Development 127, 5309-5318 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
DEV7833

Apterous mediates development of direct flight muscles autonomously and indirect flight muscles through epidermal cues

Arjuman Ghazi, Sumita Anant and K. VijayRaghavan*

National Centre for Biological Sciences, TIFR, Bangalore 560065, India
*Author for correspondence (e-mail: vijay@ncbs.res.in)

Accepted 5 October; published on WWW 14 November 2000

SUMMARY

Two physiologically distinct types of muscles, the direct and indirect flight muscles, develop from myoblasts associated with the *Drosophila* wing disc. We show that the direct flight muscles are specified by the expression of Apterous, a Lim homeodomain protein, in groups of myoblasts. This suggests a mechanism of cell-fate specification by labelling groups of fusion competent myoblasts, in contrast to mechanisms in the embryo, where muscle cell fate is specified by single founder myoblasts. In addition, Apterous is expressed in the developing adult epidermal muscle attachment sites. Here, it functions to regulate the expression of *stripe*, a gene that is an important element of early patterning of muscle fibres, from the epidermis. Our results, which may have broad implications, suggest novel mechanisms of muscle patterning in the adult, in contrast to embryonic myogenesis.

Key words: *apterous*, Myogenesis, Flight muscles, *stripe*, *Drosophila*

INTRODUCTION

Myogenesis can arbitrarily be classified into events common to most muscles and others that confer muscle- or fibre-specific identities. Myoblast proliferation, migration, cell cycle exit and fusion are common to most muscles. In addition, specific properties of muscles determine the biochemical and physiological characteristics of each fibre (Bate, 1990, 1993; Bernstein, 1993).

During differentiation, these general and specific events must finally be integrated – myoblast fusion is a directed process that results in the formation of fibres with predictable, location specific properties. How is this specificity regulated? In the *Drosophila* embryo, the general and specific aspects of myogenesis have been integrated in the properties of the founder cell that directs the fusion process, while also conferring identity to the developing fibre (Rushton et al., 1995; Ruiz-Gomez et al., 1997). Other myoblasts, the feeders, fuse to founders but are incapable of fusing to each other. Founder identity is specified by the autonomous function of specific transcription factors (Dohrmann et al., 1990; Ruiz-Gomez et al., 1997; Ruiz-Gomez and Bate, 1997; see Baylies et al., 1998 and Frasch, 1999 for reviews). It would therefore appear that, in the *Drosophila* embryo, feeder myoblasts do not require their identity to be specified and 'merely' serve to increase fibre size. This is exemplified by examining the pattern of innervation, location and function of differentiating founders, in a situation where fusion is inhibited (Rushton et al., 1995).

Flight muscle development has features that suggest that other mechanisms may operate during myogenesis (Roy and VijayRaghavan, 1999). Adult myoblasts are set aside during embryogenesis and are siblings of embryonic founder cells (Bate et al., 1991). However, unlike their siblings, they proliferate during larval stages and are closely apposed, in the case of the dorsal mesothorax, to the region of the wing disc epithelium that forms the adult notum (Bate et al., 1991). These myoblasts are the progenitors of the indirect flight muscles (IFMs) and the direct flight muscles (DFMs). The development of the IFMs has been well studied (Fernandes et al., 1991; Fernandes and VijayRaghavan, 1993; Roy et al., 1998; Roy and VijayRaghavan, 1998) but development of the DFMs has been poorly examined (see Fig. 1 for a schematic of muscle development in the dorsal mesothorax).

What are the mechanisms that determine cell fate in adult flight muscles? Specifically, how are identities of these very different muscle fibre groups, the DFMs and the IFMs determined? We show that *apterous* (*ap*), which encodes a LIM-homeodomain protein and is a muscle 'founder cell identity' marker in the embryo (Bourgouin et al., 1992), is expressed in the progenitors of DFMs during pupal development. Using loss- and gain-of-function experiments we show that *ap* is also necessary for DFM development.

In addition to its early expression and role in DFM development, *ap* also has another, nonautonomous function, in patterning the IFMs. It regulates the early expression of *stripe* (*sr*), a gene that is a crucial determinant of epidermal muscle attachment sites (Volk and VijayRaghavan, 1994; Lee et al., 1995; Fernandes et al, 1996). We show that *sr* expression is absent or reduced in *ap* mutant wing discs, and *ap* and *sr* genetically interact with each other to affect IFM development. Further, *ap* mutant animals have adult thoracic muscle defects. We demonstrate tissue-specific requirements of *ap* by showing independent rescue of DFM and IFM phenotypes by mesodermal and epidermal *ap* expression, respectively.

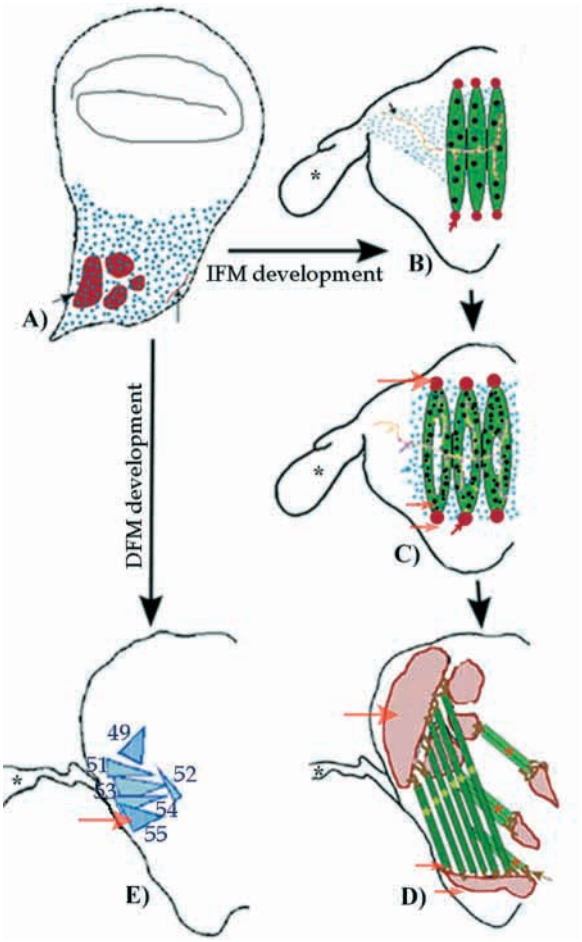

Fig. 1. Schematic representation of thoracic myogenesis. (A) Myoblasts (blue dots) on the wing disc are associated with the presumptive notum and proliferate during larval life. *sr* expression (red regions indicated by arrows) on the presumptive notum marks the future epidermal attachment sites of thoracic muscles. (B-D) IFM development; anterior is towards the top and dorsal midline towards the right. (B) Myoblasts migrate on the everting disc epithelium (schematic represents 7-12 hours APF) onto three larval muscles (green fibres; nerves in yellow) that escape histolysis and serve as templates that attach to *sr* expressing epidermal attachment sites (red spots). (C) As myoblasts (blue) fuse to the templates, they begin to split longitudinally between 14-18 hours APF. By 19 hours APF, six dorsal longitudinal muscles (DLMs) are in place. (D) IFMs in an adult heminotum with their attachment sites. Six DLMs (green with yellow asterisks) run anteroposteriorly and attach to *sr*-expressing attachment domains (red). DVMs run dorsoventrally (green with red asterisks). (E**)** Direct flight muscles (DFMs) are also derived from wing disc-associated myoblasts. Muscles 49 and 51-55 followed in our study are shown in blue. In C-E, the expression of *ap* has been indicated with bright orange horizontal arrows. Wing disc shows low levels of *ap* expression through out the presumptive notum region. Wing bud (B,C) and adult wing nub (D,E) are indicated by asterisks.

Our report analyses the mechanisms underlying the dual roles for *ap* in adult myogenesis: autonomously for DFM development and an extrinsic epidermal function in patterning early events in IFM development. We discuss our results in the context of cell-fate specification in adult myogenesis where embryonic founder markers are expressed in groups of myoblasts rather than in single cells and relate these to possible similarities with vertebrate myogenesis.

MATERIALS AND METHODS

Strains and reagents

Canton-S (CS) was used as wild type. *ap lacZ* (Cohen et al., 1992) is a hypomorphic enhancer trap insertion just 5′ of the *ap* coding region. *ap Muscle Specific lacZ (ap MS lacZ)* has been used by Becker et al. (1997) and was from Maria Capovilla and Juan Botas. *P1618* is a P-element *lacZ* insertion in the *sr* locus (Callahan et al., 1996). sr^{G11} is an embryonic null mutation (Frommer et al., 1996). The multiply marked *ru, cu, ca/ru, cu, ca* stock (Lindsley and Zimm, 1992), carrying the sr^{1} allele that, in a homozygous condition, gives rise to a dark midnotal stripe and *md237pnrGAL4* were obtained from Bloomington Stock Centre (IN). An *ap^{4}/CyO MHC lacZ; P1618/TM6 Tb* stock was generated for this study. *UAS-ap* strains were gifts of John Thomas (Salk Institute, CA). Reporter gene activity in *Ac(88F)lacZ* (Hiromi et al, 1986) has been developmentally profiled (Fernandes at el., 1991). The *srGAL4* strain is from Gines Morata (Madrid) and was recombined with *UAS-GFP (srGAL4UAS, GFP/TM6 Tb)* by Daniela Pistillo and Pat Simpson (Strasbourg).

For the epidermal rescue experiment, *ap^{4}/CyO MHC lacZ; UAS-ap/TM6 Tb* flies were crossed to *ap^{4}/CyO MHC lacZ; pnrGAL4/TM6 Tb* flies. The experiment was conducted at 18°C. Non *Tb* pupae were separated and eclosing adults scored for absence of wings. Thoracic muscle morphology of these flies was checked and compared with the wingless *ap^{4}* homozygotes. For the mesodermal rescue experiment, *1151/Y; ap^{4}/CyO MHC lacZ* males were crossed to *ap^{4}/CyO MHC lacZ; UAS-ap/TM6 Tb* virgins. The cross was grown at 18°C and eclosing wingless females scored for muscle integrity.

Twi antibody (Seigfried Roth, Cologne) was used at 1:5000 dilution. Anti-β-galactosidase and anti-MHC antibodies were used at dilutions of 1:1000 and 1:500, respectively. Anti-Ap antibody (John Thomas, Salk Institute, CA), was preadsorbed overnight and used at a dilution of 1:500. For horseradish peroxidase (HRP) immunohistochemistry, biotinylated secondary antibodies were used and visualised using an standard A+B kit (Vector Chemicals). For fluorescent reactions FITC (green), Alexa 568 (red) or Alexa 488 (green) secondary antibodies were used. Confocal analysis was carried out on a Bio-Rad Model 1024 confocal microscope.

Dissections

Larvae and pupae were dissected in phosphate-buffered saline (PBS), fixed in 4% paraformaldehyde in PBS and stained with X-gal (Fernandes et al,. 1991) or relevant antibodies. All preparations, except fluorescent samples, were mounted in 70% glycerol. Fluorescent preparations were mounted in Vectashield mounting medium (Vector Chemicals). For adult hemithoraces, nota were cut sagitally, dehydrated through 70%, 90% and absolute ethanol grades, cleared in methyl salicylate, mounted in Canada Balsam and observed under polarised light.

RESULTS

Adult *ap* expression suggests roles in direct flight muscles and muscle epidermal attachment

In examining the adult expression pattern of embryonic muscle founder markers, we observed expression of an *ap* reporter (Cohen et al., 1992) in a pattern that suggested specific roles in myogenesis. The DFMs, located dorsolaterally in each hemisegment of the adult mesothorax, showed reporter gene activity. No staining was seen in the

IFMs, which constitute the bulk of the muscles of the dorsal mesothorax. Amongst DFMs, the most conveniently identifiable ones are muscles 49-58 (Miller, 1950; Bate, 1993). Muscles 49 and 51-55, showed staining for the *ap lacZ* reporter gene in different planes of foci (Fig. 2A-D), and are schematically represented in Figs 1E, 2E.

Epidermal attachment sites of muscles in the adult fly are identifiable by anatomical examination and by expression of *stripe* (*sr*). *sr* marks all muscle attachment cells, both in the embryo and the adult (Volk and VijayRaghavan, 1994; Fernandes et al., 1996). The pattern of *sr* expression during pupal development has been studied (Fernandes et al., 1996) and the attachment sites for the IFMs identified (Fig. 1D). *ap lacZ* adult expression is also seen in the thoracic epidermis in the regions where muscles attach (Fig. 2F).

ap expression in the developing notum changes from a broad domain to localisation to muscle attachment sites

On the third instar wing imaginal disc the presumptive notum showed low *ap lacZ* (Fig. 3A) and Ap protein expression (data not shown) distinct from the high levels seen in the presumptive dorsal wing. Although the presumptive notum is a dorsal structure, *ap* does not seem to have a selector function to define 'dorsalness' in the mesothoracic trunk as it does in the dorsal wing blade (Cohen et al., 1992; Blair et al., 1994).

ap expression in the pupal epidermis changed temporally beginning with an early expression in broad regions of the dorsal notal epidermis and a subsequent localisation to restricted domains including the attachment sites of the DLMs. At 18 hours after puparium formation (APF), *ap lacZ* expression was seen in regions that included the developing anterior attachment sites of the DLMs (Fig. 3D). This expression eventually narrowed down to the anterior attachment sites and very closely abutted the posterior attachment sites of the DLMs (Fig. 3E). This can be observed by simultaneous labelling of developing pupae for *ap* and *sr* (Fig. 3F-H). By 36 hours APF, when a complete set of DLM fibres is in place, *ap* co-localisation with individual muscle-attached *sr*-expressing tendon cells was clearly visualised (Fig. 3I-K). This attachment site expression continued in the adult as shown in Fig. 2F. The same pattern was seen on labelling with Ap-specific antibodies (data not shown).

ap is not expressed in myoblasts on the third instar wing imaginal disc

We examined the developmental origins of the adult DFM-specific expression of *ap*. Three simple possibilities suggest themselves:

(1) *ap* is expressed in all myoblasts on the third larval instar wing disc and later, perhaps during metamorphosis, becomes 'switched off' in IFMs or their progenitors.

(2) *ap* is expressed in a subset of third larval instar wing disc associated myoblasts destined to form DFMs.

(3) *ap* expression is absent from all the myoblasts on the third instar wing disc and begins later, during pupal development, in the developing DFMs.

We find no evidence of *ap* expression in the wing disc myoblasts. Several lines of evidence substantiate this. *ap lacZ* wing imaginal discs were double labelled with antibodies against the β-galactosidase protein and against Twist, which marks all myoblasts (Bate, 1990; Currie and Bate, 1991; Fernandes et al., 1991). Although epidermal expression of *ap* was clear, no co-localisation of *ap lacZ* expression with Twist was observed. This was confirmed by both confocal microscopy (Fig. 3A-C) as well as HRP immunohistochemistry and examination with Nomarski optics (data not shown). Wild-type wing discs labelled with anti-Ap antibody did not show any myoblast staining, nor did wing discs of a muscle-specific *lacZ* strain labelled with anti-β-galactosidase (data not shown). The dorsal cells of the disc epithelium were labelled with anti-Ap antibody as expected. These data suggest that *ap* expression in the DFMs or their progenitors begins during pupal development.

An embryonic muscle-specific *ap lacZ* strain shows DFM-restricted expression during adult muscle development

We examined the expression of an embryonic muscle (lateral transverse muscles LT1-4) specific *ap* reporter strain (see Materials and Methods) during adult muscle development and

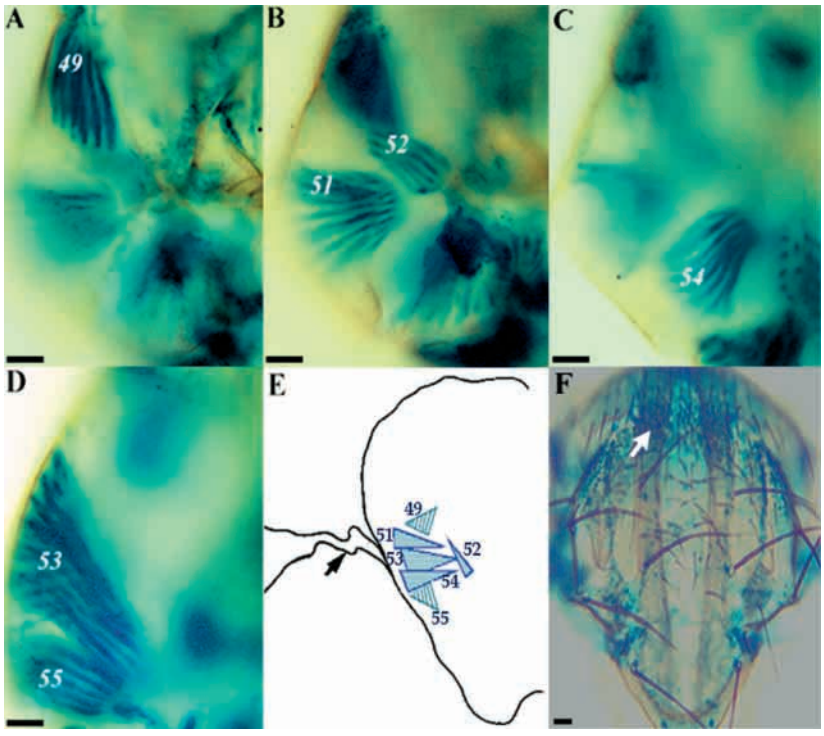

Fig. 2. *ap* expression in DFMs and adult epidermis. *ap* expression in adult thoracic tissues observed by β-galactosidase activity in *ap lacZ* animals. (A-D) Some of the DFMs analysed in our study. A-C are images from the same hemithoracic preparation observed at different planes of foci, to show muscles 49, 51-52 and 54, respectively. D shows DFMs 53 and 55 from a separate heminotum. Muscle fibres have been numbered in each panel. (E) Schematic representation of these DFMs showing their position in the notum; arrow shows wing hinge region. (F) Epidermal attachment site staining of *ap* (arrow indicates anterior dorsal staining). Anterior is towards the top. Scale bars: 10 μm.

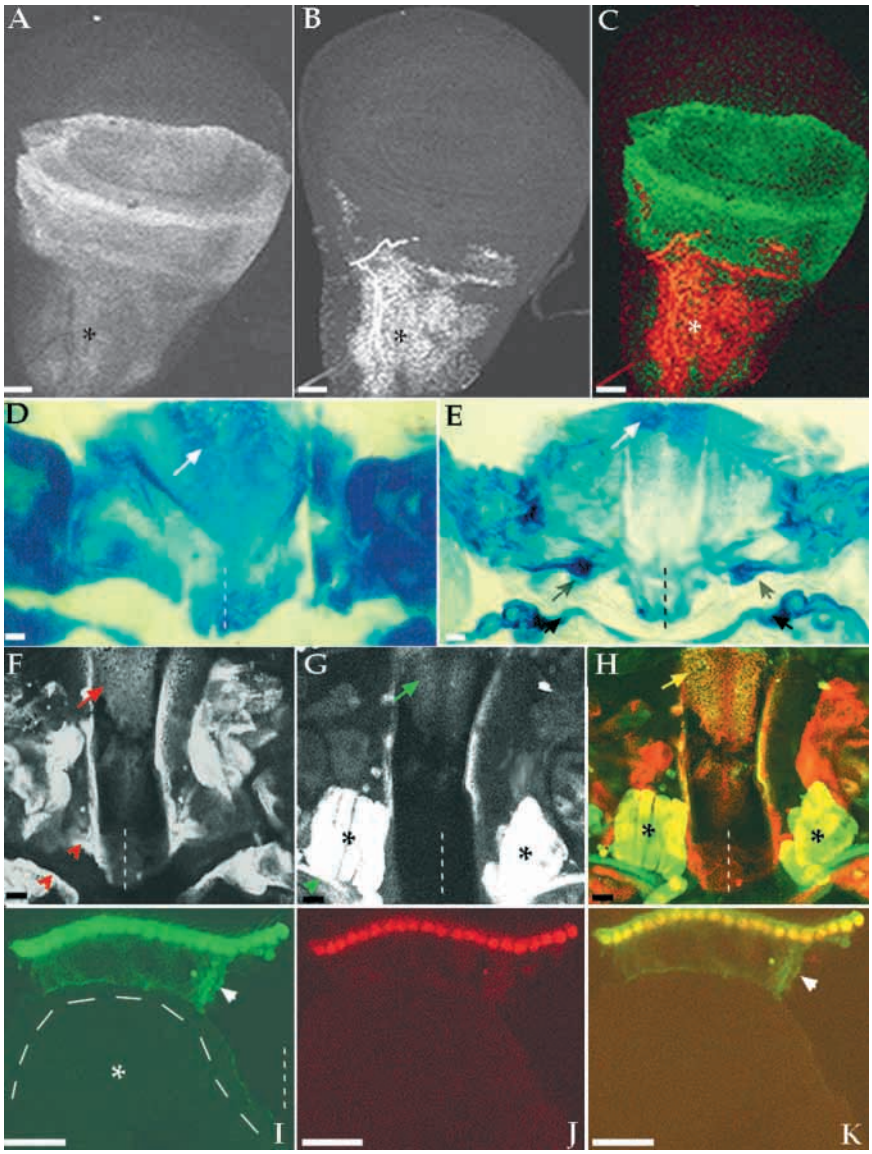

Fig. 3. *ap* is absent in wing imaginal disc myoblasts and present in epidermal attachment sites. (A,B) Confocal images of *ap lacZ* wing discs labelled with anti-β-galactosidase (A) and anti- Twist (B) antibodies. Low levels of *ap lacZ* expression observed in presumptive notal epidermis (asterisk in A). Twist marks all wing disc-associated myoblasts (asterisk in B). (C) Merged image of A and B shows that Twist (green) and *ap-lacZ* (red) are not co-localized (white asterisk). (D,E) *ap* expression in the pupal epidermis visualised using β-galactosidase activity on *ap lacZ* animals. (D) 18 hours APF, *ap lacZ* thorax shows broad regions of epidermal staining (arrow indicates region of anterior attachment sites). (E) 36 hours APF *ap lacZ* thorax shows restriction of early epidermal staining (D) to muscle attachment sites. Anterior DLM attachment sites (white arrow) and regions that abut posterior attachment sites (black and grey arrows) are indicated. (F-K) Co-localisation of *ap* with some of the IFM attachment sites. (F) 26 hours APF *ap lacZ/+; srGAL4, UAS-GFP/+* pupa labelled with anti-β-galactosidase to show *ap* expression (arrow and arrowheads). (G) The same preparation as in F observed for GFP fluorescence that marks *sr*-expressing cells (arrow indicates the anterior and arrowhead the posterior attachment sites). (H) A merged image of F and G shows co-localisation of *ap* and *sr* in anterior DLM attachment sites clearly (yellow arrow), whereas *ap* can be seen present on either side of posterior attachment sites (see region of arrowheads in F and G, and compare with H). The muscle in the preparation was marked using anti-MHC antibody, labelled with a fluorescent-conjugated secondary that is excited in the same range as GFP and fluoresces green (asterisks). DLMs can hence be seen in the same panel as *sr* GFP fluorescence (G) and are marked by asterisks. Their yellowish green colour is caused by the very high intensity of signal from muscle and is not a consequence of co-localisation of *ap* and Myosin (compare F and G). (I-K) Co-localisation of *ap* and *sr* expression in anterior attachment sites at 36 hours APF in *ap lacZ/+; srGAL, UAS-GFP/+* pupa labelled for *sr* by exciting for GFP fluorescence (I) and labelled for *ap* by anti-β-galactosidase staining (J). K is a merged image of the two and shows complete co-localisation of *ap* and *sr*. DLMs are marked by an asterisk in I; their outline is seen as background in all three panels (broken line). Apodemes, epidermal processes connecting muscle to adhesion sites, are indicated by arrowheads in I and K. In A-C, anterior is towards the left; in D-K anterior is towards the top. In D-K, dorsal midline is indicated by a vertical broken line. Scale bars: 10 μm.

compared it with the expression of the *ap lacZ* strain described above and with Ap antibody labelling.

The embryonic muscle-specific *ap lacZ* (*apMS lacZ*) showed an adult DFM-restricted pattern of expression in a manner similar to reporter insertions in the *ap* locus. However, there was no detectable expression in the presumptive dorsal wing blade or in the presumptive notum (data not shown). The pupal attachment site expression observed with *ap lacZ* and Ap antibody staining was not seen (however, in the adult, staining was seen in the dorsocentral bristles). As in the embryo, the 'muscle-specific enhancer' showed *ap* expression in the developing mesoderm but, unlike the embryo, in clusters of myoblasts and not in single 'founder' cells. This is an important difference, the implications of which are discussed later.

We used this mesodermal expression of *apMS lacZ* to follow *ap* expression during adult development and this allowed a close observation, uncluttered by epidermal staining, of the dynamic expression pattern of *ap* in the developing DFMs. It also provided us with a broad developmental analysis of DFMs.

Myoblast expression of *apMS lacZ* was seen at 12-14 hours APF in clusters of cells all over the dorsal notum (Fig. 4A-C). The three larval muscles that escaped histolysis and served as templates for DLM formation did not express the reporter gene or Ap protein at detectable levels in our assays (Fig. 4A,B). The extent of *ap* myoblast expression as seen by reporter gene activity increased from 19-21 hours APF onwards, until about 24-26 hours APF and a number of clusters of myoblasts were seen. Between 26-28 hours APF to 34-36 hours APF, these clusters began to arrange themselves into distinct fibres (Fig.

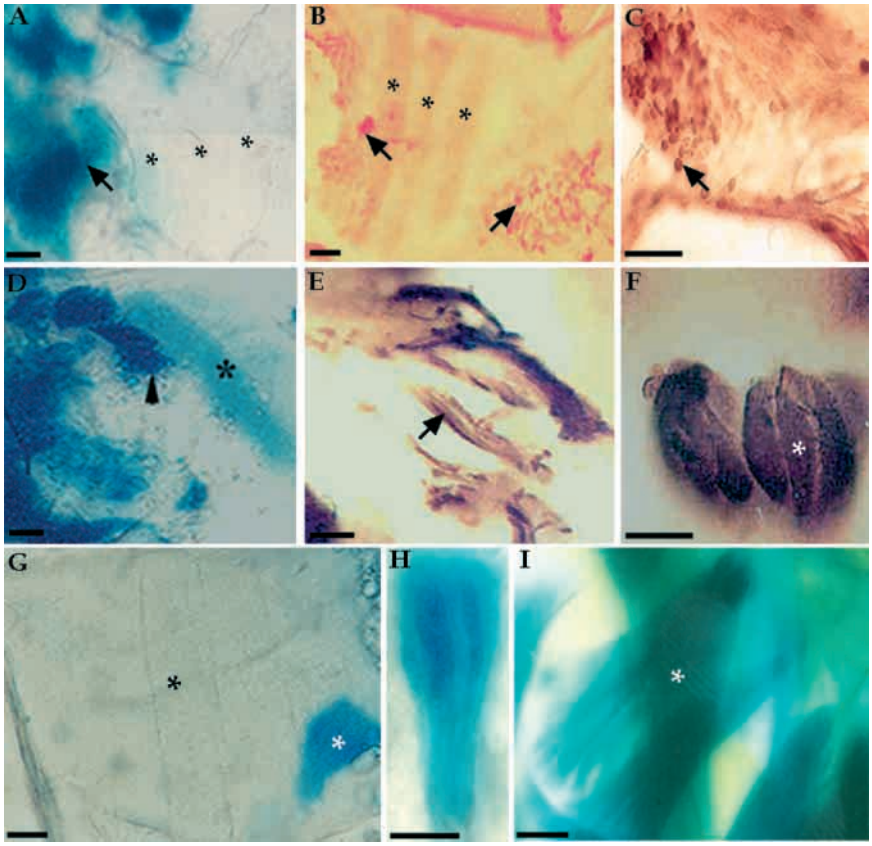

Fig. 4. *ap* expression during DFM development. DFM development followed by assaying for β-galactosidase activity (A, D,G-I) and anti-β-galactosidase antibody labelling (B,C,E,F) on *apMS lacZ* pupae. (A) 14 hours APF the pupa shows myoblast clusters (arrow). Positions of larval templates for DLMs (which do not stain) are indicated by asterisks. (B) Anti-β-galactosidase labelling on 14 hours APF thorax confirms absence of *ap* from templates (asterisks), whereas myoblast clusters are labelled (arrows). Left-hand arrow indicates a cluster that eventually forms one of the muscles analysed in this study (DFMs 49, 51-55). Strong signal from myoblasts, compared with templates, is observed in the cluster indicated by the right-hand arrow. (C) 14 hours APF thorax shows myoblast clusters staining strongly for *ap* (arrow). (D) 28 hours APF thorax shows β-galactosidase activity in DFM clusters (arrowhead). Asterisk indicates one of the DVMs that shows colour caused by leaching of stain. (E) Anti-β-galactosidase labelling at 28 hours APF shows developing DFM clusters (arrow). (F) Anti-β-galactosidase labelled 36 hours APF thorax shows a completely formed DFM cluster (asterisk marks muscle 52). (G) 36 hours APF thorax stained for β-galactosidase activity. Six DLMs seen without any staining (black asterisk) in background of a DFM (white asterisk), which is possibly muscle 55 and is magnified in H. (I) DFMs 51 (asterisk) 53 and 54 from an adult showing β-galactosidase activity. Anterior is towards the top and dorsal midline towards the right, except in H and I, which have anterior towards the left and dorsal towards the top. Scale bars: 10 μm.

4D,E) and by 36 hours APF completely formed DFMs were in place (Fig. 4F). Fibres increased in size considerably after 36 hours APF until about 48 hours APF at which time the complete complement of DFMs could be identified. Adults continued to express *apMS lacZ* in the DFMs (Fig. 4I). Immunohistochemistry with anti-Ap antibodies confirmed this (data not shown).

Many of the clusters of myoblasts that express *ap* can be identified as progenitors of specific DFMs. These inferences are based on the positions of these clusters and their correlation with Miller's numbering scheme (Miller, 1950). One cluster is destined to form muscle 55 (Fig. 4G,H), based on position and orientation, as the developing fibre in this region is always noticed at the lateral edge of the last DLM fibre and corresponds to the position occupied by DFM 55 in the adult. Another cluster that is consistently noticeable is present below the DLMs and prefigures muscle 52 (Fig. 4F).

ap is required for both DFM and IFM development

To decipher the functional significance of the *ap* expression pattern, we examined flight muscles of several viable *ap* alleles. Homozygous ap^4 animals showed the strongest defects in the thoracic musculature. In particular, the DFMs were severely affected. For our study, we have concentrated on four of the DFMs: 51, 52, 53 and 54 (Fig. 5A). Most ap^4 mutants showed a sliver of a fibre instead of four distinct muscles (Fig. 5C). Defects in *ap lacZ* homozygotes were less severe (Fig. 5B). IFM defects included a characteristic reduction in the width of the posterior attachment sites of the DLMs giving them a thin and 'tapering' appearance (Fig. 5E). This is consistent with prominent *ap* expression closely abutting the posterior attachment sites of the DLMs. Another IFM defect was the incomplete splitting of templates for DLM formation, resulting in three instead of six fibres (Fig. 5E). A third phenotype was formation of a single fibre (Fig. 5F). The DVMs also showed defects and were either absent (Fig. 5I) or reduced in size (Fig. 5H). The severity of phenotypes could be ordered as DFMs>DLMs>DVMs. A common feature of all mutants was a striking decrease in overall muscle size and volume.

DFM defects in *ap* mutants can be rescued by muscle-specific expression of *ap*

We attempted rescue of DFM mutant phenotypes by expressing *ap* selectively in the mesoderm in a viable *ap* mutant background. *ap* was expressed in the developing adult muscles of ap^4 homozygous animals using the GAL4/UAS system (Brand and Perrimon, 1993). We used the *1151 GAL4* driver, which shows reporter gene expression in all disc associated myoblasts until adult muscle differentiation (Roy et al, 1997; Roy and VijayRaghavan, 1997, 1998; Anant et al., 1998). A significant rescue of the DFM phenotypes in *ap* mutants was seen (compare Fig. 6A-C). While 68% (n=63) of mutant hemithoraces showed DFMs with different degrees of disorganisation, only 6% (n=18) of the rescued progeny showed disorganised hemithoraces. There was no corresponding rescue of the IFM phenotype (data not shown).

A large number of rescued progeny showed a single large muscle mass instead of four discrete fibres (Fig. 6C). This was noticed in 16/18 hemithoraces of the rescue progeny and 11/63 mutant hemitoraces analysed. We observed a similar phenomenon in the epidermal rescue experiment (described later). Our results strongly suggest that *ap* functions autonomously in DFM development.

IFM defects in *ap* mutants can be rescued by epidermal expression of an *ap* transgene

We provided *ap* epidermally in the ap^4 mutant background using the *pannier* (*pnr) GAL4* driver. *pnr* is expressed in dorsal cells of the presumptive notum on the disc (Calleja et al., 1996) and the adult expression continues in the same region of the dorsal notum. No mesodermal expression is observed (A. G. and K. V. R., unpublished observations). *pnr* is also required for normal fusion of the two heminota – high levels of *ap* produced at 25°C, in the midline, caused a mid-notal cleft and IFM abnormalities (data not shown). To circumvent this and still produce *ap* in sufficient amounts to mediate a rescue, animals were shifted to 18°C during second instar stages. This resulted in eclosion of flies with a normal notum that could be screened for rescue. We screened the DLMs for morphology and muscle size.

There is a significant rescue of the DLM defects in *ap* mutants upon epidermal expression of *ap* (Fig. 6F), while DFM fibres continued to remain disorganised (data not shown). There is also a very striking restoration of muscle size (Fig. 6F). A sizeable population of rescued progeny shows DLMs arranged in one large mass instead of clearly separated fibres.

While 17% (n=60) mutants showed normal DLMs, 80% (n=45) of the rescued progeny had normal DLMs. 89% (n=45) of rescued progeny showed normal muscle size while this category was restricted to 27% (n=60) in mutants. These results substantiated the epidermal requirement of *ap* for patterning IFMs.

Ectopic expression of *ap* in the IFMs disrupts their differentiation

We examined the effects of misexpression of *ap* in IFM development using the *1151* GAL4 driver. IFM differentiation was assayed using an IFM specific *Actin (88F) lacZ* marker (Hiromi et al., 1986; Fernandes et al., 1991). When wild-type *ap* was expressed under control of *1151*, the DLM templates showed either an absence of splitting or a delay. Fig. 7B shows a 15 hours APF pupa with obvious signs of degeneration (muscle clusters into a clump: compare with three normal long fibres in wild type control of same stage in Fig. 7A). Where splitting did occur, there was a marked lowering of the levels of *IFM Actin* staining as seen in Fig. 7D (compare with Fig. 7C). These phenotypes were seen most strongly when flies were transferred to 29°C.

ap in the epidermis is required for regulation of *sr*, an early marker for attachment sites

Expression of *ap* in epidermal attachment sites of muscles and the phenotypes noticed in *ap* mutants suggested a regulatory role for the gene in the development of muscle attachment sites. We chose *stripe* (*sr*), the earliest known marker for epidermal attachment sites, as a potential target for regulation by *ap* in mediating its epidermal function and examined its expression in wing discs of ap^4 homozygotes. *sr* encodes a zinc finger-containing DNA-binding protein, a member of the vertebrate early growth response (Egr) family of transcription factors and is crucial for differentiation of epidermal cells into muscle-attached tendon cells (Lee et al., 1995; Frommer et al., 1997; Vorbruggen and Jackle, 1997; Becker et al., 1997). *sr* is expressed in discrete domains in the wing disc (Fig. 8A), which will form the attachment sites of adult thoracic muscles (Fernandes et al., 1996). *sr* expression in the disc commences very late in third larval instar (A. G. and K. V. R., unpublished observations) and

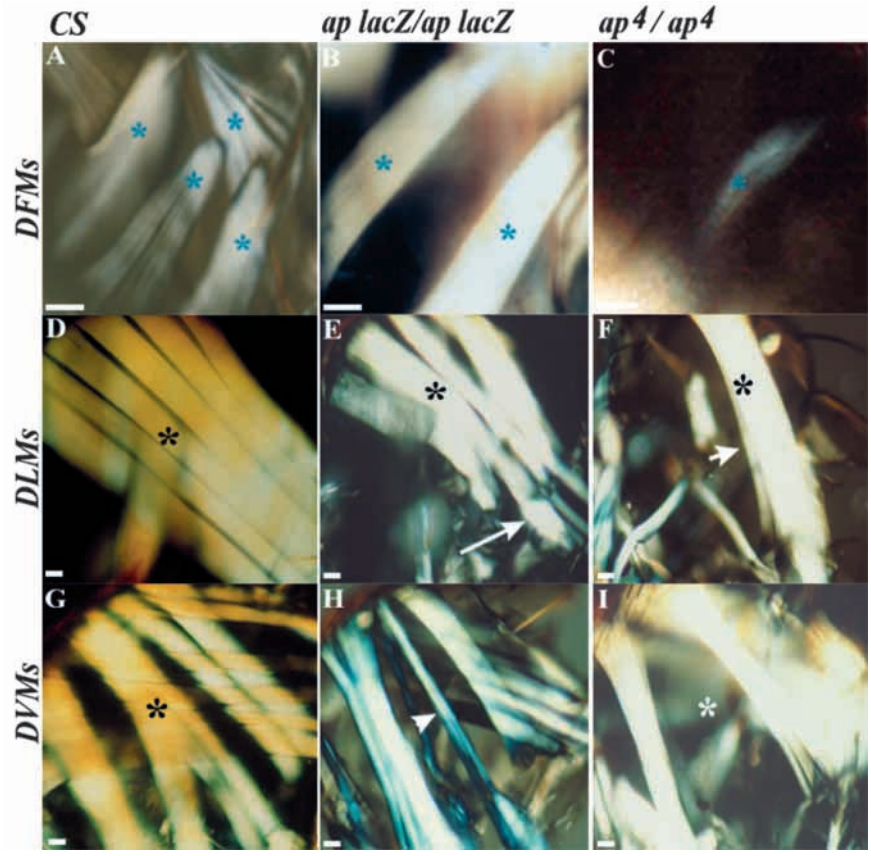

Fig. 5. Muscle phenotypes of *ap* mutants. DFM defects of *ap* mutants, ap^4 and *ap lacZ*, were observed using polarised light optics. (A) DFMs observed in *CS* are marked by asterisks. (B) *ap lacZ/ap lacZ*: two muscles missing and remnants are indicated by asterisks. (C) ap^4/ap^4: a thin fibre (asterisk) remains instead of four muscles. DLMs shown in *CS* (D), *ap lacZ/ap lacZ* (E) and ap^4/ap^4 (F). Compare six distinct fibres in D with the three in E and single strand in F (asterisk and arrow). Posterior attachment sites are tapered in these mutants (arrow in E). DVMs observed in *CS* (G), *ap lacZ/ap lacZ* (H) and ap^4/ap^4 (I). Normal DVM II (asterisk in G) is reduced in H (arrowhead) and absent in I (asterisk). Anterior is towards the left and dorsal is towards the top. Scale bars: 10 μm.

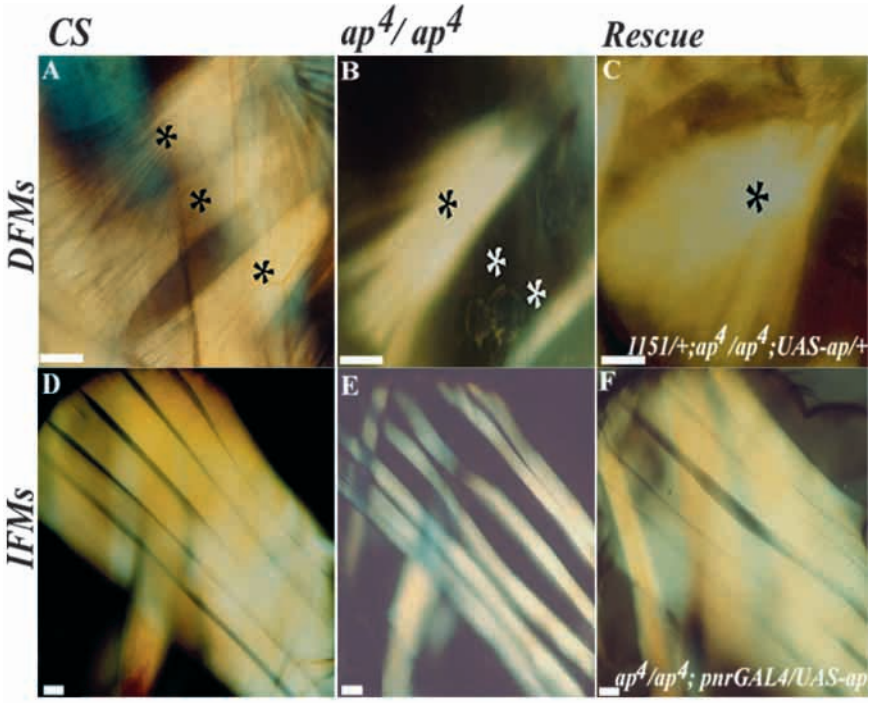

Fig. 6. Tissue specific rescue of *ap* phenotypes. Rescue of DFM phenotypes by mesodermal expression of *ap*. Muscles observed using polarised optics. *CS* (A), ap^4/ap^4 (mutant, B) and *1151/+;* ap^4/ap^4*; UAS-ap/+* (rescue, C). Asterisks indicate different muscles. Compare discrete fibres in A with absent fibres in B (white asterisks) and a mass in C. Rescue of IFM phenotypes by epidermal expression of *ap*. Muscles observed using polarised optics. *CS* (D), ap^4/ap^4 (mutant, E) and ap^4/ap^4; *pnrGAL4/UAS-ap* (rescue, F). Loss of volume seen in mutants is strikingly restored in rescued progeny (compare fibre size between E and F). Both were grown at 18°C. Anterior is towards the left and dorsal towards the top. Scale bars: 10 μm.

is consistently seen as pupation is initiated. Hence the 0 hours APF white prepupal stage was chosen for examination of *sr* expression in *ap* mutants. *sr* expression was either completely lost or drastically reduced in ap^4 wing discs (Fig. 8B). Animals that reached pupal stages showed severe reduction in *sr* levels (compare Fig. 8D with wild-type control in 8C).

Our results were further strengthened by the observation that *ap* and *sr* interact with each other genetically to affect IFM development. The ap^4 mutation is completely recessive, as is sr^{G11}, a recessive lethal at the *sr* locus (Frommer et al., 1997). In a transheterozygous combination, the two alleles showed defective IFMs in a significant population of animals (Fig. 8G). Further, *P1618*, an enhancer trap insertion at the *sr* locus that shows a very mild recessive phenotype, enhanced the IFM defects of ap^4 (Fig. 8H) and such animals also showed a dark midnotal stripe that was characteristic of strong, viable *sr* alleles (Fig. 8I-K). This suggests that *ap* functions in IFM patterning by influencing attachment site development by the regulation of *sr*.

DISCUSSION

In this study, we have defined the functions of *ap* in adult myogenesis, in the development of two distinct thoracic muscle types, the DFMs and the IFMs. The IFMs have been well studied (Fernandes et al., 1991; Fernandes and VijayRaghavan, 1993; Roy et al., 1997; Roy and VijayRaghavan, 1998) but the development of the tubular DFMs has not been significantly analysed. *ap* is very likely the first transcriptional regulator protein that, by its presence in the DFM progenitors, discriminates between the DFMs and IFMs during development. Ewg, another transcription factor, has been shown previously to be expressed in IFM

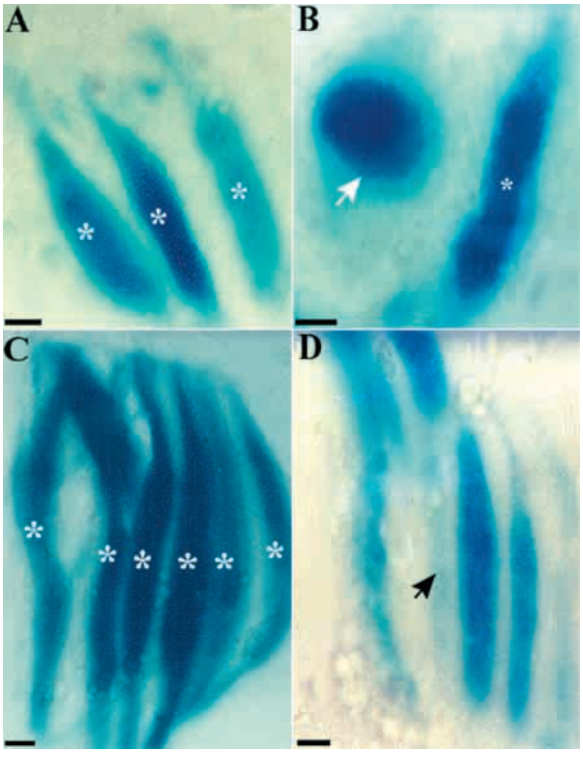

Fig. 7. Ectopic expression of *ap* in IFMs. Ectopic expression of *ap* in IFMs affects their differentiation. Visualised by β-galactosidase staining for IFM specific *Actin (88F) lacZ*, shown here in wild-type (A,C) and *ap* misexpression *(1151/+; UAS-ap/+)* progeny (B,D). A,B are 15 hours APF pupal preparations. Control animals show three fibres (asterisks in A), whereas misexpression progeny shows degenerating clusters (arrow in B) and a single fibre (asterisk in B). C,D are 19-20 hours APF preparations of the same genotypes. Six fibres in control are indicated by asterisks, whereas in the misexpression progeny Actin levels are remarkably low (arrow in D). Anterior is towards the top and midline towards the right. Scale bars: 10 μm.

development during similar stages as *ap* (DeSimone et al., 1995)

There are important features of *ap* expression in DFMs that merit analysis: its adult spatiotemporal expression, differences in expression domains between the embryo and the adult, and the mechanism of mesodermal regulation.

Mesodermal *ap* expression starts early during pupal development and is absent on disc associated myoblasts. Expression is seen in close proximity to the developing epidermis that will overly the DFMs. *ap* expression is not seen in the developing IFMs.

Using the *ap-lacZ* reporter strains we have been able to track DFM development. The process shows similarities with IFM development. Both muscle groups are completely established by 36 hours APF, after which there is only a noticeable increase in size. There is a broad division into four temporal domains. Between 12-14 hours APF and 17-19 hours APF myoblast expression of *ap* becomes noticeable. Next, from 19-21 hours APF to about 24-26 hours APF an increase in *ap* expression takes place and myoblasts begin to arrange themselves in clusters. Subsequently, from 26-28 hours APF to 34-36 hours APF, distinct fibres appear. By 36 hours APF all DFMs are completely formed and subsequently there is only an increase in fibre size.

The expression pattern of *ap* in clusters of myoblasts in the developing DFMs is in striking contrast to the mesodermal expression of *ap* in the embryo where it is expressed in single muscle founder cells (Bourgouin et al, 1992). We would like to suggest that muscle identity in the adult could be determined by expression of genes such as *ap* in *groups of 'feeder'* myoblasts, as opposed to the situation in the embryo where identity is specified by expression of such genes in a single *founder myoblast*. This hypothesis thus suggests a role for feeder myoblasts that is not present in the embryo but could be similar to the role that vertebrate myoblasts may have – in vertebrate myogenesis, groups of myoblasts express myogenic genes in response to epidermal cues (see Tajbakhsh and Cossu, 1997, and Yun and Wold, 1996 for reviews).

The phenotypes observed by ectopic expression of *ap* in the developing IFMs are revealing. The reduction in *IFM Actin lacZ* staining suggests that *ap* may be imposing a DFM identity on the IFMs and this may impede their differentiation and cause the subsequent degeneration or failure to attach correctly. However, an absence of well characterised markers of DFM differentiation prevents immediate confirmation of this hypothesis.

What are the signals that regulate the specific onset of mesodermal *ap* expression temporally and spatially, and where do these signals come from? We have no clear answer to this question at present. However, a significant amount of circumstantial evidence suggests that most aspects of DFM development are configured by the developing epidermis (Roy

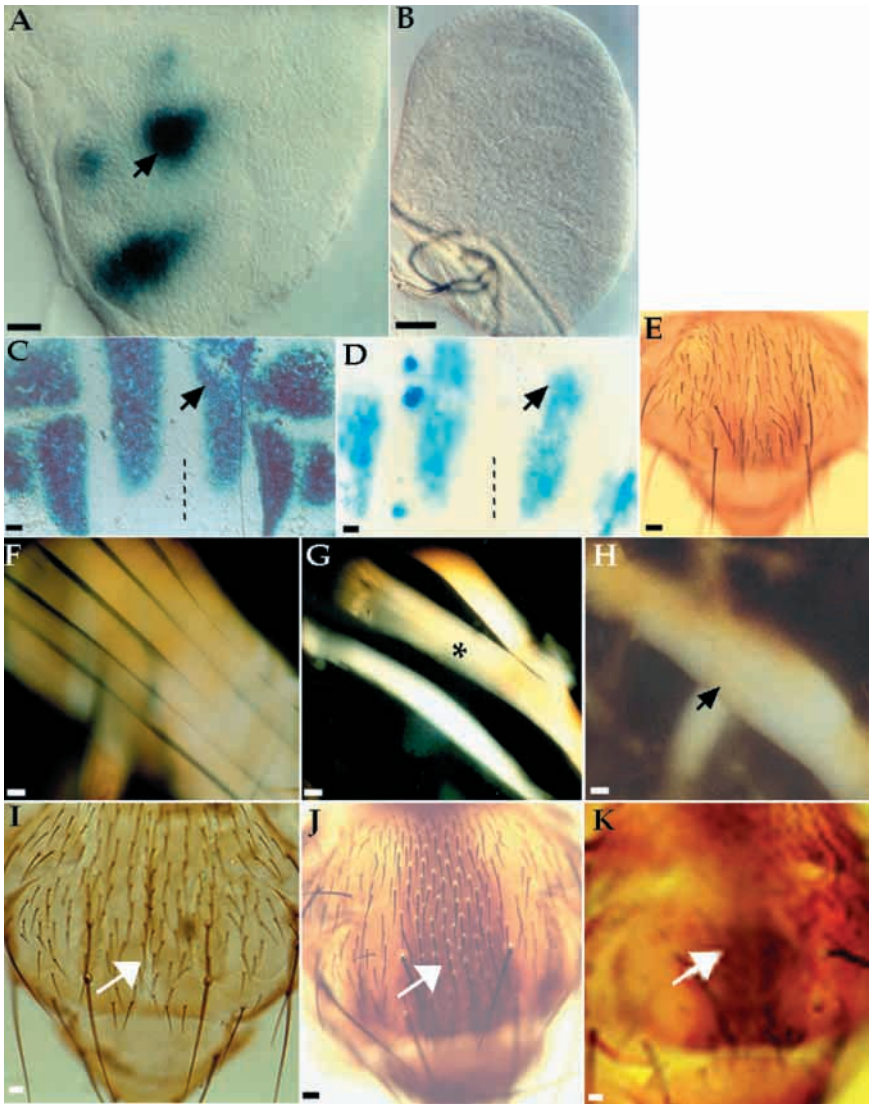

Fig. 8. Regulation of *sr* by *ap*. *sr* regulation by *ap* is shown in A-D. (A) Presumptive notum region of 0 hours APF *ap^4/Cyo MHC lacZ; P1618/TM6 Tb* control disc. β-galactosidase staining shows *sr* expression (arrow). (B) *ap^4/ap^4; P1618/TM6 Tb* 0 hours APF wing disc shows complete absence of *sr* expression. (C) *P1618/P1618* 24 hours APF pupa showing *sr* expression, which is reduced in *ap^4/ap^4; P1618/TM6 Tb* 24h APF pupa (D; compare arrows between D and C). (E) Dorsal notum *ap^4/ap^4* adult shows normal morphology except for absence of wings, eliminating the possibility that the effect on *sr* is due to general patterning defects of *ap* mutants. Genetic interaction between *sr* and *ap* is shown in F-K. DLMs observed under polarised light in *CS* (F), *ap^4/+; sr^{G11}/+* (G, six DLMs reduced to three (asterisk)) and *ap^4/ap^4; P1618/P1618* (H, enhancement of *ap^4* IFM phenotype (arrow)). Dorsal notum of *CS* (I), *sr^1/sr^1* (J) and *ap^4/ap^4; P1618/P1618* (K) animals. Arrows indicate the dark midnotal stripe characteristic of *sr^1/sr^1* viable mutants (J) that is also manifested in animals showing a genetic interaction between *ap* and *sr* (K), and is absent in wild-type nota (I). In A,B, anterior is towards the left; in C,D, anterior is towards the top and the midline is marked by a broken line; in F-H, anterior is towards the left and dorsal towards the top; and in E,I-K, anterior towards the top (viewed dorsally). Scale bars: 10 μm.

et al., 1997; Anant et al., 1998). Potential mediators of epidermal regulatory cues are members of the Wnt signalling pathway. In vertebrates, there is strong evidence for Lim-Hd proteins being controlled by Wnts. *Lmx1b* which is so closely related to *ap*, specifies dorsal cell fate in the chick limb under control of *Wnt7a* (Vogel et al., 1995; Riddle et al., 1995). We do find DFM development to be affected in gain- and loss-of-function experiments with downstream components of the Wg pathway. Misexpression of a dominant negative *TCF* transgene (V. Sudarshan, unpublished observations) and a constitutively activated *armadillo* transgene (A. G. and K. V. R., unpublished observations) results in DFM defects.

A feature of epidermal patterning crucial for adult muscle development is the specification of attachment sites. This, interestingly, happens in the late third larval instar, although attachment sites themselves are used later during pupation (Fernandes et al., 1996). Experiments that follow the development of both the muscle and epidermal side of developing IFMs have shown the importance of the early association of developing muscle fibres with their attachment sites (Fernandes et al., 1996). This is particularly easily seen in DLMs, which use larval templates for their development. Their close association with attachment sites from the very early stages of their adult specific activity is striking. The expression of *sr* on the third instar disc is, therefore, the earliest marker of epidermal attachment sites and is an important step in fibre patterning. We have shown that *sr* expression requires *ap* for its onset and maintenance.

ap is expressed and functions in the dorsal wing blade and acts as a 'selector' gene for dorsal wing cells (Cohen et al., 1992; Diaz-Benjumea and Cohen, 1993; Blair et al., 1994). In the presumptive notum on the third instar wing disc, *ap* is expressed at low levels. Indeed, absence of wing disc *sr* expression in *ap* mutants is the best indicator of its role in the notum. Viable alleles of *ap* have no effect on notal patterning and clones of cells mutant for null alleles also have no externally visible phenotype (Blair et al., 1994).

The expression pattern of *ap* during pupal development changes dynamically to co-localise with the anterior dorsal *sr*-expressing cells, the presumptive anterior attachment sites for the DLMs. It closely juxtaposes, on either side, the posterior attachment sites too. In addition, expression is seen in the developing dorsocentral bristles. Consistent with this expression pattern, *ap* mutants have muscle defects and defects in bristle development (data not shown). *ap* encodes a Lim homeodomain protein that requires a co-factor (*Chip*) for its functioning (Morcillo et al., 1997; Fernandez-Funez et al., 1998) and is negatively regulated by a Lim-only protein dLMO (Bx – FlyBase; Milan et al., 1998; Shoresh et al., 1998; Milan and Cohen, 1999; van Meyel et al., 1999). Despite widespread, albeit low level, expression of *ap* on the presumptive notum the specificity of its interaction with *Chip* and *dLmo* could restrict the domain of *ap* action to activate *sr*. Indeed, both *wingless* (*wg*) loss- and gain-of-function mutants affect the domains, but not the onset of *sr* expression, on the wing imaginal disc (A. G. and K. V. R., unpublished observations), which could happen by altering the domain of *ap* activation.

In its adult thoracic expression, *ap* shows similarities with members of the Lim protein family that lack the homeodomain and have only Lim domains or Lim domains accompanied with other structural motifs. The most remarkable correlation is with the *Caenorhabditis elegans* protein UNC-97 and its *Drosophila* homologue dPINCH (Stck – FlyBase). UNC-97 co-localises with the β-integrin PAT-3 to the focal adhesion-like attachment sites and is required for the structural integrity of the dense bodies – *Drosophila* equivalents of muscle-attachment sites (Hobert et al., 1999). dPINCH resembles *ap* in its muscle and tendon-cell expression in the embryo (Hobert et al., 1999); so do Mlp60A and Mlp84B, the muscle Lim proteins from *Drosophila* (Stronach et al., 1996). *ap*, by virtue of its homeodomain, functions as a transcription regulator but resembles in expression these non-homeodomain-containing Lim proteins that probably behave as cytoskeletal organisers. While these comparisons do open up possibilities of alternate functions for *ap*, presumably as an 'adaptor' protein itself, it probably functions as a transcriptional regulator of components involved in specification or differentiation.

A combinatorial code of Lim-Hd protein expression appears to exist in neural systems of vertebrates and invertebrates (Thor et al., 1999), where sets of neurons and interneurons express partially overlapping or complementary sets of Lim-Hd proteins to determine properties of individual neurones. A similar mechanism, the combinatorial expression of founder markers, operates to generate muscle diversity in the *Drosophila* embryo. *ap* is one such embryonic founder marker but is expressed in clusters of myoblasts in the developing adult. It is possible that *ap* is part of a code that mediates the diversification of the different thoracic muscles and their individual subsets. Our recent finding that *vestigial* is expressed in a subset of myoblasts that contribute to the IFMs (V. Sudarsan, unpublished observations) further strengthens the view that groups of myoblasts express specific transcription factors and contribute to different adult muscle types.

We are grateful to John Thomas for *UAS-ap* stocks and the anti-Ap antibody, Seth Blair for the *ap lacZ* strain, Gines Morata and Pat Simpson for graciously providing the *srGAL4, UAS-GFP* stock before publication, Talila Volk for *sr* alleles and Juan Botas for *ap MS lacZ*. Our thanks to Veronica Rodrigues, Michael Bate and Stephen Cohen for their comments and stimulating discussions. The suggestions of anonymous referees greatly contributed to improving the manuscript and are gratefully acknowledged. This work was supported by the Departments of Biotechnology and Science and Technology, Government of India and funds from NCBS.

REFERENCES

Anant, S., Roy, S. and VijayRaghavan, K. (1998). Twist and Notch negatively regulate adult muscle differentiation in *Drosophila*. *Development.* **125**, 136-1369.

Bate, M. (1990). The embryonic development of larval muscles in *Drosophila. Development.* **110**, 791-804.

Bate, M., Rushton, E. and Currie, D. A. (1991). Cells with persistent *twist* expression are the embryonic precursors of adult muscles in *Drosophila. Development*. **113**, 79-89.

Bate, M. (1993). The mesoderm and its derivatives. In *The Development of* Drosophila melanogaster. Vol.2 (ed. M. Bate and A. Martinez-Arias), pp. 1013-1090. New York: CSHL press.

Baylies, M. K., Bate, M. and Ruiz Gomez M. (1998). Myogenesis: a view from *Drosophila. Cell*. **93**, 921-927.

Becker, S., Pasca, G., Strumpf, D., Li, M. and Volk, T. (1997). Reciprocal signalling between *Drosophila* epidermal muscle attachment cells and their corresponding muscles. *Development*. **124**, 2615-2622.

Bernstein, S. I., O'Donnel, P. T. and Cripps, R. M. (1993). Molecular

genetic analysis of muscle development, structure and function in *Drosophila*. *Int. Rev. Cytol.* **143**, 63-144.

Blair, S. S., Brower, D. L., Thomas, J. B. and Zavotink, M. (1994). The role of *apterous* in the control of dorsoventral compartmentalization and PS integrin gene expression in the developing wing of *Drosophila*. *Development.* **120**, 1805-1815.

Brand, A. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development.* **118**, 401-415.

Bourgouin, C., Lundgren, S. E. and Thomas, J. (1992). *apterous* is a *Drosophila* LIM domain gene required for the development of a subset of embryonic muscles. *Neuron* **9**, 549-561.

Callahan, C. A., Bonkovsky, J. L., Scully, A. L. and Thomas J. B. (1996). *derailed* is required for muscle attachment site selection in *Drosophila*. *Development.* **122**, 2761-2767.

Calleja, M., Moreno, E., Pelaz, S. and Morata G. (1996). Visualization of gene expression in living adult. *Science*. **274**, 252-255.

Cohen, B., McGuffin, M. E., Pfeifle, C., Segal, D. and Cohen, S. (1992). *apterous*, a gene required for imaginal disc development in *Drosophila* encodes a member of the LIM family of developmental regulatory proteins. *Genes Dev*. **6**, 715-729.

Currie, D. and Bate, M. (1991). Development of adult abdominal muscles in *Drosophila*: adult myoblasts express *twi* and are associated with nerves. *Development.* **113**, 91-102.

DeSimone, S., Coelho, C., Roy, S., VijayRaghavan, K. and White, K. (1995). ERECT WING, the *Drosophila* member of a family of DNA binding proteins is required in imaginal myoblasts for flight muscle development. *Development.* **121**, 31-39

Diaz-Benjumea, F. J. and Cohen, S. M. (1993). Interaction between dorsal and ventral cells in the imaginal disc directs wing development in *Drosophila*. *Cell*. **75**, 741-752.

Dohrmann, C., Azpiazu, N. and Frasch, M. (1990). A new *Drosophila* homeobox gene is expressed in mesodermal precursor cells of distinct muscles during embryogenesis. *Genes Dev.* **4**, 2098-2111.

Fernandes, J., Bate, M. and VijayRaghavan, K. (1991). Development of the indirect flight muscles of *Drosophila*. *Development*. **113**, 67-77.

Fernandes, J. and VijayRaghavan, K. (1993). Development of the indirect flight muscle innervation in *Drosophila melanogaster. Development.* **118**, 215-227.

Fernandes, J., Celniker, S. E. and VijayRaghavan, K. (1996). Development of the indirect flight muscle attachment sites in *Drosophila:* role of the PS integrins and the *stripe* gene. *Dev. Biol*. **176**, 166-184.

Fernandez-Funez, P., Lu, C-H., Rincon-Limas, D. E., Garcia-Bellido, A. and Botas, J. (1998). The relative expression amounts of *apterous* and its co-factor *dLdb/Chip* are critical for dorso-ventral compartmentalization in the *Drosophila* wing. *EMBO J.* **17**, 6846-6853.

Frasch, M. (1999). Controls in patterning and diversification of somatic muscles during *Drosophila* embryogenesis. *Curr. Opin. Genet. Dev.* **9**, 522-529.

Frommer, G., Vorbruggen, G., Pasca, G., Jackle, H. and Volk, T. (1996). Epidermal egr-like zinc finger protein of *Drosophila* participates in myotube guidance. *EMBO J*. **15**, 1642-1649.

Hiromi, Y., Okamoto, Y., Gehring, W. and Hotta, Y. (1986). Germline transformation with *Drosophila* mutant *Actin* genes induces constitutive expression of heat shock genes. *Cell.* **44**, 293-301.

Hobert, O., Moerman, D. G., Clark, K. A., Beckerle, M. C. and Ruvkun. G. (1999). A conserved LIM protein that affects muscular adherens junction integrity and mechanosensory function in *Caenorhabditis elegans. J. Cell Biol.* **144**, 45-57.

Lee, J. C., VijayRaghavan, K., Celniker, S. E. and Tanouye M. A. (1995). Identification of a *Drosophila* muscle development gene with structural homology to mammalian early growth response transcription factors. *Proc. Natl. Acad. Sci. USA* **92**, 10344-10348.

Lindsley, D. L. and Zimm, G. G. (1992). The genome of *Drosophila melanogaster*, pp. 104, 135, 602 and 663. Academic Press, CA.

Milan, M., Diaz-Benjumea, F. J. and Cohen, S. (1998). *Beadex* encodes an LMO protein that regulates *apterous* LIM-homeodomain activity in *Drosophila* wing development: a model for LMO oncogene function. *Genes Dev*. **12**, 2912-2920

Milan, M. and Cohen, S. (1999). Regulation of LIM Homeodomain activity In Vitro: A tetramer of dLDB and Apterous confers activity and capacity for regulation by dLMO. *Mol. Cell.* **4**, 267-273.

Miller, A. (1950). The internal anatomy and histology of the imago of *Drosophila melanogaster*. In *The Biology of* Drosophila (ed. M. Demerec), pp. 420-534. Cold Spring Harbor Laboratory Press. John Wiley and Sons, NY.

Morcillo, P., Rosen, C., Baylies, M. and Dorsett, D. (1997). Chip, a widely expressed chromosomal protein required for segmentation and activity of a remote wing margin enhancer in *Drosophila*. *Genes Dev*. **11**, 2729-2740.

Riddle, R. D., Ensini, M., Nelson, C., Tsuchida, T., Jessell, T. M. and Tabin, C. (1995). Induction of the LIM homeobox gene *Lmx1* by *WNT7a* establishes dorsoventral pattern in the vertebrate limb. *Cell.* **83**, 631-640.

Roy, S. and VijayRaghavan, K. (1997). Homeotic genes and the regulation of myoblast migration, fusion and fibre-specfic gene expression during adult myogenesis in *Drosophila. Development.* **124**, 3333-3341.

Roy, S., Shashidhara, L. S. and VijayRaghavan, K. (1998). Muscles in the *Drosophila* second thoracic segment are patternedby induction and independent of autonomous homeotic gene function. *Curr. Biol.* **7**, 222-227.

Roy, S. and VijayRaghavan, K. (1998). Patterning muscles using organizers: larval muscle templates and adult myoblasts actively interact to pattern the dorsal longitudinal flight muscles of *Drosophila. J. Cell Biol.* **141**, 1135-1145.

Roy, S. and VijayRaghavan, K. (1999). Muscle pattern diversification in *Drosophila*: The story of imaginal myogenesis. *BioEssays* **21**, 486-498.

Ruiz-Gomez, M., Romani, S., Hartmann, C., Jackle, H. and Bate, M. (1997). Specific muscle identities are regulated by kruppel during *Drosophila* embryogenesis. *Development.* **124**, 3407- 3414.

Ruiz-Gomez, M. and Bate, M. (1997). Segregation of myogenic lineages in *Drosophila* requires *numb*. *Development.* **124**, 4857-484866.

Rushton, E., Drysdale, R., Abmayr, S. M., Michelson, A. M. and Bate, M. (1995). Mutations in a novel gene, *myoblast city*, provide evidence in support of the founder cell hypothesis for *Drosophila* muscle development. *Development.* **121**, 1979-1988.

Shoresh, M., Orgad, S., Shmueli, O., Werczberger, R., Gelbaum, D., Abiri, S. and Segal, D. (1998). Overexpression *Beadex* mutations and Loss-of-function *heldup-a* mutations in *Drosophila* affect the 3′ regulatory and coding components respectively, of the *Dlmo* gene. *Genetics* **206**, 283-299.

Stronach, B. E., Siegrist, S. E. and Beckerle, M. C. (1996). Two muscle specific LIM proteins in *Drosophila. J. Cell Biol.* **134**, 1179-1195.

Tajbakhsh, S. and Cossu, G. (1997). Establishing myogenic identity during somitogenesis. *Genes Dev.* 7, 634-641.

Thor, S., Andersson, S. G., Tomlinson, A. and Thomas, J. B. (1999). A LIM-homeodomain combinatorial code for motor-neuron pathway selection. *Nature* **397**, 76-80.

van Meyel, D. J., O'Keefe, D. D., Jurata, L. W., Thor, S., Gill, G. H. and Thomas, J. B. (1999). Chip and Apterous physically interact to form a functional complex during *Drosophila* devlopment. *Mol. Cell.* **4**, 259-265.

Vogel, A., Rodriguez, C., Warnken, W. and Izpisua Belmonte, J. C. (1995). Dorsal cell fate specified by chick *Lmx1* during vertebrate limb development. *Nature.* **378**, 716-20.

Volk, T. and VijayRaghavan, K. (1994). A central role for epidermal segment border cells in the induction of muscle patterning in the *Drosophila* embryo. *Development.* **120**, 59-70.

Vorbruggen, G and Jackle, H. (1997). Epidermal muscle attachment site specific target gene expression and interference with myotube guidance in response to ectopic *stripe* expression in the developing *Drosophila* epidermis. *Proc. Natl. Acad. Sci. USA* **94**, 8606-8611.

Yun, K. and Wold, B. (1996). Skeletal muscle determination and differentiation: story of a core regulatory network and its context. *Curr. Opin. Cell. Biol.* **8**, 877-889.