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The *Ultrabithorax* Hox gene of *Drosophila* controls haltere size by regulating the Dpp pathway

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The halteres and wings of *Drosophila* are homologous thoracic appendages, which share common positional information provided by signaling pathways. The activity in the haltere discs of the *Ultrabithorax* (*Ubx*) Hox gene establishes the differences between these structures, their different size being an obvious one. We show here that *Ubx* regulates the activity of the Decapentaplegic (Dpp) signaling pathway at different levels, and that this regulation is instrumental in establishing the size difference. *Ubx* downregulates *dpp* transcription and reduces Dpp diffusion by repressing the expression of *master of thick veins* and *division abnormally delayed* and by increasing the levels of *thick veins*, one of the Dpp receptors. Our results suggest that modulation in Dpp expression and spread accounts, in part, for the different size of halteres and wings.

KEY WORDS: Hox, *Ultrabithorax, decapentaplegic*, Size control, Imaginal disc, *Drosophila*

INTRODUCTION

Pattern formation in animals requires the concerted activity of selector genes and signaling pathways. A particular class of selector genes is formed by the Hox genes, which specify different structures along the anterior-posterior axis of metazoans (McGinnis and Krumlauf, 1992). In *Drosophila*, mutations in these genes frequently transform one structure into another keeping the coordinates provided by underlying positional information, established in part by the activity of signaling pathways. Mutations in the *Ultrabithorax* (*Ubx*) Hox gene illustrate this assertion. Wings and halteres are homologous structures located in the second and third thoracic segments, respectively. These appendages greatly differ in size and pattern, and derive from imaginal discs, the wing and haltere discs, which also differ in size but bear a similar morphology. *Ubx*, which is expressed in the haltere disc but not in the wing disc, determines the difference between these two structures: mutations in *Ubx* transform halteres into wings whereas *Ubx* ectopic expression changes wings into halteres (Lewis, 1963; Lewis, 1978; Cabrera et al., 1985; White and Akam, 1985; White and Wilcox, 1985). These transformations respect positional cues (Morata and García-Bellido, 1976) dictated by signaling pathways.

In *Drosophila*, one of the best-studied signaling pathways is that of the *decapentaplegic* (*dpp*) gene (homologous to the TGF-β in vertebrates). This pathway has been analyzed extensively in pattern formation of the imaginal discs, particularly the wing disc (reviewed by Tabata, 2001). This disc is subdivided early in development into an anterior (A) and a posterior (P) compartment (García-Bellido et al., 1973). The protein encoded by the *hedgehog* (*hh*) gene, synthesized in the posterior compartment, activates *dpp* transcription in anterior cells close to the anteroposterior (A/P) border (Posakony et al., 1991; Basler and Struhl, 1994; Capdevila and Guerrero, 1994; Tabata and Kornberg, 1994). The Dpp ligand diffuses into both A and P compartments, generating a gradient of protein concentration (Entchev et al., 2000; Teleman and Cohen, 2000). Dpp behaves as a morphogen, translating the protein concentration gradient into the restricted and overlapping expression of genes like *spalt* (*sal*) and *optomotor-blind* (*omb*) (Lecuit et al., 1996; Nellen et al., 1996). Among these, the *sal* gene is repressed by *Ubx* in the haltere pouch (Weatherbee et al., 1998; Barrio et al., 1999; Galant et al., 2002), indicating that the outcome of Dpp signaling is modified by *Ubx* in the haltere disc.

Dpp activity can be monitored with an antibody that recognizes the phosphorylated form of *Mothers against dpp* (*Mad*) (Persson et al., 1998; Tanimoto et al., 2000), a receptor-regulated Smad that transduces the *dpp* signal (Newfeld et al., 1997). The analysis of this and other Dpp pathway elements has revealed their different contribution to the formation of the Dpp ligand and activity gradients. Thus, the expression in the wing disc of one type I Dpp receptor, *thick veins* (*tkv*), is not uniform, and this unequal distribution modulates Dpp signaling along the A/P axis (Haerry et al., 1998; Lecuit and Cohen, 1998; Tanimoto et al., 2000). Similarly, the spread and activity of Dpp depends on the presence of cell-surface molecules like those encoded by the *division abnormally delayed* (*dally*) and *dally-like protein* (*dlp*) genes (Jackson et al., 1997; Nakato et al., 1995; Fujise et al., 2003; Belenkaya et al., 2004). All these elements establish the fine tuning of Dpp activity, which is crucial in determining the form and size of *Drosophila* wings (Spencer et al., 1982; Capdevila and Guerrero, 1994; Zecca et al., 1995; Lecuit et al., 1996; Nellen et al., 1996; Tsuneizumi et al., 1997; Haerry et al., 1998; Lecuit and Cohen, 1998; Campbell and Tomlinson, 1999; Jazwinska et al., 1999; Minami et al., 1999; Martín-Castellanos and Edgar, 2002; Martín et al., 2004). Therefore, the shape and size of adult derivatives can be established by adjusting the Dpp input in imaginal cells.

Ubx mutations increase the size of the halteres, transforming them into wings (Lewis, 1963) whereas *dpp* mutations reduce the size of the halteres (Spencer et al., 1982). Changes in the Dpp pathway affect wing size (reviewed by Day and Lawrence, 2000), and recent evidence indicates that cell proliferation in the wing disc is induced by reading different Dpp activity levels (Rogulja and Irvine, 2005). Although the *Ubx* and *dpp* effects (in halteres and wings) could be unrelated, the homologous nature of both appendages suggests that *Ubx* may fix haltere size by modifying the Dpp pathway. We have explored this idea and compared Dpp distribution and activity in the wing and haltere discs. We show that *Ubx* downregulates *dpp*

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expression, alters Dpp activity and reduces Dpp spread, and that the latter is achieved mainly by controlling the expression of *tkv* and *dally*. Our results have implications in the way *Ubx* establishes the different size of halteres and wings.

MATERIALS AND METHODS

Genetics

The dpp^{d12} and dpp^{d5} mutations remove regulatory regions of the *dpp* gene (St Johnston et al., 1990). $Ubx^{6.28}$ is a null allele of *Ubx* (Beachy et al., 1985), the *TM2* balancer carries the Ubx^{130} null mutation (Lewis, 1952), and the *Df109* deletion eliminates the *Ubx* gene (Lewis, 1978). The bx^3 and *pbx* mutations eliminate *Ubx* expression in the anterior and posterior compartments of the haltere disc, respectively, transforming them into the corresponding ones of the wing disc (Lewis, 1963; García-Bellido et al., 1973; Cabrera et al., 1985; White and Wilcox, 1985). The Cbx^{Twt} mutation ectopically expresses *Ubx* in the wing disc (Bender et al., 1983). *DfC1-h1* (Szidonya and Reuter, 1988) and *Df* tkv^2 (Szidonya and Reuter, 1988; Terracol and Lengyel, 1994) are deficiencies that uncover the *tkv* gene. The following reporter insertions or constructs were used: *dpp-lacZ*$^{BS3.0}$ (Blackman et al., 1991), *dpp-lacZ*^{10638} (Twombly et al., 1996), *hh-lacZ* (Lee et al., 1992) *dally-lacZ* (Nakato et al., 1995), *tkv-lacZ* (Tanimoto et al., 2000), *mtv-lacZ* (Funakoshi et al., 2001) and *omb*-lacZ (Grimm and Pflugfelder, 1996). The Gal4/UAS method (Brand and Perrimon, 1993) was used with the following Gal4 lines and UAS constructs: *dpp*-Gal4 (Morimura et al., 1996), *en*-Gal4 (Tabata et al., 1995), *ptc*-Gal4 (Hinz et al., 1994), *ap*-Gal4 (Calleja et al., 1996), *MS1096*-Gal4 (Capdevila and Guerrero, 1994), UAS-*dpp* (Capdevila and Guerrero, 1994), UAS-Dpp-GFP (Entchev et al., 2000), UAS-*tkv* (Lecuit and Cohen, 1998), UAS-tkv^{Q253D} (Nellen et al., 1996), UAS-tkv^{DN} (Haerry et al., 1998), UAS-*dally* (Tsuda et al., 1999), UAS-*mtv* (T. Tabata, unpublished), UAS-*Ubx* (Castelli-Gair et al., 1994), UAS-*dsRNA>Ubx* (Monier et al., 2005) and UAS-GFP (Ito et al., 1997). The *tub*-Gal80^{ts}/Gal4 system (McGuire et al., 2003) was used to temporally control the induction of transgenes with the Gal4/UAS method. To this aim, larvae were transferred from 17°C to 29°C during the second or third larval instars.

Clonal analysis

We used the FLP/FRT system (Xu and Rubin, 1993) to induce *Ubx* mutant clones in the haltere disc with the FRT82B $Ubx^{6.28}$ chromosome (Weatherbee et al., 1998), the MARCM method (Lee and Luo, 1999) to induce clones that lose *Ubx* and activate *tkv* in the haltere disc, and the combination of FLP/FRT and Gal4/UAS methods (Pignoni and Zipursky, 1997; Ito et al., 1997) to induce *Ubx*-expressing clones in the wing disc; in all the cases the clones were induced during the larval period. The genotypes of the larvae where the clones were induced are as follows.

Ubx$^-$ clones: *y hs-flp122*; *FRT82B* $Ubx^{6.28}$*/FRT82B Ubi-GFP*, *y hs-flp122*; *FRT82B* $Ubx^{6.28}$ *hh-lacZ/FRT82B Ubi-GFP* and *ptc*-Gal4/UAS-*flp*; *FRT82B Ubi*-GFP/*FRT82B* $Ubx^{6.28}$

Ubx$^-$ clones, *dpp-lac-Z*: *y hs-flp122*; *dpp-lacZ*$^{BS3.0}$/+; *FRT82B* $Ubx^{6.28}$*/FRT82B Ubi-GFP*

Ubx$^-$ clones, *omb-lac-Z*: *y hs-flp122/omb-lacZ*; *FRT82B* $Ubx^{6.28}$*/FRT82B Ubi-GFP*

Ubx$^-$ clones, *tkv-lacZ*: *y hs-flp122*; *tkv-lacZ*/+; *FRT82B* $Ubx^{6.28}$*/FRT82B Ubi-GFP*

Ubx$^-$ clones, *mtv-lacZ*: *y hs-flp122*; *mtv-lacZ*/+; *FRT82B* $Ubx^{6.28}$*/FRT82B Ubi-GFP*

Ubx$^-$ *tkv*$^+$ clones, *omb-lacZ*: *y hs-flp122 tub*-Gal4 UAS-GFP/*omb-lacZ*; UAS-*tkv FRT82B* $Ubx^{6.28}$*/FRT82B tub*-Gal80

Ubx$^+$ clones, *dally-lacZ*: *y hs-flp122*; *act5C>y+>*Gal4 UAS-GFP/UAS-*Ubx*; *dally-lacZ*/+.

In situ hybridization

In situ hybridization was performed as previously described (Azpiazu and Frasch, 1993; Wolff, 2000). The RNA *dpp* probe was synthesized from a BS-*dpp* plasmid containing a *dpp* cDNA (kindly provided by A. Macías), digested with KpnI and transcribed with the T3 polymerase.

Immunohistochemistry

Immunohistochemistry was carried out as previously described (Sánchez-Herrero, 1991; Estrada and Sánchez-Herrero, 2001). The antibodies used were: mouse and rabbit anti-β-galactosidase (Cappel), mouse Mab4D9 anti-En (Patel et al., 1989), rat anti-Tkv (Teleman and Cohen, 2000), rabbit anti-P-Mad (Tanimoto et al., 2000; Persson et al., 1998) [a gift of F. A. Martín and G. Morata, Centro de Biologia Molecular, Severo Ochoa (C.S.I.C.-U.A.M.), Madrid, Spain], and mouse anti-Ubx (White and Wilcox, 1984). Secondary antibodies were coupled to Red-X, Texas Red, FITC and Cy5 fluorochromes (Jackson ImmunoResearch).

Adult cuticle analysis

Flies were kept in a mixture of ethanol:glycerol (3:1) until needed. Flies were then macerated in 10% KOH at 60°C for 10 minutes, dissected, washed with water, dehydrated with ethanol and finally mounted in Euparal for inspection under a compound microscope.

Measurements in the imaginal discs

We calculated the width in haltere and wing pouches with the Measure Tool of Adobe Photoshop 8.0 using the position of the dorsoventral boundary as a reference line for these measurements.

The intensity of the Dpp-GFP dots was calculated with the MetaMorph Offline program. The final profiles of the intensities were obtained following the same procedure in bx^3*/MKRS* wing and haltere discs and in bx^3*/TM2* haltere discs. We first calculated the average value, along the A/P axis, of the GFP intensity in three different sections of a disc. This gives a mean value for the disc. We repeated this in three different discs of each type, thereby obtaining three mean values in each case. The final profile for either wing, haltere or bx^3*/TM2* haltere discs was obtained by plotting along the A/P axis the average of those three mean values obtained for each type of disc. The fixation and staining for all the discs was done simultaneously and under the same conditions. All pictures were processed under identical conditions.

RESULTS

The expression of *dpp* in the haltere disc is downregulated by *Ubx*

To monitor *dpp* transcription in the wing and haltere discs we hybridized them with a *dpp* RNA probe. *dpp* is expressed in both discs in anterior cells close to or abutting the A/P boundary (hereafter named 'anterior A/P Boundary cells' or 'AB cells'), but in the haltere disc the *dpp* stripe is weaker and less straight (Mohit et al., 2006) (Fig. 1A). To better define this expression we have used a *dpp-lacZ* reporter construct and a *dpp-lacZ* insertion that mimic *dpp* transcription in the imaginal discs (Blackman et al., 1991; Twombly et al., 1996; Weatherbee et al., 1998). Both give comparable results. In the wing pouch, the *dpp*-lacZ stripe (*dpp-lacZ*^{10638}) is about 8.1 cells wide (n=16) whereas in the haltere pouch it is about 6.0 cells (n=13; Fig. 1B-D′). Anterior *Ubx* mitotic recombination clones abutting the A/P boundary increase both the intensity of the signal and the width of the stripe (Fig. 1E,E′). To characterize how *Ubx* regulates *dpp* signal in A and P compartments, we used the *bithorax* (*bx*) and *postbithorax* (*pbx*) mutations (see Materials and methods). In *bx* (bx^3*/TM2*) haltere discs, the width of the *dpp-lacZ* stripe is about 7.9 cells (n=10; Fig. 1F,F′) approaching that of the wing disc (8.1 cells), whereas in *pbx* (*pbx/TM2*) haltere discs the average width is 6.4 cells (n=8; Fig. 1G,G′), slightly higher than in the wild type (6 cells). Therefore, the reduction in *dpp* transcription by *Ubx* depends mainly on the *Ubx* activity in the anterior compartment of the haltere disc.

Ubx controls the response to the *dpp* signal by retarding Dpp diffusion

To study whether *Ubx* governs the response to Dpp signaling, we monitored the expression of *omb*, a target of the *dpp* pathway, with an *omb-lacZ* insertion (Grimm and Pflugfelder, 1996; Lecuit et al.,

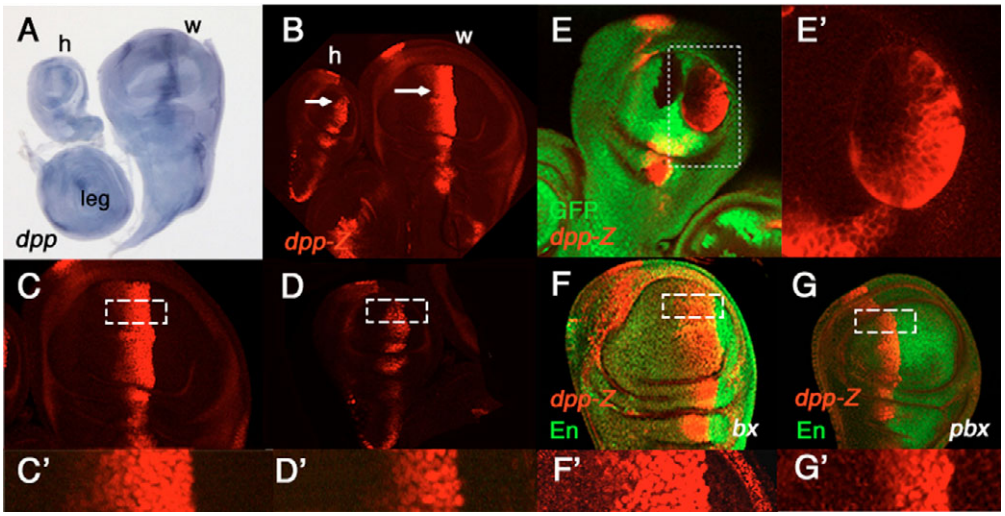

Fig. 1. *Ubx* regulates *dpp* transcription. (**A**) Wing (w) and haltere (h) imaginal discs hybridized with a *dpp* probe. In this and subsequent discs, anterior is to the left. (**B-D′**) wing (w; C,C′) and haltere (h; D,D′) imaginal discs of *dpp-lacZ*^{10638} larvae stained with an anti-β-galactosidase antibody. Note the different dpp expression in both discs. (**E**,**E′**) A *Ubx* mutant clone, marked by the absence of GFP (in green) showing expression of *dpp-lacZ*$^{BS3.0}$ (in red). Within the clone, the *dpp* band of expression widens and is more intense. (**F-G**′) Haltere discs of *dpp-lacZ*^{10638}*/+; bx*^{3}*/TM2* (F,F′) and *dpp-lacZ*^{10638}*/+; pbx/TM2* (G,G′) larvae. In the *bx* mutant haltere disc the A compartment increases its size and the *dpp* expression is like that of the anterior wing whereas in *pbx* mutant haltere discs the *dpp* expression is slightly wider but the P compartment increases its size significantly. C′-G′ are magnifications of the insets shown in C-G. The magnifications of the wing and haltere discs were done at exactly the same values.

1996; Nellen et al., 1996). As in the wing disc (Fig. 2A-A″), *omb* is expressed in both compartments of the haltere pouch (Weatherbee et al., 1998) (Fig. 2B-B″). In strong *bx* and *pbx* mutants *omb* expression in A and P compartments of the haltere disc resembles that of the corresponding compartments of the wing disc (Fig. 2C-D″). Because in *bx* mutants the *dpp* expression is like that of the wing disc (Fig. 1F) but *omb* expression in the P compartment is not (Fig. 2C), and because in *pbx* mutants *dpp* transcription in the haltere disc is only slightly increased (Fig. 1G), but *omb* signal is clearly extended (Fig. 2D), *Ubx* probably regulates the response to the Dpp signal. This conclusion is reinforced by the analysis of *omb* transcription in *Ubx* mutant clones: clones located outside the *omb* expression domain do not activate *omb* transcription. When the clones encompass the border of *omb* expression, the *omb* signal is extended further anteriorly or posteriorly. Notably, in some cases there is ectopic *omb* transcription outside the clone, indicating a non-cell-autonomous effect of *Ubx* loss on *omb* expression (Fig. 2E-F″). Taken together, these results indicate that *Ubx* represses *omb* activation in cells that receive a low amount of Dpp, but that a certain level of Dpp is required to activate *omb* even in the absence of *Ubx*.

These results suggest that *Ubx* reduces the transcriptional response of haltere disc cells to the Dpp signal. This may be achieved by limiting the spread of the Dpp product. To validate this assumption, we examined the distribution of a Dpp-GFP fusion protein expressed under the control of the *dpp*-Gal4 driver (Entchev et al., 2000; Teleman and Cohen, 2000). The GFP signal is more restricted in the haltere disc than in the wing disc (not shown). We aimed to quantify these differences and, for that, we used the *ptc*-Gal4 driver to drive Dpp-GFP expression because the *dpp*-Gal4 driver directs more irregular and variable expression in the haltere disc. The extension of Dpp-GFP expression is higher in *tub*-gal80^{ts}/*ptc*-Gal4; UAS-Dpp-GFP *bx*^{3}*/MKRS* wing discs than in haltere discs of the same genotype (Fig. 3A,B). In *tub*-gal80^{ts}/*ptc*-Gal4; UAS-Dpp-GFP *bx*^{3}*/TM2* haltere discs the expression levels and anterior extent of the fusion protein are very similar to those of the wing disc, but the spread in the posterior compartment is only slightly increased with respect to the wild type (Fig. 3C). A summary of the data is shown in Fig. 3D. This shows that the Dpp-GFP signal falls to background levels more abruptly in haltere discs than in the wing discs or in the anterior compartment of *bx* haltere discs. This difference is more evident in medial regions of both compartments: in these regions there is a graded decrease of the Dpp-GFP signal in the wing disc, whereas the distribution profile is flat in the haltere disc. A plot of the GFP-Dpp dot distribution in three different sections of a single wing or haltere disc is shown in Fig. S1 in the supplementary material. These results, together with our previous observations, strongly suggest that *Ubx* counteracts the spread of Dpp in the haltere pouch.

High levels of *tkv* in the haltere disc are induced by *Ubx* through regulation of *mtv* expression and *dpp* activity

We have just shown that *Ubx* reduces Dpp diffusion in the haltere disc, thus limiting *omb* expression. To draw a general conclusion about how *Ubx* regulates Dpp signaling we looked at the distribution of the phosphorylated form of Mad (P-Mad), a major readout of Dpp activity (Tanimoto et al., 2000). The P-Mad signal in the haltere pouch is narrower than the signal in the wing pouch, confined almost exclusively to the anterior compartment and not reduced in AB cells (Fig. 4A-C′). Hence, and also in contrast with the wing disc (Fig. 4D-D″), high levels of both Hh and Dpp signaling coincide in these cells (Fig. 4E-E″). The low Dpp signaling in central cells of the wing disc is due to the reduced expression of *tkv*, which is more strongly expressed peripherally and is particularly low in AB cells (Brummel et al., 1994; de Celis, 1997; Haerry et al., 1998; Lecuit and Cohen, 1998; Tanimoto et al., 2000) (Fig. 4F). By contrast, although the expression of *tkv* in the haltere pouch increases slightly in the periphery, it is uniform in the central region and higher than in the corresponding domain of the wing pouch (Fig. 4G). In *Ubx*$^{-}$ clones the *tkv* expression is reduced but for the clones induced in the more lateral domains (Fig. 4H-I″). Conversely, ectopic *Ubx* expression in medial regions of the wing disc of *Cbx*^{Twt} mutants increases *tkv* expression (Fig. 4J,J′). As Tkv levels are crucial for Dpp signaling and Dpp diffusion (Haerry et al., 1998; Lecuit and Cohen, 1998; Tanimoto et al., 2000; Funakoshi et al., 2001), we decided to examine in more detail the regulation of *tkv* expression by *Ubx*.

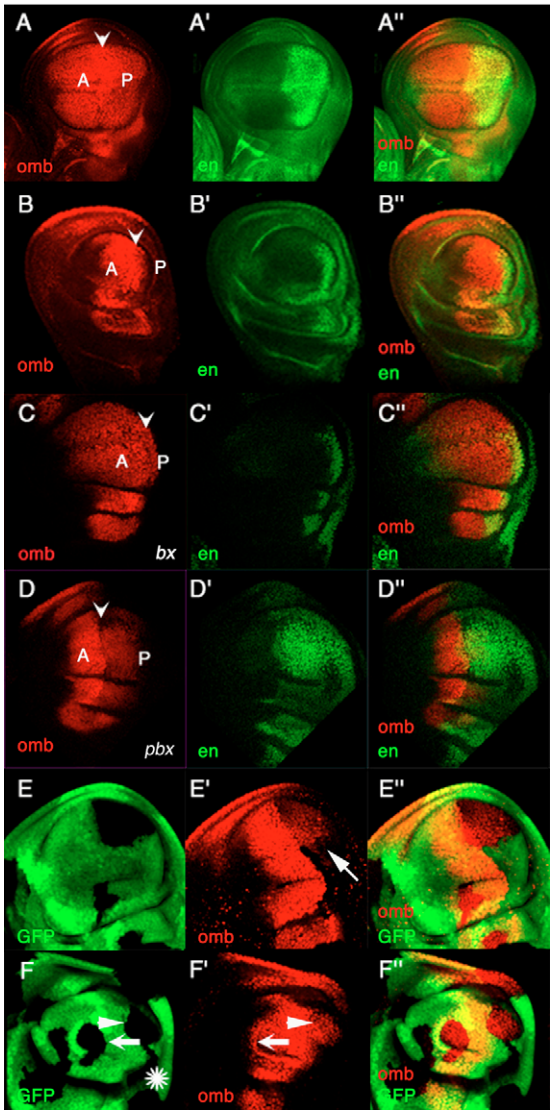

Fig. 2. *Ubx* controls the expression of Dpp targets. (**A-B″**) *omb-lacZ* (in red) and En (in green) expression in wing (A-A″) and haltere (B-B″) imaginal discs. A and P stand for anterior and posterior compartments, respectively. In bx^3/*TM2* (**C-C″**) and *pbx*/*TM2* (**D-D″**) haltere discs the *omb* expression extends significantly in the anterior (C) and posterior (D) compartments, respectively. Arrowheads in A-D mark the A/P boundary. (**E-F″**) *Ubx* mutant clones, marked by the absence of GFP expression (in green) and showing *omb-lacZ* expression (in red). Note in E′ the extended expression of *omb* in Ubx^- cells (arrow). In F-F″ there are three types of clones: a clone far from the A/P boundary does not activate *omb* (asterisk in F); clones closer to this boundary show extended *omb* expression (arrowhead in F and F′, posterior clone), and another clone (arrow in F, anterior clone) activates *omb* also outside the clone (the arrow in F′ points to non-cell-autonomous *omb* expression). Merged images in E″ and F″.

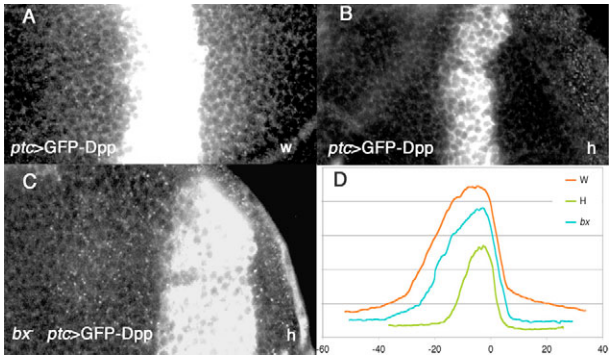

Fig. 3. *Ubx* governs Dpp spread. (**A**,**B**) Wing (A) and haltere (B) imaginal discs of *tub*-Gal80^{ts}/*ptc*-Gal4; UAS-Dpp-GFP bx^3/*MKRS* larvae, showing a more restricted spread of Dpp-GFP in the haltere disc. In *tub*-Gal80^{ts}/*ptc*-Gal4; UAS-Dpp-GFP bx^3/*TM2* haltere discs (**C**) the Dpp-GFP expression and spread in the A compartment are similar to those of the wing disc, but spread in the P compartment is much reduced compared with that of the wing disc. (**D**) A plot representing the average value of the intensity of the Dpp-GFP dots along the A/P axis in *tub*-Gal80^{ts}/*ptc*-Gal4; UAS-Dpp-GFP bx^3/*MKRS* wing and haltere discs and in *tub*-Gal80^{ts}/*ptc*-Gal4; UAS-Dpp-GFP bx^3/*TM2* haltere discs. Note the reduction in extent and the abrupt fall of the Dpp-GFP signal when *Ubx* is present. Numbers in the x-axis indicate distance in microns from the A/P boundary (0 value). The anterior compartment is to the left. w, wing disc: h, haltere disc.

In the wing pouch, the distribution of *tkv* is regulated by two mechanisms (Lecuit and Cohen, 1998; Funakoshi et al., 2001). The first mechanism depends on the activity of *master of thick veins* (*mtv*) (Funakoshi et al., 2001). In AB cells, high *mtv* expression, under control of Hh signaling, strongly reduces the *tkv* signal; in cells located in a medial position along the A/P axis, moderate *mtv* expression reduces *tkv* transcription to a basal level (Funakoshi et al., 2001). An *mtv-lacZ* reporter insertion is prominently expressed in the AB cells and in two peripheral domains of the wing pouch, and expressed at low levels in the rest of the pouch (Funakoshi et al., 2001) (Fig. 4K). In the haltere disc, only the lateral signal remains (Fig. 4L). This difference is due to *Ubx* because in bx^3/*TM2* haltere discs an A/P stripe appears (Fig. 4M) and in Ubx^- clones *mtv* is derepressed (Fig. 4N,N′). Reciprocally, ectopic *Ubx* expression in the wing disc represses *mtv* in central and medial domains (Fig. 4O,O′). Therefore, the absence of *mtv* in AB cells of the haltere pouch can explain their high levels of *tkv* expression and Dpp signaling. Consistently, in *MS1096*-Gal4; UAS-*mtv*/+ larvae, in which *mtv* is strongly expressed in the dorsal region of the haltere pouch, *tkv* levels are partially reduced dorsally except in the more lateral domains (Fig. 4P, the wild type is shown in Fig. 4G). Therefore, *Ubx* repression of *mtv* in central and medial regions of the haltere pouch contributes to their high *tkv* transcription.

The second mechanism to regulate *tkv* depends on Dpp activity. The high *tkv* levels in peripheral cells of the wing disc are reduced if Dpp signaling is augmented (Lecuit and Cohen, 1998; Tanimoto et al., 2000). Thus, in *MS1096*-Gal4; UAS- tkv^{Q253D}/+ larvae *tkv-lacZ* levels are reduced in the dorsal region (Fig. 4Q). By contrast, no such repression is observed in the haltere pouch (Fig. 4R), indicating that *Ubx* prevents the repression of *tkv* mediated by Dpp signaling. Collectively, our results show that *Ubx* promotes high levels of *tkv* in the haltere pouch by repressing *mtv* and by inhibiting the Dpp signaling-mediated downregulation of *tkv*. However, we cannot exclude a direct effect of *Ubx* on *tkv*.

The increased expression of *tkv* reverts the effect of *Ubx* loss on Dpp activity

High levels of *tkv* increase Dpp transduction cell autonomously, but also restrict Dpp spread by trapping the Dpp protein (Haerry et al., 1998; Lecuit and Cohen, 1998; Tanimoto et al., 2000). Given that

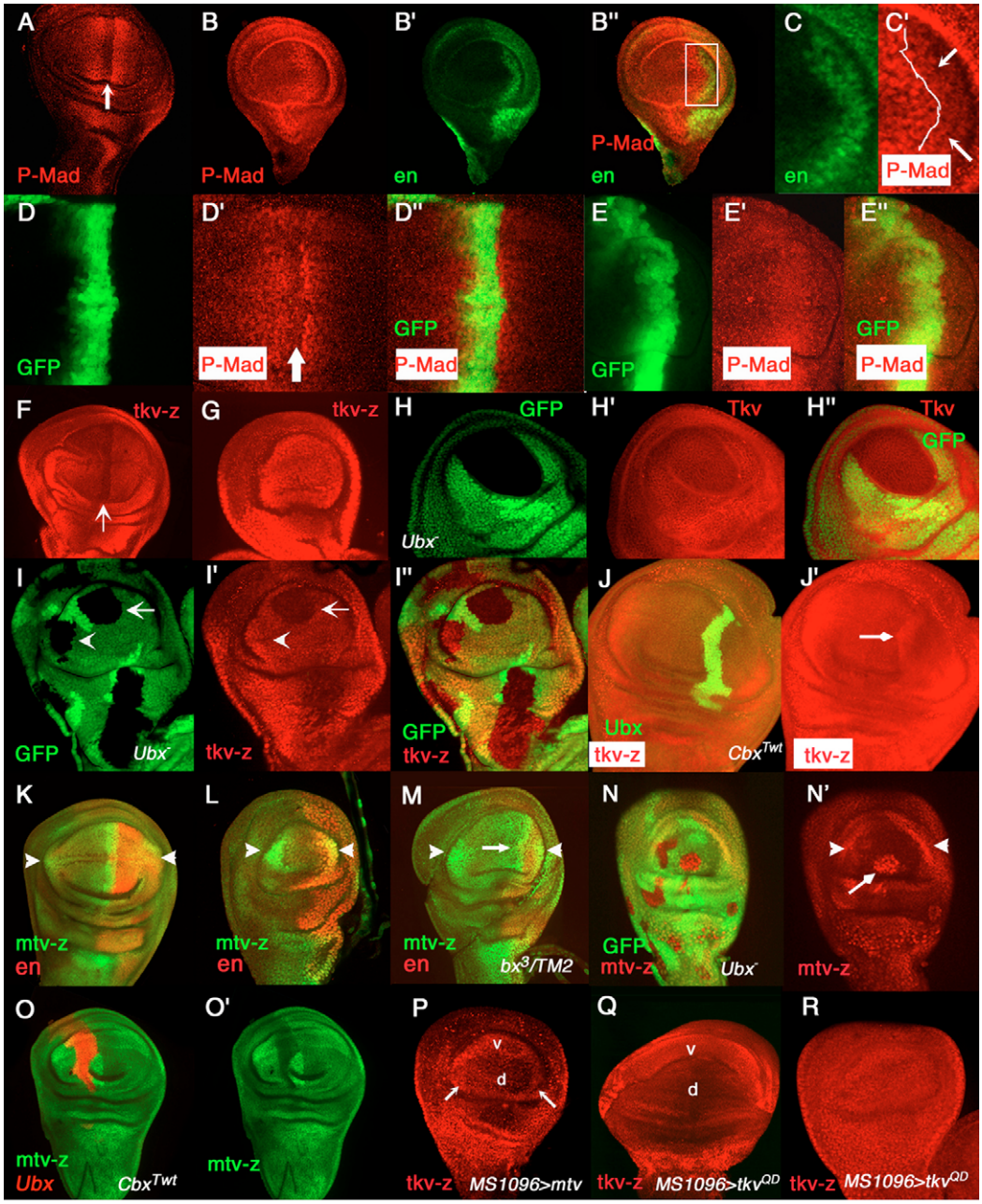

Fig. 4. *Ubx* prevents downregulation of *tkv* in the haltere pouch mediated by *mtv* expression and Dpp signaling. (**A**) In the wing pouch, P-Mad signal is strongly reduced in AB cells (arrow) (Tanimoto et al., 2000). (**B-C″**) In the haltere disc, P-Mad signal (in red in B) is narrower, but strong, in these cells (abutting the En expression domain, in green in B′). Merged image in B″. The boxed region is magnified in C,C′; the arrows point to the posterior P-Mad signal and the white line marks the A/P boundary. (**D-E″**) *ptc*-Gal4 UAS-GFP wing (D-D″) and haltere (E-E″) pouches, showing that high levels of Hh signaling (GFP signal, in green in D and E) and Dpp signaling (P-Mad, in red in D′ and E′) coincide in the haltere but not the wing disc (arrow in D′). Merged images in D″ and E″. (**F**,**G**) *tkv-lacZ* expression in the wing and haltere discs. The arrow in F marks the reduced expression in AB cells of the wing pouch. The expression detected with an anti-Tkv antibody is similar but does not show the downregulation in AB cells of the wing disc so neatly as the P-lacZ insertion. (**H-H″**) A big *Ubx*⁻ clone in the haltere disc, marked by the absence of GFP (H, in green), shows downregulation of Tkv protein expression (H′, in red). Merged image in H″. (**I-I″**). Mutant *Ubx* clones (marked by the absence of GFP, in green in I) present reduced *tkv-lacZ* expression (I′, in red) in medial (arrow) but not in lateral (arrowhead) regions of the haltere disc. Merged image in I″. (**J**,**J′**) The ectopic expression of *Ubx* in Cbx^{Twt} mutants (J, in green) increases *tkv-lacZ* signal (J′, in red). (**K**) The expression of *mtv* (*mtv-lacZ*, in green) in the wing disc is strong at the A/P boundary and in two lateral spots (arrowheads). In the haltere pouch (**L**), just these spots are observed. (**M**) In a *mtv-lacZ*/+; bx^3/*TM2* haltere disc there is *mtv* expression at the A/P boundary (arrow). (**N**,**N′**) *mtv* (in red) is also derepressed (arrow) in haltere disc *Ubx* mutant clones (arrow), marked by the absence of GFP (in green in N). The arrowheads in K-N mark the lateral spots, and En expression, marking the posterior compartment, is shown in K-M in red. (**O**,**O′**) Ectopic *Ubx* expression in Cbx^{Twt} wing discs (in red in O) strongly reduces *mtv* signal (in green) in medial regions (O,O′). (**P**) In *MS1096*-Gal4; *tkv-lacZ*/+; UAS-*mtv*/+ haltere discs the expression of *tkv* is downregulated in the dorsal (d) region, except in the periphery (arrows); compare with the expression in a *tkv-lacZ* haltere disc (G); v, ventral region. (**Q**) *MS1096*-Gal4; *tkv-lacZ*/+; UAS-tkv^{QD}/+ wing disc showing repression of *tkv* in the dorsal pouch (d). (**R**) No such repression is observed in haltere discs.

Ubx increases *tkv* expression in the haltere disc, these high levels may retain more Dpp ligand than in the wing disc, thus explaining the restricted Dpp diffusion and activity observed in the haltere pouch. To confirm this, we first induced *Ubx*$^{-}$ clones in the haltere disc and looked at the P-Mad expression pattern. In clones encompassing the border of high P-Mad staining there is an expansion of the P-Mad signal that, frequently, is observed outside the mutant territory (Fig. 5A-A″). This non-cell-autonomous effect is similar to that described previously for *omb* (Fig. 2F-F″) and suggests that Dpp extends readily through the *Ubx* mutant territory inducing high levels of signaling distally to the clone.

These observations suggest that the absence of *Ubx* elevates Dpp signaling in regions distant from the A/P boundary, and that this effect may be mediated by decreasing Tkv levels. If so, this outcome should be compensated by increasing *tkv* expression in *Ubx* mutant clones. To corroborate this inference, we made clones that simultaneously lose *Ubx* and express *tkv*, and followed Dpp activity by looking at *omb* and P-Mad expression. As shown in Fig. 5B-B‴, *Ubx*$^{-}$ *tkv*$^{+}$ clones in medial regions of the haltere disc do not show extension of P-Mad or *omb* expression, in contrast with the results observed in clones just mutant for *Ubx* (Fig. 2E-E″ and Fig. 5A-A″). This result confirms that *Ubx* regulates Dpp spread and signaling mainly by controlling Tkv levels. Strengthening this idea, we note that ectopic *Ubx* expression in the central region of the wing disc (*dpp*-Gal4/UAS-*Ubx* larvae) reduces the extent of P-Mad expression with respect to the wild type, but increases it in AB cells (Fig. 5D, compare with the wild type in Fig. 4A), and that these effects are similar to those obtained when *tkv* transcription is augmented in these same cells (Fig. 5C).

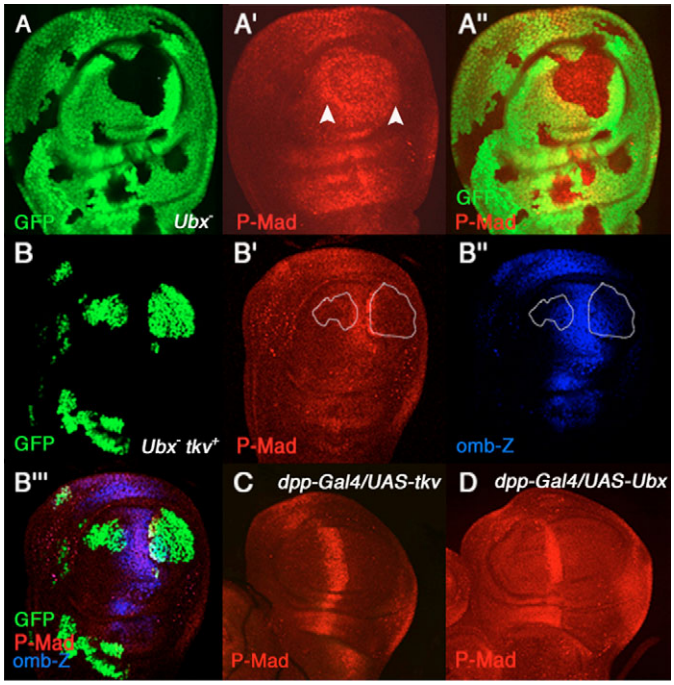

Fig. 5. *tkv* reverts the extended Dpp activity caused by Ubx loss. (**A-A″**) Two clones merged at the A/P boundary of the haltere disc (as revealed by *hh*-lacZ expression, not shown), and marked by the absence of GFP expression (in green in A), show wider P-Mad signal (A′) and non-cell-autonomous expression both anteriorly and posteriorly to the clones (arrowheads in A′). A″, merged image. (**B-B‴**) Clones mutant for *Ubx* and that simultaneously express *tkv*. The clones are marked by GFP expression (in green in B). Note that P-Mad (in red in B′) and *omb* (in blue in B″) signals are restricted to a few cells within the clones (compare with Fig. 2E′ and Fig. 5A′). Merged image in B‴. (**C**,**D**) P-Mad expression in *dpp*-Gal4/UAS-*tkv* (C) and *dpp*-Gal4/UAS-*Ubx* (D) wing discs. In both cases the extent of P-Mad signal is reduced, but the level of expression in AB cells is increased, compared with that of wild-type wing discs (Fig. 4A).

Ubx controls the expression of *dally*

The function and distribution of Dpp, like that of Wingless (Wg) or Hh, depends on the presence of a kind of cell-surface molecule named heparin sulphate proteoglycans (reviewed by Lin, 2004). Two proteoglycan members in *Drosophila* are *dally* and *dlp* (Nakato et al., 1995; Khare and Baumgartner, 2000; Baeg et al., 2001). Both are implicated in Dpp function (Fujise et al., 2001; Fujise et al., 2003; Jackson et al., 1997; Tsuda et al., 1999; Belenkaya et al., 2004) and in the transport of Dpp along the A/P axis (Belenkaya et al., 2004). *dlp* is expressed at slightly lower levels in the haltere pouch than in the wing pouch (not shown), and we have not investigated it further. However, a different *dally* expression in the two discs was patent. In the wing pouch, a *dally-lacZ* insertion shows high expression in two bands along the dorso-ventral (D/V) boundary and in the AB cells, with lower signal in the rest of the pouch (Fujise et al., 2001; Fujise et al., 2003) (Fig. 6A). By contrast, in the haltere disc the expression in AB cells is missing, the D/V signal seems to be confined to the anterior compartment (where *wg* is expressed) and there are lower levels throughout the pouch (Fig. 6B). When *Ubx* expression is reduced in the haltere pouch, the pattern of *dally* resembles that of the wing disc (Fig. 6C), and in clones that ectopically express *Ubx* in the wing disc there is a reduction in *dally* signal (Fig. 6D,D′). These results show that *Ubx* is necessary and sufficient to differentiate *dally* expression in both discs. Previous results demonstrated that the ectopic expression of *dally* in the wing disc augments Dpp activity (Fujise et al., 2003; Takeo et al., 2005). Similarly, we have observed an increase in the extent of P-Mad signal in the dorsal domain of *ap*-Gal4/UAS-*dally* haltere discs (Fig. 6E). We conclude that *Ubx* may reduce the extent of Dpp activity in the haltere disc by controlling *dally* expression.

In AB cells of the wing disc the expression of *dally* is induced by Hh signaling but can be downregulated if Dpp signaling is increased (Fujise et al., 2003). *mtv*, whose expression in these cells is also induced by Hh (Funakoshi et al., 2001), is also downregulated if Dpp activity is elevated (Fig. 6F). Therefore, we wondered if the high Dpp signaling present in the AB cells of the haltere pouch may contribute to the lack of *dally* and *mtv* expression. If this hypothesis is correct, a reduction in Dpp signaling should activate these two genes in the A/P boundary of the haltere disc. We found no such *dally* (Fig. 6G) or *mtv* (Fig. 6H) activation when Dpp activity was decreased (in *MS1096*-Gal4; UAS-*tkv*^{DN}/+ larvae). The repression of *mtv* and *dally* by *Ubx* in AB cells, therefore, is not maintained by high Dpp activity.

Haltere size depends on *Ubx* regulation of *dpp* expression and spread

A major difference between wings and halteres is their size (Fig. 7A). Although this is mostly due to the different size of wing and haltere cells (Roch and Akam, 2000), wing discs are also bigger than haltere discs, even though cell size in both structures is similar (Roch and Akam, 2000). At the end of embryogenesis, wing discs are about twice as big as haltere discs (Morata and García-Bellido, 1976; Madhavan and Schneidermann, 1977; Bate and Martínez-Arias, 1991). We have measured the size of wing and haltere

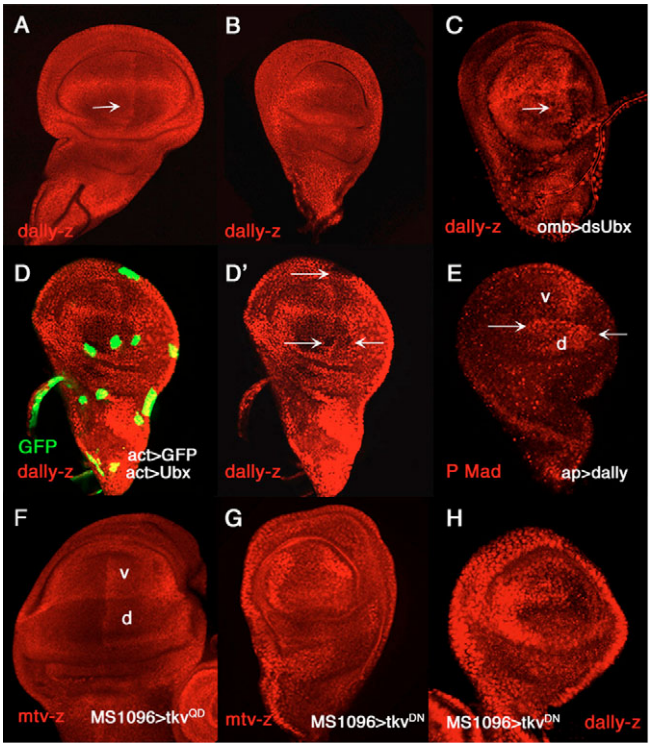

Fig. 6. *Ubx* repression of *dally* restricts the extent of Dpp activity in the haltere disc. (**A**) *dally* (*dally-lacZ*) expression in the wing disc, showing stronger signal in the dorsal-ventral and A/P boundaries (arrow) and in the lateral regions. (**B**) In the haltere disc, *dally* is not transcribed in AB cells and the signal in the dorsal-ventral boundary is restricted to the anterior compartment. There is also lower signal throughout the pouch. (**C**) An *omb*-gal4; *Df109* UAS-dsRNA>*Ubx*/+ haltere disc, showing a *dally* pattern similar to that of the wing disc. The arrow marks the A/P stripe. (**D**,**D′**) *Ubx*-expressing clones in the wing disc, marked by the GFP expression (D, in green), eliminate *dally* signal (D,D′, in red; arrows). (**E**) The expression of *dally* under the control of the *ap*-Gal4 line extends the P-Mad signal in the dorsal domain (d, arrows), where the line drives expression; v, ventral region. (**F**) The ectopic expression of activated Tkv in the dorsal (d) domain of the wing pouch (*MS1096*-Gal4 driver) downregulates *mtv* transcription; v, ventral region. (**G**,**H**) In the dorsal haltere pouch of MS1096; UAS-tkv^{DN}/+ larvae, the expression of a dominant-negative form of Tkv does not activate *mtv* (G) or *dally* (H) expression at the A/P boundary.

pouches in late third instar larvae and found the former to be about 3.5-4 times bigger than the latter. Assuming that the size difference found at the end of the embryogenesis applies equally to all regions of the disc, this implies that the wing pouch acquires around twice as many cells as the haltere pouch during the larval period. We have measured the size of *Ubx* mutant clones, and that of their twin spots, induced during the larval stages and analyzed in the haltere discs of late third instar larvae, and found that they are of similar size (Fig. 7B): the '*Ubx* clone area/twin clone area' ratio is 1.06 (n=20). This suggests that a different proliferation dynamic of *Ubx*-expressing cells is probably not responsible for the smaller size of haltere discs.

Given that *Ubx* controls the Dpp pathway and this is involved in the control of wing disc growth (Spencer et al., 1982; Capdevila and Guerrero, 1994; Burke and Basler, 1996; Lecuit et al., 1996; Nellen et al., 1996; Haerry et al., 1998; Lecuit and Cohen, 1998; Martín-Castellanos and Edgar, 2002; Martín et al., 2004), we reasoned that *Ubx* may reduce haltere size by regulating this signaling route. We tried to prove this assumption by different experiments. First, we investigated whether forcing the transcription of Dpp pathway elements, the expression of which is downregulated by *Ubx*, may impinge on haltere size. Overexpression of *dpp* in its own domain increases the size of the haltere pouch (Fig. 7C). The ectopic expression of *dally* or *mtv* also augments haltere size: we have measured the width of A and P compartments in the haltere pouches of *en*-Gal4 UAS-GFP/+, *en*-Gal4 UAS-GFP/UAS-*dally* and *en*-Gal4 UAS-GFP/UAS-*mtv* larvae (all grown at 29°C), and found that the ectopic expression of *dally* or *mtv* increases the P/A width ratio by 21% and 35%, respectively, with respect to the control discs (Fig. 7D-F,U). This difference is probably not due to the effect of *dally* on Hh and Wg signaling (reviewed by Lin, 2004) because *wg* is not expressed (Weatherbee et al., 1998) and *hh* is not active (Domínguez et al., 1996; Hepker et al., 1997) in this compartment.

As a second test, we studied whether there was a phenotypic interaction between *dpp* and *Ubx* as regards to haltere size. *dpp* hypomorphic mutations reduce the size of the distal part of the halteres (the capitellum) (Spencer et al., 1982) (Fig. 7H compare with the wild type in 7G). In a mutant background heterozygous for *Ubx*, the dpp^{-} vestigial phenotype is partially suppressed (Fig. 7I), suggesting that a reduction in *Ubx* can make up for the low Dpp levels. Several experiments argue that this interaction relies, at least in part, in the control by *Ubx* of *tkv* expression. First, wings are smaller (without apparent change in cell size, as judged by trichome size and density) if *Ubx* (Fig. 7K, compare with the wild type in 7J) or *tkv* (Haerry et al., 1998; Lecuit and Cohen, 1998; Tanimoto et al., 2000) (Fig. 7L) are present in AB cells. Second, in *Ubx*/+ adults the capitellum is enlarged (Fig. 7M), and this phenotype is stronger in sibling flies that are also heterozygous for a *tkv* deficiency (Fig. 7N). Finally, the increase in the size of the posterior or anterior compartments in *pbx*- or *bx*-mutant haltere discs (Fig. 7O,Q) is partially reverted when *tkv* levels are elevated (Fig. 7P,R): the P/A width ratio in *pbx*/$Ubx^{6.28}$ haltere discs is reduced by 37% in *en*-Gal4 UAS-GFP/+; *pbx*/UAS-*tkv* $Ubx^{6.28}$ larvae and the A/P width ratio in bx^{3}/$Ubx^{6.28}$ haltere discs is reduced by 17% in *ptc*-Gal4 UAS-GFP/+; bx^{3}/UAS-*tkv* $Ubx^{6.28}$ larvae (Fig. 7U). The latter reduction is modest probably because the *ptc*-Gal4 driver expresses *tkv* only in part of the anterior compartment and because *dpp* expression is wing-like. As a summary, our results suggest that *Ubx* reduces the size of the haltere, as compared with the wing, in part through the expression of *tkv*.

We have demonstrated that the absence of *Ubx* in mutant clones affects Dpp activity both cell autonomously and non-cell autonomously (Fig. 2F-F″ and Fig. 5A-A″), and that *Ubx* hinders Dpp spread. Therefore, local changes in *Ubx* expression may have non-cell-autonomous effects on size. To prove this, we reared larvae of the *ptc*-Gal4 UAS-GFP/+; *Df109* UAS-dsRNA>*Ubx*/*tub*-Gal80^{ts} genotype at 17°C and transferred them to 29°C at the second or early third larval stage. This procedure eliminates *Ubx* expression in the *ptc* domain (not shown). In many of the flies that underwent this treatment we observed that the anterior haltere tissue was bigger than that expected to derive from the *Ubx*-expressing region, sometimes even bigger than a whole anterior haltere compartment (Fig. 7S,S′). A similar effect was observed in flies in which the absence of *Ubx* is clonally inherited (*ptc*-Gal4/UAS-*flp*; FRT Ubx^{6-28}/FRT GFP flies; Fig. 7T). This suggests that the absence of *Ubx* in anterior border cells increases the growth of the more anterior, *Ubx*-expressing haltere region.

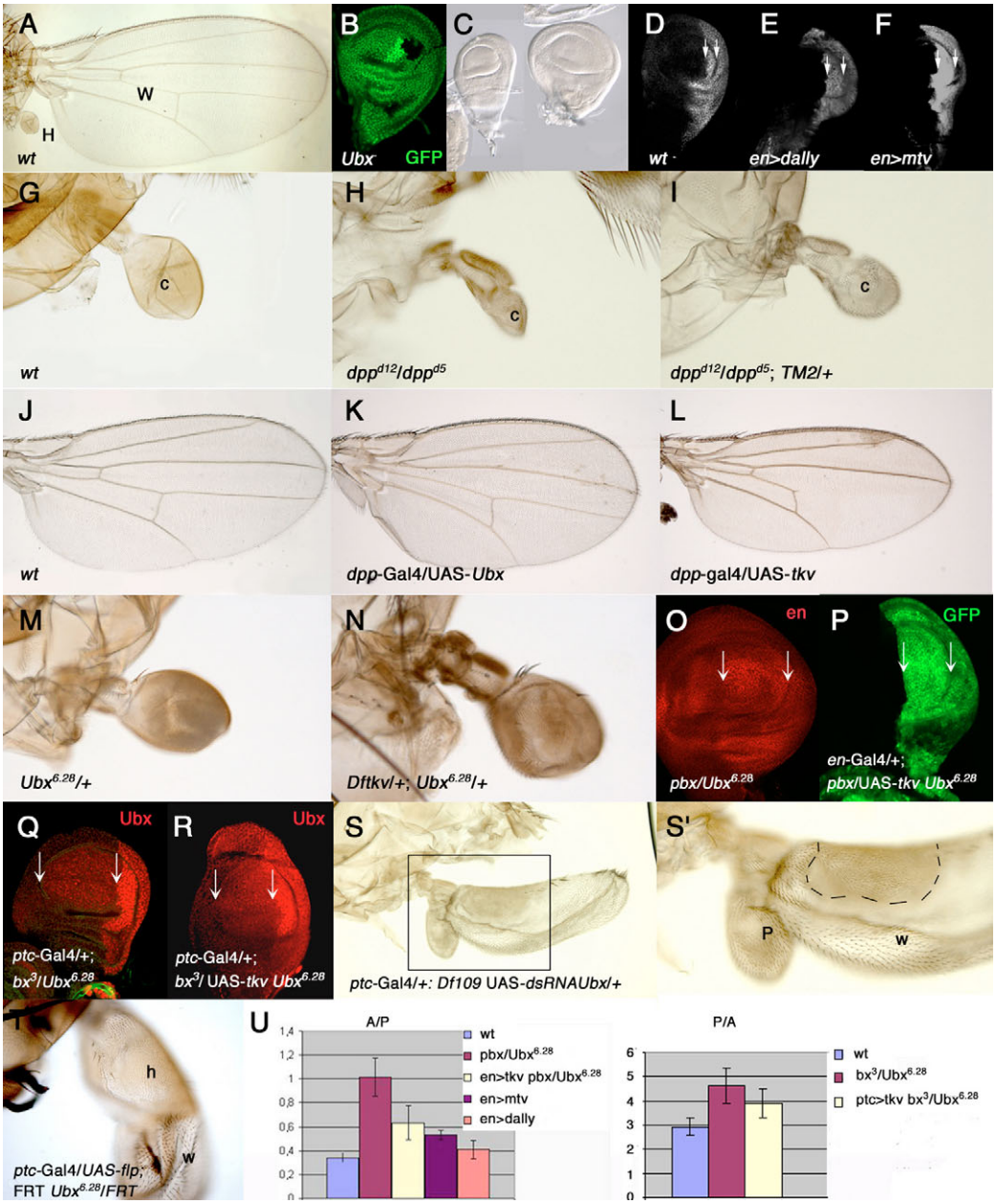

Fig. 7. The *dpp* pathway and *Ubx* regulate haltere size. (**A**) Thorax of a *Drosophila* wild-type adult, showing the different size of the wing (W) and the haltere (H). (**B**) A *Ubx* mutant clone in the haltere disc of a third instar larva showing that the size of the clone (absence of GFP expression, in green) and that of the twin spot (more stained due to the two doses of the Ubi-GFP construct) are similar. (**C**) Wild-type (left) and *dpp*-Gal4/UAS-*dpp* (right) haltere discs showing the bigger pouch of the latter. (**D-F**) The P compartments of *en*-Gal4 UAS-GFP/UAS-*dally* (E) and *en*-Gal4 UAS-GFP/UAS-*mtv* (F) haltere discs are enlarged with respect to *en*-Gal4 UAS-GFP controls (D). The three compartments are marked with GFP and delimited by arrows. (**G-I**) In dpp^{d12}/dpp^{d5} adults the size of the distal part of the haltere (the capitellum, c) is reduced (H) compared with the wild type (G), and in dpp^{d12}/dpp^{d5}; *TM2*/+ flies (I) this reduction is alleviated. (**J-L**) Wings of wild-type (J), *dpp*-Gal4/UAS-*Ubx* (K) and *dpp*-gal4/UAS-*tkv* (L) adults, showing the reduction in size of the latter two. (**M**) Haltere of a $Ubx^{6.28}$/+ adult, showing a slightly bigger haltere than in wild-type flies (G) due to the haploinsufficient phenotype of the *Ubx* locus. (**N**) In Df *tkv*/+; $Ubx^{6.28}$/+ siblings the size of the haltere is increased. (**O-R**) In *pbx*/$Ubx^{6.28}$ (O) and *ptc*-Gal4/+; bx^3/ $Ubx^{6.28}$ (Q) haltere discs there is an increase in the size of the P and A compartments, respectively (delimited by arrows). These increments are reduced if *tkv* is expressed in the posterior (*en*-gal4/+; *pbx*/UAS-*tkv* $Ubx^{6.28}$ larvae; P) or anterior (*ptc*-Gal4/+; bx^3/UAS-*tkv* $Ubx^{6.28}$ larvae; R) compartments. The P compartment is marked by anti-En antibody (in red in O), by GFP (in green in P), and by anti-Ubx (in red in Q and R). A summary of the results is shown in U. (**S**) A *ptc*-Gal4/+; *Df109* UAS-dsRNA>*Ubx*/*tub*-Gal80^{ts} fly, showing a patch of haltere tissue (delimited by the discontinuous line in S′) bigger than the anterior compartment of a wild-type haltere. Detail of the boxed region is shown in S′; w, wing territory; P, posterior compartment. (**T**) A capitellum of a *ptc*-Gal4/UAS-flp; FRT $Ubx^{6.28}$/FRT GFP adult with *Ubx* mutant clones (cells transformed into wing, w) showing more haltere tissue (h) than in the wild type (compare with G). (**U**) Histograms showing the P/A (left) or A/P (right) ratios of different genotypes. P/A ratios: wild type (wt), 0.34 (*n*=16), *pbx*/$Ubx^{6.28}$, 1 (*n*=14), *en*-gal4/+; *pbx*/UAS-*tkv* $Ubx^{6.28}$, 0.63 (*n*=11), *en*-Gal4/UAS-*mtv*, 0.53 (*n*=8), and *en*-gal4/UAS-*dally*, 0. 41 (*n*=13). A/P ratios: *wt*, 2.9 (*n*=16), *ptc*-Gal4/+; bx^3/$Ubx^{6.28}$, 4.6 (*n*=14) and *ptc*-Gal4/+; bx^3/ UAS-*tkv* $Ubx^{6.28}$, 3,9 (*n*=12).

DISCUSSION

Ubx distinguishes wings from halteres by regulating the expression of many genes, including those forming part of signaling pathways (Weatherbee et al., 1998; Barrio et al., 1999; Shashidhara et al., 1999; Galant et al., 2002; Mohit et al., 2003; Mohit et al., 2006; Pallavi et al., 2006). We show here that *Ubx* controls the Dpp signaling pathway at different levels and that this regulation contributes significantly to the different sizes of these two appendages. Similar results have also been recently reported (Crickmore and Mann, 2006).

Ubx and the *dpp* pathway

Ubx controls *dpp* transcription, Dpp spread and Dpp activity (Fig. 8A). In the wing disc, *dpp* transcription is activated by Hh signaling (Basler and Struhl, 1994; Capdevila and Guerrero, 1994; Tabata and Kornberg, 1994), and in the haltere pouch *Ubx* attenuates this activation (Mohit et al., 2006) (this report). The haltere *dpp* stripe increases slightly in *pbx* mutations, showing a non-cell-autonomous effect that is perhaps due to an increase in the numbers of cells expressing *hh*. However, the major control of *dpp* expression by *Ubx* is cell autonomous.

Ubx also governs Dpp spread and activity. A mechanism whereby *Ubx* may limit the spread of Dpp is by reducing *dally* expression. The protein encoded by this gene seems to be required to transmit the Dpp protein from cell to cell (Belankaya et al., 2004), and we have shown that *Ubx* downregulates *dally* expression, thus reducing the extent of Dpp activity. *Ubx* retards Dpp spread also by augmenting *tkv* expression (mainly at the A/P boundary and not in the periphery) because high Tkv levels retain the Dpp morphogen (Haerry et al., 1998; Lecuit and Cohen, 1998; Tanimoto et al., 2000) (Fig. 8B,C). In Ubx^{-} clones, the Dpp product can 'travel' more readily through the mutant cells, thus extending Dpp signaling not only within the clone but also in more distal cells (Fig. 8D). If we elevate *tkv* expression in these clones, Dpp spread is checked, preventing the non-cell autonomous and reducing the extent of the cell-autonomous effect on Dpp signaling (Fig. 8E).

The increased *tkv* transcription and the suppression of *dally* expression have a double effect. On the one hand, they reduce Dpp spread; on the other hand, they increase Dpp activity in AB cells. In the wing disc, Hh signaling strongly diminishes Dpp signaling in this domain by inducing *mtv* and *dally* transcription; this reduction is required for the correct pattern of the central region of the wing and for substantial *dally* and *mtv* expression (Tanimoto et al., 2000; Fujise et al., 2001; Funakoshi et al., 2001; Fujise et al., 2003) (this report). By contrast, our results demonstrate that *Ubx* allows high levels of both Dpp and Hh activity in these cells of the haltere disc, and that repression of *mtv* and *dally* is not maintained by this high Dpp signaling. This suggests that a different mechanism for patterning this domain is acting in haltere and wing discs. Finally, the *Ubx* modulation of Dpp activity is complex. Whereas *Ubx* prevents Dpp signaling from downregulating *tkv* or *mtv* in the periphery of the haltere pouch, in *MS1096*; *UAS-tkv*^{QD} haltere discs *dpp-lacZ* expression in the dorsal region is completely suppressed (not shown).

Haltere size control by the Ubx product

Several lines of evidence argue that differences in *dpp*, *tkv* and probably *dally* expression, all of them controlled by *Ubx*, may be instrumental in reducing the size of the haltere disc compared with that of the wing disc: first, *Ubx* downregulates *dpp* transcription, and the increased expression of *dpp* augments the size of haltere discs (see also Mohit et al., 2006); second, *Ubx* increases *tkv* expression, and the ectopic *Ubx* expression, or the elevated *tkv* transcription, reduces wing size (Haerry et al., 1998; Lecuit and Cohen, 1998; Tanimoto et al., 2000) (this report); third, the ectopic expression of *mtv* or *dally* (both increasing Dpp spread) in the posterior compartment of the haltere disc substantially increases its size; fourth, the reduction of *tkv* expression increases the haploinsufficient phenotype of the *Ubx* locus and, conversely, reduced *Ubx* levels partially rescue the small halteres of *dpp* hypomorphic mutations; finally, the increased size of the haltere disc observed in *pbx* or *bx* mutations is partially suppressed if Tkv levels are increased.

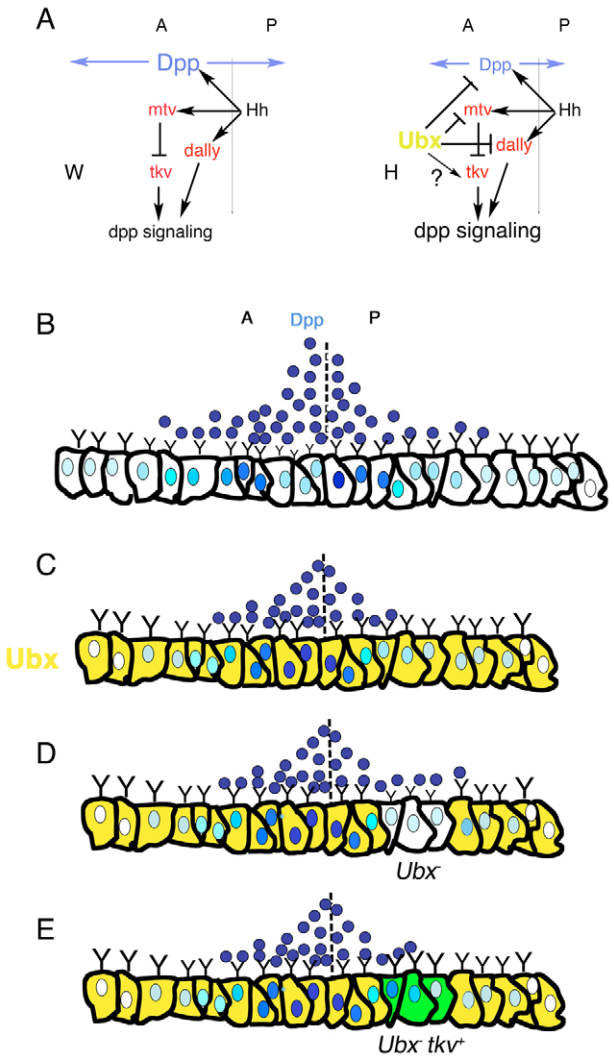

Fig. 8. Model of action of *Ubx* on the Dpp pathway. (**A**) Scheme of the relationship between *Ubx* and different elements of this pathway in the central domains of wing (W) and haltere (H) discs. In the haltere disc, *Ubx* reduces *dpp* transcription, eliminates *dally* and *mtv* expression in most of the pouch and elevates Tkv levels, thus reducing the extent of Dpp spread and activity (but increasing it in AB cells). (**B**,**C**) Cartoons representing the distribution of Dpp (blue balls) in wing (B) and haltere (C) discs, showing less Dpp and less Dpp spread in the latter. The Tkv expression is indicated by the size of the Y symbol and the colours in the nuclei represent Dpp activity. (**D**,**E**) Effect of Ubx^{-} (D) and Ubx^{-} tkv^{+} (E) mutant clones in Dpp signaling. In Ubx^{-} clones the levels of Tkv are reduced so that Dpp can travel through the clone and reach the wild-type cells in more peripheral positions (D). If *tkv* is expressed in these clones, it retains Dpp and reduces the extent of Dpp activity (E).

The control of size by *Ubx* relies on the reduction of *dpp* expression and Dpp spread. Thus, it is not surprising that it involves non-cell-autonomous effects. We have shown that if *Ubx* is removed from the central region of the haltere pouch, this domain is transformed into wing, but the remaining haltere tissue is bigger than expected. This occurs with Gal4 lines that drive a *dsRNA>Ubx* construct or when *Ubx* mutant clones are induced in the anterior compartment of the haltere disc. These results suggest that Dpp spread is increased within the mutant region so that more Dpp reaches the distal haltere domain. As a result, differences in Dpp activity between adjacent cells extend over a larger domain, and both the region that is transformed into wing and the tissue that remains as haltere, increase their size. The growth control is, in part, non-cell autonomous, but the differentiation is strictly cell autonomous (Morata and García-Bellido, 1976; Hart and Bienz, 1996; Roch and Akam, 2000). A non-cell autonomous role of *Ubx* on organ size has also been described in the development of the third leg (Stern, 2003). Our observations may explain some previous results: first, in *pbx* and *bx* mutants there is a slight increase in the size of the untransformed compartment compared with wild-type flies (González-Gaitán et al., 1990), perhaps because *dpp* expression is higher. Second, if wing and haltere tissues are confronted in the wing disc of *Contrabithorax* mutations (which partially transform wing into haltere), the haltere (transformed) tissue is also bigger than expected (González-Gaitán et al., 1990), possibly because *dpp* expression and spread are increased. Third, by changing the activity of *Ubx* during development with the temperature-sensitive bx^1 mutation, halteres bigger than normal are observed (Sánchez-Herrero and Morata, 1983), maybe because the initial growth (wing-like) and the posterior haltere differentiation are relatively uncoupled. However, although we stress the role of the Dpp pathway in regulating the size of the haltere, we are aware that other factors are also likely to contribute to this effect. For instance, *wingless* is not expressed in the posterior compartment of the haltere disc, and this absence has been correlated with its small size (Weatherbee et al., 1998).

It is unclear how these effects on the Dpp pathway are translated into a reduction in disc size as cell size is similar in both discs (Roch and Akam, 2000). Recently, Rogulja and Irvine (Rogulja and Irvine, 2005) have proposed a model to account for the proliferation in the wing disc. This model proposes that differences in Dpp signaling in the medial and lateral regions of the wing disc induce non-cell-autonomous proliferation for a short time. A similar mechanism may exist in the proliferation of the haltere discs because both discs give rise to homologous structures and rely on similar patterning cues. In the haltere disc, both the lower amount of Dpp synthesized and its lower spread result in a more narrow and sharp Dpp activity gradient. We have shown that a gradient of Dpp-GFP signal is established in medial regions of the wing but not the haltere discs. This will extend the differences in Dpp signaling between adjacent cells further in the wing disc, perhaps allowing for more cells to enter division at early larval stages. Madhavan and Schneidermann (Madhavan and Schneidermann, 1977) reported a slight delay in cells of the haltere disc reassuming division after embryogenesis compared with the wing disc, and a somewhat bigger cell-doubling time at the beginning of the second instar. Previous results indicated that the variation in clone size is similar in haltere or wing discs throughout larval stages (Morata and García-Bellido, 1976); in this experiment, however, it was assumed that each haltere cell secreted one trichome whereas a later study showed that haltere cells can secrete more than one (Roch and Akam, 2000). Our results indicate that *Ubx* does not seem to delay cell division autonomously, and that it is necessary to mutate a big region of the haltere disc to observe a clear size difference.

In the grasshopper, the increased expression of *dpp* in the metathoracic legs has been suggested to account for the larger size of these appendages (Niwa et al., 2000), and we propose that changes in *dpp* transcription and Dpp spread underlie size differences between halteres and wings. The regulation of morphogen levels and of proteins that limit the movement of the ligand (such as Tkv and Dally) by Hox genes may be a general mechanism used in evolution to differentiate size in homologous organs within a certain animal, or between homologous organs in different species. Because the Dpp pathway also controls pattern, *Ubx* may differentiate the size and pattern in halteres and wings, coordinating both processes by a single mechanism through the change in the amount and distribution of the Dpp product.

Note added in proof

Similar results to those described here have been recently reported by Makhijani, K., Kalyani, C., Srividya, T. and Shashidhara, L. S. Modulation of Decapentaplegic morphogen gradient during haltere specification in *Drosophila*. *Dev. Biol.* (in press).

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Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/133/22/4495/DC1

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