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msh specifies dorsal cell fate in the *Drosophila* wing

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SUMMARY

***Drosophila* limbs develop from imaginal discs that are subdivided into compartments. Dorsal-ventral subdivision of the wing imaginal disc depends on *apterous* activity in dorsal cells. Apterous protein is expressed in dorsal cells and is responsible for (1) induction of a signaling center along the dorsal-ventral compartment boundary (2) establishment of a lineage restriction boundary between compartments and (3) specification of dorsal cell fate. Here, we report that the homeobox gene *msh* (*muscle segment homeobox*) acts downstream of *apterous* to confer dorsal identity in wing development.**

Key words: *msh*, *apterous*, Differentiation, Identity, Selector gene, *Drosophila melanogaster*, Wing, Dorsoventral patterning

INTRODUCTION

Drosophila limbs are subdivided into adjacent cell populations, known as compartments (Garcia-Bellido et al., 1973). Compartments are specified by localized expression of transcription factors. The homeodomain proteins Engrailed and Invected specify posterior identity (García-Bellido and Santamaria, 1972; Morata and Lawrence, 1975; Tabata et al., 1995; Zecca et al., 1995). The LIM-homeodomain protein Apterous (Ap) confers dorsal identity (Diaz-Benjumea and Cohen, 1993; Blair et al., 1994). The genes that encode these transcription factors are called selector genes because their activities confer compartment-specific properties. Three distinct features of compartments have been shown to depend on selector gene activity. First, they control segregation of the two cell populations to prevent intermingling of cells at the compartment boundary. Second, they establish signaling centers at the compartment boundaries. Third, they specify compartment-specific cell differentiation.

Selector genes act in different ways in anterior-posterior (AP) and dorsal-ventral (DV) subdivision of the wing. AP subdivision of *Drosophila* limbs is mediated by the activity of the *engrailed* and *invected* genes (García-Bellido and Santamaria, 1972; Morata and Lawrence, 1975; Tabata et al., 1995; Zecca et al., 1995). *engrailed* and *invected* are responsible for all three compartment-specific properties. Posterior cells lacking *engrailed* and *invected* are able to cross the AP compartment boundary and they differentiate anterior structures. When located in the posterior compartment, mutant cells interact with normal posterior cells to induce an ectopic signaling center. *engrailed* and *invected* have overlapping although distinct functions in this process. Posterior cells lacking *engrailed* alone have milder defects than cells lacking both genes, suggesting *engrailed* and *invected* have partially overlapping functions. However, misexpression of *engrailed* or *invected* alone in anterior cells revealed distinct activities (Simmonds et al., 1995). Misexpression of *engrailed* in anterior cells induced an ectopic signaling center and a change in the mixing properties of the cells, but it caused only a mild defect in compartment identity. In contrast, misexpression of *invected* in anterior cells only induced a change in compartment identity. Thus, although both *engrailed* and *invected* are required to specify posterior cell fate, *invected* seems to play a stronger role in this process.

DV subdivision of the *Drosophila* wing is mediated by the activity of *apterous* in dorsal cells (Diaz-Benjumea and Cohen, 1993; Blair et al., 1994). *apterous* activity is required and sufficient for locating the signaling center along the DV compartment boundary, for maintaining the lineage restriction boundary and for conferring dorsal cell fate. Dorsal cells lacking *apterous* activity or ventral cells misexpressing *apterous* induce an ectopic signaling center and are able to cross the DV lineage restriction boundary. Cells expressing *apterous* differentiate dorsal structures and cells lacking *apterous* differentiate ventral structures. Here we show that *apterous* confers dorsal identity through regulation of the homeobox gene *muscle segment homeobox* (*msh*). *msh* is expressed in dorsal cells in the embryonic neuroectoderm and muscle precursors (D'Alessio and Frasch, 1996; Isshiki et al., 1997; Lu et al., 2000). In the wing disc, *msh* is expressed in dorsal cells under the control of Apterous activity. *msh* is both necessary and sufficient to confer dorsal fate in wing development. *msh* is the gene affected by the dominant mutation *Dorsal wing* (Tiong et al., 1995).

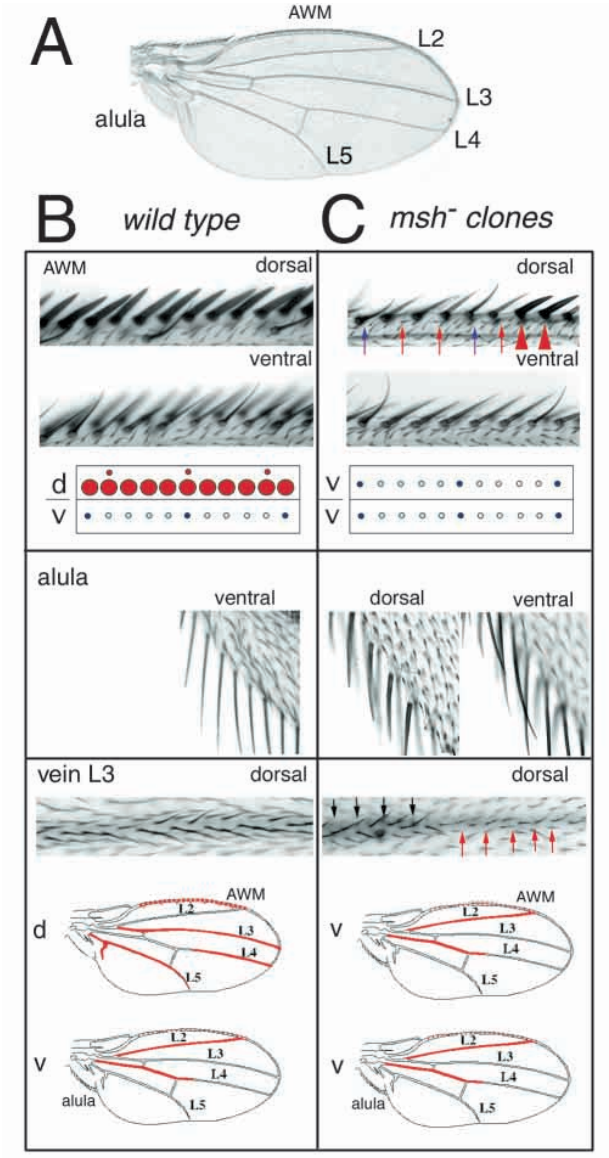

Fig. 1. Effects of removing *msh*: symmetric ventral-ventral wings. (A) Cuticle preparation of a wild-type wing. AWM, anterior wing margin; L2-L5, longitudinal veins 2-5. (B,C) Detailed views of dorsal structures of the wing in (B) wild type and (C) *msh* mutant clones. (Upper panels) Anterior wing margin (AWM); (center panels) alulas; (lower panels) veins. (B) In the wild type the AWM differentiates three rows of bristles. Two are dorsal (d); a row of thick mechanosensory bristles adjacent to the compartment boundary and a row of thin curved chemosensory bristles. The ventral row (v) is composed of thin bristles interspersed with chemosensory bristles in every fifth position. A schematic representation of the AWM is shown below. Red circles denote dorsal bristles, big circles indicate mechanosensory bristles and small circles indicate chemosensory bristles. Filled circles denote the chemosensory bristles located in the ventral surface, and open circles the mechanosensory bristles. (Center panel) The alula has a single row of bristles on the ventral surface and no dorsal bristles (not shown). (Bottom panel) Magnification of the dorsal side of vein L3. Corrugation of the L3 vein is asymmetric on dorsal (d) and ventral (v) surfaces of the wild-type wing. The corrugated surface (indicated in red in the diagrams at bottom) consists of 2-3 rows of more darkly pigmented cells. The opposite surface consists of one row of cells. In wild-type wings, veins L3, L5 and the distal part of L4 are corrugated dorsally and veins L2 and proximal L4 are corrugated ventrally. (C) Mutant clones were generated in f^{36} *hs-FLP (I); FRT 82* $msh^{\Delta 68}$*/FRT 82 P(f+)* larvae. *msh* mutant cells were marked with *forked*. In the AWM small arrows indicate the clone. The blue arrows indicate chemosensory bristles and large arrowheads indicate dorsal bristles outside the clone. A schematic representation of the AWM mutant for *msh* is shown below. Both surfaces differentiate ventral bristles (v). (Center panel) Magnification of an alula covered with clones mutant for *msh* shows that both dorsal and ventral surfaces differentiate bristles. (Lower panel) Magnification of the dorsal side of vein L3 shows part of the clone mutant for *msh* (red arrows); wild-type cells in the vein are indicated by black arrows. Note the transition from dorsal to ventral corrugation as shown in the diagram at the bottom.

MATERIALS AND METHODS

Drosophila expression constructs and strains

Fly strains for Gal4-dependent expression of *apterous*, *fringe* and *dLMO* have been described previously (Milán et al., 1998; Milán and Cohen, 1999). ap^{ugo35} is a null allele of *apterous* (Cohen et al., 1992). ap^{gal4} is a P-element insertion in the *apterous* locus (Calleja et al., 1996). *C765-Gal4* was described by Gomez-Skarmeta et al. (Gomez-Skarmeta et al., 1996). ptc^{gal4} was described by Hintz et al. (Hintz et al., 1994). $msh^{\Delta 68}$ and *uas-msh* are described in Isshiki et al. (1997). $msh^{lacZ\Delta 89}$, referred to in the text as *msh-lacZ*, is an imprecise excision of *P{lacZ}rH96*. The 5′ end, including the *lacZ* coding region, is still present (Isshiki et al., 1997). Dlw^{1} is a dominant allele (Tiong et al., 1995). Dlw^{3} is a recessive lethal (Tiong et al., 1995). Dlw^{4} is associated with transposition Tp(3R) 99B1,2; 100EF; 3R heterochromatin.

Antibodies

Anti-dLMO was raised in rats (Milán et al., 1998); rabbit anti-β-gal (Cappel).

Genotypes of larvae used for genetic mosaic analysis

f^{36} *hs-FLP (I); FRT 82* $msh^{\Delta 68}$*/FRT 82 P(f+).* Clones were marked in the adult wings by the *forked* phenotype. Clones were induced by heat shock at 38°C for 1 hour in second instar larvae (60 hours after egg laying).

RESULTS AND DISCUSSION

Distinct patterning elements in dorsal and ventral compartments

Four structural features distinguish the dorsal and ventral surfaces of the adult wing: bristle morphology in the anterior wing margin; the presence or absence of bristles in the alula; the surface on which the veins are corrugated; and fourth, the location of certain sensory organs (Fig. 1B).

The anterior wing margin (AWM; Fig. 1A) is composed of three rows of bristles, two located in the dorsal surface and one in the ventral (Fig. 1B). The dorsal wing margin differentiates a row of thick, densely aligned, mechanosensory bristles and a second row of thinner, curved, chemosensory bristles. The dorsal AWM produces one chemosensory bristle per five mechanosensory bristles. The ventral row is composed of thin bristles interspersed with chemosensory bristles in every fifth position.

The alula is located in the posterior compartment (Fig. 1A). It produces a single row of long thin bristles along the margin

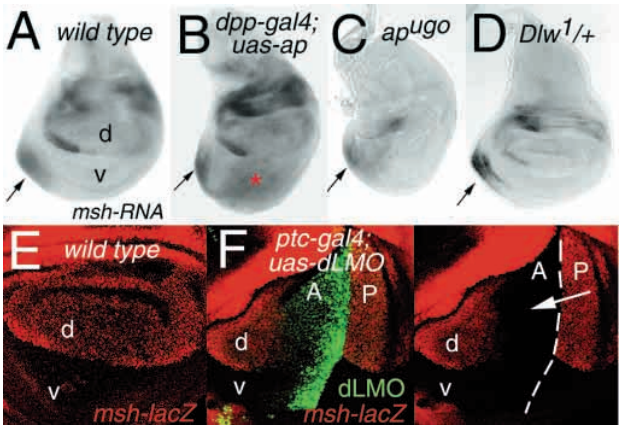

Fig. 2. Apterous regulates *msh* expression. (A-D) *msh* expression in third instar wing discs using an *msh* antisense RNA probe. d, dorsal; v, ventral. (A) Wild-type. Levels of *msh* expression are low in the dorsal compartment of the wing pouch, except along the dorsal anterior wing margin where expression is higher. In addition, a small patch of *msh* expressing cells is observed in the ventral compartment (in the anterior mesopleura, arrow) and in the dorsal notum. (B) *dpp-Gal4 UAS-apterous* disc. *dpp-Gal4* is expressed adjacent to the AP boundary in the anterior compartment. Ectopic expression of *msh* in the ventral compartment is indicated by a red star. The level is comparable to the low dorsal level. Note the difference between the ectopic *msh* expressing tissue and the normal ventral tissue adjacent to the anterior mesopleura (arrow). (C) *apterous* mutant disc. ap^{ugo} is a null allele. Expression of *msh* is lost in the dorsal compartment, but not in the anterior mesopleura (arrow) and part of the notum. (D) $Dlw^{1}/+$ disc. *msh* expression is lower in the dorsal wing pouch. In situ hybridizations to *msh* were done in parallel in A and D. (E) *msh-lacZ* expression in a wild-type late third instar wing disc visualized by anti-β-gal (red). (F) *msh-lacZ* expression in a wing disc expressing dLMO under $patched^{Gal4}$ control. $patched^{Gal4}$ is expressed in anterior cells adjacent to the AP boundary and directs high levels of dLMO expression (green). Endogenous dLMO is expressed at moderate levels in dorsal cells and at low levels in ventral cells. (Right) Repression of *msh-lacZ* in the $patched^{Gal4}$ domain is indicated by an arrow. A, anterior; P, posterior.

on the ventral surface. The dorsal surface of the alula lacks bristles (Fig. 1B).

The adult wing differentiates five longitudinal veins. L1 is located on both dorsal and ventral sides of the wing margin and L2-L5 veins are located in the wing blade (Fig. 1A). Veins L2-L5 are asymmetrical on the dorsal and ventral surfaces of the wing. One side contains more rows of tightly packed cells ("corrugated vein"). The opposite side is thinner ("ghost vein"). Corrugated veins consist of three rows of strongly pigmented and densely packed cells. Ghost veins consist of a single row of cells. Longitudinal veins L3, L5 and the distal tip of L4 are dorsally corrugated. Veins L2 and proximal L4 are ventrally corrugated (illustrated at bottom of Fig. 1B).

msh is required to confer dorsal identity

The *msh* gene belongs to the *msh/Msx* family of homeobox genes involved in dorsal cell fate specification in the *Drosophila* neuroectoderm (D'Alessio and Frasch, 1996; Isshiki et al., 1997). As *msh* is expressed in the dorsal compartment of the wing disc (D'Alessio and Frasch, 1996; Lu et al., 2000), we investigated whether *msh* is also involved in dorsal identity specification in the *Drosophila* wing. For this

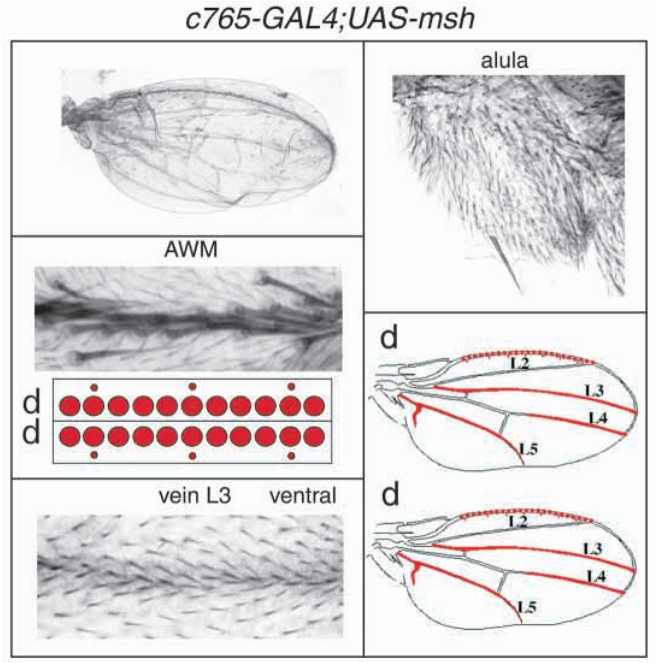

Fig. 3. Ectopic *msh* produces symmetrical dorsal-dorsal wings. *c765-Gal4/+; UAS-msh/+* wing. All features are of dorsal identity. In the anterior wing margin each surface differentiates two rows of bristles: a row of densely packed mechanosensory bristles and a row of chemosensory bristles. The pattern of vein L3 corrugation, shown in the diagram, is completely dorsal (d) on both surfaces. The alula differentiates almost no bristles in the ventral surface.

purpose, we generated *msh* mutant clones in the wing and assessed the DV identity of the bristles located along the AWM, in the alula and the DV corrugation of longitudinal veins in mutant cells. Clones mutant for *msh* had no aberrant phenotype in the ventral surface of the wing. When mutant for *msh*, the dorsal anterior wing margin differentiated ventral bristles. A single row of thin bristles interspersed with chemosensory bristles in every fifth position was observed (Fig. 1C: red arrows indicate ventral mechanosensory bristles; blue arrows indicate interspersed chemosensory bristles). Thus, the anterior wing margin differentiated a ventral pattern of bristles symmetrically on both surfaces.

When covered with mutant cells, the dorsal surface of the alula differentiated bristles (compare Fig. 1B and C). This reflects transformation to a ventralized cell fate. Absence of *msh* activity also induced a change in the pattern of corrugation of the longitudinal veins. In wild-type wings, veins L2 and L4 differentiated as 'ghost veins' on the dorsal surface. When mutant for *msh*, these veins are corrugated and differentiate three rows of strongly pigmented cells (not shown), thus mimicking a ventral-like pattern. Veins L3 and L5 were corrugated on the dorsal surface (black arrows in Fig. 1C, bottom). When mutant for *msh*, they lost pigmentation and consisted of a single row of aligned cells (red arrows in Fig. 1C). Thus veins differentiated ventral characteristics in the dorsal surface when mutant for *msh* (Fig. 1C, bottom). We conclude that *msh* is required in the dorsal compartment of the *Drosophila* wing to confer dorsal cell identity. In the absence of *msh*, symmetric wings were observed which differentiated ventral characteristics on both surfaces (Fig. 1C).

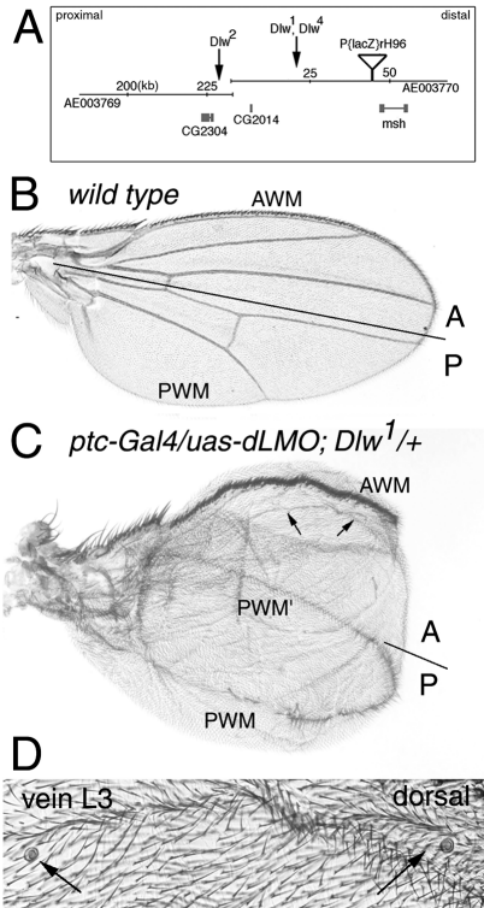

Fig. 4. *Dorsal wing* (*Dlw*) alleles. (A) Genomic organization of the *msh* gene. The *msh* transcript consists of 2 exons spanning approx. 10 kb. Arrows indicate breakpoints associated with *Dlw* alleles. Other predicted genes in the region are indicated. *msh* expression in the wing discs was monitored using $msh^{lacZ\Delta 89}$, an imprecise excision line of *P{lacZ}rH96* that keeps the *lacZ* reporter gene. (B) Wild-type wing. AWM, anterior wing margin. PWM, posterior wing margin. The AP compartment boundary is shown by a line. (C,D) $patched^{Gal4}$*/uas-dLMO;* Dlw^{1}*/+* wing. dLMO is overexpressed in the anterior compartment. dLMO inhibits Apterous activity. PWM′, ectopic posterior wing margin induced in the dorsal surface along the AP compartment boundary. The wing is overgrown owing to ectopic expression of Wg along the $patched^{Gal4}$ stripe. Dorsal and ventral surfaces do not contact normally. (D) Magnification of the dorsal side of vein L3 of the wing shown in C. In a wild-type wing, vein L3 is corrugated and has three campaniform sensillae on the dorsal surface (see Fig. 1). In the Dlw^{1}/+ wing, campaniform sensillae (arrows in C and D) and corrugation are characteristic of the dorsal surface of vein L3. Their appearance in the Dlw^{1}/+ wing indicates that Msh activity is present, despite the loss of Apterous activity.

msh is a target gene of Apterous sufficient to specify dorsal fate

Apterous is expressed in dorsal cells and is required to confer dorsal cell identity. We therefore determined whether *msh* expression in the dorsal compartment is regulated by Apterous activity. *msh* mRNA and *msh-lacZ* reporter genes were expressed in the dorsal compartment of the wing disc (Fig. 2A,E). *msh* mRNA was expressed at a low level throughout the dorsal compartment, except in the region of the anterior margin where it was expressed at higher level. Ectopic expression of Apterous in the ventral compartment under control of dppGal4 induced ectopic expression of *msh* mRNA at a level comparable to the overall low dorsal level (asterisk, Fig. 2B). In *apterous* mutant discs *msh* expression was lost from dorsal cells of the reduced wing pouch (Fig. 2C), but expression in the anterior mesopleura (arrows) and hinge region remained. Finally, overexpression of dLMO, a repressor of Apterous activity in the *Drosophila* wing (Milán et al, 1998), repressed expression of the *msh-lacZ* reporter gene (compare Fig. 2E and F). These results indicate that *msh* is indeed a target of Apterous.

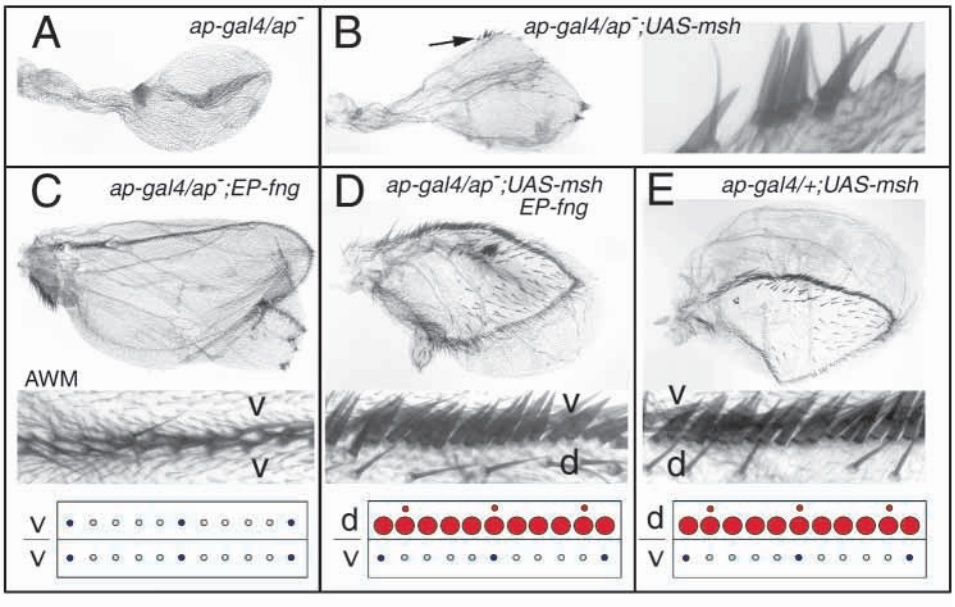

Fig. 5. *msh* restores dorsal identity in the absence of Apterous. (A) ap^{Gal4}/ap^{UGO35} wing. (B) ap^{Gal4}/ap^{UGO35} *uas-msh* wing. The arrow indicates wing margin bristles of dorsal identity shown at higher magnification on the right. (C) ap^{Gal4}/ap^{UGO35} *EP-fng* wing. The anterior wing margin (AWM) differentiated ventral-type bristles in both dorsal and ventral surfaces (middle, illustrated below). (D) ap^{Gal4}/ap^{UGO35} *EP-fng/uas-msh* wing. Bristles in the dorsal AWM had dorsal identity. AWM bristles were more densely packed than in wild type. Note also the reduced size of the dorsal compartment and the ectopic bristles in the wing blade. (E) ap^{Gal4}/+; *uas-msh* wing. The reduced size of the dorsal compartment, the ectopic bristles in the wing blade and increased bristle density in the AWM were similar to those in D. This is presumably caused by strong overexpression of *msh* under ap^{Gal4} control. Weaker overexpression using *c765-gal4* did not produce these defects (see Fig. 3). Ectopic bristles in the A compartment are mechanosensory bristles; those located in the P compartment are thin bristles. The DV identity of the posterior bristles could not be determined.

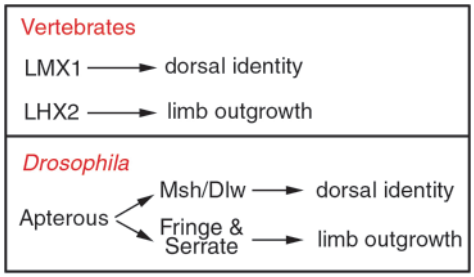

Fig. 6. Conclusions. In vertebrate limbs two related *apterous* genes, *LMX1* and *LHX2*, have been shown to act separately to define dorsal identity and limb outgrowth. In the *Drosophila* wing, Apterous induces limb outgrowth by controlling DV signaling and specifies dorsal identity. Dorsal identity is defined by the Apterous target gene *msh/Dlw.*

We next investigated whether ectopic expression of *msh* in the ventral surface had any effect on the differentiation of ventral structures. For this purpose we made use of the Gal4 driver *c765-Gal4*, which is ubiquitously expressed in the wing primordium (Gomez-Skarmeta et al., 1996). In *c765-Gal4; uas-msh* flies, the anterior wing margin differentiated dorsal-type bristles arranged in a dorsal-like pattern on both surfaces (Fig. 3). The pattern of veins was symmetric, and had a dorsal corrugation pattern on both surfaces. Finally, few bristles were recovered on the ventral surface of the alula, suggesting transformation to a dorsal fate. Thus, ectopic expression of *msh* in the ventral surface is sufficient to confer dorsal identity on ventrally located cells with respect to all characteristics examined.

Dorsal wing alleles may be regulatory mutants of the *msh* gene

The results presented thus far indicate that *msh* is necessary and sufficient to specify dorsal identity in the *Drosophila* wing. Tiong et al. (Tiong et al., 1995) identified a dominant mutation Dlw^1 that showed partial dorsalization of the AWM. Both surfaces of Dlw^1/+ AWMs had dorsal bristles, similar to what we have observed when *msh* was ectopically expressed in the ventral compartment. Interestingly, *Dlw* alleles are associated with breakpoints located 30-90 kb upstream of the *msh* gene (Fig. 4A), raising the possibility that *Dlw* alleles may be regulatory mutants of *msh*. Indeed, a lethal allele of *msh*, $msh^{\Delta 68}$, proved to be lethal when heterozygous with Dlw^1 and the recessive lethal alleles Dlw^3 and Dlw^4. Dorsal clones mutant for Dlw^3 differentiated ventral structures (Tiong et al., 1995).

The dominant phenotype of Dlw^1 might be due to Apterous independent expression of the *msh* gene in the wing pouch. This view is supported by the observation that dorsal cells lacking Apterous activity in a Dlw^1/+ wing differentiated dorsal structures despite the loss of Ap activity (Fig. 4C,D; genotype: *ptc-Gal4/UAS-dLMO;* Dlw^1/+). *ptc-Gal4* directs high levels of expression of transgenes in the region between the AP compartment boundary and vein 3 and low levels of expression between vein 3 and the anterior wing margin. In otherwise wild-type wings expressing dLMO under *ptc-Gal4* control, dorsal vein 3 adopted ventral identity. Vein 3 lost corrugation and the campaniform sensillae that normally decorate it (not shown). Campaniform sensillae and corrugation were restored on the third vein in *ptc-Gal4/UAS-dLMO;* Dlw^1/+ wings indicating that these cells had dorsal identity. These results support the proposal that the *msh* gene may be expressed in an Apterous-independent manner in Dlw^1 wings.

We have compared *msh* mRNA levels in wild-type and Dlw^1/+ wing discs. *msh* mRNA levels were reduced throughout the wing pouch in discs heterozygous for Dlw^1 (compare Fig. 2A and D). Owing to the low levels of expression in the mutant discs it was not possible to evaluate whether there was significant ectopic expression in ventral cells. We note that the low level of *msh* expression in the Dlw^1 background may explain the loss of function characteristics exhibited by the Dlw^1 allele in homozygous mutant clones (Tiong et al., 1995). Dlw^1/Dlw^1 mutant clones located in the dorsal surface of the wing differentiated ventral structures. Thus, Dlw^1 caused a dominant transformation of ventral cells to dorsal identity when heterozygous and an opposite transformation of dorsal cell to ventral identity when homozygous mutant in clones.

Interestingly, the dominant mutation *Drop*, which affects eye development, has been recently shown to be a gain-of-function allele of *msh* (Mozer, 2001). *Drop* mutants contain lesions in the same region as *Dlw* mutants (i.e. upstream of the *msh* transcription start site) and ectopic expression of *msh* in the eye phenocopies the *Drop* phenotype. However, Mozer (2001) was not able to find detectable misexpression of *msh* in *Drop* mutants. Thus, undetectably low levels of *msh* misexpression in eye and wing seem to be associated with the dominant adult phenotypes associated with the *Dlw* and *Drop* alleles of *msh*.

msh confers dorsal identity without affecting dorsal signaling properties

Apterous activity is required to confer dorsal identity and dorsal-type signaling properties. Fringe and Serrate expression in dorsal cells induce a cascade of short-range interactions between dorsal and ventral compartments that lead to the expression of the organizing molecule Wingless along the DV compartment boundary (reviewed by Irvine and Vogt, 1997; Strigini and Cohen, 1999). The results reported above suggest that *msh* confers dorsal identity without affecting DV signaling. In order to verify that this is the case, we have analyzed the ability of *msh* to restore dorsal identity and dorsal signaling properties in the absence of Apterous activity.

In ap^{Gal4}/ap^{UGO35} flies, the wing margin is reduced and the wing is considerably smaller than normal owing to reduced Apterous activity (compare Figs 5A and 1A). In the example shown, the margin was absent entirely. When present, margin bristles have ventral identity in this genotype. Expression of *msh* in ap^{Gal4}/ap^{UGO35};*uas-msh* flies did not restore outgrowth of the wing. The few margin bristles observed in the dorsal surface of these wings had dorsal identity (Fig. 5B). Growth and wing margin formation can be restored in the ap^{Gal4}/ap^{UGO35} mutant background by expression of Fringe under ap^{Gal4} control (genotype: ap^{Gal4}/ap^{UGO35}; *EP-fng*, see also Milán and Cohen, 1999; O'Keefe and Thomas, 2001). In these wings, both surfaces differentiated ventral structures: the AWM and the alula differentiated ventral bristles on both surfaces and the pattern of vein corrugation was ventral (Fig. 5C). Co-expression of *msh* with *EP-fringe* conferred dorsal differentiation in the bristles of the dorsal AWM in these

rescued wings (Fig. 5D). We also noted that overexpression of *msh* in dorsal cells reduced the size of the dorsal wing pouch, induced differentiation of ectopic bristles in the wing blade and affected vein differentiation. This was also observed in ap^{Gal4}/+; *uas-msh*/+ flies (Fig. 5E) and presumably reflect defects caused by higher than normal Msh levels in dorsal cells. Note that the endogenous levels of *msh* expression in the wing pouch are quite low (Fig. 2A). These results suggest that developmental regulation of Msh protein levels may be crucial for proper wing development and differentiation of patterning elements. All these results indicate that *msh* confers dorsal identity without affecting dorsal signaling properties.

Concluding remarks

Two *apterous* homologues, *Lmx1* and *Lhx2*, have been implicated in vertebrate limb development (Fig. 6). Interestingly, these two genes appear to have separable functions in conferring dorsal identity and limb outgrowth. *Lmx1* is expressed in the dorsal compartment of vertebrate limbs and is necessary and sufficient to confer dorsal identity (Riddle et al., 1995; Vogel et al., 1995). *Lhx2* induces *Radical-fringe* expression in the apical ectodermal ridge, which is required for limb outgrowth (Laufer et al., 1997; Rodriguez-Esteban et al., 1997; Rodriguez-Esteban et al., 1998). This contrasts with the situation in *Drosophila* where Apterous is responsible for both dorsal fate specification and for establishing the Fringe-dependent signaling center at the DV boundary (Diaz-Benjumea and Cohen, 1993; Blair et al., 1994; Irvine and Wieschaus, 1994; Diaz-Benjumea and Cohen, 1995; Kim et al., 1995; Panin et al., 1997). Our findings implicate *msh* as the principle target gene through which Apterous confers dorsal cell fate. *msh* is necessary and sufficient to induce dorsal cell fate, but has no role in DV boundary signalling. Intriguingly, the *msh*/*Msx* family of homeobox genes are also differentially expressed along the DV axis of the embryo and *msh* is required in the *Drosophila* neurectoderm to specify dorsal fate (Isshiki et al., 1997).

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