msh specifies dorsal cell fate in the Drosophila wing

Marco Milán1,*, Ulrich Weihe1,*, Stanley Tiong2,‡, Welcome Bender2 and Stephen M. Cohen1,§

1European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany
2Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA
*Present address: Exilixis Inc., 170 Harbor Way, PO Box 511, South San Francisco, CA 94083-0511, USA
†These authors contributed equally to the work
‡Author for correspondence (e-mail: cohen@embl-heidelberg.de)

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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 (García-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 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.
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). apterous is a null allele of apterous (Cohen et al., 1992). aptu35 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). ptcGal4 was described by Hintz et al. (Hintz et al., 1994). mshD68 and uas-msh are described in Isshiki et al. (1997). mshlacZ89, 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). Dhw is a dominant allele (Tiong et al., 1995). Dhw is a recessive lethal (Tiong et al., 1995). Dhw 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

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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 (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 f6 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.
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) aipterous mutant disc. ap^{en} 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) Dll^{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 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.

msh restores dorsal identity in the absence of Apterous. (A) apGal4/apUsoG035 wing. (B) apGal4/apUsoG035 uas-msh wing. The arrow indicates wing margin bristles of dorsal identity shown at higher magnification on the right. (C) apGal4/apUsoG035 EP-fng wing. The anterior wing margin (AWM) differentiated ventral-type bristles in both dorsal and ventral surfaces (middle, illustrated below). (D) apGal4/apUsoG035 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) apGal4/apUsoG035 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 apGal4 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.
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\(^d\) that showed partial dorsalization of the AWM. Both surfaces of Dhw\(^d\)/+ AWMs 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\(^68\), proved to be lethal when heterozygous with Dhw\(^d\) and the recessive lethal alleles Dlw\(^2\) and Dlw\(^d\). Dorsal clones mutant for Dlw\(^3\) differentiated ventral structures (Tiong et al., 1995).

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

We have compared msh mRNA levels in wild-type and Dlw\(^d\)/+ wing discs. msh mRNA levels were reduced throughout the wing pouch in discs heterozygous for Dlw\(^d\) (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\(^d\) background may explain the loss of function characteristics exhibited by the Dlw\(^d\) allele in homozygous mutant clones (Tiong et al., 1995). Dlw\(^d\)/Dlw\(^d\) mutant clones located in the dorsal surface of the wing differentiated ventral structures. Thus, Dlw\(^d\) 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 Dhw 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 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 (Lauer 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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REFERENCES


