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 (Garcia-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 (Garcia-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 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 f0 hs-FLP (I); FRT 82 mshD68/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). apVpos35 is a null allele of apterous (Cohen et al., 1992). aps element is a P-element insertion in the apterous locus (Callega et al., 1996). C765-Gal4 was described by Gomez-Skarmeta et al. (Gomez-Skarmeta et al., 1996). ptcGal4 was described by Hindz et al. (Hintz et al., 1994). mshD68 and uas-msh are described in Isshiki et al. (1997). mshD68 and ptds, referred to 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). Dhw2 is a dominant allele (Tiong et al., 1995). Dhw5 is a recessive lethal (Tiong et al., 1995). Dhw5 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
f0 hs-FLP (I); FRT 82 mshD68/FRT 82 P(f+) larvae. 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.
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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. apterous 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) Dl1/+ 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 patchedGal4 control. patchedGal4 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 patchedGal4 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 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).
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 activity.
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 *Dhw* that showed partial dorsalization of the AWM. Both surfaces of *Dhw*+ wing discs had dorsal bristles, similar to what we have observed when *msh* was ectopically expressed in the ventral compartment. Interestingly, *Dhw* alleles are associated with breakpoints located 30-90 kb upstream of the *msh* gene (Fig. 4A), raising the possibility that *Dhw* alleles may be regulatory mutants of *msh*. Indeed, a lethal allele of *msh*, *msh(58)*, proved to be lethal when heterozygous with *Dhw* and the recessive lethal alleles *Dlw* and *Dlw*·. Dorsal clones mutant for *Dlw* differentiated ventral structures (Tiong et al., 1995).

The dominant phenotype of *Dhw* 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 *Dhw*+ wing differentiated dorsal structures despite the loss of Ap activity (Fig. 4C,D; genotype: *ptc-Gal4/UAS-dLMO; Dhw*+). *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*+ 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* wings.

We have compared *msh* mRNA levels in wild-type and *Dlw*+ wing discs. *msh* mRNA levels were reduced throughout the wing pouch in discs heterozygous for *Dlw* (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* background may explain the loss of function characteristics exhibited by the *Dlw* allele in homozygous mutant clones (Tiong et al., 1995). *Dlw*/+ mutant clones located in the dorsal surface of the wing differentiated ventral structures. Thus, *Dlw* 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 specifies dorsal cell fate 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 *apGal4;apUGO35* 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 *apGal4;apUGO35; 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 *apGal4; apUGO35* mutant background by expression of Fringe under *apGal4* control (genotype: *apGal4;apUGO35; 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 apGal4/+; 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 (Lauffer 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 neuroectoderm to specify dorsal fate (Isshiki et al., 1997).

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REFERENCES


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