg transgene (Sampedro et al. 1993), suggesting that Wg acts in a gradient-independent manner in this tissue. Additionally, in a wg mutant background, the overexpression of a membrane-tethered form of Wg from cells that normally express the ligand can recapitulate the normal range of signaling, confirming that the restricted diffusion of Wg is dispensable for patterning of the embryonic cuticle (Pfeiffer et al. 2000). In the embryonic midgut, wg is expressed in the visceral mesoderm and directs the neighboring endoderm to differentiate into two different cell types: large, flat cells require high levels of signaling and develop immediately adjacent to the Wg source, whereas copper cells develop further away as their differentiation is repressed at high levels of Wg signaling. As the level of Wg is modified, there is a concomitant change in the domains of the large, flat cells and copper cells. Additionally, the labial gene is expressed in a graded manner in the region of copper cells, consistent with the proposed concentration-dependent effect of Wg. However, the effects of Wg have not been shown to be direct in this tissue (Hoppler and Bienz 1995). Thus, in the embryonic ectoderm and midgut, it is possible that Wg signaling does not define the pattern of the response but, rather, stabilizes patterns of gene expression that have been specified through other mechanisms.

ENDOCYTOSIS: SHAPING THE WINGLESS GRADIENT

Secreted Wg is theoretically capable of passive diffusion at a relatively fast rate in all directions over a long range. However, the graded distribution of Wg forms at a slower rate over a shorter range than that predicted through free diffusion and directionally along the epithelial surface. Moreover, the contrasting range of its activity during embryogenesis (4 cell diameters) (Bejsovec and Martinez Arias 1991; Pfeiffer et al. 2002) versus development of the wing disc (10 cell diameters) (Strigini and Cohen 2000) suggests that the movement of the Wg ligand in the extracellular environment is regulated. Recent experimental and theoretical studies favor a model in which the Wg gradient is formed and maintained through the combined effects of restricted diffusion and endocytosis. Once secreted, the Wg molecule undergoes restricted diffusion, as opposed to free diffusion, because of its interactions with lipid-based transport proteins, receptors on the surface of membranes, and heparan sulfate proteoglycans of the extracellular matrix (Lin and Perrimon 1999; Baeg et al. 2001, 2004; Lecourtois et al. 2001; Bornemann et al. 2004; Kirkpatrick et al. 2004; Han et al. 2005; Panakova et al. 2005; Piddini et al. 2005; Katanaev et al. 2008; Mulligan et al. 2012). In addition, the repeated vesicle-mediated endocytosis and resecretion of Wg, in a process called “planar transcytosis,” and the endocytosis-mediated degradation of Wg also contribute to the formation of the Wg gradient, both extracellularly and intracellularly. We here review the evidence for the endocytosis-mediated regulation of the Wg gradient in the Drosophila embryo and wing disc. At the subcellular level, Wg protein can be detected not only in the extracellular environment, but also within intracellular vesicles and multivesicular bodies in non-secreting cells. This clearly indicates that the Wg ligand is internalized and suggests that its gradient is regulated through endocytosis. The endocytic regulation of the Wg gradient has been investigated using mutations in components of the endocytic machinery. The formation of the Wg
gradient in the embryo is a result of asymmetric endocytosis and trafficking to lysosomes, and perhaps transcytosis. As previously described, during embryogenesis, the initially symmetrical Wg gradient becomes asymmetrical due to increased Wg degradation in cells posterior to its source (Fig. 1) (Sanson et al. 1999; Dubois et al. 2001). Evidence for this model comes from experiments performed using an HRP (horse radish peroxidase)–Wg fusion protein expressed under the control of the endogenous \( w^{+} \) promoter. Unlike the Wg portion of the fusion protein, the HRP moiety is stable throughout the endocytic pathway and thus serves as a tool to monitor Wg degradation in vivo. In cells anterior to the HRP-Wg source, both HRP and Wg can be detected in intracellular vesicles (presumed to be early endosomes), whereas only HRP can be detected in vesicles (presumably late endosomes) in cells posterior to the source. Moreover, posterior to the Wg source, many more HRP-positive intracellular compartments can be detected that extend beyond the Wg protein gradient, confirming that the degradation of Wg through the endocytic pathway limits the range of its gradient (Dubois et al. 2001). In deep orange (\( dor^{+} \)) mutants that have impaired trafficking to the lysosome, Wg accumulates in multivesicular bodies (Piddini et al. 2005). Lastly, in clathrin heavy chain (\( chc^{+} \)) mutants that cannot initiate clathrin-mediated endocytosis, the Wg gradient extends in the posterior direction, suggesting that clathrin is normally required for establishing and maintaining the asymmetric distribution of Wg (Desbordes et al. 2005). However, there is also contradictory evidence that suggests a role for endocytosis to the lysosome, Wg accumulates in multivesicular bodies (Piddini et al. 2005). In accordance with this hypothesis, more extracellular Wg is present within the \( shibire^{+} \) mutant clone than outside the clone (Strigini and Cohen 2000).

Notably, the majority of evidence from the embryonic epidermis and wing disc support different models of Wg transport and stability that contribute to the Wg gradient. Further studies need to be performed to resolve which mechanism of Wg distribution is predominant under physiological conditions.

WINGLESS-RESPONSIVE TARGET GENES: ACTIVATION VERSUS REPRESSION

Although the mechanistic details through which cell signaling pathways ultimately regulate gene expression to control the development of metazoans may differ, they all share at least three common, conserved features: default repression, activator insufficiency, and cooperative activation (for review, see Barolo et al. 2002; Affolter et al. 2008). Developmental signaling pathways regulate gene expression by a switch mechanism, whereby from an actively repressed state in the absence of the signal, genes are transcribed in the presence of the signal. The phenomenon of inhibition of gene transcription in the absence of signaling is referred to as “default repression.” “Activator insufficiency” and “cooperative activation” refer to the
inability of a signaling pathway to activate the same and complete set of target genes in all developmental contexts. The combination of these three features allows a signaling pathway to robustly activate specific target genes expression in response to the signal, in a context-dependent manner, while preventing target gene expression in the absence of the signal (for review, see Barolo et al. 2002; Affolter et al. 2008).

In the case of Wg signaling, default repression is exerted on the same pathway response element using the same signal-regulated transcription factor, Tcf, as is used in the presence of active signaling. In the absence of signaling, Tcf binds Wg-responsive elements within target genes along with other corepressors to suppress gene expression. When the Wg ligand is present, the same transcription factor Tcf binds Arm and recruits other coactivators to direct the expression of target genes (Brunner et al. 1997; van de Wetering et al. 1997; Lawrence et al. 2000; Schweizer et al. 2003). However, it has recently become apparent that target genes of the pathway are not only directly activated by the Wg signal, but can be directly repressed as well. Direct repression implies that following signaling, a gene’s transcription is repressed without the intermediate transcriptional induction of a repressor. Signal-induced gene repression conflicts with the principle of default repression. If gene transcription is silenced through default repression before pathway activity, there is no opportunity to repress gene transcription following signaling. Indeed, for Wg signaling to repress transcription of a gene, default repression of the gene would have to be circumvented before signaling with a switch to a default activation state. Conceptually, the reversal of this feature would allow signaling pathways to robustly repress target gene expression in the presence of the signal, in a context-dependent manner, while activating the target gene in its absence.

Several target genes including fzd, svb, rho, Ultrabithorax (Ubx), stripe (sr), and dpp have been proposed to be directly repressed by Wg signaling. However, in the cases of fzd, svb, and rho (Cadigan et al. 1998; Payre et al. 1999; Sanson et al. 1999), the molecular studies addressing the cis and/or trans regulatory elements involved in transcriptional repression are lacking, while for Ubx, it is thought that repression of gene transcription occurs indirectly and is therefore a secondary effect of Wg signaling (Waltzer et al. 2001). Only in the cases of sr in the embryonic epidermis (Piepenburg et al. 2000) and dpp in the leg imaginal disc (Theisen et al. 2007), has it been convincingly shown that these genes are, in fact, repressed by Tcf in the presence of signaling. Both of these genes contain functional Tcf-binding sites in their response elements that are required for repression, and mutation of these sites results in a failure of Wg-mediated gene silencing.

In a recent study, several genes that are repressed by Wg signaling were identified from cultured Drosophila hemocytic cells. Surprisingly, the characterization of the cis regulatory element of one of these genes, Ugt36Bc, revealed that the Tcf recognition site is markedly different from a typical consensus Tcf binding site. Furthermore, the novel Tcf binding sites were not only required for Wg-induced repression, but were also essential for the default transcription of Ugt36Bc in the absence of signaling (Blauw-kamp et al. 2008). This suggests that the nature of the Tcf binding site within the Wg-responsive element can determine the nature of the transcriptional output, both in the absence and presence of signaling, and is one possible mechanism through which Wg signaling can switch from activation to repression of certain genes.

CONCLUDING REMARKS

Over the last 30 years, the use of genetic analyses in Drosophila has elucidated both the function, in various contexts of development, and the molecular mechanism of the Wg pathway. In addition, the study of Wnt/Wg proteins first illustrated the relationship between normal development and oncogenesis. Owing to the vast array of genetic techniques available in Drosophila and its high degree of conservation, no doubt, novel components and Wg-regulated processes identified in future studies using this model system are likely to be directly applicable to vertebrate development.
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Wnt/Wingless Signaling in Drosophila


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Wnt/Wingless Signaling in *Drosophila*

Sharan Swarup and Esther M. Verheyen

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