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Homeotic genes and the regulation of myoblast migration, fusion, and fibre-specific gene expression during adult myogenesis in *Drosophila*

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SUMMARY

We have investigated the roles of homeotic selector genes in the migration and fusion of myoblasts, and in the differentiation of adult muscle fibres of *Drosophila.* Altering intrinsic homeotic identities of myoblasts does not affect their segment-specific migration patterns. By transplanting meso – and metathoracic myoblasts into the abdomen, we demonstrate that the fusion abilities of myoblasts are independent of their segmental identities. However, transplanted thoracic myoblast nuclei are 'entrained' by those of the host abdominal muscles to which they fuse and are unable to 'switch on' a thoracic muscle-specific reporter gene. This process is likely to be mediated by homeotic repression because mis-expression of an abdominal muscle-specific homeotic gene, *Ultrabithorax*, in the thoracic muscles results in the repression of the thoracic muscle-specific reporter gene. Finally, we show that removal of *Ultrabithorax* function specifically from muscle cells of the first abdominal segment, results in the expression of thoracic muscle properties. Many of these functions of homeotic genes in muscle patterning in *Drosophila* could be conserved during myogenesis in other organisms.

Key words: selector gene, myoblasts, migration, fusion, differentiation, founder cell, *Drosophila*, *Ultrabithorax*

INTRODUCTION

Several recent studies have provided clues to the molecular mechanisms that operate during myogenesis (Molkentin and Olson, 1996). The development of muscles to give rise to the precise pattern observed in the mature animal involves the function of myogenic regulatory genes common to many muscle types, and other regulatory elements that act to give each fibre its unique positional property (Donoghue and Sanes, 1994). In vertebrates, four basic-helix-loop-helix (bHLH) protein coding genes, *myoD*, *myogenin*, *myf5* and *mrf4*, act during myogenesis to define the properties of skeletal muscle (Weintraub et al., 1991; Olson and Klein, 1994). The products of these genes regulate myogenesis by interacting with members of the myocyte enhancer factor-2 (MEF2) transcription factor family, and activate the expression of other genes that encode specific structural components of differentiated muscle fibres (Molkentin et al., 1995). In addition, the bHLH myogenic genes have been shown to regulate the process of exit from the cell cycle that is required for myogenesis to proceed (Guo et al., 1995; Halevy et al., 1995). The bHLH genes are themselves regulated through inductive influences from other tissues, by mechanisms that appear to be evolutionarily conserved (Cossu et al., 1996; Molkentin and Olson, 1996).

While these experiments have illuminated the mechanisms of muscle differentiation, far less is known about how specific muscle identities are specified. Transplantation experiments with vertebral precursors have shown that structures that arise from these precursors are intrinsically determined and do not change their properties upon transplantation. However, it is not possible in this situation to distinguish between autonomous and non-autonomous roles of Hox genes in specifying their identity (Olson and Rosenthal, 1994).

Chick/quail chimeras (Ordahl and LeDouarin, 1992) and grafting experiments with cervical somites (Lance-Jones, 1988) suggest that the environment plays a important role in muscle patterning. Other experiments, however, suggest strongly that myoblasts can have heritable, cell-autonomous information about their positional identity (Donoghue et al., 1992; Grieshammer et al., 1992; DiMario et al., 1993; Donoghue and Sanes, 1994).

The molecular mechanisms that operate to specify segmental identity of muscle fibres and the positional identity of each fibre within a segment are beginning to be understood in the fruit fly *Drosophila melanogaster* (Bate, 1993; Abmayr et al., 1995). Studies on the fruit fly have demonstrated two important features: first, the high level of conservation of molecular mechanisms of specification of organ identity and second, the tremendous manipulative advantage that this organism confers in addressing questions of general value. While many aspects of muscle pattern in *Drosophila* appear to be specified by properties intrinsic to the mesoderm, there is now abundant evidence that proper muscle formation also requires that the epidermis and the nervous system send correct inductive signals to pattern many aspects of muscle development (Hooper, 1986; Lawrence and Johnston, 1986; Greig and Akam, 1993; Fernandes et al., 1994; Michelson, 1994; Roy et al., 1997).

We have investigated the functions of homeotic genes during the development of the thoracic and abdominal muscles of the adult fly (Fig. 1). We have previously shown that during pupal development, wing disc-associated myoblasts in the second thoracic segment (T2) migrate out over the pupal epithelium to reach specific muscle formation sites in the thorax (Fernandes and VijayRaghavan, 1993; Fernandes et al., 1994). Is this segment-specific migration process to seek out sites of muscle formation guided by autonomous properties of myoblasts, or are they provided with cues from the epidermis? We show that expression of an abdominal muscle-specific homeotic gene, *Ultrabithorax* (*Ubx*), in the thoracic myoblasts does not affect the migration pattern of these myoblasts.

In a pioneering experiment, Lawrence and Brower (1982) showed that myoblasts from the wing imaginal disc, which normally give rise to the dorsal muscles in T2, can contribute upon transplantation to diverse muscle types in the adult. This would suggest that myoblasts can fuse indiscriminately, independent of their segmental identity, and that many properties of a muscle fibre must be specified by cues external to them. We have recently shown that myoblasts on the wing disc in particular, and in T2 in general, do not express any homeotic selector gene of the Antennapedia (ANTP-C) or bithorax complex (BX-C) (Roy et al., 1997; Fig. 1). Therefore, one possible explanation for the results of Lawrence and Brower is that indiscriminate fusion of wing disc-derived myoblasts was observed because the donor myoblasts did not have any intrinsic homeotic identity, since they were of T2 origin. We therefore tested whether myoblasts expressing a homeotic selector gene, the *Antennapedia* (*Antp*) gene of the ANTP-C complex, can fuse with abdominal myoblasts that express other selector genes such as *Ubx*, *abdominal-A* (*abd-A*) or *Abdominal-B* (*Abd-B*). Our results demonstrate that fusion is independent of myoblast segmental identity.

Although fusion between myoblasts of different segmental origins can take place, however, the pattern of gene expression by the donor nuclei in the host syncitium is affected. We show that transplanted wing disc myoblasts that fuse with abdominal muscles do not express a thoracic muscle-specific actin reporter gene, but do so when they migrate back into the thorax and fuse with developing thoracic muscles. In another experiment, where the abdominal mesodermal selector gene, *Ubx*, is ectopically expressed in the developing mesothoracic muscles, we demonstrate the repression of the thoracic muscle-specific actin reporter gene. Finally, we examine a situation where the mesodermal identity of the first abdominal segment (A1) has been transformed to a thoracic label by removal of *Ubx* function specifically in the A1 mesodermal cells. Here we show that the A1 muscles now express the thoracic muscle-specific actin reporter, as a consequence of the removal of *Ubx*.

We interpret these results with respect to the requirements for the formation of a mature muscle fibre and discuss possible mechanisms within the broader question of how muscle development takes place in vertebrates.

MATERIALS AND METHODS

Myoblast transplantation

Wing and haltere discs were isolated from wandering third instar larvae and the notal region harbouring the imaginal myoblasts dissected as described in Lawrence and Brower (1982). These disc fragments were then transplanted into the abdomen of prepupal hosts as described before (VijayRaghavan et al., 1996). Animals that survived the transplantation process and eclosed were dissected in Ringer's solution and analysed for β-galactosidase activity or GFP expression, as described below.

Fly strains

The Canton-S strain was used as the wild-type control in all experiments unless mentioned, and also as a host strain in transplantation experiments. The *Myosin Heavy Chain-lacZ* (Hess et al., 1989) and the *Actin (88F)-lacZ/Actin (88F)-GFP* strains (Hiromi et al., 1986; Barthmaier and Fyrberg, 1995) were used as donors in transplantation experiments. Although the *Act(88F)* gene was initially thought to be indirect-flight-muscle specific, we find that at least the activity of the *lacZ* reporter gene

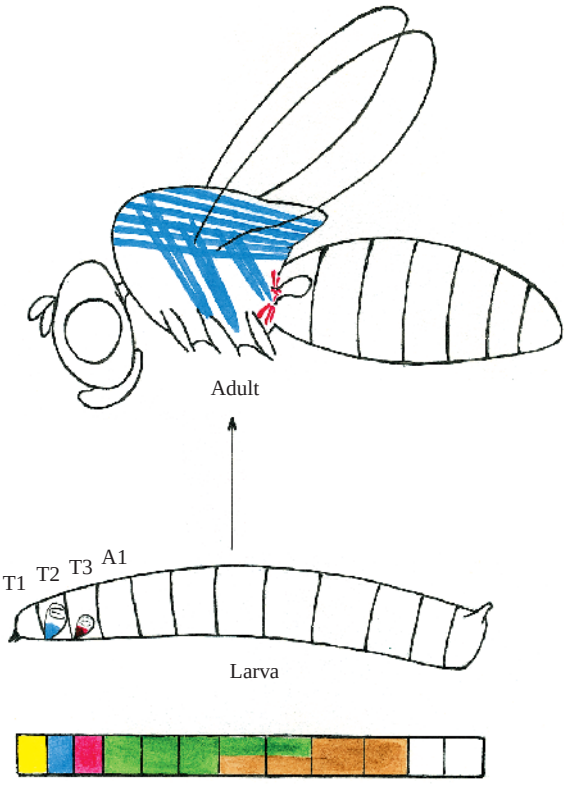

Fig. 1. Diagram illustrating the distribution of adult second (T2) and third thoracic (T3) muscle progenitors in the larva and the types of adult muscles to which they contribute. Myoblasts on the wing disc in T2 of the larva (shown in blue) contribute to the large indirect flight muscles (IFMs) of this segment in the adult (blue muscles in the adult fly), while those on the haltere disc in T3 (shown in crimson) develop into T3-specific adult muscles (crimson muscles in the adult fly). We have investigated the roles of homeotic selector genes in the migration, fusion and differentiation of these myoblasts during adult myogenesis. The normal expression patterns of homeotic selector genes in the somatic muscles in different thoracic and abdominal segments is colour coded and depicted in segmental register below the larva. Expression patterns of these genes in the muscles of the head and terminal abdominal segments have not been determined with clarity, and hence are not shown. Yellow, *Sex combs reduced* (T1); blue, No selector gene expression (T2); crimson, *Antennapedia* (T3); green, *Ultrabithorax* (A1-A5) and brown, *abdominal-A* (A4-A7). The expression patterns of homeotic selector genes shown here has been modified from Bate (1993).

from the *Act(88F)* promoter is seen in all thoracic segments; in specific muscles in T1, T2 and T3 in addition to the indirect flight muscles in T2 (VijayRaghavan et al., 1996). However we have not detected *Act(88F)-lacZ* or *Act(88F)-GFP* expression in any abdominal muscle. Thus the *Act(88F)* promoter is a reporter for adult thoracic muscles, though not all adult thoracic muscles express *Act(88F)* reporter genes. The *UAS-Ubx* strain was kindly provided by A. M. Michelson (Brigham and Women's Hospital, Boston, USA). The X-chromosome *p-GAL4* enhancer-trap insertion, *1151*, was obtained from L. S. Shashidhara (Centre for Cellular and Molecular Biology, Hyderabad, India). The expression pattern of this strain was documented using a *UAS-lacZ* reporter construct (Brand and Perrimon, 1993). The GAL4 driver expresses β-galactosidase from the *UAS-lacZ* reporter gene in the wing disc-associated myoblasts, in these myoblasts during pupal development as they spread over the developing notum, and in mature adult muscles (data not shown). The *twist-lacZ* strain, which contains the regulatory domains of the *twist* gene fused to the bacterial *lacZ*, was kindly provided by F. Perrin-Schmitt and B. Thisse (Strasbourg, France) and was used to follow the migration pattern of the myoblasts. For this experiment, *1151; twist-lacZ* flies were crossed to *UAS-Ubx* flies and the pattern of myoblast migration in the mesothorax on ectopic *Ubx* expression was followed using *twist-lacZ* expression in the pupae of the progeny.

Histochemistry and immunocytochemistry

Histochemical staining for β-galactosidase activity was done according to standard procedures. For analysis of both β-galactosidase and GFP expression, muscle fibres were first briefly fixed in 4% paraformaldehyde in phosphate buffered saline (PBS), lightly stained for β-galactosidase expression till the appearance of blue colour, washed thoroughly with several changes of PBS and then analysed for GFP expression using fluorescence optics (see below). The anti-Ubx monoclonal antibody FP 3.38 was used at a dilution of 1:50 and was a generous gift of R. White (Cambridge University, UK). The antibody reaction was developed using the Vectastain ABC Elite kit (Vector Labs, USA) according to the manufacturer's instructions.

Microscopy

Tissue preparations were either mounted directly in GelTol (Immunon, USA) or 50% glycerol, or dehydrated through ethanol grades, cleared in methyl salicylate and mounted in Canada Balsam. Preparations were viewed using polarised light (unstained muscles), Nomarski optics (stained muscles, imaginal discs and pupal tissue) or epifluorescence optics (for GFP expression in muscles) using 340-380 nm exciting light.

RESULTS

Homeotic genes do not specify migration patterns of myoblasts

During development, myoblasts migrate to muscle formation sites following stereotyped routes. During pupal development in *Drosophila*, adult muscle precursors associated with imaginal discs and nerves migrate out and reach muscle formation sites on the developing adult epidermis, following well-defined segment-specific patterns. In the mesothorax, these precursors migrate out in a broad stream of cells and reach the flight muscle formation sites on the developing dorsal notum (Fernandes and VijayRaghavan, 1993; Fig. 2A). We previously showed that in animals that exhibit a transformation of the metathoracic (T3) epidermis to the likeness of T2 due to a combination of regulatory mutations in the *Ubx* gene, the number and arrangement of T3 muscle

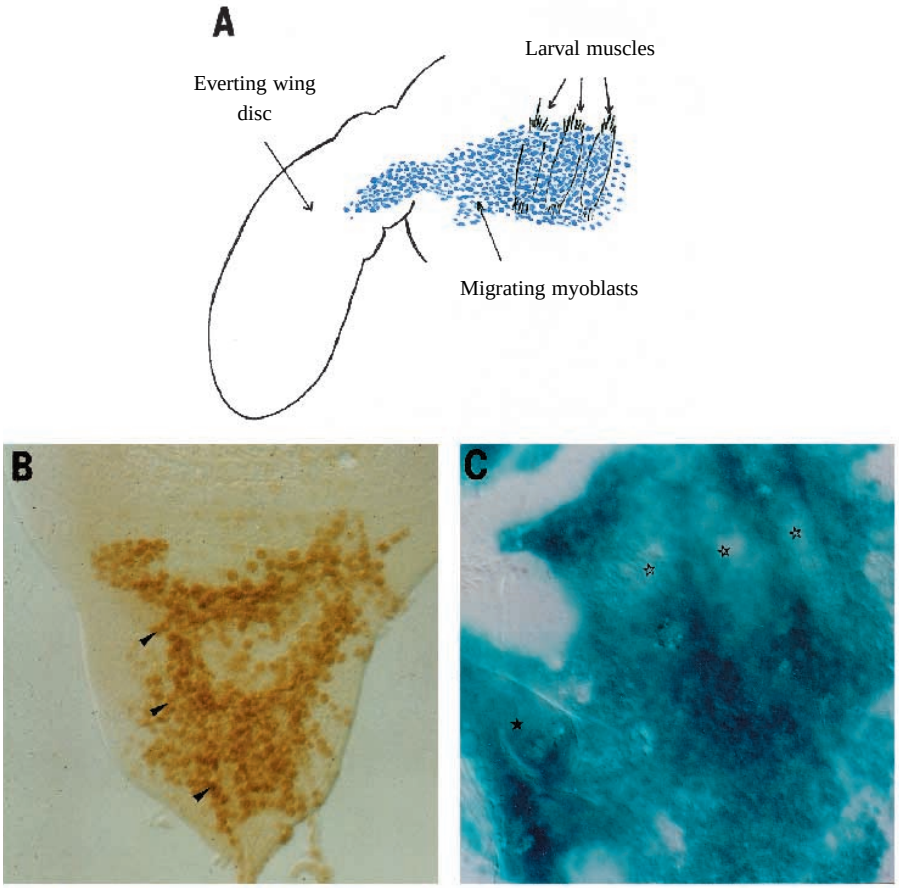

Fig. 2. Mis-expression of *Ubx*, an abdominal muscle identity homeotic gene, in the myoblasts of T2 does not alter their migration pattern. (A) Diagram showing the migration pattern of myoblasts in T2 during early pupal development. The three larval muscles indicated escape histolysis during pupation, and serve as templates for the development of one set of IFMs, the dorsal longitudinal muscles (DLMs). (For further details on the wild-type pattern of myoblast migration see Fig. 8 in Fernandes and VijayRaghavan (1993)). (B) *Ubx* expression in the myoblasts associated with the wing disc in T2 (arrowheads) from the *UAS-Ubx* transgene under the influence of the *1151* GAL4 driver. This pattern of myoblasts on the wing disc is similar to the wild-type pattern (see Fig. 3 in Fernandes et al. (1994) for wild-type pattern of myoblasts on the wing disc). (C) Migration pattern of *Ubx*-expressing (see Materials and methods) myoblasts in T2 revealed by expression of the *twist-lacZ* reporter gene (blue staining), which is expressed in all undifferentiated myoblasts. The diffuse staining is due to the cytoplasmic localisation of the β-galactosidase product in the myoblasts. The filled asterisk marks the position where one set of IFMs, the dorsoventral muscles (DVMs), will develop. The open asterisks mark the positions of the three remnant larval muscles that serve as templates for the development of the DLMs. The myoblasts are seen over the larval templates. This pattern is indistinguishable from wild type (compare with A, and Fig. 8 in Fernandes and VijayRaghavan (1993)).

precursors and their migration pattern during pupation is transformed to that of a T2 identity (Fernandes et al., 1994). This indicated that the process of myoblast migration is very likely determined by cues provided by the epidermis and is independent of the segmental identity of the migrating myoblasts themselves. In order to further clarify the role of myoblast identity in their migration process, we mis-expressed an abdominal muscle-specific selector gene *Ubx* using a GAL4 line called *1151* and the *UAS-Ubx* transgene in the wing disc-associated T2 myoblasts, and determined its effect on the migration of these cells during pupal development. We analysed the expression pattern of *1151* by crossing it to the *UAS-lacZ* reporter strain (Brand and Perrimon, 1993) and find that it is expressed in all imaginal disc and nerve-associated adult muscle precursors, and also during their differentiation and in almost all mature adult muscles (see Materials and methods). When *Ubx* is expressed with this GAL4 driver, we find that T2 myoblast migration pattern is unaffected and that these myoblasts are able to follow defined routes to reach the sites of muscle formation (Fig. 2B,C). This observation provides clear evidence that the intrinsic identity of the myoblasts is irrelevant in determining their segment-specific migration patterns, which are very likely determined entirely by the segmental identity of the epidermis.

Homeotic genes do not specify fusion properties of myoblasts

We transplanted the presumptive notum region of wing imaginal discs, harbouring myoblasts that give rise to the indirect flight muscles (IFMs) of T2, into the abdomen of prepupal hosts (Fig. 3A). Donor myoblasts were from the *Myosin Heavy Chain-lacZ* (*MHC-lacZ)* transformant strain and hosts were wild type. We used myoblasts from the *MHC-lacZ* strain because this transgene is expressed in all differentiated muscle fibres in *Drosophila* and hence would serve as a useful marker to follow the fates of the transplanted myoblasts (Hess et al., 1989). We find that myoblasts from the wing discs can fuse with the developing abdominal muscles of the host (Fig. 3B). The myoblasts on the wing imaginal discs will normally contribute to the dorsal muscles of the adult mesothorax (Lawrence, 1982; Fig. 1). The transplantation experiments show that these myoblasts, when freed of the disc epithelium, can contribute to diverse muscle types. This result essentially reconfirmed Lawrence and Brower's previous observation using wild-type wing disc-derived donor myoblasts transplanted into hosts that were mutant for a gene that affected the activity of the enzyme succinate dehydrogenase (Lawrence and Brower, 1982). However, our recent study on homeotic gene expression and function in the thoracic mesoderm has revealed that no known homeotic selector gene of the ANT-C or the BX-C is expressed or required for the specification of muscle pattern in T2 in either the embryo or the adult. Specifically, myoblasts associated with the wing imaginal disc in this segment do not express any selector gene (Roy et al., 1997). The same study also revealed that muscle pattern in T3 is specified by the activity of the selector gene *Antp*, and myoblasts associated with the haltere imaginal discs of this segment, unlike those on the wing disc in T2, express *Antp*.

We reasoned that the ability of the wing disc myoblasts to indiscriminately fuse and contribute to abdominal muscles may arise from the fact that these cells are not committed by the activity of any selector gene. We therefore transplanted portions of haltere discs that harbour the T3-specific myoblasts from *MHC-lacZ* donors into the abdomen of wild-type hosts (Fig. 3A). We predicted that if restrictions to fusion in these myoblasts are dictated by *Antp* expression, then unlike the wing disc-derived myoblasts, these cells will not be able to fuse with abdominal myoblasts and contribute to abdominal muscles. However on transplantation, haltere disc myoblasts behave in a very similar way to wing disc myoblasts and can fuse with and contribute to host abdominal muscles (Fig. 3C). These results suggest that imaginal myoblasts are not restricted by segment-specific fusion properties through the activity of homeotic genes, but are indeed quite promiscuous in their ability to participate in diverse myogenic programmes.

Repression of segment-specific gene expression in thoracic myoblast nuclei that fuse with abdominal fibres

Though thoracic imaginal myoblasts appear not to exhibit any restrictions to their fusion capabilities, it is possible that their nuclei are able to maintain a thoracic identity even on being part of an abdominal muscle. In vertebrates for instance, there is evidence that the patterns of gene activity in the nuclei of a muscle fibre can indeed be very diverse (Sanes et al., 1991). Alternatively, the transcriptional status of the donor thoracic nuclei could be controlled by the activity of selector genes expressed in nuclei of the abdominal myoblasts and be 'entrained' by them. We next examined whether thoracic muscle nuclei are able to express thorax-specific differentiation genes even while coexisting with abdominal muscle nuclei or whether they get 'entrained' to execute an abdominal muscle-specific differentiation programme.

We transplanted wing disc myoblasts doubly marked with two different transgenes: *MHC-lacZ* and *Actin (88F)-Green fluorescent protein* (*Act(88F)-GFP*). The *Actin (88F)* gene is expressed in thoracic muscles, predominantly in the IFM fibres (Hiromi et al., 1986; Barthmaier and Fyrberg, 1995; See also Materials and methods). Fig. 4A shows the expression of GFP in the IFMs of an *Act(88F)-GFP* fly. In the transplantation experiments, *MHC-lacZ* expression monitored the fusion of myoblasts and the *Act(88F)-GFP* expression would test the ability of these myoblasts to express a thorax-specific gene. We find that while wing disc myoblasts are able to fuse and contribute to abdominal muscles, as observed by the expression of the *MHC-lacZ* transgene, they are unable to express the *Act(88F)-GFP* reporter gene, suggesting that on fusion their nuclei are 'entrained' by those of the abdominal muscles to prevent *Act(88F)* gene expression (Fig. 4B,C). Since we could observe GFP fluorescence in flight muscles of *Act(88F)-GFP*; *MHC-lacZ* flies even after fixation and β-galactosidase staining (see Materials and methods), the absence of GFP fluorescence in the abdominal muscles in the transplantation experiments is unlikely to be due to quenching effects from fixation and β-galactosidase staining.

We have done similar transplantation experiments with wing disc-derived myoblasts marked with the *Act(88F)-lacZ* transgene and, in every case examined, we were unable to detect any β-galactosidase expression from the *Act(88F)* reporter in the abdominal muscles, though in these experiments, unlike the one above, for want of a suitable marker, we were unable to monitor fusion events of the thoracic myoblasts

with the abdominal muscles. However, the sensitiveness of this latter set of experiments is borne out by the fact that in cases where transplantations with *Act(88F)-lacZ*-marked wing disc myoblasts occurred close to T2, i.e. on the first or second abdominal segments (A1 and A2), some of the donor myoblasts could migrate back into T2 and fuse with the developing IFMs in that segment. In such cases, we were able to observe *Act(88F)-lacZ* expression even from single transplanted myoblast nuclei that happened to fuse with the developing IFMs. One such example is shown in Fig. 4D.

The inability of thoracic myoblast nuclei to express the *Act(88F)* reporter genes in the abdominal muscles indicates that the expression of this gene is a segmental property of the thoracic muscles, and that the thoracic myoblasts on fusion with abdominal muscles are 'entrained' into an abdominal identity, most likely by the activity of abdominal muscle-specific selector genes such as *Ubx*, *abd-A* and *Abd-B* (Bate, 1993; also see below).

Ubx mis-expression in the developing flight muscles represses the expression of the thoracic muscle-specific reporter gene, *Act(88F)-lacZ*

To test if any of the homeotic genes that are normally expressed in the abdominal muscles are negative regulators of the *Act(88F)* gene, *Ubx* was expressed in the developing IFMs using the GAL4/UAS system (Brand and Perrimon, 1993). For this mis-expression study, the GAL4 driver *1151*, which was previously used to express *Ubx* in the T2 myoblasts to determine the effect of this mis-expression on myoblast migration, was used. As mentioned earlier, this GAL4 driver was found to be expressed in the differentiating myoblasts as well as in most mature adult muscles. During normal development, expression of the *Act(88F)-lacZ* transgene is first seen at about 14-16 hours after puparium formation (APF) in the differentiating IFM fibres (Fig. 5A). Expression of *Ubx* in these developing muscles resulted in the repression of *Act(88F)-lacZ* expression (Fig. 5B). Almost no expression of this reporter gene was seen, and the indirect flight muscles failed to complete development and eventually degenerated. The absence of *Act(88F)-lacZ* expression cannot be attributed to the fact that *Ubx* expression in T2 myoblasts resulted in inappropriate cell death or inhibited myoblast differentiation, because the myoblasts were able to migrate to IFM formation sites and fuse to form developing myofibres, and at a stage in development when these myofibres should normally be expressing the *Act(88F)* reporter, the expression of this transgene was not observed in these muscle fibres (Fig. 5B). Thus, *Ubx* mis-expression in T2 myoblasts resulted in the alteration of their identity into that of an abdominal one and these myoblasts therefore were unable to execute a differentiation programme that is appropriate to that of the thoracic segment.

Removal of *Ubx* expression from abdominal muscles allows the expression of *Act(88F)-lacZ*

In animals homozygous for *anterobithorax* (*abx*), *bithorax*^{3} (*bx*^{3}), *postbithorax* (*pbx*), three mutant alleles of the *Ubx* gene of the BX-C, a transformation of the epidermis of T3 towards that of T2 is observed. Such adults have two pairs of wings. In our analysis of flight muscle development in animals carrying the above 'triple mutation' combination in *Ubx*, we also demonstrated that the mesoderm of the first abdominal segment took on properties of the third thoracic segment (Fernandes et al., 1994). Thus, while the ectoderm was transformed from T3 towards T2, the mesoderm was transformed from A1 towards T3. We also showed that in the wild-type animals, *Ubx* was expressed at its most anterior mesodermal domain in A1. In 'triple mutant' animals, *Ubx* expression in A1 mesoderm is absent (Fernandes et al., 1994). High levels of *Antp* expression in the mesoderm are seen, at its most anterior domain in T3, and the level is significantly lower in A1 mesoderm of the wild-type. In the 'triple mutant', high levels of *Antp* expression are also seen in the A1 mesoderm (Fernandes et al., 1994). On the basis of the transformation of the mesoderm observed in A1 of the 'triple mutant', the absence of transformation in the ectoderm of this segment, and the pattern of expression of *Ubx* and *Antp* in the mesoderm of wild type and mutant animals, we concluded that *Ubx* expression in the mesoderm of A1 is autonomously required for proper muscle development and that absence of *Ubx* expression leads to the transformation observed. We examined A1 muscles in wild type and 'triple mutant' animals for the expression of the *Act(88F)-lacZ* reporter. While expression of this reporter was never observed in wild-type A1 muscles, it was found that absence of *Ubx* in A1 mesoderm of the 'triple mutant' leads to defects in muscle patterning, and allows the expression of *Act(88F)-lacZ* in A1 muscles (Fig. 5C,D). Thus, while expression of *Ubx* in T2 mesoderm alters its identity into that of abdominal one and modifies its differentiation programme such that it is no longer able to express thorax-specific differentiation genes, removal of *Ubx* expression in A1 mesoderm results in the alteration of the identity of the A1 muscles into that of a thoracic label, and these muscles now follow a thoracic muscle differentiation programme and express thoracic muscle-specific genes.

DISCUSSION

We have addressed specific questions regarding the role of homeotic selector genes during muscle development. In the epidermis of *Drosophila*, where their function has been best investigated, these genes function cell-autonomously to specify the developmental identity of parasegments (Lawrence and Morata, 1994). Not only are the selector genes involved in promoting a unique pattern of differentiation of the cells that belong to a particular parasegment, but they are also involved in regulating their mixing properties and in defining cellular affinities (Lawrence, 1992). While there is evidence that homeotic genes do function autonomously in muscle cells to specify pattern, their roles in different aspects of myogenesis remain obscure. It would be reasonable, for example, to expect that as in the epidermis, homeotic genes regulate the surface properties of mesodermal cells and thereby channel them to migrate along defined paths and to participate in specific fusion programmes. However, our present study indicates that these properties of myoblasts are not regulated by the autonomous function of selector genes in these cells; a situation very different from that in the epidermis. This difference in the activity of homeotic genes in the two germ layers may have to do with the peculiar way by which muscles develop (Lawrence, 1992). It is now a well established fact, at least in the *Drosophila* embryo, that each muscle fibre is prefigured by a single cell called a 'founder cell'. One extreme view of the role

of ‘founder cells’ is that their segmental identity and positional information are necessary and sufficient to pattern the formation of a muscle fibre. Other myoblasts that fuse with the ‘founder’ are ‘feeder myoblasts’ of no distinct identity, and they will be submissive, in their pattern of gene expression, to controls by regulatory proteins made by the ‘founder cell’ nucleus. In this view, myoblasts from different segments will be able to fuse with each other, but the developing muscle fibre will take on properties decided by the ‘founder cell’ (Lawrence, 1985). An alternative extreme view could be that fibre properties are a mix of the properties of its constituent nuclei, with no nucleus being ‘more equal’. In this scheme, the property of a fibre will be determined by the identity of the majority of its nuclei. While studies from vertebrates have suggested that muscle nuclei have differential abilities to express synapse-specific genes (Sanes et al., 1991), we do not know if properties such as the segmental identity of a fibre are determined by the activity of one or many nuclei. Similarly, muscle ‘founder cells’, which have only one nucleus, send out processes that attach to specific epidermal attachment sites (Rushton et al., 1995). Is the ability to recognise these epidermal attachment sites a result of cues from the epidermis,

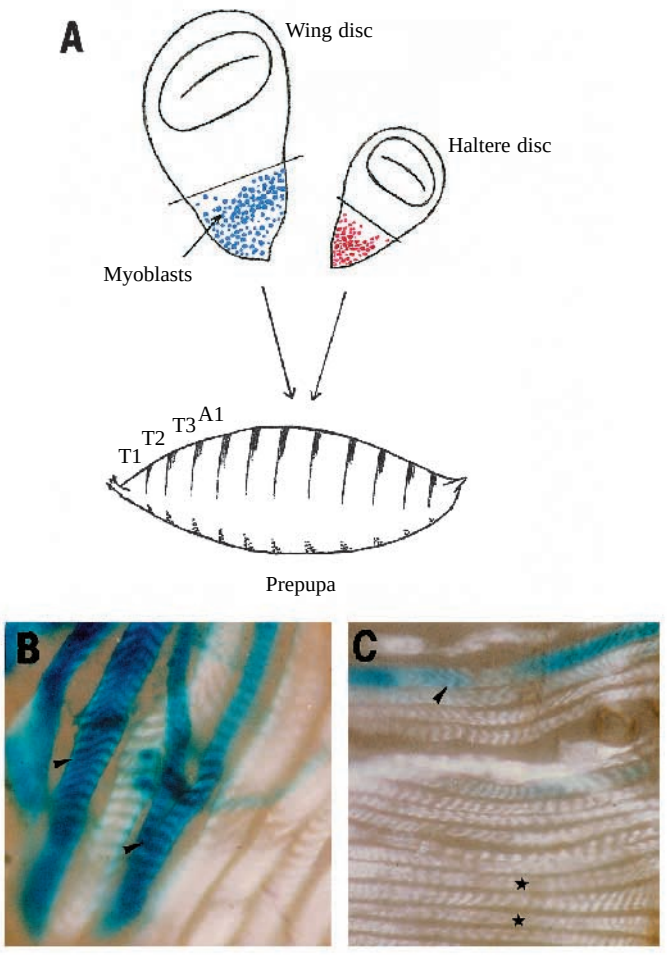

Fig. 3. When transplanted into the abdomen, thoracic myoblasts, irrespective of their homeotic identities, are able to fuse with abdominal myoblasts and form abdominal muscles. (A) Diagram illustrating the transplantation technique that was used to determine the fusion abilities of myoblasts. The notal regions of wing and haltere discs harbouring the myoblasts were dissected (the straight line across the discs represents the line of dissection) and transplanted into the abdominal regions of prepupal hosts. The myoblasts on the wing disc do not express any homeotic selector gene (represented in blue), while those associated with the haltere disc express *Antp* (represented in crimson). (B) Dorsal muscles in the fifth abdominal segment of a host fly that arose from the fusion of wing disc-derived T2-specific myoblasts (marked with the *MHC-lacZ* transgene) with abdominal myoblasts (arrowheads). Muscles which developed without fusion of *MHC-lacZ*-marked T2 myoblasts are unstained for β-galactosidase expression and are white. (C) Pleural muscles in the fifth abdominal segment of a host fly that arose from the fusion of haltere disc derived T3-specific myoblasts (marked as in above with the *MHC-lacZ* transgene) with abdominal myoblasts (arrowhead). The neighbouring unstained muscles have arisen only from the abdominal myoblasts and hence do not show β-galactosidase expression (asterisks). For pattern of adult abdominal muscles see Bate (1993).

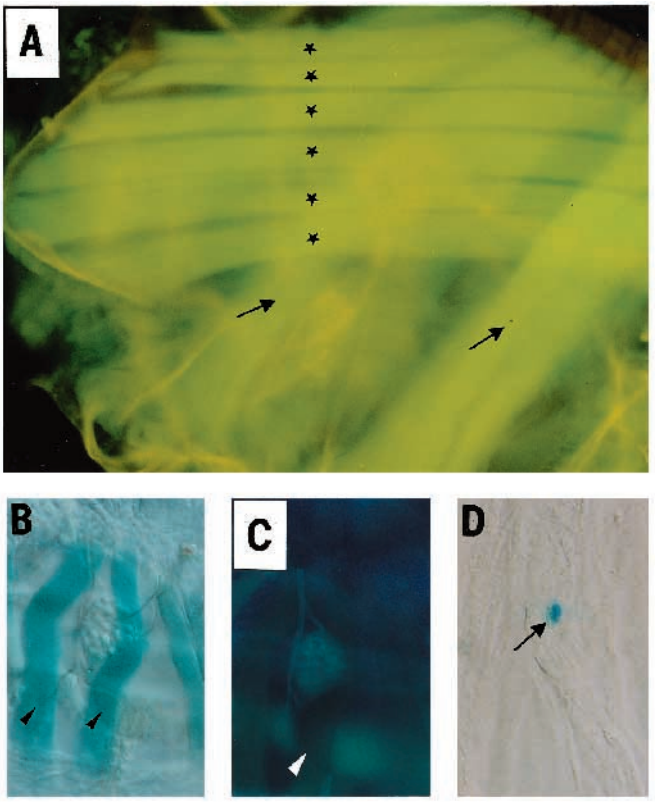

Fig. 4. On fusion, thoracic myoblast nuclei lose their segment-specific identities and are entrained by those of the host muscle. (A) *Act(88F)-GFP* expression in the IFMs of an adult fly on excitation with 340-380 nm light. The asterisks mark the six dorsal longitudinal muscle fibres (DLMs) and the arrowheads indicate two groups of dorsoventral muscles (DVMs). In this figure, anterior is to the right. (B) Muscles in the fourth abdominal segment that have developed by the fusion of T2 myoblasts from the wing disc (marked with *MHC-lacZ* and *Act(88F)-GFP*) with abdominal myoblasts (blue muscles marked with arrowheads). (C) The same muscles shown in B on excitation with 340-380 nm light do not show GFP fluorescence (arrowhead; compare with GFP expression in A). (D) β-galactosidase expression in a donor T2 myoblast nucleus (arrow) from the *Act(88F)-lacZ* strain that had migrated into the thorax from the abdomen (the site of transplantation) and had fused with the developing IFMs in T2.

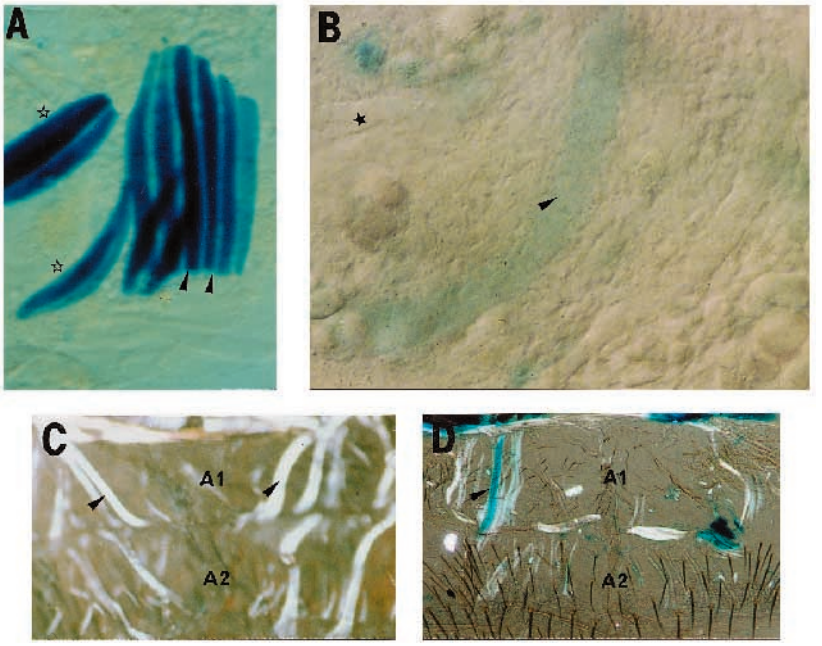

Fig. 5. Mis-expression of *Ubx* in the developing thoracic muscles results in the repression of thoracic muscle-specific *Act(88F)-lacZ* reporter gene, while loss of *Ubx* expression from abdominal muscles results in the activation of this reporter in these muscles. (A) A 24-hour APF wild-type pupal preparation, showing the expression pattern of *Act(88F)-lacZ* in the developing IFMs. The arrowheads indicate two developing DLM fibres while the asterisks mark two groups of developing DVMs. (B) *Act(88F)-lacZ* expression is not observed on mis-expression of *Ubx* in the developing IFMs at 24 hours APF. Note the extremely low level of β-galactosidase staining in one developing DLM fibre (arrowhead). The asterisk marks the position of a developing DVM. Note the complete absence of β-galactosidase expression in this muscle. The developing IFMs in this preparation are shown at a higher magnification than in A. (C) Polarised light picture of muscle fibres (arrowheads) in the A1 segment of an *Act(88F)-lacZ* transformant fly. Expression of the *Act(88F)-lacZ* transgene is normally not observed in these muscles. (D) Polarised light picture of A1 muscle fibres in a 'triple mutant' animal. Absence of *Ubx* function in A1 mesodermal cells of these mutants results the transformation of their identity to that of T3 mesoderm. This results in defects in muscle patterning, and allows the expression of the thoracic muscle-specific *Act(88F)-lacZ* transgene (arrowheads). Not all T3 muscles normally express the *Act(88F)* reporter, therefore *lacZ* expression is not observed in all homeotically transformed A1 muscles in the 'triple mutant' (See Materials and methods).

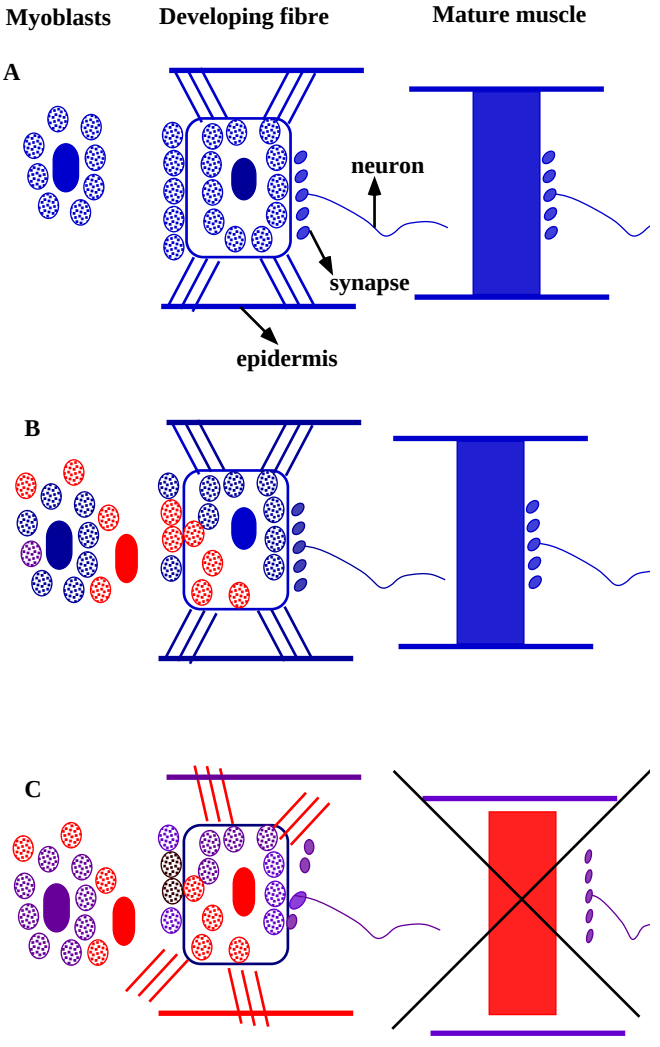

Fig. 6. Diagram illustrating the relative importance of the identities of the epidermis, the motor neuron, the 'founder cell' and the contributing myoblasts for the elaboration of segment-specific muscle patterns. (A) The development of a mature muscle fibre with proper attachment and innervation requires that the identities of the 'founder cell', the epidermis that it attaches to and the motor neuron that innervates it, are compatible. The 'founder' is represented by the oblong cell while the other myoblasts are depicted as circles. (B) When donor myoblasts from one segment (shown in red) are transplanted to the sites of muscle formation in another segment, these myoblasts are able to fuse with the resident host myoblasts of that segment (shown in blue) and form a mature fibre, as long as the 'founder' is of the right segmental identity (represented by the blue oblong cell). Even in the presence of a vast majority of donor myoblasts, the 'founder' will be able to 'entrain' them to its pattern of gene expression and make a wild-type muscle fibre. (C) However, if a 'founder' from the donor myoblast population, and hence of a wrong segmental identity (represented by the red oblong cell), nucleates the process of muscle development, it will not be able to recognise the proper attachment sites on the mismatched epidermis, nor will it be able to direct the formation of a proper pattern of innervation. This will result in derangement or degeneration of the muscle fibre.

or are factors intrinsic to each muscle also involved? If the latter, are the muscle-derived components specified by the 'founder' nucleus, with the later arriving myoblast nuclei having no effect on attachment? Analysis of mutant embryos that are specifically affected in myoblast fusion has revealed that in the absence of 'feeder myoblast' fusion, the 'founder cell' is nevertheless able to recognise its correct attachment sites, synthesise contractile proteins and get appropriately innervated, suggesting that these cells do indeed have all the information required to generate the final muscle pattern (Rushton et al., 1995). However, it remains to be seen whether during normal development such a mechanism is operative, or whether other contributing myoblasts also exert an influence on the development and identity of the mature muscle fibre.

While it is not known whether the 'founder cell' mechanism operates during adult muscle development in *Drosophila*, many features of myogenesis suggest that differences between myoblasts exist and are required for normal muscle development. It is possible that imaginal disc-associated myoblasts that we and others have used in transplantation assays represent the uncommitted 'feeder myoblasts' we have alluded to above. As in the embryo, 'founder cells' could be specified at a certain developmental stage during adult myogenesis, from among these cells, by mechanisms of cell-cell interactions together with extrinsic cues provided by interacting tissues (Bate et al., 1993; Baylies et al., 1995). It is also possible that 'founders' may be present among other groups of myoblasts such as those associated with the larval nerves, and they may also take the form of structures like persistent larval templates, as in the case of the developing dorsal longitudinal muscles in T2 (Fernandes et al., 1991) Our recent studies on the expression patterns of an e*ven skipped-lacZ* (*eve-lacZ*) transgene has revealed that it is expressed in subsets of nerve-associated adult myoblasts in the larva, but not in wing disc-associated myoblasts, making *eve* a possible candidate for a 'founder cell'-specific marker gene for adult muscles (S.R., unpublished). In keeping with this observation is the fact that *eve* is also expressed in the 'founders' of subsets of larval muscles during embryonic development (Bate, 1993). The progenitor of the ventral adult muscles in each abdominal segment has been identified, and it arises from a single 'founder-like' cell in the embryo (Carmena et al., 1995). The formation of adult fibres by descendants of this cell raises several interesting questions about the relative potential of sibling myoblasts to configure muscle pattern, and about how and when differences, if any, amongst these myoblasts could arise.

The essence of the above argument on the roles of 'founders' in muscle pattern specification is schematically summarised in Fig. 6. A 'founder' myoblast is selected from a pool of myoblasts. This 'founder' cell sends out filopodia to epidermal attachment sites, which will later develop to form the apodemes. Other myoblasts fuse to the 'founder cell' and contribute to the growth of a mature muscle fibre. Developing neurons innervate the fibre and form a defined pattern of synaptic boutons. Thus, for a mature muscle to be observed in the adult, the 'founder cell' must recognise correct attachment sites on the epidermis, i.e. muscle and epidermis must have the same 'segmental address' and the developing innervation must recognise the muscle fibre as being of the same 'address' (Fig. 6A). When myoblasts from another segment are ectopically transplanted to the site of a developing fibre, they fuse with the fibre and can contribute to the muscle that is formed. A mature fibre with a heterogeneous population of donor and host nuclei is observed only when the 'founder cell' belongs to the host. Only then will the appropriate epidermal and neuronal 'addresses' be read. A mature fibre can develop even when the majority of the myoblasts are from another segment (Lawrence, 1985). As long as the 'founder cell' is of the right segmental identity, it will be able to 'entrain' the nuclei of other myoblasts, resulting in the development of a mature fibre (Fig. 6B). However, if the 'founder cell' is formed by the contribution from the 'wrong' segment, as in Fig. 6C, we suggest that a mature muscle is unlikely to form even if a substantial number of the myoblasts are of the correct segmental identity. In surface transplant experiments of whole thoracic discs, VijayRaghavan et al. (1996) found rare situations where clumps of transplanted muscle were associated with the abdominal epidermis but expressed thoracic markers. These muscles were clearly not part of the normal pattern of abdominal musculature. While it is not possible to say how many, if any, abdominal myoblasts contributed to these fibres, such clumps of fibres could represent aborted muscles with a 'wrong' segmental identity.

Finally, what do these lessons learnt from myogenesis in flies tell us about muscle patterning in other organisms, specially vertebrates? As mentioned earlier, large gaps remain in our understanding of skeletal muscle patterning during vertebrate embryogenesis. While recent work has indicated that myoblasts in vertebrates are very diverse, much remains to be understood regarding the mechanisms of how diversity is generated, and how muscle patterns arise from the interplay between intrinsic properties of myoblasts and influences extrinsic to them (Miller, 1992). Several interesting similarities are readily apparent when our results are compared with some elegant studies of a similar nature that have been done in vertebrates. For instance, transplantation of rodent satellite myoblasts during postnatal development have shown that they can indiscriminately fuse to many different fibre types and take on the myosin heavy chain isoform expression patterns of the fibre to which they have fused (Hughes and Blau, 1992). This would suggest that satellite cells are similar to *Drosophila* 'feeder myoblasts' in that they are not committed to participate in any specific myogenic program, and that their gene expression patterns can be 'entrained' by the nuclei of the fibre to which they happen to fuse. It is possible that mammalian and avian primary myoblasts and pioneer myoblasts in teleosts are similar to 'founder myoblasts' in *Drosophila* and other insects, and it will be interesting to investigate the flexibility of their developmental potentials using similar experimental paradigms. In fact, transplantation experiments with clones of primary myoblasts and satellite cells in birds have demonstrated that fates of muscle fibres are stringently controlled by myogenic programs intrinsic to these myoblasts during early development (DiMario et al., 1993). This would suggest that cells that are involved in the earliest stages of muscle pattern formation are indeed much less permissive as far as their developmental potentials are concerned, and once the pattern has been seeded by these cells, other myoblasts which fuse with them, do exhibit a considerable degree of developmental flexibility. Like many other molecular functions that have been shown to be conserved between vertebrates and lower animals, we would like to think that much of what we have gleaned from

our experiments on the functions of homeotic genes in diverse aspects of myogenesis in *Drosophila* will also reflect the manner in which their homologs, the Hox genes, would function during pattern formation in the somatic mesoderm of vertebrates.

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