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The role of wingless in the development of the proximal wing hinge of *Drosophila*.

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Abstract

We have examined the role of Wingless (Wg) in the development of the proximal portion of the *Drosophila* wing hinge. We find that in the dorsal hinge, ectopic expression of *wg* causes duplication of Axillary Sclerite 3 (AS3) at the expense of Axillary Sclerite 1 (AS1), Axillary Sclerite 2 (AS2) and the Unnamed Plate (UP). Attenuation or removal of Wg activity causes shrinkage or deletion of AS3. Therefore Wg is necessary to establish the development of AS3 in the dorsal hinge. In the ventral hinge, ectopic expression of Wg removes the Axillary Pouch (AP) and attenuation or removal of Wg activity in the ventral hinge duplicates the AP. Therefore, Wg is necessary to restrict the formation of the AP to its particular location in the ventral hinge. Thus Wg is an important signaling molecule in the development of the proximal dorsal and ventral wing hinge.

Introduction

Wnt proteins constitute a large family of secreted glycoproteins that function as signaling molecules during the development of both invertebrate and vertebrate organisms (for review see Cadigan and Nusse, 1997). *Wnt* genes show very widespread expression and many tissues require Wnt proteins for their development.

Wg signaling studies in *Drosophila* have made great contributions to our understanding of Wnt signal transduction. Wnt proteins associate with the Frizzled (Fz) family of receptors (Bhanot *et al.*, 1996). The Wnt signal is then transduced by a cytoplasmic protein, Dishevelled (Dsh; Klingensmith *et al.*, 1994; Theisen *et al.*, 1994), resulting in repression of the Zeste-white3/Shaggy kinase (Siegfried *et al.*, 1990; Siegfried *et al.*, 1992), the ortholog of Glycogen Synthase Kinase 3 β . Repression of the Shaggy kinase involves the dissociation of a complex between Shaggy and a scaffold protein called Axin (Hamada *et al.*, 1999; Ruel *et al.*, 1999; Willert *et al.*, 1999), which also acts as a negative regulator of Wg signaling. The Shaggy/Axin complex binds to the Armadillo protein, which is the ortholog of β -catenin (Peifer and Wieschaus, 1990), and phosphorylates it (Peifer *et al.*, 1994a; Peifer *et al.*, 1994b), which marks Armadillo for degradation (Pai *et al.*, 1997) and holds it in within the cytoplasm (Tolwinski and Wieschaus, 2001). Inactivation of Shaggy stabilizes Armadillo protein (Peifer *et al.*, 1991), which translocates to the nucleus where it associates with Pangolin, the *Drosophila* LEF-1/TCF ortholog (Brunner *et al.*, 1997; van de Wetering *et al.*, 1997) to regulate gene expression. Vertebrate orthologs of these proteins have been shown to function in a similar cascade to transduce

Wnt-1-like signals (Karnovsky and Klymkowsky, 1995; Sokol *et al.*, 1995; Molenaar *et al.*, 1996; Sokol, 1996; Yang-Snyder *et al.*, 1996; Yost *et al.*, 1996; Zeng *et al.*, 1997; Behrens *et al.*, 1998).

Wg plays a crucially important role in the development of the *Drosophila* imaginal discs (Baker, 1988; Simcox *et al.*, 1989; Couso *et al.*, 1993). The wing disc in particular requires Wg at several phases of its development. In the absence of Wg activity, both the wing blade and the hinge are deleted and replaced by notum (Sharma and Chopra, 1976; Morata and Lawrence, 1977). The early expression of *wg* during the second larval instar is required to establish the wing primordium (Couso *et al.*, 1993; Williams *et al.*, 1993; Ng *et al.*, 1996). During the second instar, Wg collaborates with Notch to activate and maintain the expression of *vestigial* (*vg*) and *scalloped* (*sd*), which are essential for the growth of the wing (Williams *et al.*, 1991; Williams *et al.*, 1994; Couso *et al.*, 1995; Kim *et al.*, 1996; Halder *et al.*, 1998; Simmonds *et al.*, 1998; Klein and Arias, 1999; Varadarajan and VijayRaghavan, 1999). During the third instar at the D/V boundary, Wg and Notch signal to generate the elaborate sensory organs that decorate the adult wing margin (Couso *et al.*, 1994; Williams *et al.*, 1994; Diaz-Benjumea and Cohen, 1995; Rulifson and Blair, 1995; Neumann and Cohen, 1996b).

The role of Wg in the development of the *Drosophila* wing has been extensively studied, but the role of Wg in the development of the wing hinge has been less well studied. Wg is important for hinge development (Neumann and Cohen, 1996a; Klein and Arias, 1998). However, Wg does not signal through *vg* in the wing hinge, and the exact role of Wg in the development of the hinge is still uncertain.

In this paper we have undertaken a study of the role of Wg in the proximal wing hinge. We have used overexpression and ectopic expression studies and loss-of-function experiments to determine the role of Wg in the development of the wing hinge. We show that the dorsal hinge requires Wg for the establishment of Axillary Sclerite 3, and the ventral hinge requires Wg to restrict the formation of the Axillary Pouch.

Materials and Methods

Fly Strains: The following GAL4 lines were the main ones used in this study: *30A GAL4/CyO* (Brand and Perrimon, 1993), *MS1096 GAL4* (Capdevila and Guerrero, 1994), and *decapentaplegic GAL4/TM6 β* (Staebling-Hampton *et al.*, 1994). All GAL4 lines were used in crosses with the following UAS lines: *UASwg^{ts}* (Wilder and Perrimon, 1995), *UASwg* (Lawrence *et al.*, 1995), *UASzw3* (Steitz *et al.*, 1998), *UASdsh* (Axelrod *et al.*, 1996), *UASarm^{S10}* and *UASarm ^{Δ N}* (Pai *et al.*, 1997), *UASpan^{DN}* (van de Wetering *et al.*, 1997), *UASDfz2* and *UASDfz2-GPI* (Cadigan *et al.*, 1998). The progeny of crosses between GAL4 and UAS lines is written as GAL4 - UAS. Canton-S and *w¹¹¹⁸* were used as wild-type stocks. To visualize the expression pattern of these GAL4 lines in imaginal discs from third instar larvae, each GAL4 line was crossed to *UASlacZ* (Brand and Perrimon, 1993), and the discs from the larvae produced by these crosses were stained with X-Gal (see below). All crosses were done at 25 °C unless otherwise specified. All crosses with *UASwg^{ts}* were kept at 25 °C for the first day of development and then downshifted to 18 °C for specified periods of time.

For experiments with *wg^{ts}* (*wg^{IL114}*; Nusslein-Volhard *et al.*, 1984), *wg^{ts} pr bw / CyO ftz-lacZ* flies were crossed to *wg^{CX4} b pr / CyO hb-lacZ* (Baker, 1987; Struhl *et al.*, 1992), and the progeny kept at 18°C until late second/early third larval instar, and then upshifted to 25°C for 20-24 hours. *wg^{CX4}* is a null allele of *wg* that contains a 2.7-kb deletion that completely removes the first exon (van den Heuvel *et al.*, 1993).

Mosaic clones of *dsh* were induced using the $y^1 w^{1118} dsh^{75} FRT18A$ chromosome as described by Klingensmith *et al.*, (1994). Similarly, clones of *zw3* were made as described by Wilder and Perrimon (1995) by employing the $y^1 sgg^{D127} v f FRT18A$ chromosome. Clones of wg^{CX4} were induced as described by Neumann and Cohen (1997) by utilizing the $wg^{CX4} FRT40A$ chromosome. Mosaic clones were identified by means of the presence of the *y* mutant phenotype, which causes bristles to have a lighter color.

Direct Flight Muscles were observed with *myosin rod protein-lacZ* (Standiford *et al.*, 1997).

Immunohistochemistry: β -galactosidase (β -Gal) staining of imaginal discs was done according to the protocol of Ashburner (1989).

In situ hybridization of imaginal discs was done with DIG-labeled probes of *wg*, according to the protocol of Sturtevant and Bier (1993).

β -Gal staining of thoraces for DFM examination was done by dissecting adult thoraces and staining with X-Gal using the Ashburner protocol. For designation of individual the Direct Flight Muscles (DFMs), we followed the nomenclature and anatomical description of Miller (1950).

To stain imaginal discs with anti- β -Gal antibodies, we employed the protocol of Campbell *et al.*, (1993) and used a rabbit anti- β -Gal at 1:500 (5 Prime \rightarrow 3 Prime, Inc.). To stain Direct Flight Muscles with a rabbit anti-Myosin Rod Protein (MRP) antibody, we followed the protocol of Standiford *et al.* (1997), and also used this antibody at 1:500.

Wing Hinge Preparations: All unstained wing hinges were dissected in 70% ethanol and mounted in Hoyer's Medium (Ashburner, 1989) between two cover slips so that both the dorsal and ventral sides could be examined. The preparations were then baked at 60 °C and pressed with weights to make them flat. For the wing hinge, the nomenclature of Bryant (1978) was used.

Results

In late third-instar wing imaginal discs, the *wg* gene is transcribed in a stripe of cells across the D/V boundary, and in two concentric rings that surround the wing pouch (Baker, 1988; Couso *et al.*, 1993; Williams *et al.*, 1993; Figure 1A-B). The central stripe of *wg*-expressing cells gives rise to the wing margin (Couso *et al.*, 1994; Rulifson and Blair, 1995). The inner ring of *wg* gives rise to distal hinge structures like the Costa, Dorsal Radius and Alula (Neumann and Cohen, 1996a). The outer ring of *wg* expression maps to the proximal hinge, which generates the Axillary Sclerites, Axillary Chord, and various structures of the ventral hinge (Figure 1B; see below). More proximally lies the primordium of the mesonotum, and a stripe of *wg* expression in this region, elaborated during the third larval instar, helps to establish the pattern of sensory bristles (Phillips and Whittle, 1993).

To ascertain the effects of *Wg* in the proximal wing hinge, we used several GAL4 lines to drive expression in the hinge. GAL4 line 30A (Brand and Perrimon, 1993) drives expression throughout the dorsal and ventral hinge primordia, but not in the wing pouch (Figure 1C). *dpp*GAL4 (Staehling-Hampton *et al.*, 1994) drives expression along the anterior/posterior border, including the medial portion of the dorsal hinge, and to a lesser extent the ventral hinge (Figure 1D). *MS1096* (Capdevila and Guerrero, 1994) drives expression in the dorsal hinge and the dorsal and ventral wing pouch but is excluded from the ventral hinge (Figure 1E). Similar effects were observed with different GAL4 lines.

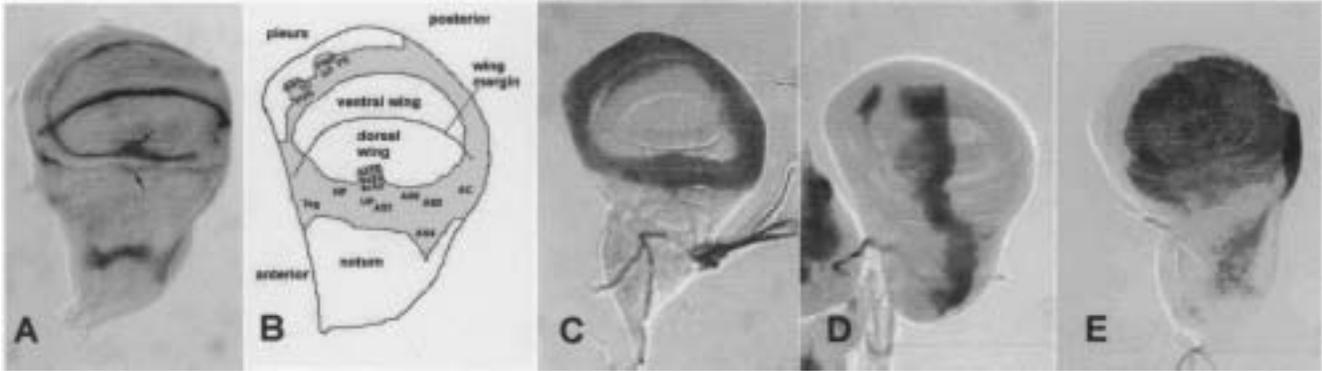


Figure 1. (A) The expression patterns of *wg* in the wing imaginal disc from late third-instar larvae, as ascertained by *in situ* hybridization. *wg* is expressed in a stripe that corresponds to the future wing margin, and in the hinge primordium (see arrows) in two concentric rings that encircle the wing pouch (Baker, 1988; Couso *et al.*, 1993; Phillips and Whittle, 1993; Williams *et al.*, 1993; Neumann and Cohen, 1996a). *wg* is also expressed in a stripe in the presumptive notum (Phillips and Whittle, 1993). (B) Fate map of the *Drosophila* wing imaginal disc from a third instar larvae (modified from Bryant, 1975). The adult wing blade, notum, and wing hinge of *Drosophila* all develop from the wing imaginal disc. The gray region depicts the hinge anlage and the notal, wing and pleural anlagen are all in white. The abbreviations used in this fate map are as follows: AC - Axillary Chord; AP - Axillary Pouch; AS1 - Axillary Sclerite 1; AS2 - Axillary Sclerite 2; AS3 - Axillary Sclerite 3; AS4 - Axillary Sclerite 4; HP - Humeral Plate; PAA - Postalar Apophysis; PS - Pleural Sclerite; PVR - Proximal Ventral Radius; PWP - Pleural Wing Process; sc12 - sensilla campaniformia 12, sc25; sensilla campaniformia 25; sc4d - sensilla campaniformia 4 on the dorsal side; Teg - Tegula; UP - Unnamed Plate; YC - Yellow Club. (C) Wing imaginal disc from a *30A - UASlacZ* larva, stained with X-GAL. This GAL4 insertion drives expression throughout the dorsal and ventral hinge anlagen. (D) Wing disc from a *dppGAL4 - UASlacZ* larva stained with X-GAL. This line drives expression along the anterior/posterior border, including the medial region of the dorsal hinge anlage, and in the ventral hinge to a lesser extent. (E) Wing disc from a *MS1096 - UASlacZ* larva stained with X-GAL. This line drives expression in the dorsal hinge anlage, and the dorsal and ventral wing pouch as well. It is excluded from the ventral hinge anlage.

The wild-type dorsal hinge has many salient features (Figure 2A-B; Bryant, 1978). Four cuticular plates, called Axillary Sclerites, form a folding structure that allows the wing to move in three dimensions, and one of these attaches this apparatus to the body wall. The Axillary Sclerites are recognizable by their structure, position relative to each other, and the absence of hairs. Axillary Sclerite 1 (AS1) is a C-shaped plate, the cleft of which contains Axillary Sclerite 2 (AS2). Anterior to AS2 is an oblong cuticular plate termed the Unnamed Plate (UP) that contacts both AS2 and AS1. Posterior to AS2 is a large, flat plate called Axillary Sclerite 3 (AS3) that articulates with AS2. The integrity of the contact between AS2 and AS3 is important for wing movement and wing position (Miyan and Ewing, 1985; Wissler, 1987; Wissler, 1988). AS3 is connected to Axillary Sclerite 4 (AS4), which is a long thin cuticular plate that links AS1, AS2, AS3 and the UP to the body wall (Figure 2A-B). The connection between AS3 and AS4 is also important for proper wing position.

Excessive Wg activity causes duplication of AS3 in the dorsal wing hinge.

Misexpression of *wg* in the hinge produces dramatic phenotypes, primarily characterized by duplication of AS3 with concomitant reduction or elimination of AS1, AS2, and the UP (Figure 2C-D). While in most cases it seems that duplication of AS3 occurs at the expense of the other structures, we

have observed diminution or loss of AS1, AS2, and the UP without duplication of AS3, especially at lower levels of ectopic *wg* expression. AS4 is also sometimes deformed, apparently as a consequence of its detachment from AS3. Duplication or loss of these cuticular elements also results in duplication or loss of the direct flight muscles that attach to them (not shown).

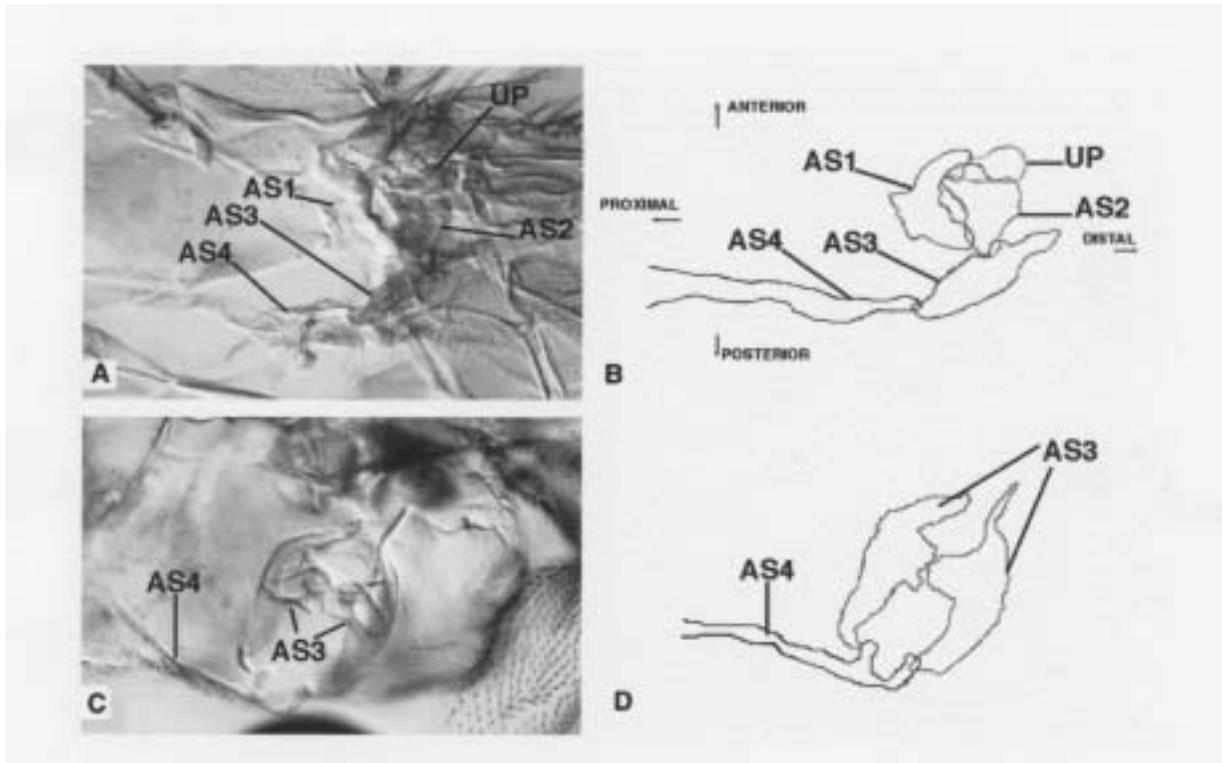


Figure 2. (A) Dorsal hinge from a wild-type adult *Drosophila*. In all hinge photographs, anterior is up, and proximal is to the left. (B) A cartoon tracing of the salient features found in Figure 2A. The cartoon tracings sometimes represent a synthesis of multiple focal planes. (C) A dorsal hinge from a *dppGAL4* - *UASwg* pharate adult grown at 25 °C until the early third instar, and then downshifted to 18 °C for the remainder of its larval/pupal life (n = 23). AS1, AS2, and the UP have all been removed and AS3 is duplicated. Sometimes AS1, AS2, and the UP are missing without duplication of AS3 (13%; not shown). (D) A cartoon tracing of Figure 2C.

Ectopic expression of *dsh* (Axelrod *et al.*, 1996) or an activated form of *arm* (*arm^{ΔN}*; Pai *et al.*, 1997) produces hinge phenotypes indistinguishable from those produced by ectopic expression of *wg* (not shown). Ectopic expression of *Dfz2* does not affect the hinge unless *wg* is concomitantly expressed with it. However, co-expression of *Dfz2* with *wg* tended to make the hinge more sensitive to Wg. For example, *30A* - *UASwg^{ts}* flies rarely show effects on AS3, but *30A* - *UASDfz2* *UASwg^{ts}* flies show consistent diminution of AS3 (not shown).

Loss of *zw3* mimics ectopic *wg* activation in imaginal discs (Diaz-Benjumea and Cohen, 1994; Wilder and Perrimon, 1995). Large mitotic clones of *zw3* that cover most of the dorsal hinge cause duplication of AS3 at the expense of AS1, AS2 and the UP (not shown).

Excessive Wg activity eliminates the Axillary Pouch in the ventral hinge.

The ventral hinge contains several cuticular landmarks that are easily recognizable (Figure 3A-B). For our analysis, the two most relevant structures are the Axillary Pouch (AP) and the Pleural Sclerite (PS). The AP is a cuticular pouch that is recognizable by its size and by the hairs that cover it. The PS is a plate that juxtaposes the AP.

Misexpression of *wg* causes loss of the AP and expansion of the PS (Figure 3C-D). Loss or reduction of the AP is the most reproducible effect of ectopically expressing or overexpressing *wg* in the ventral wing hinge. Mitotic clones of *zw3* that cover this portion of the ventral hinge also cause reduction or loss of the AP, depending on the size of the clone (not shown).

Overexpression of *dsh*, or a constitutively activated *armadillo* allele (*arm*^{S10}) also produces phenotypes that are indistinguishable from those generated by the ectopic expression of *wg* (not shown).

Reduction of Wg signaling in the dorsal wing hinge removes AS3.

A temperature-sensitive allele of *wg* (*wg*^{IL114}) was employed in order to assay the effect of lowering Wg activity in the hinge. If *wg*^{IL114} / *wg*^{CX4} animals were upshifted to the restrictive temperature during the late second or early third larval instar, such animals produced pharate adults with hinge defects that consistently showed reductions of AS3 (Figure 4 A-B).

dsh mutant clones induced during the first instar exhibit dramatic phenotypes in the dorsal hinge. Dorsal hinge structures, with the exception of AS1, are obliterated if they fall within the clone (not shown). Mitotic clones of *wg*^{CX4} do not cause hinge phenotypes (not shown), since the surrounding wild-type tissue secretes Wg and non-autonomously rescues the mutant tissue (Li *et al.*, 1995).

Phenotypes produced by overexpression of *zw3* (Steitz *et al.*, 1998) or *pan*^{DN} (van de Wetering *et al.*, 1997) are generally opposite in nature to those produced by misexpression of *wg*. Overexpression of *zw3* causes reduction or elimination of AS3 (Figure 4C-D), as does ectopic expression of *Dfz2-GPI* or *pan*^{DN} (not shown).

Reduction of Wg signaling in the ventral hinge causes duplication of the AP.

Ventrally, since hyperactivation of Wg signaling in the ventral hinge causes loss of the AP, reduction or elimination of Wg signaling in the ventral hinge should cause enlargement or duplication of the AP. Mitotic clones of *dsh* that are large enough to include the proximal hinge result in duplication of the AP and the YC (Figure 4E-F). Phenotypes resulting from expression in more restricted domains were consistent with cell autonomous effects of this gene (not shown).

Ectopic expression of *zw3*, which attenuates Wg activity, also causes duplication of the AP (Figure 4G-H). Ectopic expression of *Dfz2-GPI* or *pan*^{DN} produced similar results (not shown). These results are consistent with the mediation of Wg signaling by Dsh, Arm, Pan, and Zw3 in the hinge.

Discussion

Analysis of the phenotypes induced by hyperactivation or attenuation of Wg activity in the wing hinge has shown that Wg is required for the formation of AS3 in the dorsal hinge and the restriction of the AP in the ventral hinge.

In the wing hinge, the Wg protein seems to have three distinct tasks: establishment of the wing field, delineation of the hinge anlage from the wing anlage, and establishment of specific structures in the proximal and distal portions of the hinge.

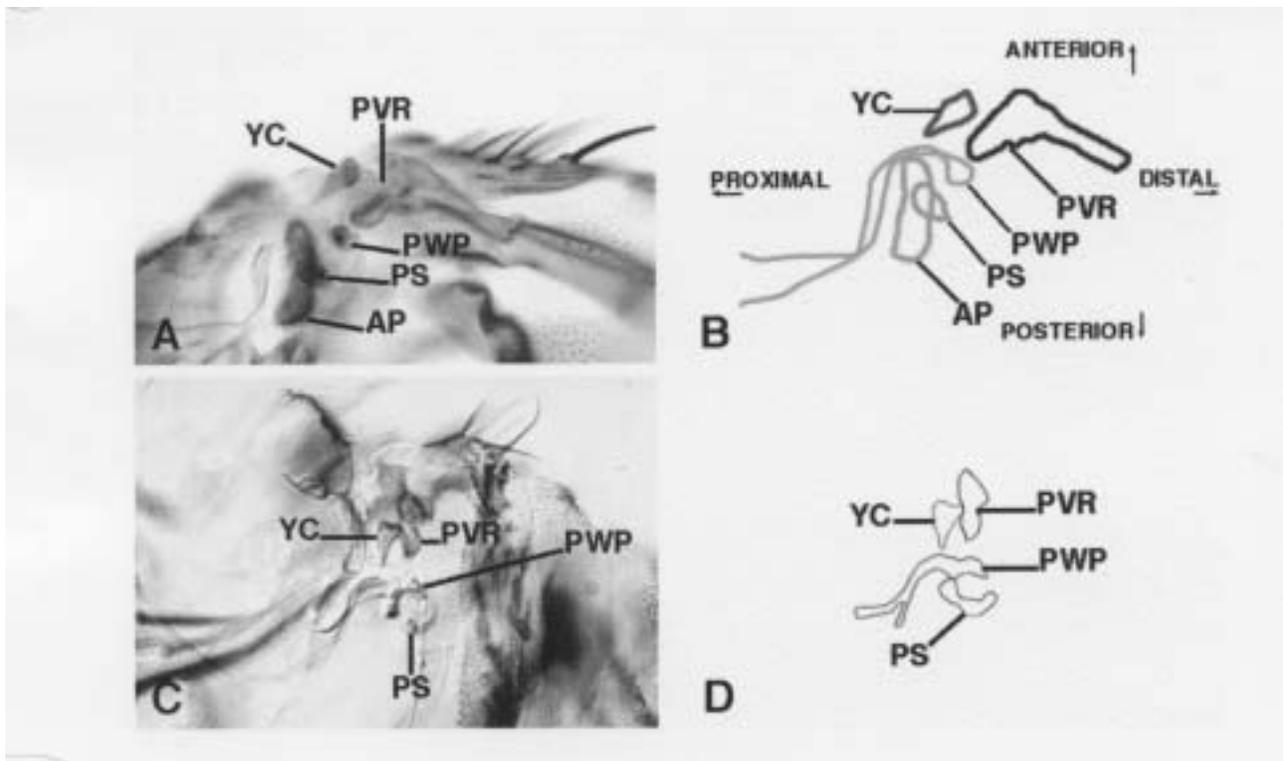
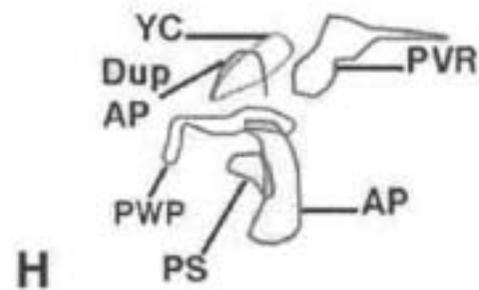
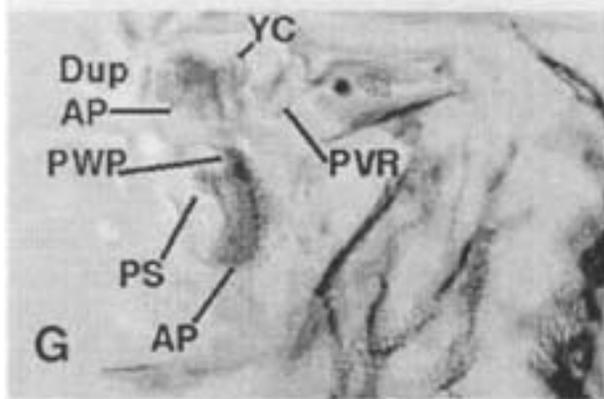
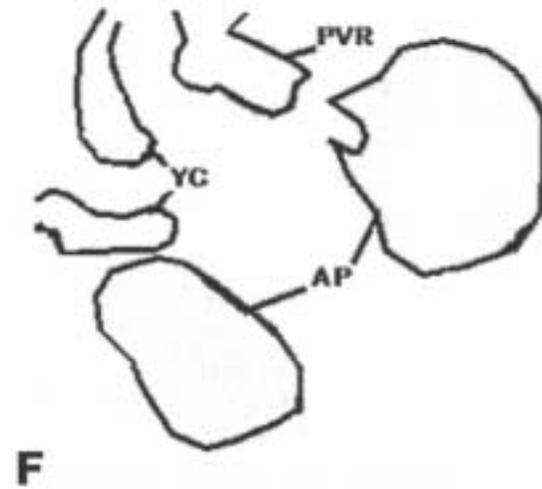
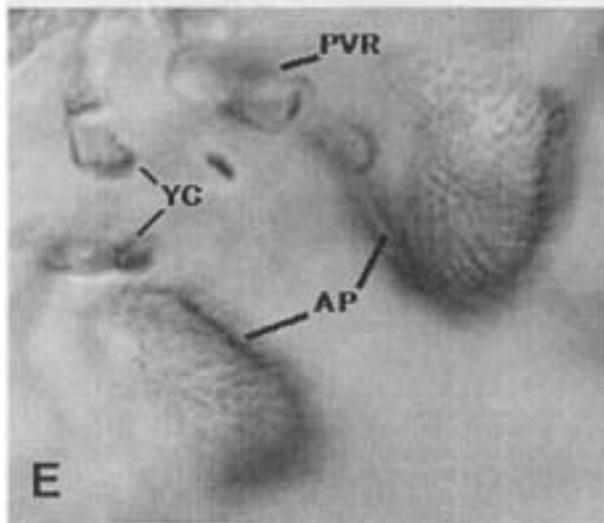
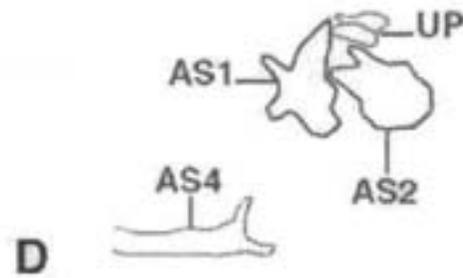
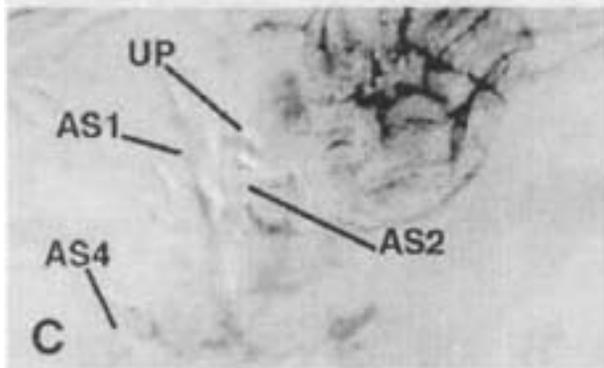
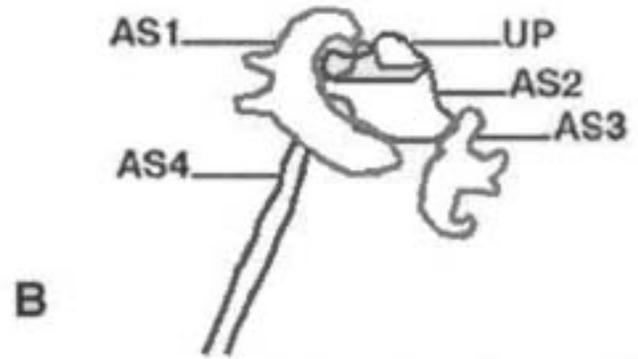
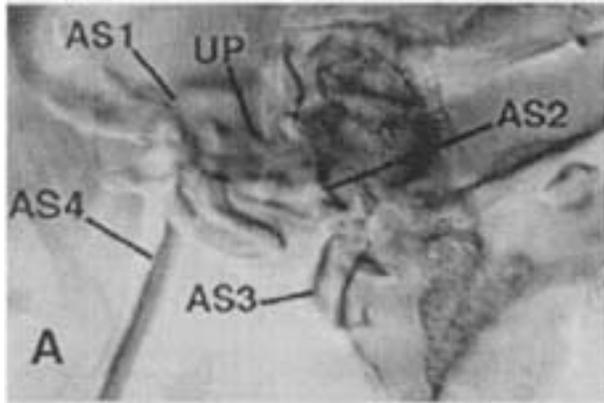


Figure 3. (A) Ventral hinge from a wild-type adult. Ventrally, the confluence of wing veins #1, #2, and #3 terminates in a structure called the Proximal Ventral Radius (PVR), which bears three groups of sensory structures that contain four (sc4v), three (sc3) and five (sc5) campaniform sensillae respectively (Bryant, 1978). Just proximal to the PVR is the Yellow Club (YC), which is the most conspicuous ventral hinge structure. Posterior to the YC is a series of plates and a pouch. The Axillary Pouch (AP) is a large outpouching of the hinge cuticle, which is covered by hairs. Just anterior to the AP lies the Pleural Wing Process (PWP). The PWP arches around the AP to form the pleural suture, which separates the pleura into the mesopleura and the pteropleura. The head of the PWP articulates with the ventral side of AS2. Posterior to the PWP is the Pleural Sclerite (PS). The PS is hidden from view in some preparations by the AP, which overlies and obscures it. (B) A cartoon tracing of Figure 3A. (C) Ventral hinge from a *30A - UASwg* pharate adult. The AP is missing and the PS is enlarged. (D) A cartoon tracing of Figure 3C.

Figure 4 (next page). (A) A dorsal hinge from a *wg^{ts} / wg^{CX4}* pharate adult that was grown as described (See Materials and Methods). All proximal hinge elements are normal with the exception of AS3, which is smaller than normal and fails to articulate with AS4 (n = 13). (B) A cartoon tracing of Figure 4A. (C) Dorsal wing hinge from a *MS1096 - UASzw3* adult (n = 31). AS3 is missing, and AS1, AS2, AS4, and the UP are unaffected. Wings from *MS1096 - UASzw3* animals are very small and devoid of marginal structures (data not shown). (D) A cartoon tracing of Figure 4C. (E) Ventral wing hinge from a *y^I w¹¹¹⁸ dsh⁷⁵ FRT18A / w¹¹¹⁸ arm-lacZ FRT18A; hsflp³⁸ / +* animal, in which a large *dsh* clone has been induced. The AP and YC are both duplicated and the PWP and PS are missing (n = 12). (F) A cartoon tracing of Figure 4E. (G) A ventral hinge from a *dppGAL4 - UASzw3* pharate adult (n = 21). All the ventral hinge structures are intact, but the AP is duplicated. The duplicate AP lies just posterior to the PAA. (H) A cartoon tracing of Figure 4G.



Wg is required for the establishment of the wing field.

During the second instar, *wg* is expressed in a patch of cells nested within the anterior and ventral portions of the wing disc (Couso *et al.*, 1993; Williams *et al.*, 1993; Ng *et al.*, 1996). Without *Wg* activity during the second instar, the entire wing field, both the wing blade and the hinge, is absent and is replaced by notum (Sharma and Chopra, 1976; Morata and Lawrence, 1977). Therefore *Wg* is essential to establish the anlagen that give rise to both the wing pouch and the hinge primordium.

Wg is necessary for delineation between the wing and hinge primordia.

Wg helps establish the wing blade by directing the expression of the wing-specific gene *vg*. Proper activation of *vg* in the wing pouch requires a collaboration between *Wg*, the Notch receptor, which is activated by interactions between dorsal and ventral cells at the D/V boundary (Williams *et al.*, 1994; Kim *et al.*, 1995; Kim *et al.*, 1996), and Decapentaplegic (*Dpp*), the TGF- β ortholog in *Drosophila* (Padgett *et al.*, 1987), which is secreted by the cells along the anterior/posterior (A/P) border (Blair, 1994; Kim *et al.*, 1996; Zecca *et al.*, 1996; Kim *et al.*, 1997; Neumann and Cohen, 1997). *Wg*, which is secreted from the D/V boundary cells, induces *vg* expression at a distance from the D/V boundary (Zecca *et al.*, 1996; Neumann and Cohen, 1997). This generates a gradient of *Vg*, with peak levels at the D/V boundary.

Outside the wing pouch, *Wg* activates targets other than *vg*. One of the targets of *Wg* signaling within the hinge is the *hth* gene, which, during the third instar, shows an expression pattern that is restricted to the hinge primordia and is required for hinge development (Azpiazu and Morata, 2000; Casares and Mann, 2000). Clones of *hth* and *extradenticle* (*exd*), show similar effects in the hinge (Gonzalez-Crespo and Morata, 1995; Rauskolb *et al.*, 1995). In imaginal discs, *extradenticle* shows uniform expression (Rauskolb *et al.*, 1995) but is only active in those cells where it shows nuclear localization (Mann and Abu-Shaar, 1996; Aspland and White, 1997), and *Hth* activates the nuclear localization of *Exd* (Rieckhof *et al.*, 1997; Pai *et al.*, 1998). Presently, it is not clear if *Hth* has any function independent of *Exd*.

Wg and *Dpp* activate the expression of the *vg* gene, and in the wing pouch, *Vg* represses *hth* expression (Azpiazu and Morata, 2000). Initially, the wing disc, which is derived from the second thoracic segment, shows high and uniform levels of *hth* expression (Rieckhof *et al.*, 1997). The repression of *hth* by *Vg* prevents the nuclear accumulation of *Exd* in the cells of the wing pouch, which prevents the wing pouch cells from forming more proximal structures. Therefore, *Wg* establishes the hinge by activating *hth* in the hinge and directing the expression of *Vg*, which prevents the expression of *hth*, in the wing. In this way, *Wg* helps delineate between the hinge and wing primordia.

The specification of notum rather than hinge appears to come from the notum-specific expression of the *Iroquois-C* (*Iro-C*) gene (Diez del Corral *et al.*, 1999).

Wg establishes specific structures in the proximal and distal hinge.

Once the wing field has been established and the hinge primordium delineated, *Wg*, *Hth* and another gene that shows hinge-specific expression in the wing disc called *teashirt* (*tsh*; Fasano *et al.*, 1991; Soanes *et al.*, 2001) distinguish the distal and proximal regions of the hinge. In the hinge, *Wg* and *Tsh* seem to maintain the high levels of *hth* expression that were inherited from the embryo (Azpiazu and Morata, 2000; Casares and Mann, 2000). Expression of *tsh* is restricted to the proximal hinge, and it is possible that the combination of *Tsh*, *Wg* and *Hth* generates the proximal hinge, whereas the distal

hinge is specified by the combination of Wg, Hth, and some of the wing pouch-specific genes whose expression domains spill into the distal hinge, like *nubbin* (Azpiazu and Morata, 2000).

Within these two molecular contexts, Wg specifies the development of particular hinge structures. The inner ring of *wg*-expressing cells is missing in wing imaginal discs from animals homozygous for *wg^{spd-fg}*. Flies homozygous for *wg^{spd-fg}* or *wg^{IL114}* / *wg^{CX4}* animals grown at the restrictive temperature during the third instar, show deletion of part of the Dorsal Radius, Costa and Alula (Phillips and Whittle, 1993; Neumann and Cohen, 1996a).

Likewise, if Wg activity is attenuated during development of the proximal hinge, AS3 fails to develop and the AP duplicates. While other axillary sclerites in the dorsal hinge are also affected by the absence of Wg signaling, AS3 is the most sensitive. The same can be said for the YC in the ventral hinge, which can also be affected by levels of Wg signaling, but is much less sensitive than the AP (data not shown). Therefore, even though AS3 and the AP are the structures most readily affected by variations in Wg signaling, they are by no means the only structures that respond to Wg, and the role of Wg in the proximal hinge is almost certainly more complex than just the specification of these two structures.

Wg does not function simply as a mitogen in the developing hinge.

Neumann and Cohen (1996a) concluded that Wg acts as a mitogen for the hinge since overexpression of Wg causes enlargement of the hinge, and a decrease in Wg expression in the hinge causes loss of hinge tissue. Our results are essentially consistent with their conclusions, but we find that the mitogenic effect of Wg only applies to specific areas of the hinge, since other regions of the hinge do not overproliferate in response to ectopic Wg. AS1, AS2, the AP, and the UP shrink or disappear rather than enlarge when Wg is ectopically expressed. In addition, duplication of AS3, which is a characteristic result of ectopically expressing Wg with *dppGAL4*, occurs at the expense of these other structures in the dorsal hinge. Ventrally, several structures appear to require Wg activity in order to form, but, with the exception of the PS, overexpression of Wg does not enlarge them. Dorsally, the primary role of Wg seems to be in the formation of AS3 and sc12 (Neumann and Cohen, 1996a).

Our data indicate that even in cells where Wg apparently increases proliferation, it is also associated with changes in cell fate. Therefore, the role of Wg in the development of the hinge is complex, and includes mitogenic and cell determination activities.

Concluding Remarks

The wing hinge of *Drosophila* is a tissue that requires Wg for its development. Particularly two of its structures; AS3 in the dorsal hinge requires Wg for its development, and the AP in the ventral hinge requires Wg for restriction of its development. It is particularly interesting that portions of the hinge respond differently to this protein, but the molecular contexts in which Wg is expressed probably determine the effects it has on the cells that experience it.

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