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Regulation of Wingless and Vestigial expression in wing and haltere discs of *Drosophila*

Prasad Mohit, Ruchi Bajpai and L. S. Shashidhara*

Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, 500 007, India
*Author for correspondence (e-mail: shashi@ccmb.res.in)

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SUMMARY

In the third thoracic segment of *Drosophila*, wing development is suppressed by the homeotic selector gene *Ultrabithorax* (*Ubx*) in order to mediate haltere development. Previously, we have shown that *Ubx* represses dorsoventral (DV) signaling to specify haltere fate. Here we examine the mechanism of *Ubx*-mediated downregulation of DV signaling. We show that Wingless (Wg) and Vestigial (Vg) are differentially regulated in wing and haltere discs. In wing discs, although Vg expression in non-DV cells is dependent on DV boundary function of Wg, it maintains its expression by autoregulation. Thus, overexpression of Vg in non-DV cells can bypass the requirement for Wg signaling from the DV boundary. *Ubx* functions, at least, at two levels to repress Vestigial expression in non-DV cells of haltere discs. At the DV boundary, it functions downstream of Shaggy/GSK3β to enhance the degradation of Armadillo (Arm), which causes downregulation of Wg signaling. In non-DV cells, *Ubx* inhibits event(s) downstream of Arm, but upstream of Vg autoregulation. Repression of Vg at multiple levels appears to be crucial for *Ubx*-mediated specification of the haltere fate. Overexpression of Vg in haltere discs is enough to override *Ubx* function and cause haltere-to-wing homeotic transformations.

Key words: *Drosophila*, Haltere, *Ultrabithorax*, Armadillo, DV signaling

INTRODUCTION

In the fruitfly *Drosophila melanogaster*, wings and halteres are the dorsal appendages of the second and third thoracic segments, respectively. In the third thoracic segment, wing development is suppressed by the homeotic selector gene *Ultrabithorax* (*Ubx*) in order to mediate haltere development (Lewis, 1978). Loss of *Ubx* function from developing haltere discs induces haltere-to-wing transformations, whereas ectopic expression of *Ubx* in developing wing discs leads to wing-to-haltere transformations (Lewis, 1978; Cabrera et al., 1985; White and Akam, 1985). The differential development of wings and halteres thus constitutes a good genetic system with which to study cell fate determination. They also give insight into the evolutionary trend that has established the differences between fore and hind wings in insects, wings and legs in birds and fore- and hindlimbs in mammals.

Growth and patterning during fly wing development are mediated by signaling from the dorsoventral (DV) organizer. Interactions between dorsal and ventral cells of the wing pouch set up the organizer by activating Notch (N) at the DV boundary (Diaz-Benjumea and Cohen, 1993; Diaz-Benjumea and Cohen, 1995; Williams et al., 1994; Irvine and Wieschaus, 1994; Kim et al., 1995; de Celis et al., 1996b). N, in turn, activates Wingless (Wg), Cut (Ct) and Vestigial (Vg) at the DV boundary (Couso et al., 1995; Kim et al., 1995; Rulifson and Blair, 1995; Kim et al., 1996; Neumann and Cohen, 1996). Wg is known to diffuse to non-DV cells from the DV boundary to act as a morphogen (Zecca et al., 1996; Neumann and Cohen, 1997). High levels of Wg are required for activating Achaete (Ac), whereas moderate levels are sufficient to activate Distal-less (Dll) and low levels to activate Vg (Neumann and Cohen, 1997). Thus, Vg is expressed in both DV and non-DV cells. It has been shown that two different promoters regulate Vg expression in DV and non-DV cells (Kim et al., 1996). They are *vg*-boundary enhancer (*vg*-BE) and *vg*-quadrant enhancer (*vg*-QE).

Previously, we have shown that *Ubx* downregulates DV signaling to specify haltere fate (Shashidhara et al., 1999). In haltere imaginal discs, Wg and Ct are expressed only in the anterior compartment (Weatherbee et al., 1998; Shashidhara et al., 1999). However, none of the three targets of Wg (i.e. Ac, Dll and *vg*-QE) is expressed in the haltere disc (Gorfinkiel et al., 1997; Weatherbee et al., 1998; Shashidhara et al., 1999). As expression of Wg itself is robust in the anterior DV boundary of haltere discs, downregulation of its targets, in this compartment at least, could be due to the repression of event(s) downstream of Wg, such as transduction of Wg signaling from the DV boundary. Consistently, although overexpression of Ubx in the wing disc DV boundary results in loss of Wg only in DV boundary cells of the posterior compartment, it causes loss of *vg*-QE in non-DV cells of both the anterior and posterior compartments (Shashidhara et al., 1999). We show that Ubx functions at multiple levels to repress Vg in non-DV cells, including enhanced degradation of Arm in the haltere pouch. Repression of Vg at multiple levels appears to be crucial for

Ubx-mediated specification of the haltere fate. Overexpression of Vg in haltere discs overrides Ubx function and thereby induces haltere-to-wing homeotic transformations.

MATERIALS AND METHODS

Recombinant chromosomes and combinations of GAL4 drivers, UAS lines, different mutations and/or markers were generated by standard genetic techniques. The GAL4-UAS system (Brand and Perrimon, 1993) was used for targeted misexpression of gene products. The FLP-FRT method (Xu and Rubin, 1993) was used for generating mitotic clones of *arm* and *vg*. P[FRT]18A $arm^{H8.6}$ has been reported previously (Neumann and Cohen, 1997); we recombined vg^{1} to P[FRT]42 πMyc. Clones were generated with the help of hsFLP using either *arm-lacZ* or *Ubi*-GFP as clonal markers. The original second chromosome *vg*-quadrant enhancer-*lacZ* [*vg*-QE (Kim et al., 1996)] was mobilized to obtain first and third chromosome insertions by crossing to a genetic source of transposase. We selected new insertions that showed original expression patterns in all stages of wing development. UAS lines used in this study were *UAS-Flu-ΔArm* (Zecca et al., 1996), $UAS\text{-}arm^{S2}$ and $UAS\text{-}arm^{S10}$ [both of which are Myc tagged (Pai et al., 1997)], *UAS-DN-TCF/pan* (van der Wetering et al., 1997), *UAS-Dsh* (Neumann and Cohen, 1996), *UAS-APC/CBD* (Bhandari and Shashidhara, 2001), $UAS\text{-}N^{intra}$ (Fortini et al., 1993), *UAS-Ubx* (Castelli-Gair et al., 1994), *UAS-Vg* (Kim et al., 1996), and *UAS-Wg* (Lawrence et al., 1995). GAL4 strains used were *dpp-GAL4* (Morimura et al., 1996), *en-GAL4* (A. Brand, personal communication to FlyBase, 30 June 1997), *omb-GAL4* (M. Calleja, personal communication to FlyBase, 16 October 1996) and *vg*-GAL4 (Simmonds et al., 1995). *N23-GAL4* was used to express genes of interest in non-DV cells of wing and haltere discs. This GAL4 line was identified in the lab in an enhancer-trap screen (Shashidhara et al., 1999). Although its activation in non-DV cells is dependent on N signaling in the DV boundary, it is not dependent on Wg or Vg (R.B. and L.S.S., unpublished).

Histology

X-gal and immunohistochemical staining was performed essentially as described by Ghysen and O'Kane (Ghysen and O'Kane, 1989) and Patel et al. (Patel et al., 1989), respectively. The primary antibodies used were, monoclonal anti-Arm (Riggleman et al., 1990), anti-Ct (Blochlinger et al., 1993), anti-Salm (de Celis et al., 19666a), anti-Wg (Brook and Cohen, 1996) and anti-β-galactosidase (Sigma) and polyclonal anti-Vg (Williams et al., 1991), anti-Arm (Ruel et al., 1999) and anti-β-galactosidase (Sigma). Monoclonal anti-Arm and anti-Wg antibodies were obtained from the Development Studies Hybridoma Bank, University of Iowa, USA. Confocal microscopy was carried out on Meridian Ultima. The adult appendages were processed for microscopy as described previously (Shashidhara et al., 1999).

RESULTS

Regulation of Wg and Vg expression in wing discs

We designed several experiments to test the working model of Wg and Vg regulation (which is essentially based on studies on wing imaginal discs) in haltere discs. However, information on certain aspects of Wg and Vg regulation in wing discs is limited. For example, autoregulation of Vg in non-DV cells is not well understood. Understanding these events was a pre-requisite to interpret the results related to the mechanism of Ubx-mediated repression of Wg and Vg in haltere discs.

Wg is required for the maintenance of Vg expression in the DV boundary

A mutant version of TCF/pan protein, which lacks the N-terminal Arm interaction domain, functions as a dominant negative for both TCF/pan and Arm (van der Wetering et al., 1997). We overexpressed DN-TCF/pan using *vg-GAL4* to downregulate Wg signaling in the DV boundary. We observed loss of Vg in both DV and non-DV cells when Wg signaling is downregulated (monitored by anti-Vg antibody, *vg*-BE and *vg*-QE staining; Fig. 1D-F). This is contrary to the earlier reports that Wg activity is not required for the expression of Vg at the DV boundary (Neumann and Cohen, 1996). We further tested the cell-autonomy of this phenomenon by generating mitotic clones of *arm*. As loss-of-function clones of null alleles of *arm* are lethal, we used $arm^{H8.6}$, a temperature-sensitive hypomorphic allele (Neumann and Cohen, 1997). We monitored Vg expression in small $arm^{H8.6}$ clones, survival of which were confirmed by DAPI staining. Clonal loss of Arm at the DV boundary resulted in cell-autonomous loss of Vg expression (Fig. 1G), confirming a role for Wg in the maintenance of Vg expression in DV cells. It has been reported previously that ectopic expression of Vg or Scalloped (Sd; a co-factor of Vg in the nucleus) causes ectopic Wg expression (Go et al., 1998; Klein and Martinez-Arias, 1998; Klein and Martinez-Arias 1999; Liu et al., 2000). Thus, Wg and Vg may interact to maintain each other's expression in the wing disc DV boundary.

Autoregulation of Vg in non-DV cells of wing discs

It has been shown that Sd binds to *vg*-QE and thus regulates its expression (Halder and Carroll, 2001). Since Sd and Vg are known to physically interact (Simmonds et al., 1998; Halder et al., 1998), Vg may regulate its own expression in non-DV cells by modulating Sd function. To test the autoregulation of Vg, we generated mitotic clones of *vg* and examined the status of *vg*-QE. vg^{-} clones grow very slowly and often they are replaced by the neighboring cells (Kim et al., 1996). We monitored *vg*-QE expression in small vg^{1} clones, the survival of which was confirmed by DAPI staining. Clonal loss of Vg resulted in loss of *vg*-QE expression (Fig. 2A), thus confirming autoregulation of Vg in non-DV cells.

To answer the question of whether Vg autoregulation is dependent on Wg, we overexpressed Vg in non-DV cells in the absence of endogenous Wg. In vg^{1} wing discs, both Vg and Wg are absent at the DV boundary (Fig. 2B,C) and no *vg*-QE expression is seen (data not shown). We overexpressed Vg using the *N23-GAL4* driver, which is expressed only in non-DV cells of both wild-type (Fig. 2D) and vg^{1} (Fig. 2E) wing discs. Overexpression of Vg in non-DV cells of vg^{1} wing discs was enough to rescue *vg*-QE expression (Fig. 2G) as well as adult wing phenotypes (Fig. 2I). Rescued wing discs did not show any Wg expression in the presumptive DV boundary (Fig. 2G). The absence of Wg is also reflected in the absence of margin bristles in the rescued adult wing blades (Fig. 2I). These results suggest that Vg in non-DV cells is necessary and sufficient to activate its quadrant enhancer.

Wg signaling is required, but is not sufficient, to activate *vg*-QE

Although Vg is capable of activating *vg*-QE in both wild-type

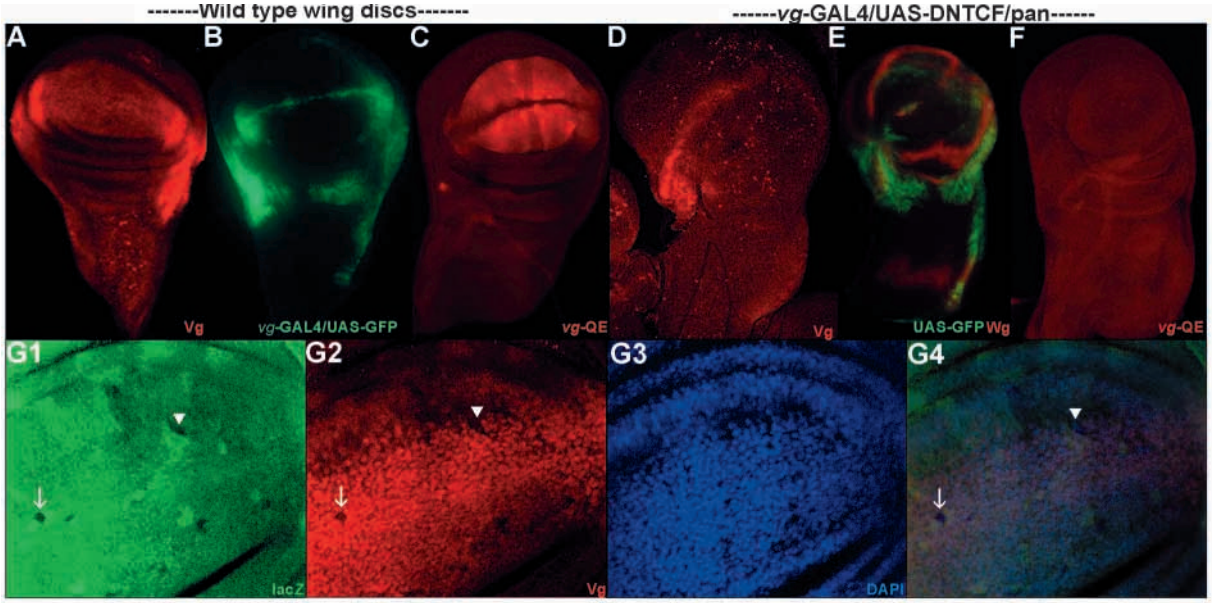

Fig. 1. Wg is required for the maintenance of Vg expression in the DV boundary. (A) Wild-type expression pattern of Vg in wing discs. Vg is expressed in both DV and non-DV cells. (B,C) Vg expression in DV and non-DV cells is regulated by *vg*-BE (B) and *vg*-QE (C), respectively. (D-F) *vg-GAL4/UAS-DN-TCF/pan* wing discs stained with anti-Vg antibodies (D), *vg*-BE (E) and *vg*-QE (F). Misexpression of DN-TCF/pan in wing discs downregulates Vg expression in both DV and non-DV cells. (G) Wing disc with $arm^{H8.6}/arm^{H8.6}$ mitotic clones. (G1) *lacZ* marker (*arm-lacZ* P[FRT]18A); loss of *lacZ* expression marks $arm^{H8.6}/arm^{H8.6}$ cells. (G2) Vg expression pattern in the same disc. (G3) DAPI staining in the same disc. (G4) Merge image of G1-G3. Note that in a representative $arm^{H8.6}/arm^{H8.6}$ clone at the DV boundary (arrow), Vg expression is downregulated. Downregulation of Vg in non-DV cells (arrowhead) in arm^{-} clones is also shown.

and vg^{1} backgrounds (Fig. 2G), ectopic expression of Wg or activated Arm does not induce ectopic *vg*-QE expression (Nagaraj et al., 1999). This is contrary to the non-cell autonomous loss of both Vg and *vg*-QE by the ectopic expression of *DN-TCF/pan* at the DV boundary (Fig. 1F), and cell-autonomous loss of Vg in arm^{-} mitotic clones generated in non-DV cells (Neumann and Cohen, 1997) (Fig. 1G).

Ectopic expression of activated N using *dpp-GAL4* resulted in non-cell autonomous activation of Vg in the wing pouch (Fig. 3C). As N specifies DV boundary activity and *vg*-QE expression is inhibited in N-expressing cells (Klein and Martinez-Arias, 1999), cell-autonomous activation of Vg might correspond to the activation of *vg*-BE and non-cell autonomous component might correspond to *vg*-QE. As, ectopic Wg also causes non cell-autonomous activation of Vg (Neumann and Cohen, 1997), ectopic N might first cell-autonomously activate Wg, which in turn would activate Vg in neighboring cells. Consistent with this, ectopic N^{intra}-induced cell-autonomous activation of Wg (Fig. 3D) and activated Arm resulted in cell-autonomous activation of Vg in wing discs (Fig. 3E).

We therefore examined the status of *vg*-QE in arm^{-} clones. We observed downregulation of *vg*-QE expression in arm^{-} clones (Fig. 3F). Thus, Wg signaling is required, but is not sufficient to activate *vg*-QE. As Vg alone was sufficient to activate *vg*-QE, Wg signaling might activate Vg either indirectly or by activating some other enhancer of Vg (see Discussion). Once Vg is activated, it maintains its own expression by autoregulation, which is mediated through its quadrant enhancer.

Regulation of Wg and Vg expression in haltere discs

With the new insights into the mechanism of Wg and Vg expression in wing discs, we studied the mechanism by which Ubx represses their expression in haltere discs. Wing and haltere discs employ similar genetic pathways for pattern formation along the A/P and DV axes (Williams et al., 1993; Williams et al., 1994). However, although Wg is expressed at the anterior DV boundary, Vg is not expressed in non-DV cells of haltere discs (Weatherbee et al., 1998; Shashidhara et al., 1999). Thus, Ubx may repress event(s) downstream of Wg to inhibit Vg expression in non-DV cells.

Ubx inhibits stabilization of Arm

Stabilization of cytoplasmic Arm is a key step in the transduction of Wg signaling. Although Arm is present in all cells, cytoplasmic levels of Arm, which transduces Wg signaling, are higher only in cells in which Wg signaling is active (Peifer et al., 1994). For example, cells immediately adjacent to the wing disc DV boundary show higher levels of Arm than do non-DV cells (Fig. 4A,B). In the absence of Wg signaling, cytoplasmic Arm is subjected to Ubiquitin-mediated degradation.

In haltere discs, Arm levels are uniform in the entire pouch (Fig. 4C,D). In particular, we did not observe increased levels of Arm in cells surrounding the DV boundary. This is true for both the anterior compartment (in which Wg is expressed at the DV boundary) and the posterior compartment, which suggests that Ubx interferes with Arm stabilization. Interestingly, cells neighboring Wg-expressing hinge cells showed increased levels of Arm (Fig. 4C,D), similar to those

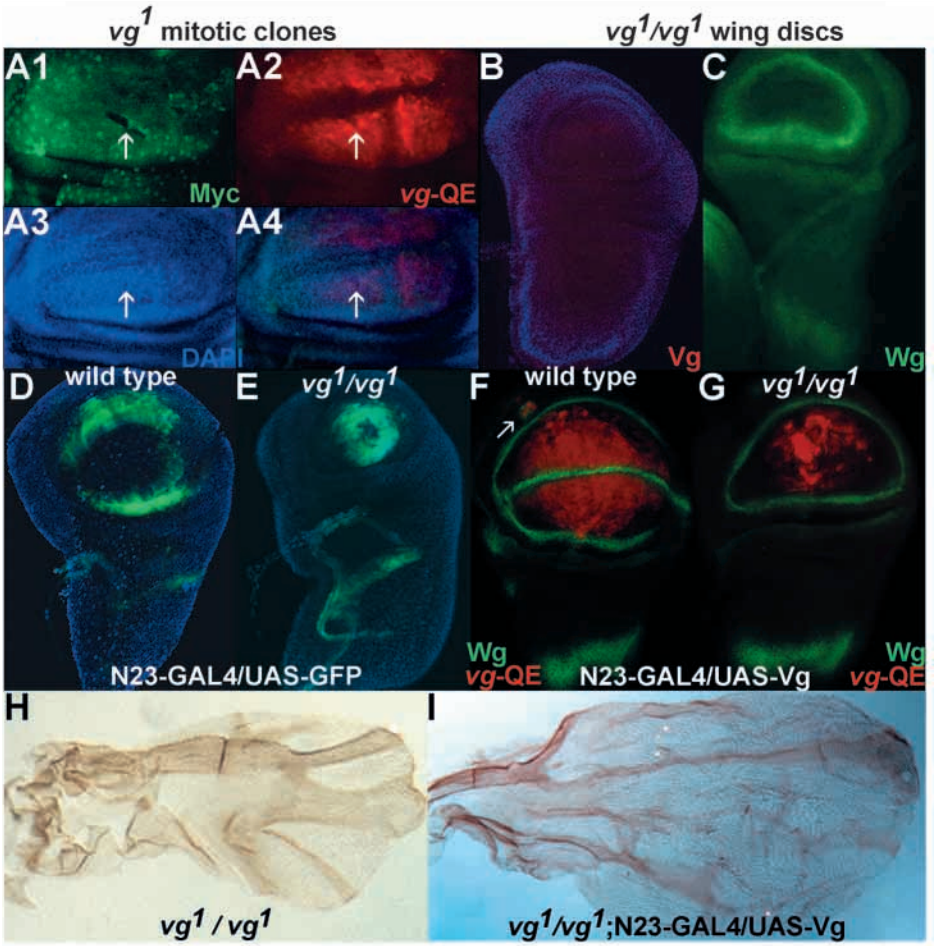

Fig. 2. Autoregulation of Vg in non-DV cells of wing discs. (A1-3) Wing disc with vg^1/vg^1 clones. (A1) πMyc marker (P[FRT]42 πMyc); loss of πMyc marks vg^1/vg^1 cells. (A2) Expression pattern of *vg*-QE in the same disc. (A3) DAPI staining in the same disc. (A4) Merged image of A1-A3. Note the representative vg^1/vg^1 clones in non-DV cells (arrows), which are positive for DAPI, indicating the survival of the clones. *vg*-QE is not expressed in these clones, which provides genetic evidence for Vg autoregulation. (B-C) vg^1/vg^1 wing discs stained with anti-Vg antibodies and DAPI (B), and anti-Wg antibodies (C). Note no Vg expression is seen in vg^1/vg^1 wing discs. Wg expression at the DV boundary is also absent. (D,E) Wild-type (D) and vg^1/vg^1 (E) wing discs showing the expression pattern of N23-GAL4. The discs are also stained with DAPI. Note that N23-GAL4, which is expressed only in non-DV cells, still shows separation of dorsal and ventral compartments at the presumptive DV boundary of vg^1/vg^1 discs. Thus, loss of Wg seen in vg^1/vg^1 wing discs is not caused by loss of the DV boundary per se. (F) N23-GAL4/UAS-Vg wing discs stained for *vg*-QE (red) and Wg (green). Note activation of both *vg*-QE and Wg (arrow) outside the wing pouch. In all such cases, Wg expression always surrounded but did not overlap *vg*-QE expression [similar observations have been made by Liu et al. (Liu et al., 2000)]. (G) vg^1/vg^1; N23-GAL4/UAS-Vg stained for *vg*-QE (red) and Wg (green). Note the very high levels of *vg*-QE activity in the pouch. No Wg expression was seen in the DV boundary, suggesting that autoregulation of Vg through its quadrant enhancer is independent of Wg. Note also that the size of the wing pouch is nearly normal. (H) vg^1/vg^1 adult wing blade. (I) vg^1/vg^1; N23-GAL4/UAS-Vg adult wing blade showing partial rescue of vg^1 phenotype.

in wing discs (Fig. 4A,B). These observations suggest that Ubx inhibits the stabilization of Arm specifically to downregulate DV signaling during haltere development. This is further supported by the observation that misexpression of Ubx at the wing disc DV boundary causes downregulation of Arm (Fig. 4E). In both anterior and posterior compartments there was a severe reduction in Arm levels, although Wg was suppressed only in the posterior compartment (Shashidhara et al., 1999).

Enhanced degradation of Arm in haltere discs

To further test if Arm degradation is enhanced in haltere discs, we used Myc-tagged degradation-resistant and degradation-sensitive forms of Arm [Arm^{S10} and Arm^{S2} (Pai et al., 1997)]. Arm^{S10} has an internal deletion of 43-87 residues at the N terminus. This deletion removes residues that are normally phosphorylated by Sgg, thus making it degradation resistant. Arm^{S2} expresses normal protein and is susceptible to the degradation machinery. *arm*-mutant embryos rescued by Arm^{S2}, secrete normal denticle belts and also have normally patterned naked cuticle (Pai et al., 1997). Thus, similar to endogenous Arm, Arm^{S2} is stabilized only in Wg signaling cells. Thus, relative levels of Arm^{S2} at the DV boundary of wing and haltere discs can be used as an estimate of the relative efficiency of the Arm-degradation machinery. We expressed Arm^{S10} and Arm^{S2} using the *omb-GAL4* driver, and stained wing and haltere discs with anti-Myc and anti-Arm antibodies. We observed uniform levels of the degradation-resistant form of Arm in both wing (Fig. 4F) and haltere discs (Fig. 4G). However, degradation-sensitive Arm^{S2} accumulated in the DV boundary of wing discs (Fig. 4H) but not of haltere discs (Fig. 4I). This suggests that Arm is degraded more efficiently at the DV boundary of haltere discs than at the DV boundary of wing discs.

Ubx functions downstream to Sgg to enhance the degradation of Arm

In DV cells of the wing disc, in which Wg signaling is active, Arm degradation is inhibited owing to inhibition of the degradation machinery. Dsh functions immediately downstream of Wg, and inhibits Sgg activity and thereby stabilizes Arm. Overexpression of Dsh at the haltere DV boundary did not induce the stabilization of Arm (Fig. 5A) suggesting that the Ubx-mediated inhibition is downstream of Dsh function. One possibility is that Ubx interferes with Dsh-

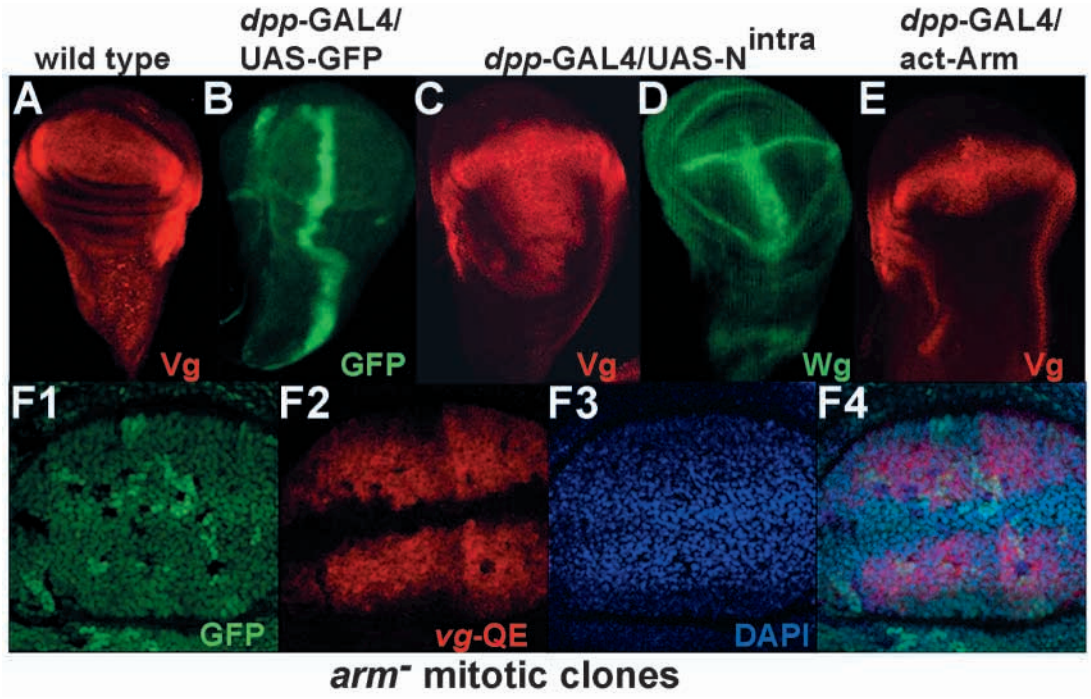

Fig. 3. Wg signaling is required, but is not sufficient to activate vg-QE. (A-B) Wild-type wing disc showing the expression pattern of Vg (A) and *dpp-GAL4* driver (B). (C,D) *dpp*-GAL4/UAS-N^{intra}-wing discs stained for Vg (C) and Wg (D). Activation of Vg by ectopic N^{intra} expression is non-cell autonomous whereas that of Wg is cell autonomous. (E) Dpp-GAL4/UAS-activated Arm showing cell-autonomous activation of Vg. (F1-4) Wing disc with $arm^{H8.6}/arm^{H8.6}$ mitotic clones. (F1) GFP marker (*Ubi*-GFP P[FRT]18A), (F2) *vg*-QE and (F3) DAPI staining. (F4) Merged image of F1-F3. Loss of GFP expression marks $arm^{H8.6}/arm^{H8.6}$ cells. *vg*-QE is not expressed in these clones.

mediated inhibition of Sgg activity, thus keeping Sgg active and causing degradation of Arm. To test this hypothesis, we overexpressed the human colon cancer gene *APC* in wing and haltere discs. In both *Drosophila* and mammalian cells, it has been shown that APC binds to Arm/β-catenin even when Wg/Wnt is active (Papkoff et al., 1996; Bhandari and Shashidhara, 2001). In those cells, APC sequesters Arm/β-catenin, rather than recruiting it to the degradation machinery. For example, overexpression of APC in wing discs sequesters Arm only in DV cells (Bhandari and Shashidhara, 2001) (Fig. 5B). In other cells, overexpressed APC participates in the Arm-degradation machinery and hence no change in Arm expression was observed. Thus, the amount of Arm sequestered by overexpressed APC could be an assay for the level of Wg/Wnt activity. As only unphosphorylated Arm is sequestered and not the phosphorylated form (Munemitsu et al., 1996), such an assay could also be used to obtain a relative estimate of Sgg activity. When we overexpressed human APC at the haltere DV boundary using *vg-GAL4*, we observed increased levels of Arm (owing to sequestration) in the anterior compartment (Fig. 5C) but not in the posterior compartment, indicating that Sgg is inactive in the anterior compartment and active in the posterior compartment. Thus, Ubx-mediated inhibition of Arm stabilization in the anterior compartment is downstream of Sgg.

As Wg itself is repressed in the posterior compartment, it was expected that Sgg would be active in that compartment. We further examined whether overexpression of Dsh in the posterior compartment was capable of inhibiting Sgg activity. We co-expressed Dsh and APC and monitored the sequestration of Arm, with the assumption that, if Dsh inhibits Sgg activity, overexpressed APC would be able to sequester Arm in the posterior compartment. Indeed, overexpression of Dsh and APC together resulted in the sequestration of Arm in the posterior compartment at levels similar to those in the anterior compartment (Fig. 5D).

Wg is not autoregulated at the haltere disc DV boundary

Although levels of Arm were much lower in haltere discs than in wing discs, it is possible that available amounts of Arm are sufficient to transduce Wg signaling. We used Wg-autoregulation as a test for Arm function at the DV boundary of haltere discs. It has been shown that Wg is autoregulated and Arm is necessary for this process (Hooper, 1994; Yoffe et al., 1995). For example, ectopic activation of Arm function in leg discs induces ectopic Wg expression (Bhandari and Shashidhara, 2001). We observed repression of Wg at the DV boundary when we overexpressed DN-TCF/pan in wing discs using *vg-GAL4* (Fig. 6A). However, we did not observe any such loss of Wg at the haltere DV boundary (Fig. 6B), nor was there any change in the size of haltere pouch. These results suggest that Arm function is indeed downregulated at the haltere DV boundary.

In haltere discs too Wg expression is dependent on Vg function

We then examined how, in the absence of autoregulation, Wg expression is maintained at the anterior haltere DV boundary. In vg^1/vg^1 haltere discs, in which Vg is not expressed at the DV boundary (Fig. 6C), Wg expression is completely absent

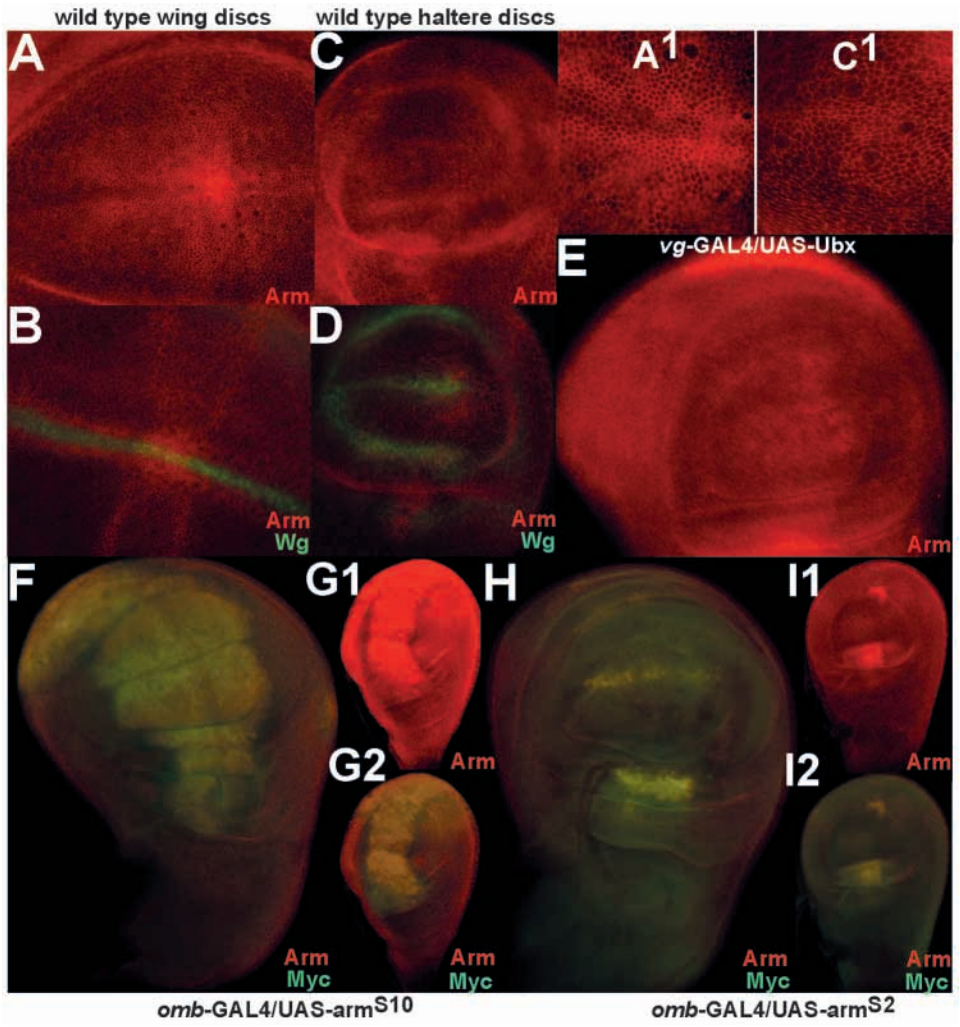

Fig. 4. Enhanced degradation of Arm in haltere discs. (A-D) Wild-type expression of Arm in wing (A,B) and haltere (C,D) discs. (A1,C1) Higher magnification images of wing (A1) and haltere pouch (C1). In wing discs, cells surrounding Wg-expressing DV boundary cells show higher levels of Arm (A,A1,B). In haltere discs, levels of Arm at the DV boundary are indistinguishable from those of non-DV cells (C,C1,D). In a few haltere discs, we observed somewhat higher levels of Arm in the cells that intersect the A/P and DV boundaries (D). (E) *vg-GAL4/UAS-Ubx*-wing disc. Ectopic Ubx downregulates Arm levels in the DV cells of the wing disc. (F,G) *omb-GAL4/UAS-arm^{S10}*-wing (F) and -haltere (G) discs. Wing and haltere discs show comparable levels of degradation-resistant Arm expressed from *UAS-arm^{S10}*. (H,I) *omb-GAL4/UAS-arm^{S2}*-wing (H) and -haltere (I) discs. In wing discs, overexpression of wild-type Arm from *UAS-arm^{S2}* leads to accumulation of Arm only in the presumptive DV boundary and hinge cells. However, no significant accumulation of wild-type Arm (from *arm^{S2}*) is seen in the DV boundary of haltere discs, although, as in wing discs, hinge cells accumulate large amounts of wild-type Arm. This suggests that Ubx enhances degradation of Arm in the haltere pouch.

(Fig. 6D). Thus, the maintenance of Wg expression at the anterior haltere DV boundary, even when Arm function, and thereby Wg autoregulation is inhibited by Ubx, could be attributed to Vg function. This raises the question of why Wg is not expressed in the posterior compartment, in spite of robust expression of Vg. This is particularly intriguing because in wing discs it has been observed that ectopic Vg is capable of activating Wg even in the absence of N signaling (Klein and Martinez-Arias, 1999). We further tested the ability of DV boundary cells in the posterior compartment to express Wg by ectopic expression or overexpression of activated N (*UAS-N^{intra}*), Dsh and activated Arm using the *vg-GAL4* driver. None of these positive regulators induced Wg expression in the posterior compartment. It is likely that Ubx (probably with a posterior-specific co-factor) directly inhibits Wg expression.

Vg expression at the haltere DV boundary is not dependent on Wg

In haltere discs, in which Wg is not expressed in the posterior compartment, Vg is still expressed all along the DV boundary, suggesting that Vg is independent of Wg function in the posterior compartment. In the anterior compartment also, Vg might not be dependent on Wg as Arm function is downregulated by Ubx. Indeed, expression of DN-TCF/pan at the haltere DV boundary did not affect Vg expression in haltere discs (Fig. 6E). The possibility that DN-TCF/pan did not downregulate Vg in haltere discs owing to its late expression (we used *vg-GAL4*) is ruled out because in wing discs it downregulated Vg in both DV and non-DV cells (Fig. 1D-F). This suggests that Vg expression at the DV boundary of haltere discs is independent of Wg function.

Ubx-mediated repression of Vg in non-DV cells is downstream of Arm and upstream of Vg-autoregulation

Overexpression of N, Wg or activated Arm (both Flu-ΔArm and Arm^{S10}) at the haltere DV boundary using the *vg-GAL4* driver did not induce activation of Vg in non-DV cells (monitored by both anti-Vg antibody and *vg*-QE staining) in haltere discs, nor did they induce any adult haltere phenotypes (data not shown). This suggests that Ubx inhibits additional events downstream of DV signaling.

We then examined the events in non-DV cells that might contribute to the suppression of Vg in the haltere pouch. We observed cell-autonomous activation of Vg in non-DV cells when we expressed activated N using *dpp-GAL4* (Fig. 7A). However, unlike in wing discs (Fig. 3D), ectopic N expression failed to activate Wg in haltere discs (Fig. 7B). Furthermore, overexpression of Wg, Dsh or activated Arm directly in non-DV cells using *dpp-GAL4*, *omb-GAL4* or *N23-GAL4* drivers did not activate Vg (monitored by both anti-Vg antibody and

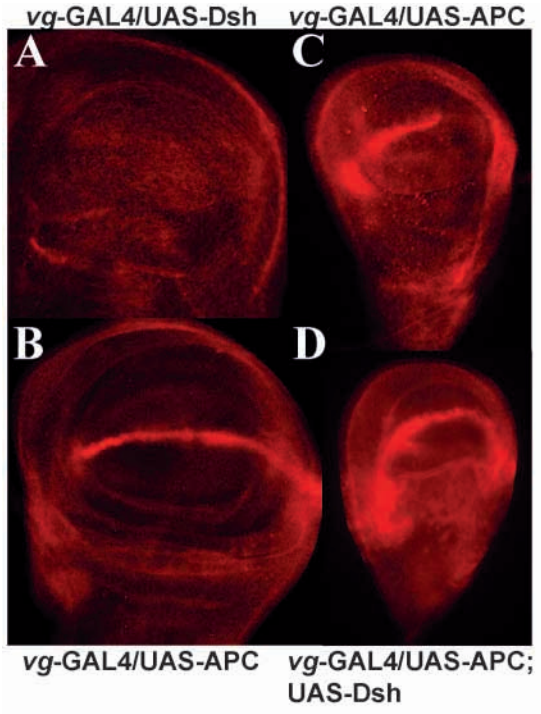

Fig. 5. Ubx-mediated inhibition of Arm stabilization is downstream of Sgg function. All discs in this figure are stained with anti-Arm antibodies. (A) *vg-GAL4/UAS-Dsh*-haltere disc. Overexpression of Dsh does not enhance Arm levels at the haltere DV boundary, suggesting that Ubx functions downstream of Dsh. (B,C) *vg-GAL4/UAS-APC/CBD*-wing (B) and -haltere (C) discs. Misexpression of human APC sequesters Arm only in cells where Sgg is inactive (Bhandari and Shashidhara, 2001): for example, at the DV boundary of the wing disc (B). In haltere discs, APC sequesters Arm only in the anterior compartment (C). This suggests that Sgg is inactive in the anterior compartment and active in the posterior compartment. (D) *vg-GAL4/UAS-Dsh; UAS-APC/CBD*-haltere disc. Overexpression of both Dsh and APC together causes sequestration of Arm in both anterior and posterior compartments. This is owing to the Dsh-mediated inhibition of Sgg activity followed by APC-mediated sequestration of Arm.

vg-QE staining; data shown only for Dpp-GAL4/UAS-activated Arm; Fig. 7C). These results further suggest that in addition to repressing DV signaling, Ubx downregulates event(s) downstream of Arm in both anterior and posterior non-DV cells.

As Vg is not expressed in non-DV cells of haltere discs, we examined the effect of its 'ectopic expression' in those cells. In haltere discs the *N23-GAL4* driver is expressed only in the posterior compartment (Fig. 7E), in which Wg is not expressed. Ectopic expression of Vg in non-DV cells using *N23-GAL4* activated its own expression in haltere discs, as seen with *vg*-QE staining (Fig. 7G). Even in a vg^1 background, ectopic expression of Vg in non-DV cells was sufficient to activate *vg*-QE (Fig. 7H). This suggests that in non-DV cells, Ubx functions downstream of Arm and upstream of Vg-autoregulation.

Haltere-to-wing homeotic transformation by ectopic Vg

vg is a pro-wing gene: ectopic expression of Vg induces ectopic wing development (e.g. ectopic Vg induces ectopic wing tissue on T2 legs) (Kim et al., 1996). Interestingly, ectopic Vg in T3 leg discs induces ectopic haltere development (Weatherbee et al., 1998). Activation of *vg*-QE (Fig. 7G) by ectopic Vg in non-DV cells of haltere discs results in homeotic transformation, albeit only partial. Ubx regulates haltere development by modifying wing-patterning events at multiple levels (Weatherbee et al., 1998; Shashidhara et al., 1999). As haltere discs express several other wing-patterning genes (including *vg* at the DV boundary), ectopic expression of Vg might override Ubx function in non-DV cells of haltere discs but not in T3 leg discs. We therefore expressed Vg in haltere discs using several GAL4 lines. We observed a high degree of haltere-to-wing homeotic transformations when Vg was expressed using *omb-GAL4* (Fig. 8B). In addition, we observed enhanced homeotic transformations when Vg was expressed in a *Ubx*-heterozygous background (Fig. 8D). Ectopic expression or overexpression of Wg, Dsh or activated Arm in haltere discs did not induce homeotic transformation (data not shown). This is consistent with the inability of Wg, Dsh and activated Arm to activate *vg*-QE or Vg protein expression in the haltere pouch.

All the reported haltere-to-wing homeotic transformations at the cuticle level are associated with the loss of Ubx protein. The only exception is a minor sensory bristle phenotype induced by the overexpression of Ac (Weatherbee et al., 1998). In this context, we tested if ectopic Vg downregulated Ubx levels. Anti-Ubx antibody staining of *omb-GAL4/UAS-vg* haltere discs did not reveal any reduction in Ubx protein levels (data not shown). Furthermore, we did not observe any wing-margin bristles (Fig. 8B), which is the characteristic phenotype of both null (manifested in heterozygous flies) and hypomorphic alleles (manifested in homozygous flies) of *Ubx*. We also examined the expression pattern of Salm, which is a direct target of Ubx in the haltere pouch (Weatherbee et al., 1998; Galant et al., 2002). Salm expression remained repressed in the haltere pouch (Fig. 8F), which suggests that Vg-induced phenotypes are caused by a reversal of Ubx function and not due to downregulation of Ubx itself.

DISCUSSION

Suppression of hind-wing development marks the evolution of dipteran flies from their ancestral four-winged insects. However, Ubx, the master regulatory gene that specifies haltere development in *Drosophila*, is expressed during lepidopteran hind-wing development (Warren et al., 1994; Weatherbee et al., 1999). It is therefore likely that Ubx functions by repressing a few key genes required for wing development rather than by acting as a global repressor (Weatherbee et al., 1999). Previous reports suggest that Wg and Vg, the two genes that play crucial roles during *Drosophila* wing development, are targets of Ubx activity during haltere development (Weatherbee et al., 1998; Shashidhara et al., 1999). We have examined the mechanism by which Ubx modifies Wg and Vg expression and thereby downregulates DV signaling.

Absolute requirement for Vg in non-DV cells for its quadrant enhancer activation

We designed experiments to test the current model of Wg and Vg regulation (which is essentially based on studies on wing

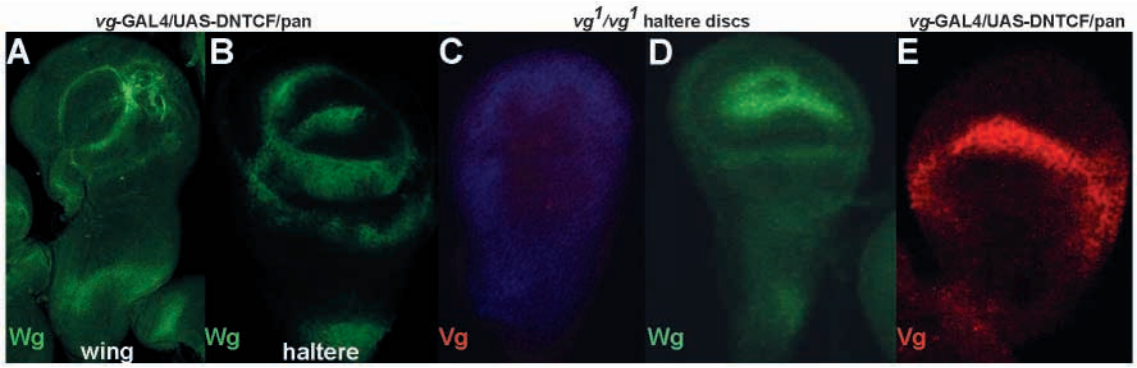

Fig. 6. Differential regulation of Wg and Vg in wing and haltere discs. (A,B) *vg-GAL4/UAS-DN-TCF/pan*-wing (A) and -haltere (B) discs stained with anti-Wg antibodies. Misexpression of DN-TCF/pan downregulates Wg expression at the wing disc DV boundary, but not in haltere discs. (C,D) vg^1/vg^1 haltere discs stained for Vg (C) and Wg (D). Note the absence of Wg expression at the DV boundary, which suggests that Wg expression in haltere discs is dependent on Vg. (E) *vg-GAL4/UAS-DN-TCF/pan*-haltere disc stained for Vg. DN-TCF/pan does not have any affect on Vg expression, which suggests that its expression at the haltere DV boundary, unlike in wing discs, is independent of Wg signaling.

imaginal discs) in haltere discs. In wing discs, both Wg and Vg are subjected to an elaborate regulatory circuit, the understanding of which would help us to unravel crucial events during wing development. To examine the Wg and Vg interactions further in DV and non-DV cells, we carried out experiments that are essentially complementary to those reported previously.

The experiments described in this report further suggest that Wg and Vg interact to maintain each other's expression at the DV boundary. We have shown that Vg-mediated activation of Wg is independent of Arm and TCF/pan function, which suggests that Vg may activate Wg either directly or through the N signaling pathway. We have also shown that Vg is capable of specifying wing development, even in the absence of Wg signaling. Overexpression of Vg in a vg^1/vg^1 background (in which no Wg or Vg is expressed) was sufficient to rescue wing phenotypes. This is particularly significant because we expressed Vg in this experiment only in non-DV cells. Our results also suggest that Vg cell-autonomously regulates its own expression through its quadrant enhancer. Clonal analysis of *arm* suggested that Wg is required to activate *vg*-QE and Arm was not able to activate this enhancer in vg^1 background. Wg signaling might activate Vg either indirectly or by activating some other enhancer of Vg. Once activated, Vg might maintain its expression by autoregulation, which is mediated through its quadrant enhancer (Fig. 8G).

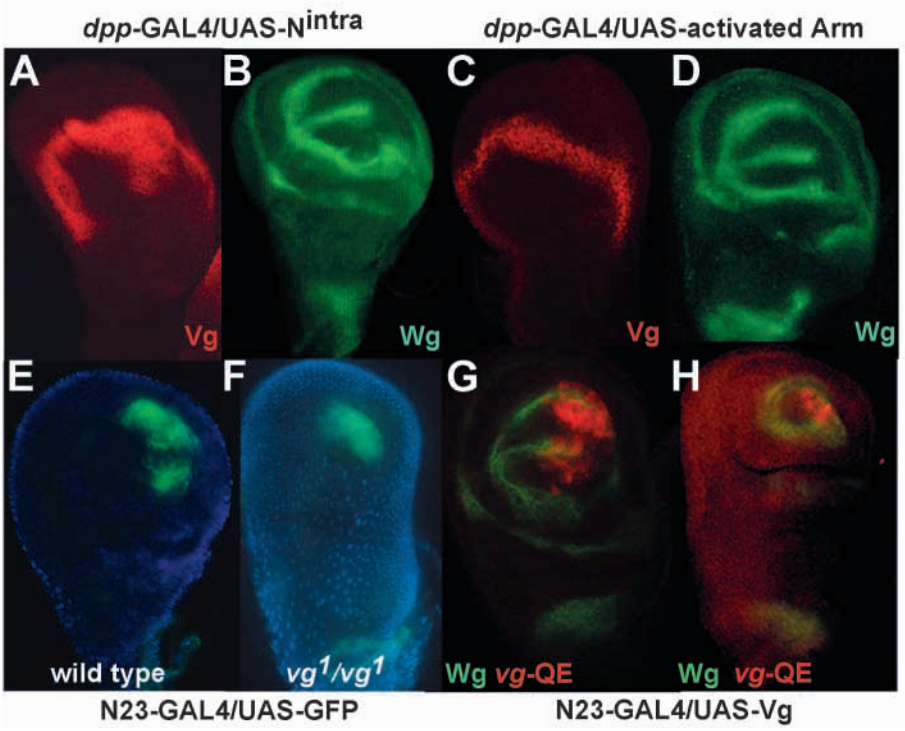

Fig. 7. Ubx-mediated repression of Vg in non-DV cells is downstream to Arm and upstream to Vg-autoregulation. (A,B) *dpp-GAL4/UAS-*N^{intra}-haltere discs stained for Vg (A) and Wg (B). Unlike in wing discs, activation of Vg by ectopic N^{intra} is cell autonomous and Wg is not activated in haltere discs. (C,D) *dpp-GAL4/UAS-*activated Arm-haltere discs stained for Vg (C) and Wg (D). No activation of Vg and Wg was observed. Compare this with Fig. 3E, which shows cell-autonomous activation of Vg in wing discs by ectopic Arm. (E,F) Wild-type (E) and vg^1/vg^1- (F) haltere discs showing the expression pattern of N23-GAL4. As in wing discs, the *GAL4* driver is expressed in non-DV cells of haltere discs, but only in the posterior compartment. (G,H) *N23-GAL4/UAS-vg-* (G) and vg^1/vg^1; *N23-GAL4/UAS-vg-* (H) haltere discs stained for *vg*-QE (red) and Wg (green). Note that Vg is capable of activating its quadrant enhancer in both wild-type and vg^1/vg^1 backgrounds. This suggests that downregulation of Vg by Ubx in non-DV cells in wild-type haltere discs is upstream of Vg-autoregulation.

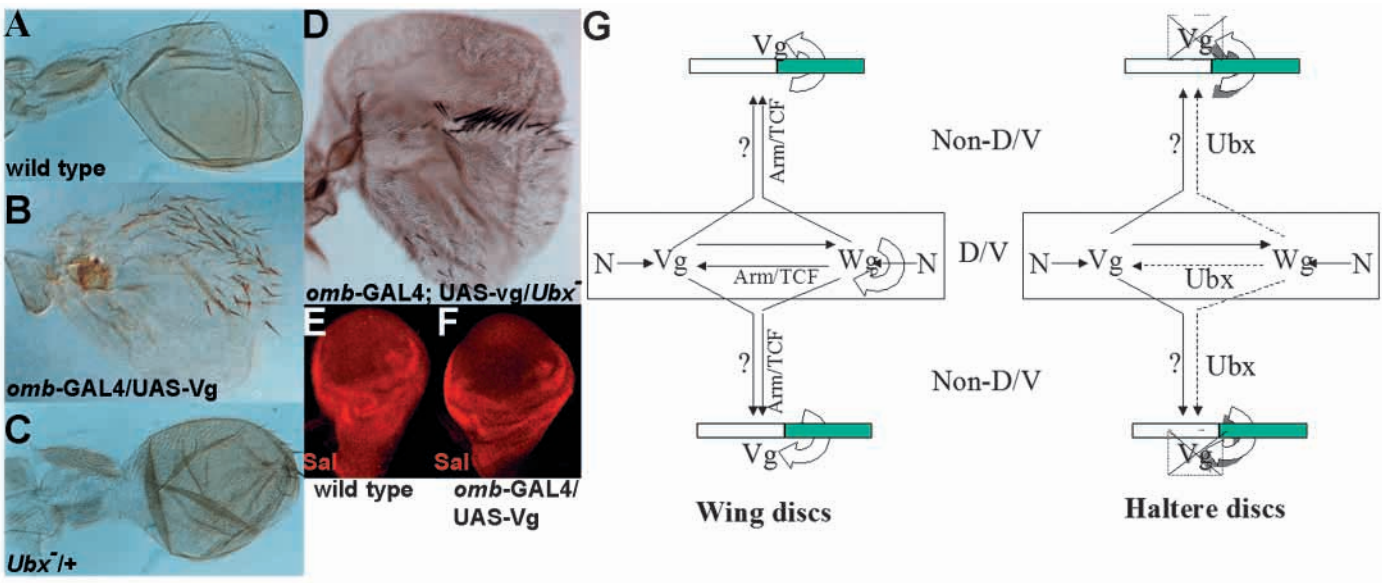

Fig. 8. Haltere-to-wing homeotic transformations induced by ectopic Vg. (A) Wild-type haltere. (B) *omb-GAL4; UAS-vg* haltere showing significant transformation of haltere capitellum to wing blade. Note the wing-like trichomes, which are larger, flatter and more pigmented and sparsely arranged than capitellum cells. (C) Ubx^{-}/Ubx^{+} halteres showing mild haltere-to-wing transformation. This is generally marked by the appearance of one or two wing-margin bristles. (D) *omb-GAL4; UAS-vg/*Ubx^{-} haltere showing enhanced homeotic transformation in a *Ubx* heterozygous background. The increase in the number of margin-bristles could be caused by the additive effects of increased growth, upregulated Wg signaling by overexpressed Vg and sensitized genetic ($Ubx^{-/+}$) background. This further confirms that Vg is required for the correct interpretation of Wg signaling (Klein and Martinez-Arias, 1999). (E,F) Wild-type (E) and *omb-GAL4; UAS-vg-* (F) haltere discs stained with anti-Salm antibodies. Salm is not normally expressed in the haltere pouch (E), nor is it induced by ectopic Vg (F). (G) Wg and Vg regulation in wing and haltere discs. Figure shows how DV signals activate Vg in non-DV cells in wing discs, and the events that are downregulated by Ubx in haltere discs. Regulatory elements of Vg are represented in two boxes: green box, *vg*-quadrant enhancer; white box, other enhancers of Vg that respond to Wg and probably one more, hitherto unknown, DV signal. Once activated, Vg maintains its expression by autoregulation, which is mediated through its quadrant enhancer. The discontinuous lines shown for haltere discs are the steps inhibited by Ubx during haltere specification. At the top of the hierarchy, Ubx downregulates Wg expression at the DV boundary of the posterior compartment (not shown). Although Vg-autoregulation per se is not affected, in the absence of initial activation of Vg by Wg signaling *vg*-QE is not activated in haltere discs.

This could ensure the maintenance of Vg expression in non-DV cells, once it is activated by Wg signaling. It might also explain how the Wg gradient is translated into uniformly higher levels of Vg in non-DV cells.

However, the above-mentioned model does not reconcile the observation that Vg, and not Wg, is capable of activating *vg*-QE in Ser^{-} background (Klein and Martinez-Arias, 1999). As the *vg* gene is intact in Ser^{-} background, ectopic expression of Wg using *dpp-GAL4* should have activated one of the enhancers to induce Vg expression, which in turn would activate *vg*-QE. A model that reconciles all the results would, therefore, include a third component, which may act either in parallel to or downstream of Wg and Vg at the DV boundary (Fig. 8G). The presence of such a signaling molecule downstream of Vg has been previously predicted (Neumann and Cohen, 1996). Although there is no direct evidence for the existence of such a molecule, the fact that *N23-GAL4* expression in non-DV cells is dependent on N function and independent of Vg and Wg function (R.B. and L.S.S., unpublished observations) suggests such a possibility.

Mechanism of *Ubx*-mediated downregulation of DV signaling in haltere discs

We also studied possible mechanisms by which Ubx regulates expression of Wg and Vg in haltere discs. One important finding was the downregulation of Wg signaling by Ubx at the level of Arm stabilization. We have further shown that Ubx inhibits stabilization of Arm by acting on event(s) downstream of Sgg. Normally, the Arm degradation machinery is very efficient and can degrade even overexpressed Arm. This is evident from the fact that embryos overexpressing Arm (from arm^{S2}) secrete normal denticle belts (Pai et al., 1997). If a downstream component functions with enhanced efficiency (either by direct enhancement of its expression by Ubx or owing to repression of a positive component of Wg signaling), residual activity of Sgg may be sufficient to cause enhanced degradation of Arm. Thus, enhanced degradation of Arm in haltere discs provided us with a new assay system to identify additional components of Wg signaling. For example, in microarray experiments to identify genes that are differentially expressed in wing and haltere discs, we observed that several transcripts of known (e.g. Casein kinase) and putative (e.g. Ubiquitin ligase) negative regulators of Wg signaling are upregulated in haltere discs (M.P. and L.S.S., unpublished).

Our results suggest that Wg and Vg regulation in haltere discs is different from that of wing discs. We have observed that Wg is not autoregulated in haltere discs. In addition, Vg expression at the haltere DV boundary is independent of Wg function. However, in both wing and haltere discs, Wg expression at the DV boundary is dependent on Vg. Wg

expression at the anterior DV boundary of haltere discs could be redundant because overexpression of DN-TCF at the haltere DV boundary shows no phenotype. However, Vg at the DV boundary appears to have an independent function. *vg*^{1} flies exhibit much smaller halteres than do wild-type flies (Williams et al., 1991). As Wg function (and expression in the posterior compartment) is already repressed in haltere discs, reduction in haltere size in *vg*^{1} flies suggests Wg-independent long-range effects of Vg from the DV boundary. This could be one of the reasons why Ubx does not affect Vg expression at the DV boundary but represses Vg expression in non-DV cells. In wing discs too, Vg may have such a function on cells at a distance (Neumann and Cohen, 1996).

One way to test the requirement of Ubx in DV and non-DV cells directly is by removing *Ubx* only from the haltere DV boundary or from non-DV cells. We have previously reported that clonal removal of *Ubx* solely from the haltere DV boundary does not induce cuticle phenotype in the capitellum (Shashidhara et al., 1999). However, we could not ascertain the effect on *vg*-QE because of haploinsufficiency, *Ubx*$^{-}$-heterozygous haltere discs themselves show activation of *lacZ* in the entire haltere pouch (data not shown). The activation of *vg*-QE in *Ubx*$^{-/+}$ haltere discs could be a result of reduced Ubx function at the DV boundary, or in non-DV cells, or in both. We had previously shown that misexpression of Ubx at the wing disc DV boundary causes non-cell-autonomous reduction in *vg*-QE expression (Shashidhara et al., 1999). Our current results suggest that Ubx represses additional event(s) in non-DV cells to downregulate Vg expression. This is consistent with the recent report on cell-autonomous repression of *vg*-QE by ectopic Ubx in wing discs (Galant et al., 2002). We propose that Ubx inhibits the activation of Vg in non-DV cells at three different levels (Fig. 8G): (1) Wg in the posterior compartment; (2) event(s) downstream of Sgg that inhibit the stabilization of Arm; and (3) additional event(s) downstream of Arm in non-DV cells. In wing discs, as discussed above, Wg and a hitherto unknown DV component may function together to activate Vg in non-DV cells. As Vg-autoregulation is not inhibited in haltere discs, it is possible that Ubx represses Vg activation in non-DV cells by interfering with the Wg-mediated activation of Vg and/or by repressing the activity of the unknown DV-signal molecule in the haltere.

We have also provided evidence that repression of Vg in non-DV cells by Ubx is crucial for haltere development. Overexpression of Vg in haltere discs causes haltere-to-wing transformations. This is particularly significant considering the fact that haltere-to-wing homeotic transformations are always associated with loss of *Ubx*, by direct removal of *Ubx*, by activation of its repressors (e.g. polycomb proteins) or by suppression of its activators (e.g. trithorax proteins). Mitotic clones of *Ubx*$^{-}$ alleles in the haltere capitellum normally 'sort out' and often remain as an undifferentiated mass of cells (Morata and Garcia-Bellido, 1976; Shashidhara et al., 1999). This is attributed to differential cell-adhesion properties of transformed (*Ubx*$^{-}$) and non-transformed (*Ubx*$^{+}$) cells. No such sorting out of wing-like trichomes was observed in halteres overexpressing Vg. This implies that cells surrounding the wing-like trichomes are also transformed, at least at the level of cell-adhesion properties. This is consistent with our earlier observations that removal of *Ubx* from the DV boundary or over-growth caused by mutations in the tumor-suppressor gene *fat* confers wing-like cell-adhesion properties to capitellum cells (Shashidhara et al., 1999). As DV signaling is closely associated with the activation of Vg in non-DV cells and Vg is primarily a growth-promoting gene, it is likely that the cell-sorting behaviour of *Ubx*$^{-}$ clones is linked to their changed growth properties.

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REFERENCES

Bhandari, P. and Shashidhara, L. S. (2001). Studies on human colon cancer gene *apc* by targeted expression in *Drosophila*. *Oncogene* **20**, 6871-6880.

Blochlinger, K., Jan, L. Y. and Jan, Y. N. (1993). Postembryonic patterns of expression of *cut*, a locus regulating sensory organ identity in *Drosophila*. *Development* **117**, 441-450.

Brand, A. and Perrimon, N. (1993). Targeted expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Brook, W. J. and Cohen, S. M. (1996). Antagonistic interactions between *Wingless* and *Decapentaplegic* responsible for dorsal-ventral pattern in the *Drosophila* leg. *Science* **273**, 1373-1377.

Cabrera, C. V., Botas, J. and Garcia-Bellido, A. (1985). Distribution of proteins in mutants of bithorax complex genes and its transregulatory genes. *Nature* **318**, 569-571.

Castelli-Gair, J., Greig, S., Micklem, G. and Akam, M. (1994). Dissecting the temporal requirements for homeotic gene function. *Development* **120**, 1983-1995.

Couso, J. P., Knust, E. and Martinez Arias, A. (1995). *Serrate* and *wingless* cooperate to induce *vestigial* gene expression and wing formation in *Drosophila*. *Curr. Biol.* **5**, 1437-1448.

de Celis, J. F., Barrio, R. and Kafatos, F. C. (1966a). A gene complex acting downstream of dpp in *Drosophila* wing morphogenesis. *Nature* **381**, 421-424.

de Celis, J. F., Garcia-Bellido, A. and Bray, S. J. (1996b). Activation and function of Notch at the dorsal-ventral boundary of the wing imaginal disc. *Development* **122**, 359-369.

Diaz-Benjumea, F. J. and Cohen, S. M. (1993). Interaction between dorsal and ventral cells in the imaginal disc directs wing development in *Drosophila*. *Cell* **75**, 741-752.

Diaz-Benjumea, F. J. and Cohen, S. M. (1995). Serrate signals through Notch to establish a Wingless-dependent organizer at the dorsal/ventral compartment boundary of the *Drosophila* wing. *Development* **121**, 4215-4225.

Fortini, M. E., Rebay, I., Caron, L. A. and Artavanis-Tsakonas, S. (1993). An activated Notch receptor blocks cell-fate commitment in the developing *Drosophila* eye. *Nature* **365**, 555-557.

Galant, R., Walsh, C. M. and Carroll, S. B. (2002). Hox repression of a target gene: extradenticle-independent, additive action through multiple monomer binding sites. *Development* **129**, 3115-3126.

Ghysen, A. and O'Kane, C. (1989). Neural enhancer-like elements as specific cell markers in *Drosophila*. *Development* **105**, 35-52.

Go, M. J., Eastman, D. S. and Artavanis-Tsakonas, S. (1998). Cell proliferation control by Notch signaling in *Drosophila* development. *Development* **125**, 2031-2040.

Gorfinkiel, N., Morata, G. and Guerrero, I. (1997). The homeobox gene *Distal-less* induces ventral appendage development in *Drosophila*. *Genes Dev.* **11**, 2259-2271.

Halder, G. and Carroll, S. B. (2001). Binding of the Vestigial co-factor switches the DNA-target selectivity of the Scalloped selector protein. *Development* **128**, 3295-3305.

Halder, G., Polaczyk, P., Kraus, M. E., Hudson, A., Kim, J., Laughon, A. and Carroll, S. (1998). The Vestigial and Scalloped proteins act together

to directly regulate wing-specific gene expression in *Drosophila*. *Genes Dev.* **12**, 3900-3909.

Hooper, J. E. (1994). Distinct pathways for autocrine and paracrine Wingless signalling in *Drosophila* embryos. *Nature* **372**, 461-464.

Irvine, K. D. and Wieschaus, E. (1994). Fringe, a boundary-specific signaling molecule, mediates interactions between dorsal and ventral cells during *Drosophila* wing development. *Cell* **79**, 595-606.

Kim, J., Irvine, K. D. and Carroll, S. B. (1995). Cell recognition, signal induction, and symmetrical gene activation at the dorsal-ventral boundary of the developing *Drosophila* wing. *Cell* **2**, 795-802.

Kim, J., Sebring, A., Esch, J. J., Kraus, M. E., Vorwerk, K., Magee, J. and Carroll, S. B. (1996). Integration of positional signals and regulation of wing formation and identity by *Drosophila vestigial* gene. *Nature* **382**, 133-138.

Klein, T. and Martinez-Arias, A. (1998). Different spatial and temporal interactions between Notch, wingless, and vestigial specify proximal and distal pattern elements of the wing in *Drosophila*. *Dev. Biol*. **194**, 196-212.

Klein, T. and Martinez-Arias, A. (1999). The Vestigial gene product provides a molecular context for the interpretation of signals during the development of the wing in *Drosophila*. *Development* **126**, 913-925.

Lawrence, P. A., Bodmer, R., Vincent, J. P. (1995). Segmental patterning of heart precursors in *Drosophila. Development* **121**, 4303-4308.

Lewis, E. B. (1978). A gene complex controlling segmentation in *Drosophila. Nature* **276**, 565-570.

Liu, X., Grammont, M. and Irvine, K. D. (2000). Roles for scalloped and vestigial in regulating cell affinity and interactions between the wing blade and the wing hinge. *Dev. Biol*. **228**, 287-303.

Morata, G. and Garcia-Bellido, A. (1976). Developmental analysis of some mutants of the bithorax system of *Drosophila. Roux's Arch. Dev. Biol.* **179**, 125-143.

Morimura, S., Maves, L., Chen, Y. and Hoffmann, F. M. (1996). decapentaplegic overexpression affects *Drosophila* wing and leg imaginal disc development and wingless expression. *Dev. Biol.* **177**, 136-151.

Munemitsu, S., Albert, I., Rubinfeld, B. and Polakis, P. (1996). Deletion of an amino-terminal sequence beta-catenin in vivo and promotes hyperphosporylation of the adenomatous polyposis coli tumor suppressor protein. *Mol. Cell. Biol.* **16**, 4088-4094.

Nagaraj, R., Pickup, A. T., Howes, R., Moses, K., Freeman, M. and Banerjee, U. (1999). Role of the EGF receptor pathway in growth and patterning of the Drosophila wing through the regulation of vestigial. *Development* **126**, 975-985.

Neumann, C. J. and Cohen, S. M. (1996). A hierarchy of cross-regulation involving *Notch*, *wingless*, *vestigial* and *cut* organizes the dorsal/ventral axis of the *Drosophila* wing. *Development* **122**, 3477-3485.

Neumann, C. J. and Cohen, S. M. (1997). Long-range action of Wingless organizes the dorsal-ventral axis of the *Drosophila* wing. *Development* **124**, 871-880.

Pai, L. M., Orsulic, S., Bejsovec, A. and Peifer, M. (1997). Negative regulation of Armadillo, a Wingless effector in Drosophila. *Development* **124**, 2255-2266.

Papkoff, J., Rubinfeld, B., Schryver, B. and Polakis, P. (1996). Wnt-1 regulates free pools of catenins and stabilizes APC-catenin complexes. *Mol. Cell. Biol.* **16**, 2128-2134.

Patel, N. H., Martin-Blanco, E., Coleman, K. G., Poole, S. J., Ellis, M. C., Kornberg, T. B. and Goodman, C. S. (1989). Expression of engrailed proteins in arthropods, annelids and chordates. *Cell* **58**, 955-968.

Peifer, M., Sweeton, D., Casey, M. and Wieschaus, E. (1994). Wingless signal and Zeste-white 3 kinase trigger opposing changes in the intracellular distribution of Armadillo. *Development* **120**, 369-380.

Riggleman, B., Schedl, P. and Wieschaus, E. (1990). Spatial expression of the *Drosophila* segment polarity gene *armadillo* is postranscriptionally regulated by *wingless*. *Cell* **63**, 549-560.

Ruel, L., Stambolic, V., Ali, A., Manoukian, A. S. and Woodgett, J. R. (1999). Regulation of the protein kinase activity of Shaggy (Zeste-white3) by components of the wingless pathway in Drosophila cells and embryos. *J. Biol. Chem.* **274**, 21790-21796.

Rulifson, E. J. and Blair, S. S. (1995). Notch regulates *wingless* expression and is not required for reception of the paracrine *wingless* signal during wing margin neurogenesis in *Drosophila*. *Development* **121**, 2813-2824.

Shashidhara, L. S., Agrawal, N., Bajpai, R., Bharathi, V. and Sinha, P. (1999). Negative regulation of dorsoventral signaling by the homeotic gene *Ultrabithorax* during haltere development in *Drosophila*. *Dev. Biol*. **212**, 491-502.

Simmonds, A. J., Brook, W. J., Cohen, S. M. and Bell, J. B. (1995). Distinguishable functions for *engrailed* and *invected* in anterior-posterior patterning in the *Drosophila* wing. *Nature* **37**, 424-427.

Simmonds, A. J., Liu, X., Soanes, K. H., Krause, H. M., Irvine, K. D. and Bell, J. B. (1998). Molecular interactions between Vestigial and Scalloped promote wing formation in *Drosophila. Genes Dev.* **15**, 3815-3820.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., Peifer, M., Mortin, M. and Clevers, H. (1997). Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene *dTCF*. *Cell* **88**, 789-799.

Warren, R. W., Nagy, L., Selegue, J., Gates, J. and Carroll, S. (1994). Evolution of homeotic gene regulation and function in flies and butterflies. *Nature* **372**, 458-461.

Weatherbee, S. D., Halder, G., Kim, J., Hudson, A. and Carroll, S. (1998). *Ultrabithorax* regulates genes at several levels of the wing-patterning hierarchy to shape the development of the *Drosophila* haltere. *Genes Dev*. **12**, 1474-1482.

Weatherbee, S. D., Nijhout, H. F., Grunert, L. W., Halder, G., Galant, R., Selegue, J. and Carroll, S. B. (1999). Ultrabithorax function in butterfly wings and the evolution of insect wing patterns. *Curr. Biol.* **11**, 109-115.

White, R. A. H. and Akam, M. (1985). Contrabithorax mutations cause inappropriate expression of *Ultrabithorax* in *Drosophila. Nature* **318**, 567-569.

Williams, J. A., Bell, J. B. and Carroll, S. B. (1991). Control of *Drosophila* wing and haltere development by the nuclear *vestigial* gene product. *Genes Dev.* **5**, 2481-2495.

Williams, J. A., Paddock, S. W. and Carroll, S. B. (1993). Pattern formation in a secondary field: a hierarchy of regulatory genes subdivides the developing *Drosophila* wing disc into discrete sub-regions. *Development* **117**, 571-584.

Williams, J. A., Paddock, S. W., Vorwerk, K. and Carroll, S. B. (1994). Organization of wing formation and induction of a wing-patterning gene at the dorsal/ventral compartment boundary. *Nature* **368**, 299-305.

Xu, T. and Rubin, G. M. (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* **117**, 1223-1237.

Yoffe, K. B., Manoukian, A. S., Wilder, E. L., Brand, A. H. and Perrimon, N. (1995). Evidence for engrailed-independent wingless autoregulation in *Drosophila*. *Dev. Biol*. **170**, 636-650.

Zecca, M., Basler, K. and Struhl, G. (1996). Direct and long-range action of a Wingless morphogen gradient. *Cell* **87**, 833-844.