Muscle pattern diversification in *Drosophila* is determined by the autonomous function of homeotic genes in the embryonic mesoderm

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SUMMARY

Muscle diversification in the *Drosophila* embryo is manifest in a stereotyped array of myofibers that exhibit distinct segment-specific patterns. Here it is shown that the homeotic genes of the Bithorax complex control the identities of abdominal somatic muscles and their precursors by functioning directly in cells of the mesoderm. Whereas *Ultrabithorax* (*Ubx*) and *abdominal-A* (*abd-A*) have equivalent functions in promoting the formation of particular muscle precursors in the anterior abdominal segments, *Abdominal-B* (*Abd-B*) suppresses the development of these same myogenic cells in the posterior region of the abdomen. When expressed in the same mesodermal cells, however, either *UBX* or *ABD-A* can override the inhibitory influence of ABD-B, suggesting that these factors may compete in the regulation of common downstream genes. Furthermore, targeted ectopic expression of *Ubx* or *abd-A* indicates that these homeotic genes influence muscle cell fates by autonomous action in mesodermal cells. Muscle identity also appears to be sensitive to the level of UBX in myogenic precursors. Finally, these experiments reveal that homeotic cues specific to both the mesoderm and the ectoderm cooperate to specify the pattern of muscle attachment sites.

Key words: Bithorax complex, myogenesis, homeodomain, mesoderm, *Drosophila*

INTRODUCTION

The *Drosophila* homeotic selector genes specify the unique properties that distinguish the various segments of the embryonic and adult body plans. These genes are clustered at two sites in the *Drosophila* genome, the Antennapedia complex (ANT-C) and the Bithorax complex (BX-C), and each member is expressed and exerts its effects in a distinct domain along the anteroposterior axis of the organism (McGinnis and Krumlauf, 1992).

The phenotypes of gain- and loss-of-function mutations of homeotic genes are characterized by transformations of the structural characteristics of one or more metameric units into those of another (McGinnis and Krumlauf, 1992). Most of the studies leading to these conclusions have been confined to the analysis of ectodermal derivatives, notably the epidermis (Lewis, 1978; Sanchez-Herrero et al., 1985) and to some extent, the nervous system (Jimenez and Campos-Ortega, 1981; Teugels and Ghysen, 1985). Mosaic analysis clearly indicates that homeotic genes have an autonomous function in epidermal development (Morata and Garcia-Bellido, 1976; Minana and Garcia-Bellido, 1982), a finding that is consistent with the expression patterns of these genes (Beachy et al., 1985; White and Wilcox, 1985; Karch et al., 1990; Macias et al., 1990). Although homeotic genes are also expressed in cells derived from other germ layers and these derivatives exhibit distinct segmental differences (Hooper, 1986; Bate, 1990), relatively few studies have addressed the localized requirement for homeotic gene function in these tissues.

In one case, it was shown that the development of a particular adult male-specific abdominal muscle is regulated by the BX-C (Lawrence and Johnston, 1984, 1986). Certain BX-C mutations led to the duplication of this muscle in abdominal segments where it is not normally located (Lawrence and Johnston, 1984). Furthermore, mosaic analysis demonstrated that this regulatory effect is not due to BX-C gene function in either this muscle or the epidermis to which it attaches. Rather, it is the genotype of the innervating motoneuron that appears to specify the identity of this particular muscle (Lawrence and Johnston, 1986). However, such a nonautonomous influence of a homeotic gene on adult muscle development may be exceptional since the opposite result has recently been obtained for the *twist*-expressing precursors of several adult thoracic muscles (Greig and Akam, 1993). In this case, though, an effect on the pattern of mature muscles and their epidermal attachments was not investigated.

Indirect evidence has also been presented that a homeotic gene acts autonomously in the mesoderm to determine the diversity of the embryonic/larval musculature (Hooper, 1986). This conclusion was based on the finding that ectodermal and muscle transformations associated with *Ultrabithorax* (*Ubx*) mutations were out of register with each other. Although analysis of genetic mosaics has provided some evidence that BX-C genes may function autonomously in muscle development (Lawrence, 1985), this point has not been rigorously
established. Since Ubx and other members of this class of genes are expressed in many different cell types in a given segment and since mutations of these genes are associated with distinct ectodermal transformations, it is not possible to completely eliminate indirect effects of the BX-C on myogenesis. The possibility of nonautonomy of homeotic gene function in the mesoderm is further underscored by the fact that the body wall muscles develop in close proximity to the epidermis and central nervous system (Bate, 1990) and ultimately form epidermal insertions. Thus, although innervation does not play a significant role in establishing muscle identity in the embryo (Johansen et al., 1989; Bate, 1990; Broadie and Bate, 1993), alterations in the segment-specific properties of the overlying epidermis could profoundly affect the somatic muscle pattern (Williams and Caveney, 1980; Bier et al., 1990). Additional effects of ANT-C and BX-C genes on midgut visceral mesoderm development have also been described (Tremml and Bienz, 1989; Immergluck et al., 1990; Panganiban et al., 1990; Reuter and Scott, 1990; Reuter et al., 1990), but again the question of germ layer autonomy of homeotic gene action has not been definitively established.

The present study employed a novel gene expression system recently described by Brand and Perrimon (1993) to directly assess the function of BX-C genes on mesoderm development in the Drosophila embryo. Targeted misexpression of Ubx or abdominal-A (abd-A) throughout the embryonic mesoderm led to abdominal transformations of both mature somatic muscles and at least a subset of their precursors in the thoracic segments. Since these thoracic muscle transformations were not associated with abnormalities of the corresponding cuticle, and since no ectopic homeotic gene expression was observed in thoracic ectodermal derivatives, it is concluded that BX-C genes must function autonomously in mesodermal cells.

MATERIALS AND METHODS

Drosophila strains

The following BX-C mutant strains were employed in this study: Ubx$^{22}$, a null allele that deletes most of the UBX homeodomain (Weinzierl et al., 1987), Df(3R)P10, a deficiency that eliminates the entire Ubx and the 3′-end of the abd-A coding sequences (Karch et al., 1985), Abd-B(3)$^{3}$, a strong m-r allele of Abd-B (Casanova et al., 1986) and Df(3R)94 that deletes the entire BX-C (Karch et al., 1985). Oregon R was the wild-type strain used for controls. The GAL4 24B enhancer trap and UAS-lacZ transformant lines have been described and were kindly provided by Andrea Brand and Norbert Perrimon (Brand and Perrimon, 1993). All flies were grown on standard yeast-cornmeal-agar medium at 25°C.

Plasmid constructions

A Ubx form la cDNA (Mann and Hogness, 1990) was cloned as a 2.2 kb EcoRI fragment downstream of the GAL4 UAS and hsp70 TATA box in the EcoRI site of pUAST (Brand and Perrimon, 1993). Similarly, a 1.77 kb EcoRI fragment from abd-A cDNA clone 1dB-5 (Karch et al., 1990) was inserted into pUAST. Clones containing the sense orientations of these inserts were identified by appropriate restriction digests and were designated pUAS-Ubxla and pUAS-abdA, respectively. Plasmids were purified by CsCl ultracentrifugation and dialyzed extensively prior to embryo injections.

Germline transformation

Transgenic lines were generated by injecting either pUAS-Ubxla or pUAS-abdA into embryos derived from the y w strain using standard techniques (Spradling, 1986). Homozygous and balanced insertion stocks were established by appropriate genetic crosses.

Immunohistochemical staining of embryos

Embryos were collected on molasses agar plates containing a small amount of yeast paste, aged for the requisite times, dechorionated in 50% bleach, fixed for 15-20 minutes in a 1:1 mixture of heptane and 4% formaldehyde in 100 mM Pipes, pH 6.9, 2 mM MgSO$_4$, 1 mM EGTA, and devitellinized by shaking with heptane/methanol (1:1). After a 20 minutes pretreatment with 1.5% H$_2$O$_2$ in methanol, embryos were rehydrated in PBT (phosphate-buffered saline containing 0.2% Tween-20), blocked for at least 1 hour in PBT containing 2% goat serum and incubated overnight at 4°C with primary antibody at the following dilutions: affinity-purified polyclonal rat α-NAU (1:50); pre-adsorbed polyclonal rabbit α-β-galactosidase (Cappel; 1:500); mouse monoclonal α-UBX (ascites diluted 1:1000); α-ABD-A (ascites diluted 1:2000) and α-MHC (FMM5 hybridoma culture supernatant diluted 1:500). The α-NAU antibody has been described previously (Abmayr et al., 1992) and was generously provided by Susan Abmayr and Ming Tian. Appropriate biotinylated secondary antibodies were pre-adsorbed and used at 1:500 dilutions. Histological staining was achieved using horseradish peroxidase Vectastain Elite ABC reagents (Vector Laboratories) and diaminobenzidine and H$_2$O$_2$ as substrates in the presence of NiCl$_2$. Embryos were cleared in methylsalicylate, mounted in DPX (Fluka) and photographed using Nomarski optics on a Zeiss Axioshot microscope.

Embryo in situ hybridization

Whole-mount embryo in situ hybridization was carried out as described by Tautz and Pfleifle (1989) and modified by Michelson et al. (1990). A dpp cDNA fragment from PBElh was used as the hybridization probe (St. Johnston et al., 1990). Stained embryos were mounted for photography in 90% glycerol.

Cuticle preparations

Cuticles were prepared from those embryos that failed to hatch and were mounted in Hoyer’s medium, as described (Wieschaus and Nusslein-Volhard, 1986). Cuticles were examined and photographed using phase contrast optics.

RESULTS

Domains of BX-C gene function in the somatic mesoderm

Previous studies have established that each member of the BX-C is expressed in a region of the embryonic mesoderm that is shifted somewhat posteriorly from its corresponding ectodermal domain (Beachy et al., 1985; White and Wilcox, 1985; Celniker et al., 1989; Delorenzi and Bienz, 1990; Karch et al., 1990; Macias et al., 1990). Despite the availability of this information, relatively little is known about the functional role of these genes in development of the somatic mesoderm. Such an analysis was therefore undertaken using nautilus (nau) (Michelson et al., 1990; Paterson et al., 1991) a Drosophila member of the MyoD family of myogenic regulators (Weintraub et al., 1991), as a marker for particular embryonic muscle precursors.

nau is normally expressed in only a subset of embryonic muscle precursors at any given stage of development (Michelson et al., 1990; Paterson et al., 1991; Abmayr et al., 1992). For example, at the end of germ band shortening when the entire complement of embryonic muscle precursors has
formed (Bate, 1990), a polyclonal antibody raised against a NAU bacterial fusion protein detects this gene product in only a few of these cells (Abmayr et al., 1992; Fig. 1A). This pattern is indistinguishable from that previously determined for nau mRNA by whole-mount in situ hybridization (Michelson et al., 1990). Distinct arrangements of NAU-expressing cells are evident in each of the three thoracic segments, and yet another, invariant pattern is present in the first 7 abdominal segments. Of particular note is the absence of NAU-positive muscle precursors in the ventral region of T1-T3 and the presence of a single such cell (the precursor to abdominal muscle 26) in the ventral region of A1-A7. In contrast, no NAU expression is seen in A8 at this developmental stage, although later embryos exhibit several NAU-positive cells in a unique pattern in this segment (Abmayr et al., 1992). These segmental differences in the NAU expression pattern anticipate the subsequent segment-specific identities of the mature embryonic muscles.

In embryos homozygous for a null mutation in Ubx, the first
two abdominal segments undergo a transformation of the NAU pattern to a T3-like identity (Fig. 1B). This is seen most clearly for the loss of the ventral muscle 26 precursor from A1 and A2. However, the more posterior abdominal segments maintain a wild-type NAU pattern in this genetic background. These results are in agreement with the earlier observations of Hooper that partial loss of Ubx function leads to the absence of mature larval muscle 26 in A1 and A2 (Hooper, 1986), and further establish that this alteration occurs at the precursor stage of development. Embryos lacking both Ubx and abd-A functions have a similar thoracic transformation that extends from A1 to A7, though A8 retains its wild-type appearance in being devoid of NAU expression at an equivalent developmental stage (Fig. 1C). Loss of Abd-B function alone leads to an A1 to A7-like pattern of NAU expression in A8 while the more anterior segments remain unaffected (Fig. 1D). Whereas loss of abd-A function alone is not associated with any detectable change in the NAU pattern (data not shown), when the entire BX-C is deleted there is a thoracic transformation of NAU expression in all of the abdominal segments (Fig. 1E). These results are summarized schematically in Fig. 1F. Ubx and abd-A appear to have equivalent functions in promoting an identical pattern of nau expression in A1-A7 while Abd-B has the opposite effect of suppressing the formation of NAU-positive muscle precursors in the most posterior region of the abdomen at the end of germ band retraction.

**Effect of ectopic mesodermal expression of UBX on development of the somatic muscles**

The muscle precursor transformations associated with loss of BX-C gene function could be due to homeotic effects directly within the mesoderm or could be secondary to transformations of ectodermal derivatives with which early myogenic cells make intimate contacts (Bate, 1990). To distinguish between these two possibilities, the mesodermal effects of Ubx expression in the thoracic mesoderm but not the corresponding ectoderm were investigated. The occurrence of homeotic transformations of thoracic muscles in this situation would establish that Ubx functions autonomously in mesodermal cells.

Misexpression of UBX in the embryonic mesoderm was achieved using the GAL4 targeting system developed by Brand and Perrimon (1993). In this approach, a *Drosophila* line in which the yeast transcriptional activator GAL4 is expressed in a particular temporal and spatial pattern is crossed to a second line containing a gene of interest that has been cloned downstream of GAL4-responsive regulatory elements, the so-called upstream activating sequence or UAS. This results in the activation of the target gene in the same cells that express GAL4. The system employed in the present study is shown in Fig. 2. Enhancer trap line 24B (Brand and Perrimon, 1993) expresses GAL4 throughout the mesoderm of early stage 11 embryos (Fig. 2B), and by stage 14 expression is observed in most if not all somatic muscle precursors (Fig. 2E) and the entire visceral mesoderm of the midgut (Fig. 2H). The only ectodermal expression in line 24B occurred in a few epidermal cells just posterior to the stomodeal opening (Fig. 2B,C) and in a small number of pharyngeal cells later in embryogenesis (Fig. 2H,I; data not shown). When line 24B was crossed to a UAS-Ubx transformant line, expression of UBX was found in all mesodermal cells of the germ band extended embryo, including those of the thoracic segments (Fig. 2C). This ectopic expression persisted in thoracic muscle precursors and the entire visceral mesoderm following germ band retraction (Fig. 2F,J). The total pattern of UBX expression in this situation appears to be a direct superimposition of the GAL4 enhancer trap pattern with that of endogenous UBX at all developmental stages (Fig. 2A,D,G). Furthermore, ectopic UBX does not seem to modulate the expression of GAL4 in this line. Neither was ectopic expression of UBX evident in either parental line (data not shown).

The consequences of ectopic mesodermal expression of UBX on development of the somatic muscles and their precursors are shown in Fig. 3. The thoracic segments underwent an abdominal transformation of the NAU pattern, as most easily seen with the appearance of an abdominal muscle 26 precursor in the ventral region of T1-T3 (Fig. 3A,B). In other embryos from the same experiment, a partial transformation was observed in which only T2 and T3 or T3 alone exhibited the abdominal-like NAU-expressing cells (data not shown). In addition, in some but not all embryos that ectopically expressed UBX, a NAU-positive precursor formed in the ventral region of A8 (Fig. 3B and data not shown). Transformation of the mature muscle patterns of the thoracic segments and of A1 also occurred with ectopic mesodermal expression of UBX (Fig. 3C-F). For example, the ventral longitudinal muscles of T1-T3 assumed an abdominal identity (Fig. 3E,F). In addition, T3 acquired a muscle having the shape, location and attachments characteristic of abdominal muscle 26 for which there is no normal thoracic counterpart (Fig. 3F). Other changes in thoracic muscle identity occurred but a precise correlation with an equivalent abdominal muscle is more difficult to ascertain (arrowheads in Fig. 3F; see Discussion).

Although A1 has a nearly identical arrangement of somatic muscles as A2-A7, there are several notable exceptions (Bate, 1990). Instead of three posteriorly directed ventral oblique muscles (muscles 15, 16 and 17), A1 normally has only two (Fig. 3C,E). In addition, a wild-type A1 lacks muscle 25 and possesses an extra internal ventral longitudinal muscle (see below). With GAL4 line 24B-induced ectopic expression of UBX, A1 acquired the two abdominal muscles that it usually lacks (Fig. 3F). The same results were obtained using two independent UAS-Ubx transformants. Thus, a transformation occurred in response to ectopic UBX even in a segment where the affected cells normally express this homeotic product (Hooper, 1986; Fig. 2D).

**Ectopic ABD-A expression has the same effect as UBX on somatic muscle development**

The loss-of-function phenotypes described above suggested that Ubx and abd-A exert equivalent effects in specifying the muscle pattern of the first seven abdominal segments. Given the gain-of-function myogenic phenotype observed for Ubx (Fig. 3), the potential equivalence between Ubx and abd-A was tested further by ectopically expressing abd-A under control of the GAL4 24B enhancer trap line.

As was the case for Ubx, crossing line 24B to a UAS-abd-A transformant resulted in ectopic expression of ABD-A in all mesodermal cells of early (Fig. 4B) and late stage (data not shown) embryos. The wild-type pattern of ABD-A expression (Fig. 4A) appeared to be additive to that induced by the GAL4
Fig. 2. Ectopic expression of GAL4-responsive genes induced by enhancer trap line 24B. GAL4 enhancer trap line 24B (Brand and Perrimon, 1993) was crossed to lines transformed with either UAS-lacZ or UAS-Ubx constructs. Embryos were then collected, fixed and stained with antibodies directed against β-galactosidase (B,E,H) or UBX (C,F,I). Wild-type embryos stained for expression of UBX are shown in A,D and G. Lateral views with anterior to the left and dorsal up are shown in A,B and C (stage 11) and D,E and F (stage 14). Horizontal optical sections through stage 14 embryos are illustrated in G,H and I. Line 24B directs target gene expression in all mesodermal cells of germ band extended embryos (B,C), and later in the complete complement of somatic muscle precursors (E,F) and the entire visceral mesoderm (H,I). Ectopic expression of UBX appears to be superimposed on the endogenous pattern. Note also that line 24B drives expression of β-galactosidase and UBX in a small cluster of ectodermal cells posterior to the stomodeal opening (arrowheads in B,C) and in a few cells of the pharynx (H,I). However, there is no expression of target genes in the ectoderm of the thoracic or abdominal segments (see also Fig. 7).
enhancer trap (Fig. 4B). The ectopic mesodermal expression of ABD-A was associated with an identical transformation of the NAU pattern as observed for UBX. In some of these embryos, an abdominal-like NAU-expressing muscle precursor was present in the thoracic segments but not in A8 (Fig. 4C), while in others there was a similar transformation of both the thorax and the posterior abdomen (Fig. 4D). As had been observed for ectopic UBX, the number of transformed thoracic segments was variable with ectopic ABD-A (data not shown).

Alterations of the mature muscle patterns of the thoracic segments and of A1 were also associated with ectopic mesodermal ABD-A expression (Fig. 4E,F). These included a change in the thoracic ventral longitudinal muscles to a more abdominal-like identity, acquisition of an abdominal muscle 26 in T3 and an extra ventral oblique muscle (muscle 17) in A1, as well as the presence of atypical ventral muscles in T1 and T2 (Fig. 4F). Some but not all embryos exhibiting these muscle pattern alterations also had a muscle 25 in A1 (data not shown). In at least some cases, it was possible to establish that these changes represented authentic transformations since there was not only the gain of one muscle but also the asso-

Fig. 3. Thoracic to abdominal muscle transformations are associated with ectopic mesodermal expression of UBX. Expression of NAU (A,B; stage 13) and myosin heavy chain (C-F; stage 16) was examined in wild-type embryos (A,C,E) and embryos derived from a cross between GAL4 line 24B and a UAS-Ubx transformant (B,D,F). Targeted expression of UBX in the embryonic mesoderm resulted in an abdominal transformation of the NAU pattern in the thoracic segments. In addition, a muscle 26 precursor formed in A8 under the influence of ectopic UBX (arrow in B). Transformations of the mature muscles in A1 and T1-T3 toward A2 identities were also evident in response to ectopic UBX (D,F; see the text for details). Note the formation of atypical myofibers in T1 and T2 (arrowheads in F) that cannot be identified as particular wild-type thoracic or abdominal muscles.
associated loss of another. This was the case for ventral longitudinal muscle 12 in T3, which has a different characteristic shape than its counterpart in A1 (Figs 3E,F, 4F). An even more dramatic example of a muscle transformation occurred in A1. For both ectopic UBX and ABD-A, the gain of muscle 17 in A1 was associated with the loss of A1-specific muscle 31 (Fig. 5). In summary, identical transformations of mature muscles and NAU-expressing muscle precursors were observed when either UBX or ABD-A was ectopically expressed solely in the mesoderm. These observations underscore the equivalent myogenic functions of UBX and ABD-A and establish that these two homeotic factors must influence muscle pattern formation by autonomous action in mesodermal cells.

The thoracic cuticle is unaffected by ectopic mesodermal expression of UBX and ABD-A

The temporal occurrence and morphological appearance of transformed muscles in embryos with ectopic mesodermal expression of UBX or ABD-A indicates that the altered myofibers have not only developed as fused syncytia but also have formed attachments to the overlying epidermis. This conclusion is reinforced by observations of vigorous thoracic movements of affected embryos within their vitelline
membranes (A. M. M., unpublished results). In some cases, the transformed muscles have attached appropriately, while in others, aberrant insertions have formed (see above and Discussion). To determine if the ectoderm is more extensively altered in these embryos, cuticle preparations were examined (Fig. 6). Ectopic ABD-A in the mesoderm was not associated with any cuticular abnormalities of either the head or thorax (Fig. 6A,B); the entire abdominal cuticle also appeared completely normal (data not shown). This was consistent with the ability of 50% of these embryos to hatch, although all of these died as first instar larvae. The variation in the capacity to hatch most likely reflected differences in the extent of the muscle transformations and correlated with the degree of movement of late-stage embryos (A. M. M., unpublished observations). Thus, as predicted for autonomous mesodermal function of ABD-A in these experiments, the epidermis (with the apparent exception of some muscle attachment sites) is unaffected.

Expression of UBX, driven by the GAL4 24B line, yielded a quite different result despite having the same ectopic expression pattern as ABD-A. Although all components of a normal head could be identified, there was partial inhibition of head involution, improper development of the head skeleton with distortion of the pharynx and lateral displacement of the sense organs, and formation of ectopic abdominal-like denticles in the pharyngeal wall (Fig. 6C,D). These defects are most likely secondary to ectopic UBX in a small number of epidermal cells in the vicinity of the stomodeum and later in the developing pharynx (Fig. 2C,I; data not shown), but are significantly less severe than those associated with ectopic expression of UBX in all cells of the embryo (Gonzalez-Reyes and Morata, 1990; Mann and Hogness, 1990). The abnormal head development that occurred in response to ectopic UBX was associated with a much higher rate of embryonic lethality than was observed for ABD-A expressed in an identical ectopic pattern (93% of the progeny of a cross between GAL4 line 24B and a UAS-Ubx line died as embryos with the remainder not progressing past the first larval instar). Significantly, the cuticle of the thoracic and abdominal segments was normal in the embryos with ectopic mesodermal expression of UBX, although in some cases there were occasional enlarged denticles in one of what was otherwise a wild-type thoracic denticle belt (data not shown). Thus, as for ABD-A, the muscle transformations associated with line 24B-induced expression of UBX are not simply a consequence of altered ectodermal differentiation but are indicative of autonomous mesodermal function of this homeotic gene.

**Homeotic transformations of the embryonic muscle pattern occur independently of direct effects on innervation**

Mosaic analysis had previously established that the identity of one adult muscle is determined by the genotype of its innervating motoneuron (Lawrence and Johnston, 1986). A similar effect is unlikely to explain the present results for the embryonic muscles since at least some of the transformations were observed for NAU-expressing precursors at a stage prior to contact with motor axon growth cones (Johansen et al.,...
Furthermore, no ectopic expression of either UBX or ABD-A was induced by GAL4 line 24B in the central nervous system of those segments that exhibit muscle transformations (Fig. 7). Thus, it seems unlikely that the primary effect of BX-C genes on muscle patterning observed in the current study is mediated by altered innervation. On the contrary, changes in the arrangement of motoneurons has probably occurred as a consequence of the homeotically induced muscle transformations (Sink and Whittington, 1991; Cash et al., 1992; Chiba et al., 1993).

Altered development of the midgut in response to ectopic mesodermal expression of UBX

BX-C genes participate in gut morphogenesis by acting in a cascade of regulatory interactions involving both the mesoderm and endoderm (Immergluck et al., 1990; Panganiban et al., 1990; Reuter et al., 1990). Ubx is expressed in parasegment 7 of the midgut where it is required for the activation of decapentaplegic (dpp) expression and for subsequent formation of the second midgut constriction (Immergluck et al., 1990; Panganiban et al., 1990; Reuter et al., 1990). dpp is also expressed in a small anterior portion of the visceral mesoderm, which corresponds to the primordium of the gastric caeca (Fig. 8A). It was therefore of interest to determine the effects of ectopic UBX expression in the visceral mesoderm. GAL4 line 24B-induced synthesis of UBX led to the activation of dpp expression in a continuous band in the midgut mesoderm anterior to and including parasegment 7 (Fig. 8B). However, no dpp transcripts were detected in more posterior regions of the visceral mesoderm, presumably due to repression by ABD-A (Immergluck et al., 1990; Reuter et al., 1990). Neither was dpp mRNA found in the somatic mesoderm where UBX is abundantly expressed in these experiments (Fig. 2FJ), indicating that UBX alone is not sufficient to activate transcription of dpp. These changes in UBX and dpp expression were associated with the absence of the gastric caeca and the first midgut constriction (Fig. 8C,D). Identical findings have been reported for global ectopic expression of UBX induced under control of a heat shock Fig. 6. Cuticle phenotypes associated with GAL4 line 24B-induced misexpression of UBX and ABD-A. (A) Wild-type cuticle showing the normal head skeleton, thorax-specific sense organs (vp, ventral pit; ko, Keilin’s organ) and characteristic denticle belts of T1-A1. (B) Cuticle from an embryo expressing ABD-A under the control of GAL4 line 24B. The head and thorax appear entirely wild type. (C,D) Cuticle from an embryo with line 24B-induced ectopic expression of UBX. Head involution is incomplete, the head skeleton is distorted and large denticles having an abdominal appearance are present within the pharynx (arrows in the higher magnification view shown in D). However, the thoracic cuticle of this embryo is entirely normal.
promoter (Reuter et al., 1990). However, the present results obtained with targeted mesodermal expression of UBX unambiguously establish that this homeotic gene acts autonomously in cells of the visceral mesoderm.

When similar experiments were undertaken with GAL4 line 24B-activated expression of ABD-A, no effect was observed on midgut development and there was no repression of the parasegment 7 expression of UBX in the visceral mesoderm (data not shown). Thus, under the conditions of this experiment, gain of ABD-A function did not perturb midgut morphogenesis or the expression of other regulatory factors in visceral mesodermal cells in a manner predicted by the corresponding loss-of-function phenotypes (Immergluck et al., 1990; Reuter et al., 1990).

**DISCUSSION**

*Autonomous function of homeotic genes in embryonic mesoderm development*

Targeted misexpression of either UBX or ABD-A to the mesoderm of the thoracic segments resulted in transformations of embryonic somatic muscles and their precursors to an abdominal identity. Since in these experiments neither the epidermis nor the central nervous system of the thorax ectopically expressed the BX-C genes and the thoracic cuticle remained wild type, the effects on muscle development must be due to autonomous function of the homeotic factors in mesodermal cells. Thus, the present experiments clearly separate the developmental effects of the BX-C genes in two germ layers and establish that the observed muscle transformations are not simply a consequence of altered ectodermal differentiation.

The present results provide direct support for previous suggestions that BX-C genes act autonomously in the mesoderm to specify embryonic muscle identity (Lawrence, 1985; Hooper, 1986). The difference between thoracic and abdominal segments in the number of *twist*-expressing adult muscle precursors is also determined by the autonomous action of a homeotic gene in the embryonic mesoderm, although transformations of the mature muscle pattern were not established in this study (Greig and Akam, 1993). These findings are in contrast to the nonautonomous influence of the BX-C on the development of a male-specific adult muscle (Lawrence and Johnston, 1984, 1986). Thus, homeotic effects on muscle patterning can be mediated by either intrinsic or extrinsic mechanisms depending on the developmental context.

Both UBX and ABD-A are expressed in the mesoderm prior to the formation of fused muscle precursors (Beachy et al., 1985; White and Wilcox, 1985; Bate, 1990; Karch et al., 1990; Macias et al., 1990). These homeotic factors are therefore poised to exert an early influence on muscle identity. Such early mesodermal action does appear to occur, since transformations associated with both gain and loss of BX-C gene...
function were observed in the pattern of NAU-expressing muscle precursors. Although only a subset of the entire complement of muscle precursors expresses NAU at the end of germ band shortening, it is expected that the identities of most if not all abdominal myogenic cells are also specified by BX-C genes. The additional transformations of the mature muscles that were observed in the present GAL4 experiments and in Ubx mutant larvae (Hooper, 1986) are consistent with this proposal. By analogy, members of the ANT-C are likely to exert similar functions in the mesoderm of the thoracic segments.

The time course of BX-C gene expression and action in the embryonic mesoderm raises the possibility that nau is a direct downstream target for regulation by these homeotic factors. The results of both in vitro and in vivo experiments to define sequences responsible for nau transcription are consistent with this idea (A. M. M., S. Abmayr and T. Maniatis, unpublished observations). Thus, the global effects on segmental identity that are exerted by homeotic genes may be mediated through the control of genes encoding tissue-specific regulators such as nau.

Although Ubx has a well-characterized role in development of the midgut (Tremml and Bienz, 1989; Immergluck et al., 1990; Panganiban et al., 1990; Reuter et al., 1990), only indirect evidence has suggested that this effect is autonomous to the mesoderm (Reuter et al., 1990). The present results unambiguously establish that this homeotic gene acts intrinsically in the visceral mesoderm to control dpp expression and to influence midgut morphogenesis. When a similar experiment was undertaken with abd-A, no effect was observed either on Ubx expression in parasegment 7 of the visceral mesoderm or on formation of the midgut (A. M. M., unpublished observations). Thus, whereas loss of abd-A function is associated with a distinct gut phenotype and with a posterior expansion of the visceral mesodermal domain of UBX expression (Tremml and Bienz, 1989; Immergluck et al., 1990; Panganiban et al., 1990; Reuter et al., 1990), the ectopic expression of ABD-A in this system is not sufficient to exert a reciprocal effect. Perhaps additional factors are required to cooperate with ABD-A in the visceral mesoderm and these are confined to cells that normally express ABD-A. Alternatively, factors in more anterior regions might antagonize the potential influence of ectopic ABD-A in the visceral mesoderm.

**Equivalent functions of Ubx and abd-A in somatic mesoderm development**

Although differences were observed in the effects of Ubx and abd-A in the visceral mesoderm, equivalent functions were apparent for somatic muscle diversification. Thus, Ubx and abd-A were found to direct an identical pattern of nau expression in A1-A7. In fact, despite the partial overlap of their expression domains in the somatic mesoderm, the BX-C loss-

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**Fig. 8. Effects of UBX misexpression in the visceral mesoderm.** Stage 14 embryos were hybridized in situ with a digoxigenin-labeled dpp probe (horizontal optical sections are shown in A and B). Stage 16 embryos were stained with an antibody directed against myosin heavy chain and photographed in a nearly midsagittal plane of focus (C,D). Wild-type embryos are shown in A and C, while those in B and D misexpress UBX in the enhancer trap pattern of GAL4 line 24B. The domain of dpp expression in the visceral mesoderm is expanded anterior to parasegment 7 (PS7) in response to ectopic UBX. This is associated with a loss of both the first midgut constriction (the normal constrictions are labeled 1, 2 and 3 in C) and the gastric caeca (GC).
of-function phenotypes indicated that these two homeotic genes are able to substitute for each other in this regard. The gain-of-function experiments confirmed this conclusion since identical abdominal transformations of the thoracic segments were observed with ectopic expression of either UBX or ABD-A. This equivalent regulatory effect of Ubx and abd-A is consistent with the similarity of the wild-type muscle and muscle precursor patterns of the anterior abdominal segments where these genes are differentially expressed (Bate, 1990).

Identical actions of Ubx and abd-A in development of larval and adult ectodermal derivatives have also been described (Karch et al., 1990). A molecular explanation for these related functions of UBX and ABD-A can be found in the sequence similarity and DNA binding specificities of their homeodomains (Samson et al., 1989; Scott et al., 1989). Furthermore, it was recently established that these two homeotic factors can bind to the same regulatory sites in defined target genes where they appear to exert common effects in multiple ectodermal cell types (Vachon et al., 1992; Appel and Sakonju, 1993). A similar result is anticipated for mesoderm-specific target genes.

**Distinct role of Abd-B in the somatic mesoderm**

In contrast to the activation of nau in the anterior abdominal segments under the influence of Ubx and abd-A, Abd-B was found to prevent expression of this muscle-specific gene in A8 early after the completion of germ band retraction. However, high levels of ectopic UBX or ABD-A were found to overcome this repression of nau by Abd-B. This argues against a strict hierarchy of homeotic gene function in which posteriorly expressed members of the BX-C suppress the phenotypic effects of those members expressed in more anterior domains (Gonzalez-Reyes and Morata, 1990; Gonzalez-Reyes et al., 1990). Rather, the present results are consistent with a dose-dependent competition among several homeotic proteins for the regulation of a common mesodermal target gene (Lamka et al., 1992). The observed variation between embryos in the posterior influence of UBX and ABD-A could be due to small individual differences in the ectopic expression level of these factors and the existence of a requisite threshold concentration for the competitive effect. The suggested competition could occur at the level of DNA binding or, given the probable differences in sequence recognition specificities of these divergent homeodomains (Scott et al., 1989), at an independent transcriptional step (Appel and Sakonju, 1993).

It must be emphasized that at various embryonic stages nau is expressed in the somatic mesoderm of all segments but in distinct patterns that vary along both the anteroposterior and dorsoventral axes. In the absence of BX-C function, nau expression is not lost from the abdominal segments but rather is transformed into a thoracic pattern, presumably under the influence of derepressed ANT-C genes. Thus, homeotic factors primarily serve to modulate the metameric identity of the nau pattern that is additionally regulated by as yet unidentified determinants.

**Origin of the unique muscle pattern of A1**

There are several unique features that distinguish the muscle pattern of A1 from that of A2-A7 (Bate, 1990). These include the presence of an additional inner ventral longitudinal muscle and the absence of two neighboring ventral muscles. Ectopic expression of either UBX or ABD-A led to the complete transformation of the A1-specific muscle pattern to that of A2. These transformations are in agreement with the relationships that have been suggested to exist among the relevant muscle precursors involved in establishing the variant arrangement of muscles in A1 (Bate, 1990).

The response of A1 to ectopic UBX occurred even though this factor is normally present in all muscles of this segment (Fig. 2D; Hooper, 1986). This apparent paradox can be explained by a differential sensitivity of muscle identity to varying levels of UBX and/or ABD-A in the individual abdominal segments. The amount of UBX in the muscles of A1 is lower than that of A2 (Hooper, 1986) and ABD-A is not expressed in A1. Ectopic expression of either UBX or ABD-A would eliminate this concentration difference and this could drive muscle precursor development toward an A2 specificity. This idea is in agreement with the equivalence of function of these two homeotic factors, as previously discussed. Since the amount of UBX declines as the ABD-A domain is encountered and the nau pattern of this region is not transformed in a Ubx mutant, the level of ABD-A alone must be sufficient to maintain abdominal muscle identity in the more posterior segments. Effects of Ubx dosage on the identities of both embryonic and adult ectodermal structures have also been described (Gonzalez-Reyes and Morata, 1990; Smolik-Ultaut, 1990).

Whereas the acquisition of muscle 17 in A1 in response to ectopic UBX or ABD-A expression is accompanied by the loss of muscle 31, the novel ventral muscles that form aberrant attachments in the thoracic segments of these embryos are not associated with the loss of a normal thoracic neighbor. The latter situation does not represent an identity change between two mature embryonic myofibers. Nevertheless, the new muscles must derive from a transformation in mesodermal cell identity. The source of the requisite myoblasts for their formation may be the pool of twist-expressing adult muscle precursors that are lost from the thorax when a BX-C gene is ectopically expressed in the mesoderm (Greig and Akam, 1993). This suggests that an early function of homeotic genes is to influence the segment-specific partitioning of particular mesodermal cells into embryonic and adult muscle lineages.

**Interactions between the mesoderm and ectoderm**

Ectopic expression of UBX and ABD-A led not only to the development of NAU-expressing abdominal muscle precursors in the thorax but also to transformations of the mature muscle pattern in segments anterior to and including A1. Some of the transformed muscles appeared to have the equivalent epidermal attachments as their counterparts in wild-type abdominal segments, despite their ectopic anterior locations. However, in other cases the transformed muscles did not attach to the wild-type epidermis in a manner dictated by their abdominal identities. The latter situation is exemplified by the abovementioned atypical ventral thoracic muscles. These myofibers, which on the basis of their directionality and ventral position probably possess abdominal muscle 26 identity, have attachments that are characteristic of neither the thorax nor the abdomen. This aberrant situation must reflect the outcome of juxtaposing an abdominal muscle and an epidermis that retains its thoracic identity. Thus, the pattern of muscle attachments cannot be determined exclusively by either the mesoderm or
the ectoderm but must be the product of reciprocal interactions between the segment-specific properties of both myogenic and neighboring epidermal cells. The complete transformation of the A1 muscle pattern by ectopic UBX is consistent with this idea since the strong expression of UBX in the wild-type epidermis of this segment provides a precise match between mesodermal and ectodermal cues.

Innervation does not play a significant role in determining embryonic muscle identity (Johansen et al., 1989; Bate, 1990; Broadie and Bate, 1993). Rather, the pattern of motoneuron connectivity is directly influenced by changes in the arrangement of somatic muscles (Sink and Whitington, 1991; Cash et al., 1992; Chiba et al., 1993). Based on these findings and the presence of muscle contractions in the affected segments of living embryos ectopically expressing UBX or ABD-A (A. M. M., unpublished observations), it is likely that motor synapses were formed on the ectopic muscles induced in the present experiments. This is consistent with the view that synaptic recognition is affected by the properties of particular myofibers (Halpern et al., 1991; Chiba et al., 1993). Thus, a complex interplay between mesodermal and ectodermal derivatives is responsible for the definitive pattern of mature, innervated muscles.

Positional information and muscle pattern formation

The muscles of all species must acquire not only general differentiated properties such as the production of contractile proteins and the formation of motor synapses, but also the unique features that distinguish individual myogenic cells. In vertebrates, the latter process of muscle fiber diversification is dependent on both intrinsic and extrinsic factors (Stockdale, 1992; Blau et al., 1993). In this report, it has been established that the intersegmental diversity of muscle fibers in the Drosophila embryo is determined by homeotic genes acting autonomously in mesodermal cells. This represents a clear example of how positional information that is established early in development can be utilized at later times to modulate a morphogenetic process. Additional regulatory factors must influence the individual properties of muscles within each segment.

A role for positional information in mammalian myogenesis has also recently been demonstrated (Donoghue et al., 1992; Grieshammer et al., 1992; Cheng et al., 1993). However, in these cases the molecular nature and origin of the relevant cues have not been established. Perhaps homeotic genes play a key role in specifying myogenic cell identity in mammals as they do in Drosophila. Additional work in both vertebrate and invertebrate systems will be required to further delineate this and other components of the muscle patterning machinery and should lead to a better understanding of evolutionarily shared mechanisms of organogenesis.

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