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Persistent myogenic capacity of the dermomyotome dorsomedial lip and restriction of myogenic competence

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SUMMARY

The dorsomedial lip (DML) of the somite dermomyotome is the source of cells for the early growth and morphogenesis of the epaxial primary myotome and the overlying dermomyotome epithelium. We have used quail-chick transplantation to investigate the mechanistic basis for DML activity. The ablated DML of chick wing-level somites was replaced with tissue fragments from various mesoderm regions of quail embryos and their capacity to form myotomal tissue assessed by confocal microscopy. Transplanted fragments from the epithelial sheet region of the dermomyotome exhibited full DML growth and morphogenetic capacity. Ventral somite fragments (sclerotome), head paraxial mesoderm or non-paraxial (lateral plate) mesoderm tested in this assay were each able to expand mitotically in concert with the surrounding paraxial mesoderm, although no myogenic potential was evident. When ablated DMLs were replaced with fragments of the dermomyotome ventrolateral lip of wing-level somites or pre-somitic mesoderm (segmental plate), myotome development was evident but was delayed or otherwise limited in some cases. Timed DML ablation-replacement experiments demonstrate that DML activity is progressive throughout the embryonic period (to at least E7) and its continued presence is necessary for the complete patterning of each myotome segment. The results of serial transplantation and BrdU pulse-chase experiments are most consistent with the conclusion that the DML consists of a self-renewing population of progenitor cells that are the primary source of cells driving the growth and morphogenesis of the myotome and dermomyotome in the epaxial domain of the body.

Key words: Myotome, Muscle patterning, Chick/quail, Experimental embryology, Somite, Dermatome, Competence, Confocal microscopy, Epaxial, Stem cell, Progenitor cell, Asymmetry

INTRODUCTION

The epaxial myotome, the earliest skeletal muscle tissue to appear during avian and mammalian embryonic development, arises from progenitor cells located in the medial half of each somite, while development of hypaxial muscle occurs later during embryogenesis from progenitor cells located in the lateral half of the somite (Ordahl and Le Douarin, 1992). Recent work from several laboratories shows that the patterning of the epaxial myotome occurs as a result of distinct growth phases. The primary myotome grows in a lateral-to-medial direction through progressive addition of new myocytes along its medial border (Denetclaw et al., 1997; Ordahl et al., 2000). Later phases of myotome development involve addition of myocytes from the dermomyotome (Kahane et al., 1998b; Denetclaw and Ordahl, 2000; Kahane et al., 2001). The subsequent development of multisegmental epaxial muscle tissue occurs through mechanisms that are incompletely understood. Although recent progress has been made in understanding the growth and morphogenesis of the primary myotome, other aspects of the early embryonic development of this tissue remain controversial.

One unresolved issue is the extent to which intrinsic or extrinsic influences govern the initiation and maintenance of early muscle development. The results of tissue recombination experiments in vitro and in vivo have led to proposals that myogenic fate is initially induced in 'naïve' somite cells and later maintained by extrinsic signals emanating from tissues surrounding the somite (reviewed by Borycki and Emerson, 2000; Hirsinger et al., 2000). Prospective candidates for the initial inductive signals include sonic hedgehog (Shh) from the neural tube/notochord complex (Borycki et al., 1998; Borycki et al., 1999) and Wnt signals from the dorsal neural tube and adjacent dorsal ectoderm (Stern and Hauschka, 1995; Tajbakhsh et al., 1998; Wagner et al., 2000). Subsequent maintenance of myogenic patterning and differentiation has been attributed to the BMP4 antagonist noggin (Hirsinger et al., 1997; Reshef et al., 1998) and members of the TGF, bFGF and IGF families (Stern et al., 1997; Pirskanen et al., 2000).

Alternatively, exogenous sonic hedgehog has been proposed to serve as a survival/proliferation factor for early myotomal progenitor cells rather than as an inducer per se (Teillet et al., 1998; Cann et al., 1999; Marcelle et al., 1999). This is consistent with the finding that myogenically competent cells (cells that are capable of generating differentiated muscle in an ectopic,

permissive environment in vitro or in vivo) are present within the primitive streak and node as well as in the pre-gastrula blastoderm (Krenn et al., 1988; George-Weinstein et al., 1994; Stern and Hauschka, 1995). The presence of such cells is further supported by the demonstration that messenger RNAs encoding myogenic determination factors (MDFs), such as MyoD, can be detected in the blastoderm, primitive streak and node prior to the appearance of paraxial mesoderm (Gerhart et al., 2000; Kiefer and Hauschka, 2001). Finally, determined myogenic progenitor cells (cells that are capable of creating muscle tissue in an antagonistic signalling environment in vivo) are present in the pre-segmented mesoderm and newly formed somites prior to the onset of overt myogenic differentiation (Williams and Ordahl, 1997). Thus, myogenically competent cells appear prior to and maintain their state of determination in the absence of signals that putatively induce them.

Another issue concerns the location and timing of asymmetric cell division in the formation of the myotome. Clonal analysis of myotome development in mouse embryos shows that myotomal myocytes in multiple adjacent segments along the body axis arise from mitotic division of individual ancestral cells in the primitive node and streak or even earlier blastoderm (Nicolas et al., 1996; Eloy-Trinquet et al., 2000). The conserved lateral-to-medial position of such myocytes within multiple segments has led these workers to hypothesise that myotome progenitor cells are mediolateral pre-patterned by the time the somite buds off the rostral end of the pre-somitic mesoderm (Eloy-Trinquet and Nicolas, 2002). In this latter hypothesis, mitotic activity of myogenic progenitor cells after somite formation would serve to expand the existing pre-pattern but repeated asymmetric division of such cells could not generate, de novo, the medio-lateral position of cells within each myotome segment.

This conclusion is inconsistent with recent results from our laboratory indicating that both primary myotome and dermomyotome cells emerge from the dermomyotome dorsomedial lip (DML) in a time- and position-dependent fashion (Denetclaw et al., 1997; Denetclaw et al., 2001). Furthermore, surgical transplantation experiments showed that replacement of quail DML with that of chick (or vice versa) resulted in medially located myotome and dermomyotome cells of exclusively donor origin and sharply demarcated from laterally located host cells (Ordahl et al., 2001). These results indicate that mediolateral position within the myotome (and dermomyotome) is a time- and position-dependent function of DML activity, rather than pre-patterned position that is already established at the time of somite formation.

That such a small portion of tissue, and therefore a limited number of cells, can generate both myotome and dermomyotome leads to the hypothesis that the DML contains a pool of self-renewing stem-like cells, herein referred to as progenitor cells. Division of these progenitor cells produces daughters that ultimately emerge from the DML as myotome and/or dermomyotome cells. According to this hypothesis, asymmetric cell division and/or timing of differentiation within the DML, rather than pre-patterned cell location in the newly formed somite, is responsible for the lateral-to-medial position of myotome cells. In the present report, we use experimental embryological methods to test these hypotheses. The results support the conclusion that asymmetry within the DML is responsible for the growth and patterning of the primary myotome and indicate further that DML activity is required throughout the embryonic period for the complete development of each myotomal segment. In addition, the capacity to form primary myotome is restricted to the DML very early in development.

MATERIALS AND METHODS

Tissue transplants

Fertilised chicken eggs (*Gallus domesticus*) and Japanese quail eggs (*Coturnix coturnix japonica*) were obtained from Petaluma Farms (Petaluma, CA) and from Strickland Quail Farms (Pooler, GA), respectively. After timed incubation at 39°C transplant procedures were carried out. DML transplants were carried out as described previously (Ordahl et al., 2001). For non-DML transplants, donor tissue was isolated from the donor embryo using electrolytically sharpened tungsten knives and pancreatin enzyme treatment and transported to a DML-ablated host embryo using a glass micro-pipette charged with Tyrodes solution. Fig. 1 schematically represents the transplant procedure and shows examples of DML structure as used for evaluation. Host embryos were re-incubated at 39°C after re-sealing with Parafilm for the time periods indicated.

Serial DML transplants

DML transplants were carried out as described above and a nearby somite was marked with carbon for identification purposes. After overnight incubation, the primary host was harvested and the donor DML excised and transferred into a DML ablated secondary host (illustrated in Fig. 6A, see Results). The secondary host was sealed and reincubated overnight at 39°C. In some cases, fluorescent tracer dyes were injected into the DML prior to the sequential transplants. Embryos were microinjected with 1,1′, di-octadecyl-3,3,3′,3′,-tetramethylindocarbocyanine perchlorate (DiI) prior to transplant to the primary host and with 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO) solution as described elsewhere (Denetclaw et al., 1997) before the secondary transplant. After a second overnight reincubation, embryos were harvested and fixed in PBS containing 4% paraformaldehyde.

BrdU labelling

After overnight incubation 50 μl of a 10 mM solution of BrdU (Sigma) was pipetted around the heart of DML transplanted embryos. Embryos were sealed and reincubated for 2 or 4 hours before harvest. Embryos were fixed in a 4% paraformaldehyde solution and cryo-embedded in PBS containing 15% sucrose and 7.5% gelatin. Serial transverse sections were collected through the transplanted area at 10 μm intervals using a Leica CM1900 cryostat.

Immunohistochemistry and microscopy

Embryo whole-mount specimens and tissue sections were processed for immunohistochemistry as described previously (Ordahl et al., 2001). Primary antibodies used were mouse anti-qcpn, 1:50 (Developmental Studies Hybridoma Bank), rabbit anti-desmin, 1:500 (Sigma) and mouse anti-BrdU, 1:500 (Caltag). Whole-mount embryos were mounted in PBS in well slides and sections mounted in glycergel (Sigma). Confocal microscopy was carried out using a Nikon E600FN microscope fitted with a PCM 2000 laser-scanning unit (Nikon) and C-Imaging Simple 32 software (Compix). Optical sections were collected at between 1 and 5 μm intervals. Individual and partial *z*-series were examined and compiled using NIH image 1.61ppc and Adobe Photoshop 5.5.

RESULTS

Competence to mimic DML activity

The competence of non-DML tissues to mimic DML activity

Table 1. Non-DML transplants

A Donor tissue	B Donor/level	C Host/level	D Overall growth	E Segmental growth	F Myotome growth	G Donor fibres	H DML formed
LPM							
1.01	20s/cPSM	26s/ssIX	Yes	Yes	Truncated	No	No
1.02	23s/m PSM	24s/ssXII	No		Truncated	No	No
1.03	23s/mPSM	25s/ssXIV	Yes	Yes	Truncated	No	No
1.04	26s/mPSM	26s/ssXI	Yes	Yes	Truncated	No	No
1.05	23s/rPSM	19s/ssXIII	Yes	Yes	Truncated	No	No
1.06	20s/rPSM	25s/ssVIII	Yes	Yes	Truncated	No	No
1.07	23s/rPSM	24s/ssXIII	Yes	Yes	Truncated	No	No
1.08	21s/ss-I	21s/ssX	Yes	Yes	Truncated	No	No
1.09	21s/ss0	19s/ssXIII	Yes	Yes	Truncated	No	No
1.10	23s/ss0/I	19s/ssXI	Yes	Yes	Truncated	No	No
1.11	15s/ssI	25s/ssIX	Yes	Yes	Truncated	No	No
1.12	26s/ssII	27s/ssXI	No		Truncated	No	No
1.13	23s/ssII	15s/ssX	Yes	Yes	Truncated	No	No
1.14	15/ssII	22s/ssVIII	No		Truncated	No	No
1.15	15s/ssII	25s/ssVIII	Yes	Yes	Truncated	No	No
1.16	23s/ssII/III	21s/ssXII	No		Truncated	No	No
1.17	20s/ssIII	26s/ssXI	Yes	Yes	Truncated	No	No
1.18	20s/ssIV	28s/ssVIII	Yes	Yes	Truncated	No	No
CPM							
2.01	15s	24s/ssXIV	No		Truncated	No	No
2.02	20s	19s/ssXI	No		Truncated	No	No
2.03	20s	16s/ssVIII	Yes	Yes	Truncated	No	No
2.04	20s	32/ssVIII	Yes	Yes	Truncated	No	No
2.05	20s	26s/ssVII	Yes	Yes	Truncated	No	No
Sclerotome							
3.01	21s/ssVIII	22s/ssXIII	Yes	No	Truncated	No	No
3.02	22s/ssV	25s/ssXII	Yes	Yes	Truncated	No	No
3.03	24s/ssIV	24s/ssXIV	Yes	Yes	Truncated	No	No
3.04	23s/ssIV	23s/ssXV	Yes	Yes	Truncated	No	No
3.05	23s/ssVI	22s/ssXIII	Yes	Yes	Truncated	No	No
3.06	30s/ssV	22s/ssXI	Yes	Yes	Truncated	No	No
3.07	30s/ssV	21s/ssXII	Yes	Yes	Truncated	No	No
3.08	30s/ssVI	23s/ssXIII	Yes	Yes	Truncated	No	No
3.09	30s/ssIV	24s/ssXI	Yes	Yes	Truncated	No	No
DM sheet							
4.01	22s/ssXII	24s/ssXI	Yes	Yes	Yes	Yes/central	Yes
4.02	22s/ssXIII	15s/ssX	Yes	Yes	Yes	Yes/central	Yes
4.03	23s/ssXVI	17s/ssXII	Yes	Yes	Yes	Yes/central	Yes
4.04	23s/ssXVIII	16s/ssXI	Yes	Yes	Yes	Yes/central	Yes
4.05	38s/ssXXII	21s/ssXI	Yes	Yes	Yes	Yes/central	Yes
VLL							
5.01	23s/ssIV	26s/ssXV	Yes	Yes	Yes	Yes/central	Yes
5.02	23s/ssV	24s/ssXIV	Yes	Yes	Yes	Yes	Yes
5.03	26s/ssVI	22s/ssXII	Yes	Yes	Yes	Yes	Yes
5.04	33s/ssVIII	26s/ssXII	Yes	Yes	Yes	Yes/central	Yes
5.05	33s/ssIX	23s/ssXII	No		Truncated	No	
5.06	32s/ssXVII	28s/ssXIV	Yes	Yes	Truncated	No	No
5.07	34s/ssXIX	21s/ssXIV	No quail		Yes	No	
5.08	34s/ssXVIII	22s/ssXII	Yes	Yes	Truncated	No	No
5.09	34s/ssXVII	24s/ssXIV	No		Truncated	No	
5.10	32s/ssXV	27/ssXIII	Yes	Yes	Truncated	No	No
PSM							
6.01	23s/ss-I	18s/ssXIII	Yes	Yes	Yes	Yes	Yes
6.02	15s/ss-I	23s/ssIX	Yes	Yes	Yes	Yes	Yes
6.03	23s/mPSM	26s/ssXII	Yes	Yes	Yes	Yes	Yes
6.04	15s/mPSM	24s/ssXIV	Yes	Yes	Yes	Yes/central	Yes
6.05	15s/mPSM	27s/ssXI	Yes	Yes	Yes	Yes	No
6.06	23s/cPSM	19s/ssX	Yes	Yes	Gap	Yes	Yes
6.07	23s/cPSM	17s/ssXI	Yes	Yes	Truncated	No	No

Transplants of various quail tissues to replace the host DML. Donor tissue type and embryo number are indicated in A. B and C show donor and host details, respectively. Embryo age and level of tissue transplant/collection are recorded as somite number (s) and by somite stage (ss), respectively. Overall growth of transplanted tissue is shown in D. Retention within the host somite boundaries is shown in E. F shows myotome growth, rated as 'truncated' (at level of DML ablation) or 'yes' (growth from donor). For PSM transplants 'gap' indicates that donor-derived myocytes were not present immediately abutted to host myocytes. Myocyte nuclei aligned at the axial midpoint of the somite is noted (central) in G. The formation of a 'DML-like' structure at the dorsomedial limit of the somite is indicated (H). LPM, lateral plate mesoderm; PSM, presomitic mesoderm (the prefixes c, m and r indicate caudal, middle and rostral, respectively); CPM, cranial paraxial mesoderm; DM sheet, central dermomyotome sheet; VLL, ventrolateral lip of the dermomyotome.

was tested by replacing the somite DML of a chick host with tissue from a quail donor (Table 1; Fig. 1). DML replacement with lateral plate mesoderm (LPM) (Fig. 2A) resulted in a truncation of the myotome of the operated segment (Fig. 2B, arrowheads). No quail derived myocytes were observed in any of the LPM transplants carried out (Table 1, 1.01-1.18). The donor LPM tissue expanded in concert with host tissue in neighbouring somites (Fig. 2A) and was therefore able to grow in the ectopic DML site. Interestingly, the transplanted LPM growth was restricted to the segmental boundaries of the host somite (broken line in 2A) and did not expand beyond the intersomitic space. Although factors deriving from LPM have been shown to inhibit myogenesis (reviewed by Hirsinger et al., 2000) myotome development in adjacent somites appeared to be unaffected by the ectopic LPM.

Cranial paraxial mesoderm (CPM) has been shown to give rise to muscles of the head (Noden, 1983; Couly et al., 1992; Trainor et al., 1994). CPM used to replace an ablated DML was able to expand within the dorsomedial somite position (Fig. 2C; Table 1), but no quail-derived myocytes were present and the host myotome of the operated somite was truncated (Fig. 2D, arrowheads). As with the LPM transplants, the CPM remained within host somite boundaries although some quail cells extended slightly beyond these boundaries in both rostral and caudal directions at the dorsomedialmost aspect of the tissue (Fig. 2C, arrows).

Sclerotome has been previously shown to have myogenic capacity at later embryonic stages (Dockter and Ordahl, 1998). All nine cases in which DMLs were replaced with sclerotome fragments resulted in truncated myotomes and no donor derived myocytes (Table 1, 3.01-3.09). Additionally, donor tissue expanded within the host somite boundaries comparable with growth of neighbouring somites (not shown).

Paraxial mesoderm transplants to the DML position

Transplant and tracing experiments have shown dermomyotome tissue lateral to the DML has myogenic potential (Williams and Ordahl, 2000) and contributes fibres to the myotome (Denetclaw et al., 1997; Kahane et al., 1998a; Huang and Christ, 2000; Kahane et al., 2001). The central region of quail dermomyotome sheet was transplanted to replace the DML in a chick host (Fig. 3A,B). In each case (Table 1, 4.01-4.05), the donor tissue was able to give rise to myotomal muscle that occupied the medial-most myotome position, was axially aligned and restricted to the host somite segment (Fig. 3B). Additionally, myocyte nuclei were arranged along the centres of the myotome fibres (Fig. 3A, arrow). Finally, the cut edge of the transplanted dermomyotome tissue positioned adjacent to the neural tube had reformed an epithelial DML. Thus, the central dermomyotome sheet is able to functionally replace an ablated DML.

The dermomyotome ventrolateral lip (VLL) of wing-level somites was similarly tested. VLLs were harvested from wing-level somites between somite stages (ss) IV-VIII, prior to the time at which wing muscle precursors have migrated into the limb mesenchyme (Chevallier et al., 1977; Christ et al., 1977; Jacob et al., 1978) and used to replace the DML in a chick host somite (Fig. 3C,D). In four cases (Table 1, 5.01-5.04), transplanted VLL tissue from young somites formed an epithelial DML structure that formed both myotome and dermomyotome epithelium. Several notable aspects of

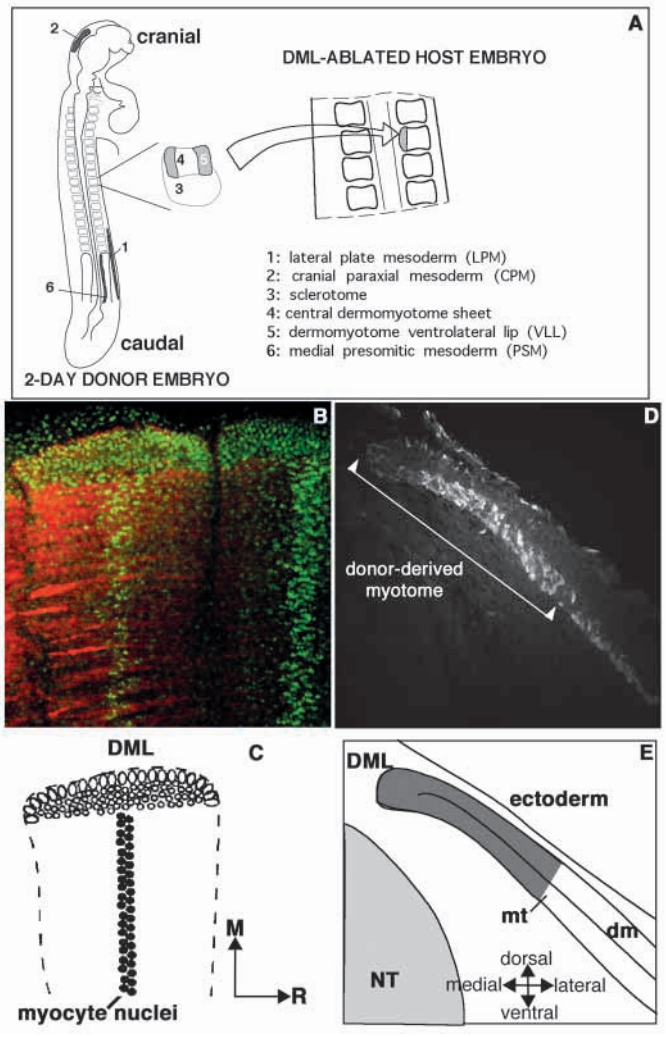

Fig. 1. Transplant scheme and DML evaluation. (A) Schematic representation of the tissues used to replace an ablated DML. (B) Confocal image stack of an E2.5 quail embryo immunolabelled with anti-desmin (red) and qcpn (green) antibodies. (C) Schematic of B showing the DML as a cluster of quail epithelial cells at the dorsomedial limit of the somite, which are also lightly labelled by the desmin antibody. (D) Transverse section of an E3 chick somite that received a quail DML transplant 24 hours prior to harvest. Desmin immunoreactivity marks the myotome and some cells within the DML and lateral dermomyotome. The bar indicates the extent of donor-derived myotome as determined by the presence of quail nuclei in an adjacent serial section (shown in Fig. 4). (E) Schematic of D showing the position of the myotome, dermomyotome and DML with areas comprising quail nuclei shaded. Abbreviations: CPM, cranial paraxial mesoderm; dm, dermomyotome; DML, dorsomedial lip of the dermomyotome; LPM, lateral plate mesoderm; M, medial; mt, myotome; NT, neural tube; PSM, presomitic mesoderm; R, rostral; VLL, ventrolateral lip of the dermomyotome. The rostral direction is towards the right in B,C and in subsequent whole-mount images.

myotome growth from the transplanted VLL differed from that of either normal DML reported elsewhere (Ordahl et al., 2001) or the dermomyotome sheet reported above. First, some rostral and caudal encroachment of donor cells was evident at the dorsomedial limit of the transplant (Fig. 3C). Positioning of

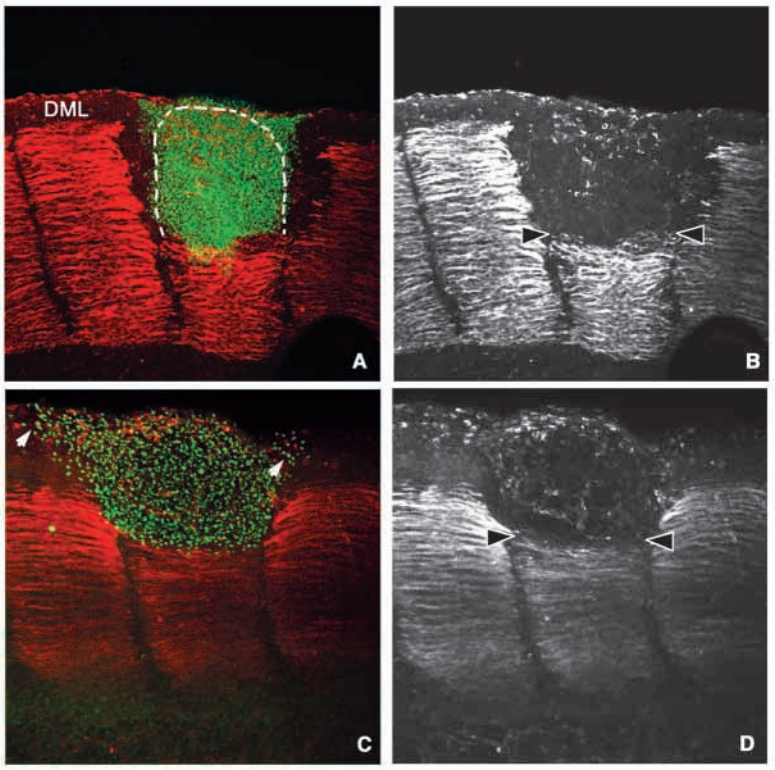

Fig. 2. Transplant of non-dermomyotome tissues to a DML position. (A-D) Confocal *z*-series images immunolabelled with anti-desmin and qcpn antibodies to label the myotome (red) and quail (green), respectively. (A,B) Transplant of LPM from the level of the rostral PSM (Table 1, 1.07). (A) The expansion of the transplanted quail tissue is comparable with that of neighbouring myotomes. The quail tissue remained within the rostral and caudal boundaries of the host somite (outlined with a broken line) and expanded dorsomedially, but no epithelial DML structure was apparent. (B) Desmin labelling shows truncation of the myotome in the operated somite from the level of the DML ablation (arrowheads). Note robust growth of the neighbouring myotomes. (C,D) CPM transplant (Table 1, 2.04). (C) The quail tissue expanded dorsomedially in concert with the neighbouring somites and has largely remained within the somite boundaries. Arrows indicate encroachment of quail nuclei in cells that have crossed somitic boundaries. (D) The myotome of the operated somite is truncated in comparison to neighbours corresponding to the site of the DML ablation (arrowheads).

nuclei within myocytes was determined by scan-by-scan analysis of individual sections of a confocal *z*-series. In the example shown VLL-derived myocyte nuclei were dispersed along the axial length of the myotome and not constrained to the central area (Fig. 3C, arrows). In other VLL transplants, however, donor myocyte nuclei were aligned in the centre of the myotome (Table 1). VLLs from somites older than ssIX transplanted to replace the DML did not generate primary myotome (Table 1, 5-05-5.10) and host myotomes were truncated from the point of the DML ablation.

The competence of presomitic mesoderm (PSM) to respond to the DML environment was similarly tested using somite sized pieces of rostral, mid or caudal regions of PSM [or classified according to Pourquié and Tam (Pourquié and Tam, 2001)], transplanted into a DML ablated somite. In all rostral and mid-level PSM transplants (Table 1, 6.01-6.05) donor-derived myotome was produced after overnight incubation, transplanted tissue remained within the host somite boundaries and formed a DML-like structure.

Caudal PSM was used to replace the DML in two cases (Fig. 3E,F; Table 1, 6.06, 6.07) and in both cases grew within the somite boundaries of the host. One transplant formed a structured DML and produced myocytes, although a gap was evident between the host myotome and the donor-derived myocytes (Fig. 3F, arrows). The second caudal PSM transplant neither formed a DML-like structure nor produced myocytes (Table 1 and data not shown).

Time and position dependent entry of newly replicated DML cells into the myotome

DML transplants were carried out on E2 embryos as described previously and exposed to a single pulse of BrdU either 2 or 4 hours before harvest after overnight reincubation. Cryosections of 2 hour BrdU-treated DML transplant embryos (three out of three cases) reacted with anti-quail antibody revealed a double layer of quail nuclei within the dermomyotome and underlying myotome (Fig. 4A,D). High-density BrdU labelling was evident in the dermomyotome (Fig. 4B,C) but the quail-derived myotome was almost completely devoid of nuclei that had incorporated BrdU. A few quail nuclei in the myotome layer were labelled with BrdU [Fig. 4C (arrows), Fig. 4D (black dots)]. The nuclei at the extreme medial edge of the dermomyotome (see Fig. 4D) had not incorporated BrdU (Fig. 4C). Sections from other levels of this somite and other somites showed cells in comparable locations that had incorporated BrdU.

In DML transplanted embryos harvested 4 hours after BrdU administration (Fig. 5), BrdU-labelled quail myocyte nuclei were evident at the medial aspect of the myotome [Fig. 5A,C (asterisk) and Fig. 5D (schematic representation)], while more laterally positioned quail myonuclei were not labelled (Fig. 5C, arrow). The presence of BrdU labelled nuclei at the medial aspect of the myotome with a 4 hour chase period demonstrates that these, newer, myocytes were derived from DML cells that were synthesising DNA 4 hours prior to harvest.

Sustainability of myogenic output of DML progenitor cells

To determine if the DML is a continuous source of primary myotome and dermomyotome cells, quail DMLs were subjected to serial transplantation through two chick host embryos (Fig. 6A). A quail DML was harvested and transplanted to replace the DML ablated from a chick primary host (Table 2, s-03). After an overnight re-incubation the donor (quail) DML was re-harvested and transplanted into a second DML ablated host and re-incubated overnight.

Immunohistochemical labelling of the primary chick host showed that quail derived myocytes within the recombinant somite (Fig. 6B) extended medially from the level of original DML ablation/transplant (Fig. 6B, asterisks). In this primary host, quail-derived myocytes were arranged normally with nuclei aligned at the axial centre of the operated somite. The dermomyotome and myotome of the operated somite had expanded in concert with their neighbouring segments, as determined by visual examination at the time of re-harvesting of the quail DML and the extent of quail dermomyotome/myotome detected by immunolabelling (Fig. 6B).

After a second overnight re-incubation, in the secondary host, the transplanted DML had again expanded dorsomedially in concert with its neighbours (Fig. 6C). The dorsomedialward growth of the myotome is indicated by the extent of quail nuclei in the operated somite. The DML and medially located myotome comprised quail cells (Fig. 6C,D), as did the corresponding overlying dermomyotome (data not shown). The presence of quail nuclei within myocytes was observed within individual optical sections. In contrast to the primary host, donor-derived myocyte nuclei in the second host were not arranged centrally within the somite. Therefore, throughout the time of the serial transplant the cells of the DML retained their competence for myotome and dermomyotome growth and morphogenesis.

DiI injections at the axial midpoint of the donor DML [Site B according to Denetclaw et al. (Denetclaw et al., 1997)] gave rise to DiI-labelled myofibres in the myotome of the primary host after overnight re-incubation (data not shown) and as described previously (Denetclaw et al., 1997). A second DiO injection at the time of the second transplant showed that myofibres continued to be deposited from the donor DML in the myotome of the secondary host (Fig. 6E). The presence of DiI labelled fibres in the myotome of the secondary host (Fig. 6E) showed that labelled cells from the original, DiI labelled, donor DML continued to generate myocytes in both the primary and secondary hosts and therefore throughout the 2-day period of the serial transplant experiment.

Longevity of DML myogenic output

To examine the longevity of DML myogenic output through later stages of development, embryos were re-incubated for longer periods after DML transplants at E2. Re-incubation of a DML transplanted embryo for 2 days, through to E4, after surgery showed that the entire medial region of the myotome continued to be derived from the transplanted quail DML (Fig. 7A). Myocytes spanned a single segment with centrally aligned nuclei. The confocal image in Fig. 7A shows the myotome layer of the operated somite; therefore, dermis that originates from the transplanted DML is largely omitted. However, some dermis nuclei can be seen at the caudal part of the operated

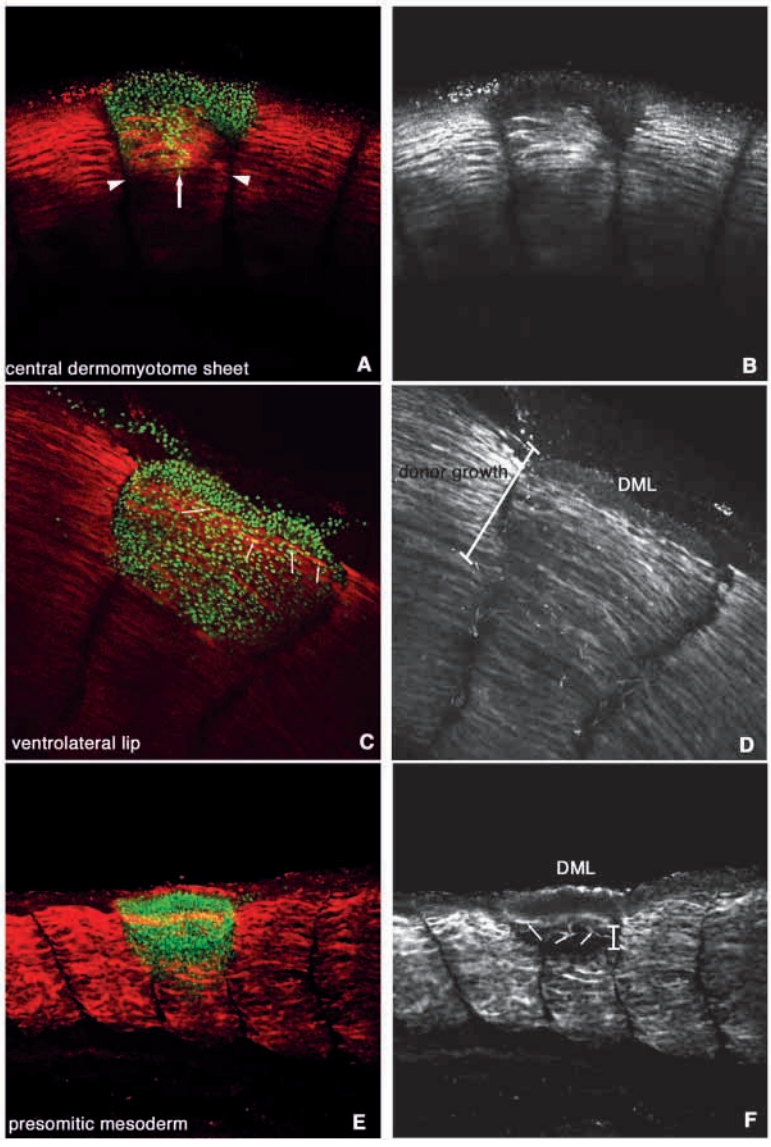

Fig. 3. Transplant of somitic and presomitic tissue to replace the DML. (A-F) Confocal *z*-series of transplanted embryos immunolabelled for desmin (red) and quail (green). (A,B) Transplant of the dermomyotome sheet (Table 1, 4.01). (A) Composite through the myotome region shows dorsomedial expansion of the transplanted tissue and centrally aligned nuclei in quail derived myotome (arrow). The arrowheads indicate the level of the DML ablation. An epithelial DML has reformed at the correct position and is composed of quail cells. (B) The desmin immunoreactivity of A showing the medially located, donor-derived, myotome has grown to a similar extent as its neighbours. (C,D) Transplant of the VLL (Table 1, 5.02). (C) A single optical section in the plane of the myotome of the operated somite shows quail nuclei associated with donor derived myotome. The transplanted tissue has remained within the segment boundaries, a few cells at the medial limit have extended past the segment boundaries. In this example, quail myocyte nuclei (arrows) are not centrally localised but spread along the axial myotome length. (D) The desmin channel of C. The growth of the donor myotome is consistent with that of neighbours (bar) and an epithelial DML structure is evident in the correct location. (E,F) Transplant of caudal PSM (Table 1, 6.06). (E) The quail tissue has expanded in concert with the neighbouring somites and formed a DML. (F) The desmin channel of E highlights the DML structure and shows myofibres situated just lateral to the DML. There is a gap (bar) between the host and the older, laterally situated, donor myofibres (arrows). Donor fibres correspond to the axial length of the transplanted fragment.

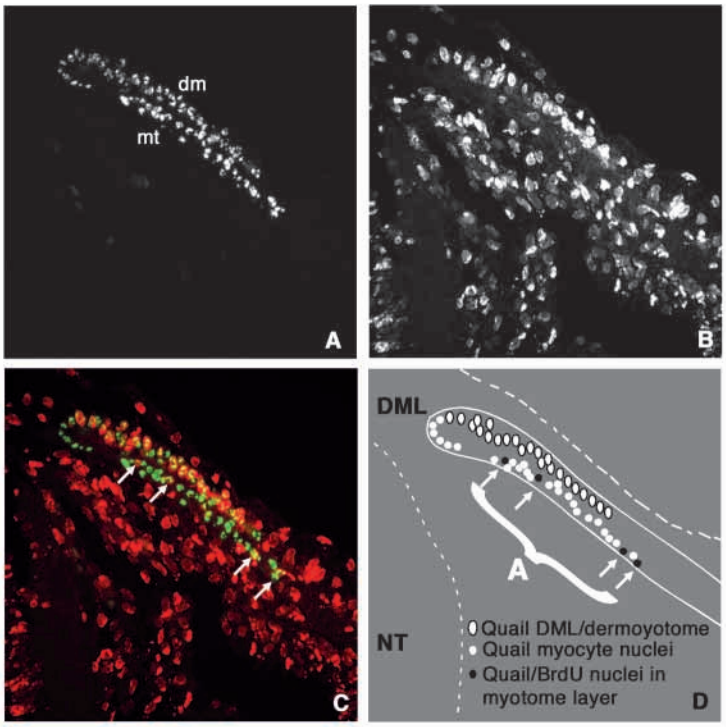

Fig. 4. BrdU incorporation into the epaxial myotome (2 hours post administration). (A-C) Transverse cryosections through an embryo that received a DML transplant from ssXIII of a 25 somite quail embryo to ssXI at 26 somites and was harvested the following day, 2 hours after BrdU administration. (A) Section through the axial mid region of the recombinant somite at the plane of the myocyte nuclei reacted to label quail cells. Two layers of quail nuclei can be seen; the medialmost (lower) layer corresponds to the myotome (mt) and the more superficial layer the dermomyotome (dm). (B) The same section as A showing BrdU-labelled cells. (C) A composite of A and B, showing BrdU co-localised with quail immunoreactivity in the dermomyotome and predominantly non-BrdU-labelled quail nuclei in the myotome layer. A few BrdU-labelled quail cells are present in the myotome layer (arrows). (D) Schematic representation of the section shown in A-C. The extent of non-BrdU-labelled donor-derived nuclei in the myotome layer are indicated (bracket, A). Comparable data were obtained in three out of three treated embryos. DML, dorsomedial lip; NT; neural tube.

somite and in the middle of the next caudal somite (Fig. 7A, arrowheads). Therefore, upon de-epithelialisation of the dermomyotome, DML-derived dermal cells migrate past their original axial somite boundaries.

In transplanted embryos reincubated until E7, the epaxial musculature had developed distinct multisegmental and unisegmental muscles (Fig. 7B). The operated side of these embryos showed normal epaxial muscle architecture in comparison with the opposite, non-operated, left-hand side, although the medial limit of the operated somite (Fig. 7B, asterisk) appeared slightly reduced along the rostrocaudal axis. A high magnification view of the medial limit of the operated somite showed quail myocyte nuclei (Fig. 7C, arrows), a small level of background fluorescence from the red (desmin) channel highlights that the quail nuclei are contained within muscle fibres.

The desmin labelling in E7 embryos resulted in brighter fluorescence intensity at the medial part of the muscle (Fig. 7B, arrowheads), reminiscent of the pattern noted in younger embryos where the youngest (dorsomedial) primary myotome fibres were more intensely labelled than older, laterally situated

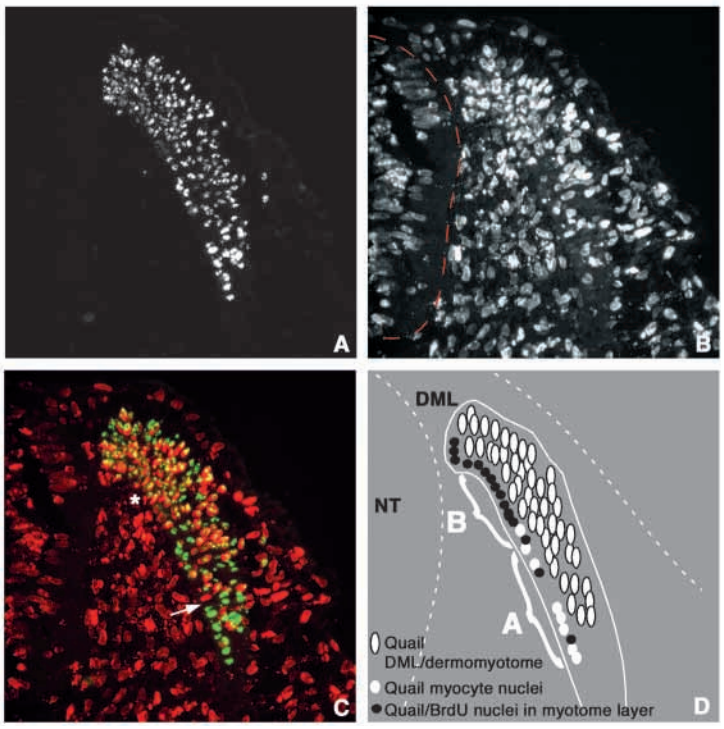

Fig. 5. BrdU incorporation into the epaxial myotome (4 hour post administration). (A,B) A section from a chick embryo that received a ssXV DML from a 30 somite quail embryo transplanted at ssXIV (23 somites) after overnight re-incubation and harvesting 4 hours after BrdU administration. The section is through the plane of the myocyte nuclei, a single layer at the inner extent of the quail nuclei. The slightly oblique plane of section results in the myotome layer being depicted truly as a single cell layer while the dermomyotome appears stratified. (A) Quail derived dermomyotome and myotome labelled with qcpn antibody. (B) Same section as A showing BrdU-labelled nuclei. Broken line outlines the neural tube. (C) Composite image of A,B. BrdU-labelled nuclei (red) and quail nuclei (green). The asterisk marks the medial part of the myotome comprising dual labelled nuclei and the arrow marks the medial boundary of quail myonuclei that have not incorporated BrdU. One or two quail nuclei are present in the dermis overlying the lateral donor-derived dermomyotome. (D) Schematic interpretation of the section in A-C highlighting the medial position of BrdU-labelled quail myocyte nuclei (bracket, B) and the non-BrdU labelled quail myotome nuclei located laterally (bracket, A). The same distribution of BrdU and/or quail nuclei was seen in three out of three embryos. DML, dorsomedial lip; NT; neural tube.

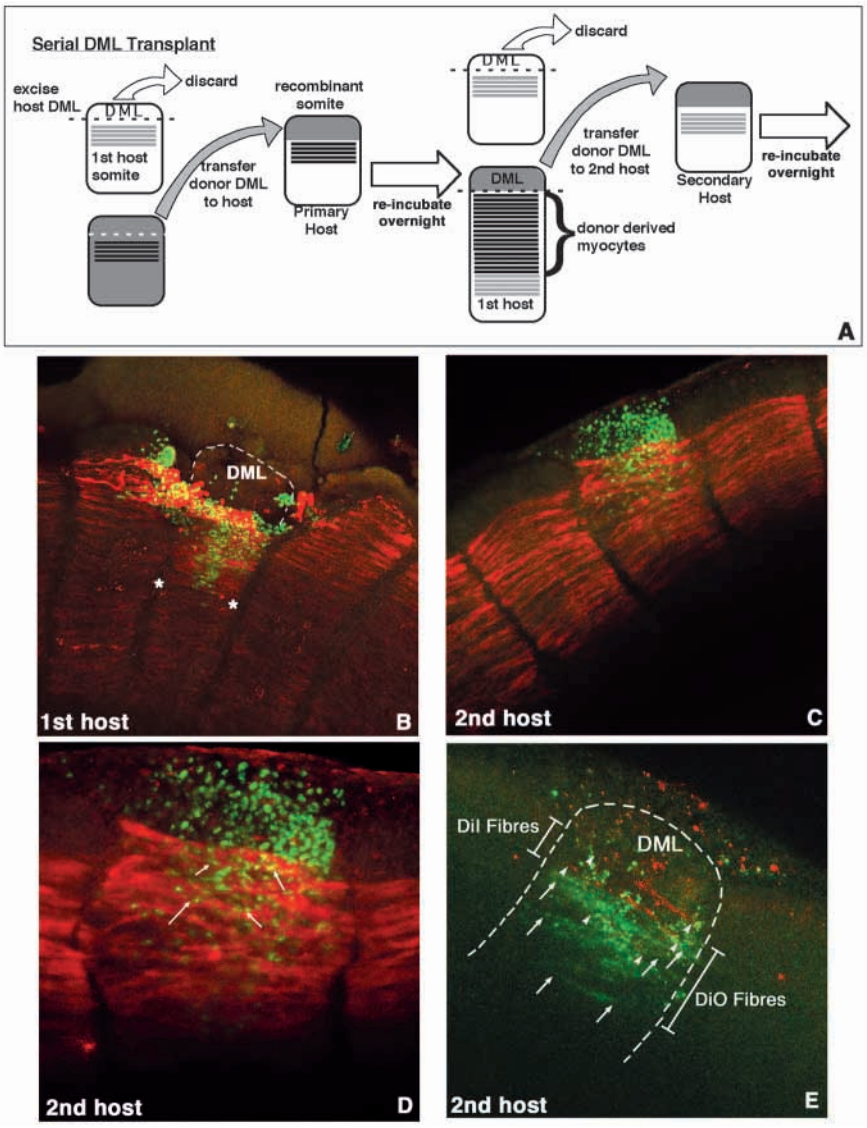

Fig. 6. Serial DML transplant. (A) Schematic outline of the serial lip experimental procedure. The example shown is embryo s-03 (Table 2). (B-D) Composite confocal images of whole-mount embryos labelled with anti-desmin antibody (red) and anti-quail antibody (green). (B) Primary host after reincubation. The operated segment is highlighted by the broken line that indicates the position of the quail DML prior to harvest for transplantation into the second host. DML harvest caused the apparent disruption to somite morphology. The operated somite developed normally with a clear boundary between host and donor myotome (asterisks); centrally aligned quail nuclei are evident in the medial myotome. Some quail cells are apparent in adjacent somites. This is an artefact resulting from the harvest of the quail DML. (C) The secondary host after overnight re-incubation following DML transplant. The operated somite myotome grew medially to a similar extent as its neighbours and quail cells are evident at the DML and within the medial myotome. The quail-derived myocytes are not running completely parallel to the body axis, although they still remain within the somitic boundaries. (D) A higher magnification of the secondary host somite showing individual quail nuclei in myocytes (arrows). (E) A composite image of fluorescent dye labelling of transplanted tissue. Dye-labelled fibres in the myotome of the secondary host resulting from donor DML dye injections of DiI and DiO prior to transplant in the primary and secondary hosts, respectively. Some DiI-labelled fibres (arrowheads) are evident medial to DiO-labelled fibres (arrows) with some overlap.

Table 2. Serial DML transplants

Number	Primary host	Donor embryo	Secondary host	Secondary host myotome	Donor-derived fibres?	Notes (second host)
s-01	27s/ssXII	19s/ssXII	24s/ssXI	Normal	Yes	
s-02	21s/ssXV	25s/ssXIV	22s/ssXIII	Normal	Yes	
s-03	21s/ssXIII	23s/ssXIII	19s/ssXII	Normal	Yes	
s-04	27s/ssXV	21s/ssXII	22s/ssXIV	Truncated	No	No quail
s-05	21s/ssXII	24s/ssXI	20s/ssXIII	Normal	No	No quail
s-06	21s/ssXII	24s/ssXI	20s/ssVIII	Truncated	No	Few quail nuclei

Of 13 serial transplants attempted, 11 secondary hosts survived. Of these embryos, the wrong lip was transplanted to the second host on five occasions. The remaining data are shown here. The second host contained no or few quail nuclei in three cases (s-04, s-05, s-06), despite transplant of the correct DML from the primary host. For the remaining three embryos (s-01, s-02, s-03), the myotome of the second host was normal and comprised quail myocytes medially.

counterparts (Ordahl et al., 2001). If the unisegmental medial muscle was the most recently laid down, then a DML ablation carried out in older embryos should result in absence of the medial muscle. To test this hypothesis, DML ablations were carried out at low cervical or wing-level of E4 embryos and embryos reincubated for 2 or 3 days until E6 or E7, respectively. An example of an embryo that had a DML ablated from the rostral wing-level and was re-incubated until E7 is shown in Fig. 8A, where normal muscle pattern is indicated by the unoperated left-hand side of the embryo. By contrast, at the operated level the medial muscle is absent (Fig. 8A,B, filled bar) and some disruption to the laterally situated multisegmental muscle is evident. Analysis of individual optical scans confirmed the absence of the deeper, unisegmental, medial muscle and that it

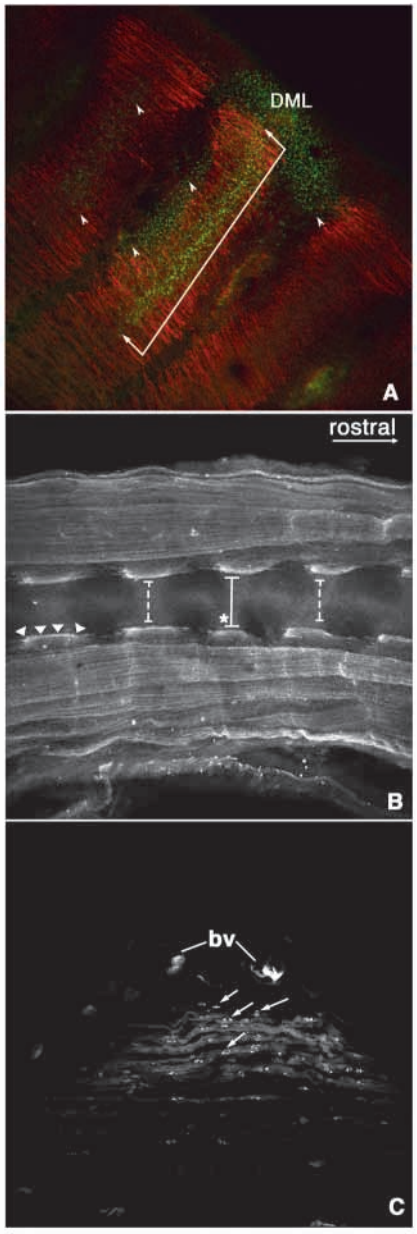

Fig. 7. Continuing development of the epaxial musculature. (A) A composite of four consecutive *z*-scans through the plane of the myotome of an E4 embryo transplanted at ssXV at 20 somites with the ssXIV DML of a 21-somite quail embryo. Extensive dorsomedial expansion of quail myotome is evident (bar) and a dense cluster of quail nuclei are located at the DML position. The myotome has retained its uni-segmental organisation and central myocyte nuclei alignment. Some quail dermis cells (arrowheads) are present over the neighbouring somite and at the caudal edge of the transplanted somite. (B) Dorsal view of desmin immunoreactivity composite of a *z*-series through an embryo that received a DML transplant of ssX at 20 somites with a ssXV DML from a 25 somite quail and was re-incubated for 5 days. Broken bars mark, and join, corresponding neighbouring segments. The unbroken bar joins the transplanted segment (asterisk) to its contralateral counterpart. Medially, the musculature is unisegmental while the overlying more laterally positioned muscle extends over several segments. Arrowheads indicate more intensive immunolabelling at the medial limit of the musculature. (C) A higher magnification view of the medial limit of the transplanted segment. Quail nuclei are evident within myofibres (arrows). bv, blood vessels.

was the more superficial, multisegmental muscle that was pulled slightly towards the medial direction. Control embryos, which were treated similarly except that the DML was not ablated, retained the medialmost muscle (Fig. 8C).

At earlier stages of myotome development, the primary myotome comprises extensively mononucleate myocytes. It is interesting to note, therefore, that even at E7 the youngest DML-derived unisegmental muscle also contains myocytes that comprise a single large nucleus located medial to multinucleate myotubes (Fig. 8D).

DISCUSSION

The DML contains a progenitor cell population that is self-renewing

Previous work showed that the DML of somites from ssVIII to XV was both necessary and sufficient to provide the cells that form the primary myotome following myotome initiation from E2 to E4 (Ordahl et al., 2001). The results here support the conclusion that the cells entering the myotome from the DML are daughters of a self-renewing progenitor cell system as determined by several criteria.

First, DML transplanted at E2 is sufficient to complete the epaxial myotome from the point at which the host myotome is truncated as a consequence of host DML ablation (Fig. 7). The fact that donor-derived myotome cells occupy the entire medial position relative to that start point is consistent with previous work showing that primary epaxial myotome expansion proceeds in a lateral-to-medial direction (Denetclaw et al., 1997; Venters et al., 1999; Denetclaw and Ordahl, 2000; Ordahl et al., 2001). In addition, even at E7, new myocytes laid down at the extreme dorsomedial limit of the myotome are of donor DML origin. Taken together, these findings indicate that the DML retains potency to generate all of the cells comprising the primary epaxial myotome throughout the entire embryonic period.

Second, a prediction that devolves from the above conclusion is that DML ablation at any point during myotome formation should cause cessation of primary myotome development from that point onwards. Consistent with this prediction, ablation of the DML at E4 resulted in medially restricted truncation of the myotome that was evident at the end of the embryonic period (E7). Therefore, progressivity of primary myotome development is dependent upon an intact DML.

Third, the DML retains its potency to generate new myotome even after serial transplantation through two host embryos. Therefore, the DML continues to be the source of new cells entering the myotome, either directly or indirectly via DML-derived dermomyotome sheet, from the point of transplant.

Finally, it is mitotic activity in the DML that results in new cells entering the myotome because BrdU-labelled nuclei are chased into the myotome layer after a BrdU pulse administered to the embryo. Moreover, the appearance of BrdU-labelled myotome nuclei is time-dependent as few, if any, BrdU-

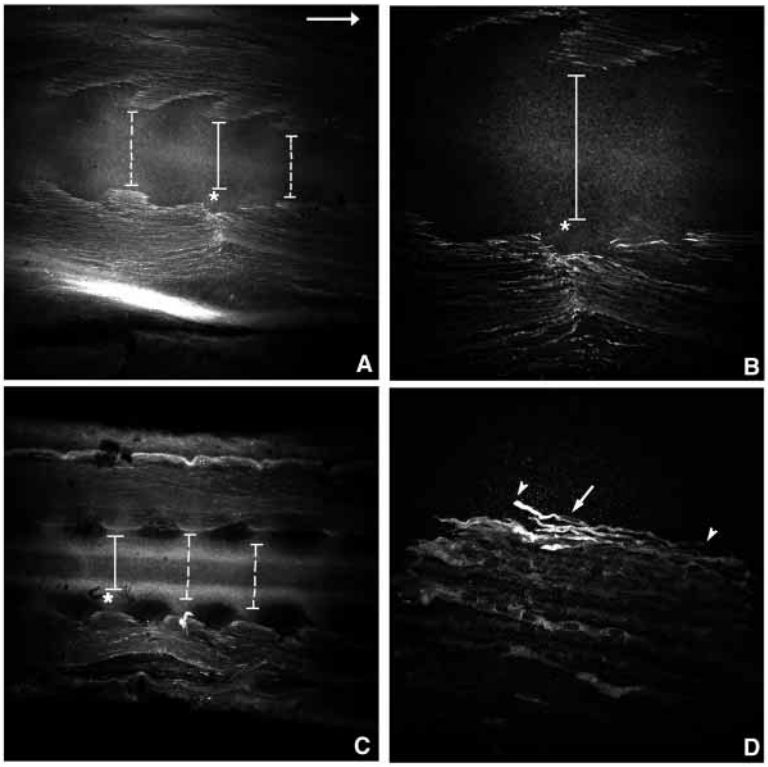

Fig. 8. Late ablation of the DML. (A) Confocal image of a desmin-labelled embryo in which a DML at the wing-level was ablated at E4 and the embryo reincubated for 3 days. Bars extend between corresponding segments on opposing sides of the embryo, the unbroken bar marks the DML ablated segment (asterisk) and broken bars run between non-operated neighbours. The medial unisegmental muscle is absent at the level of the DML ablation when compared with the unoperated control side. (B) A higher magnification of the operated area from the embryo shown in A. (C) An embryo in which a wing-level DML was exposed and treated with enzyme but not ablated at E4, and the embryo reincubated for 3 days. Corresponding segments are marked as above. The medial segmented muscle is present, some disruption is evident in the more superficial, lateral multisegmental muscle at the operated site. The mock-operated control embryo shown in C is slightly older than that shown in A,B. (D) High-magnification view of the medial limit of one segment of the embryo shown in C. A mononucleate myocyte (an arrow marks the nucleus and arrowheads mark its rostral and caudal ends) is present at the medial extremity and multinucleate myotubes are apparent laterally.

labelled cells enter during a 2 hour pulse-chase experiment but many labelled myotome cells are seen after a 4 hour pulse-chase in accord with earlier studies (Langman and Nelson, 1968). Labelled myotome cells are predominantly restricted to the dorsomedial aspect of the myotome. Such distribution of BrdU-labelled myotome nuclei highlights the flow of daughter cells from mitotic DML progenitors to the dorsomedial aspect of the myotome.

The few BrdU-labelled myotome cells that appear during a 2 hour pulse-chase are scattered randomly throughout the primary myotome layer. This random positioning indicates that these cells did not arise directly from the DML during the 2-hour pulse chase, but either entered the myotome earlier (where they remained both undifferentiated and mitotically quiescent) or were derived from mitotically active precursors in the dermomyotome sheet and entered from the cranial and/or caudal lips of the dermomyotome (Marcelle et al., 1995; Kahane et al., 1998b; Kahane et al., 2001). In either case, these few cells represent the beginnings of secondary myotome formation (Kahane et al., 1998a), whose precursors were derived from the DML at an earlier stage of myotome formation.

The results presented here are also consistent with previous transplant and dye studies, indicating that the epaxial dermomyotome epithelium is itself DML derived. The above conclusions regarding morphogenetic potential of primary myotome progenitor cells in the DML are also valid for both myogenic and dermal, and possibly other, precursor cells contained within DML-derived dermomoyotome sheet.

Permanence of DML myogenic progenitor cell pool

Recent analysis of myotome formation in mouse embryos (Eloy-Trinquet and Nicolas, 2002) concluded that there is no 'permanent' stem cell population that gives rise to the myotome, a conclusion in apparent conflict with that reached from the experiments reported here. The notion of 'permanence', in this instance, implies that once a stem cell begins producing myotomal daughters it must continue to do so throughout the period of myotome development. There is, however, no reason to rule out the possibility that the DML contains stem cells with varying longevity [see fig. 4 in Denetclaw et al. (Denetclaw et al., 2001)]. Thus, while some DML stem cells may give rise to myotome daughter cells during the entire embryonic period (and thus be 'permanent' in the strict sense outlined above), the results of Nicolas and co-workers show that such stem cells are probably rare, while most generate myotomal daughters over a limited period of time and space (Eloy-Trinquet et al., 2000; Eloy-Trinquet and Nicolas, 2002). Predictions that opposing stem cell systems in the DML and its ventrolateral counterpart would result in an increase in labelled progeny at medial and lateral myotome extremities, respectively, do not take into account the dynamic nature of dermomyotome expansion demonstrated here. The expansion of these lips in opposing directions results instead in a corresponding deposition of myocytes in progressively medial, or lateral, positions. Therefore, such coherent opposing growth vectors (from dorsomedial and ventrolateral dermomyotome lips) may play an active role in maintenance of a proposed active clonal boundary between epaxial and hypaxial myotome clones (Eloy-Trinquet and Nicolas, 2002). Because DML transplant analyses involve tracking daughters of the entirety of the progenitor cell pool, the absolute contribution of individual cells is impossible to predict. We propose, therefore, that the progenitor cells in the DML constitute a 'dynamically stable' stem cell pool where the majority of mother cells may produce myogenic daughters for

limited but overlapping periods during the development of the entire myotome. We refer to them as embryonic progenitor cells because they are restricted to development of a limited range of tissue and cell types, and in order to distinguish them from embryonic stem cells (which have much broader developmental potential).

Dynamic aspects of the inter-relationships among the progenitor cells within the DML and their daughters entering the myotome and dermomyotome remain undefined. For example, although many or all of the DML-derived dermomyotome epithelial cells remain both mitotically active and pluripotent the presence of such cells within the myotome can, at present, only be speculated upon. While differentiated myotome cells do not incorporate BrdU, the possibility that mitotically quiescent, undifferentiated cells may reside in the myotome cannot be ruled out. If such cells are generated during DML activity, they could undergo mitoses at a later point to generate muscle tissue. In addition, the ability of a given DML progenitor cell to give rise to both myotome and dermomyotome daughters remains undefined. Thus, any given DML cell may give rise to only one type of daughter (e.g. myotome) for a limited period and to the other type of daughter (dermomyotome) during other periods. Finally, some progenitor cells may remain quiescent (giving rise to neither type of daughter) for unknown periods, while being carried along with actively dividing neighbours during the dorsomedial expansion of the DML. Taken together, these considerations suggest that the DML progenitor cell population is complex and contains cells with various and possibly changeable phenotypic and mitotic potentialities.

Myogenic competence is restricted to paraxial mesoderm

Previous work from numerous laboratories has shown that competence for myogenic development is restricted to the paraxial mesoderm (Christ et al., 1979; Wachtler et al., 1982) and shown a role for neighbouring structures in promoting somitic myogenesis (reviewed by Borycki and Emerson, 2000; Hirsinger et al., 2000). We therefore used non-paraxial mesoderm tissue as a control donor tissue to assess the extent to which the DML environment could induce growth and myogenicity. As predicted from those previous experiments, no myogenic potential was ever observed in donor tissue from the lateral plate mesoderm. In addition, CPM transplanted into the DML position also lacked myogenic potential. This was unexpected as CPM does form muscle tissue in the head (Noden, 1983; Couly et al., 1992; Trainor et al., 1994). However, the retention of cranial muscle in *Myf5*/*Pax3* null mice (Tajbakhsh et al., 1997) and recent work describing discrete control of head versus body musculature (Mootoosamy and Dietrich, 2002) argue for inequivalence between precursors of these populations. It remains possible, however, that longer re-incubation would yield a myogenic response by CPM in a DML position, simply reflecting the intrinsic differentiation schedule of cranial musculature (Noden et al., 1999).

An interesting finding, however, was that all of the donor tissues that were negative for myogenic potential were able to respond to the DML environment by vigorously growing (expanding) in concert with adjacent, unoperated host paraxial mesoderm tissue. We conclude from this that the growth factor environment surrounding the DML is sufficient to support division and hypertrophy of many cell and tissue types, as suggested for sonic hedgehog during somitic myogenesis (Teillet et al., 1998; Charrier et al., 2001; Kruger et al., 2001). However, the growth factor environment is not sufficient to impart myogenic potential to non-paraxial mesoderm tissue.

The ventrally located sclerotome was also found to be non-myogenic in the test used here. Classical somite rotation experiments (Aoyama and Asamoto, 1988) showed that the ventral, pre-sclerotome regions of newly formed somites (ssI-ssII) are capable of forming normal myotomes while those from ssIII and older somites were not. The sclerotome transplant experiments reported here employed donor somites ssIV and older and so these conclusions are consistent with those of earlier workers. Other sclerotome transplantation experiments in which donor tissue fragments were placed dorsal to the DML (Dockter and Ordahl, 1998) showed that sclerotome from somites older than ssIII retains myogenic competence, but only after E4 during the period of multi-segmental muscle formation. Thus, the sclerotome loses capacity for primary myotome growth and morphogenesis, while retaining capacity to participate in later muscle patterning and growth.

The dermomyotome lateral to the DML retains myogenic competence and is able to form primary myotome when placed in the DML position. Because cells in this region of the dermomyotome are derived from the DML (Denetclaw et al., 1997; Ordahl et al., 2001) they represent symmetric daughters of the DML stem cell pool. These DML daughters within the dermomyotome retain latent myogenic competence for primary myotome formation that can be initiated when these cells are placed in the DML position. Finally, myogenic progenitor cells from the VLL of wing-level somites are similarly competent to mimic the DML role in primary epaxial myotome formation. Interestingly, older stage VLLs are unable to fulfil such a role, suggesting depletion of myogenically competent cells with migration from the somite into the limb field (Chevallier et al., 1977; Christ et al., 1977; Jacob et al., 1978; Jacob et al., 1979) (for a review, see Birchmeier and Brohmann, 2000).

In conclusion, the results of the studies conducted here are consistent with the hypothesis that the dermomyotome DML contains a population of self-renewing cells, which simultaneously give rise to daughters that contribute to the myotome and dermomyotome. These daughters emerge from the DML in a topologically asymmetrical fashion, whereby mother cells are displaced medially in a process that results in the lateral-to-medial expansion of both the myotome and dermomyotome epithelium. Asymmetric division of mother cells and/or asymmetric timing of terminal differentiation of daughters combined with morphogenetic movements of cells emerging from the DML accounts for the growth and morphogenetic patterning of the myotome, and is also likely to govern patterning of other dermomyotome-derived tissues such as the dermis. Earlier in vitro experiments also implicated asymmetric, stem-cell-like divisions in the development of hypaxial muscle lineages (Quinn et al., 1984; Quinn et al., 1985). DML-directed growth and morphogenesis bears similarity to the blastematic growth of regenerating limb mesenchyme. A clear difference between the regenerating limb blastema and the DML is that the latter is epithelial, although serial transplanation experiments reported here indicate that

the epithelial nature of the DML may not be necessary for proper growth and morphogenesis. We therefore propose that the DML is an 'epithelial blastema', which governs unidirectional growth and patterning through asymmetrical division of progenitor cells within the DML and asymmetrical morphogenetic cell movements of their daughters.

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REFERENCES

Aoyama, H. and Asamoto, K. (1988). Determination of somite cells: independence of cell differentiation and morphogenesis. *Development* **104**, 15-28.

Birchmeier, C. and Brohmann, H. (2000). Genes that control the development of migrating muscle precursor cells. *Curr. Opin. Cell Biol.* **12**, 725-730.

Borycki, A. G., Mendham, L. and Emerson, C. P., Jr (1998). Control of somite patterning by Sonic hedgehog and its downstream signal response genes. *Development* **125**, 777-790.

Borycki, A., Brunk, B., Tajbakhsh, S., Buckingham, M., Chiang, C. and Emerson, C. P. (1999). Sonic hedgehog controls epaxial muscle determination through Myf5 activation. *Development* **126**, 4053-4063.

Borycki, A. G. and Emerson, C. P., Jr (2000). Multiple tissue interactions and signal transduction pathways control somite myogenesis. *Curr. Top. Dev. Biol.* **48**, 165-224.

Cann, G. M., Lee, J. W. and Stockdale, F. E. (1999). Sonic hedgehog enhances somite cell viability and formation of primary slow muscle fibers in avian segmented mesoderm. *Anat. Embryol.* **200**, 239-252.

Charrier, J. B., Lapointe, F., le Douarin, N. M. and Teillet, M. A. (2001). Anti-apoptotic role of Sonic hedgehog protein at the early stages of nervous system organogenesis. *Development* **128**, 4011-4020.

Chevallier, A., Kieny, M. and Mauger, A. (1977). Limb-somite relationship: origin of the limb musculature. *J. Embryol. Exp. Morphol.* **41**, 245-258.

Christ, B., Jacob, H. J. and Jacob, M. (1977). Experimental analysis of the origin of the wing musculature in avian embryos. *Anat. Embryol.* **150**, 171-186.

Christ, B., Jacob, H. J. and Jacob, M. (1979). Differentiating abilities of avian somatopleural mesoderm. *Experientia* **35**, 1376-1378.

Couly, G. F., Coltey, P. M. and le Douarin, N. M. (1992). The developmental fate of the cephalic mesoderm in quail-chick chimeras. *Development* **114**, 1-15.

Denetclaw, W. F., Jr, Christ, B. and Ordahl, C. P. (1997). Location and growth of epaxial myotome precursor cells. *Development* **124**, 1601-1610.

Denetclaw, W. F. and Ordahl, C. P. (2000). The growth of the dermomyotome and formation of early myotome lineages in thoracolumbar somites of chicken embryos. *Development* **127**, 893-905.

Denetclaw, W. F., Berdougo, E., Venters, S. J. and Ordahl, C. P. (2001). Morphogenetic cell movements in the middle region of the dermomyotome dorsomedial lip associated with patterning and growth of the primary epaxial myotome. *Development* **128**, 1745-1755.

Dockter, J. L. and Ordahl, C. P. (1998). Determination of sclerotome to the cartilage fate. *Development* **125**, 2113-2124.

Eloy-Trinquet, S., Mathis, L. and Nicolas, J. F. (2000). Retrospective tracing of the developmental lineage of the mouse myotome. *Curr. Top. Dev. Biol.* **47**, 33-80.

Eloy-Trinquet, S. and Nicolas, J. F. (2002). Clonal separation during development of the medial and lateral myotomes in the mouse embryo. *Development* **129**, 111-122.

George-Weinstein, M., Gerhart, J. V., Foti, G. J. and Lash, J. W. (1994). Maturation of myogenic and chondrogenic cells in the presomitic mesoderm of the chick embryo. *Exp. Cell Res.* **211**, 263-274.

Gerhart, J., Baytion, M., DeLuca, S., Getts, R., Lopez, C., Niewenhuis, R., Nilsen, T., Olex, S., Weintraub, H. and George-Weinstein, M. (2000). DNA dendrimers localize MyoD mRNA in presomitic tissues of the chick embryo. *J. Cell Biol.* **149**, 825-834.

Hirsinger, E., Duprez, D., Jouve, C., Malapert, P., Cooke, J. and Pourquié, O. (1997). Noggin acts downstream of Wnt and Sonic Hedgehog to antagonize BMP4 in avian somite patterning. *Development* **124**, 4605-4614.

Hirsinger, E., Jouve, C., Dubrulle, J. and Pourquié, O. (2000). Somite formation and patterning. *Int. Rev. Cytol.* **198**, 1-65.

Huang, R. and Christ, B. (2000). Origin of the epaxial and hypaxial myotome in avian embryos. *Anat. Embryol.* **202**, 369-374.

Jacob, M., Christ, B. and Jacob, H. J. (1978). On the migration of myogenic stem cells into the prospective wing region of chick embryos. A scanning and transmission electron microscope study. *Anat. Embryol.* **153**, 179-193.

Jacob, M., Christ, B. and Jacob, H. J. (1979). The migration of myogenic cells from the somites into the leg region of avian embryos. An ultrastructural study. *Anat. Embryol.* **157**, 291-309.

Kahane, N., Cinnamon, Y. and Kalcheim, C. (1998a). The cellular mechanism by which the dermomyotome contributes to the second wave of myotome development. *Development* **125**, 4259-4271.

Kahane, N., Cinnamon, Y. and Kalcheim, C. (1998b). The origin and fate of pioneer myotomal cells in the avian embryo. *Mech. Dev.* **74**, 59-73.

Kahane, N., Cinnamon, Y., Bachelet, I. and Kalcheim, C. (2001). The third wave of myotome colonization by mitotically competent progenitors: regulating the balance between differentiation and proliferation during muscle development. *Development* **128**, 2187-2198.

Kiefer, J. C. and Hauschka, S. D. (2001). Myf-5 is transiently expressed in nonmuscle mesoderm and exhibits dynamic regional changes within the presegmented mesoderm and somites I- IV. *Dev. Biol.* **232**, 77-90.

Krenn, V., Gorka, P., Wachtler, F., Christ, B. and Jacob, H. J. (1988). On the origin of cells determined to form skeletal muscle in avian embryos. *Anat. Embryol.* **179**, 49-54.

Kruger, M., Mennerich, D., Fees, S., Schafer, R., Mundlos, S. and Braun, T. (2001). Sonic hedgehog is a survival factor for hypaxial muscles during mouse development. *Development* **128**, 743-752.

Langman, J. and Nelson, G. R. (1968). A radioautographic study of the development of the somite in the chick embryo. *J. Embryol. Exp. Morphol.* **19**, 217-226.

Marcelle, C., Wolf, J. and Bronner-Fraser, M. (1995). The in vivo expression of the FGF receptor FREK mRNA in avian myoblasts suggests a role in muscle growth and differentiation. *Dev. Biol.* **172**, 100-114.

Marcelle, C., Ahlgren, S. and Bronner-Fraser, M. (1999). In vivo regulation of somite differentiation and proliferation by Sonic Hedgehog. *Dev. Biol.* **214**, 277-287.

Mootoosamy, R. C. and Dietrich, S. (2002). Distinct regulatory cascades for head and trunk myogenesis. *Development* **129**, 573-583.

Nicolas, J. F., Mathis, L. and Bonnerot, C. (1996). Evidence in the mouse for self-renewing stem cells in the formation of a segmented longitudinal structure, the myotome. *Development* **122**, 2933-2946.

Noden, D. M. (1983). The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. *Am. J. Anat.* **168**, 257-276.

Noden, D. M., Marcucio, R., Borycki, A. G. and Emerson, C. P., Jr (1999). Differentiation of avian craniofacial muscles: I. Patterns of early regulatory gene expression and myosin heavy chain synthesis. *Dev. Dyn.* **216**, 96-112.

Ordahl, C. P. and le Douarin, N. M. (1992). Two myogenic lineages within the developing somite. *Development* **114**, 339-353.

Ordahl, C. P., Williams, B. A. and Denetclaw, W. (2000). Determination and morphogenesis in myogenic progenitor cells: an experimental embryological approach. *Curr. Top. Dev. Biol.* **48**, 319-367.

Ordahl, C. P., Berdougo, E., Venters, S. J. and Denetclaw, W. F. (2001). The dermomyotome dorsomedial lip drives growth and morphogenesis of both the primary myotome and dermomyotome epithelium. *Development* **128**, 1731-1744.

Pirskanen, A., Kiefer, J. C. and Hauschka, S. D. (2000). IGFs, insulin, Shh, bFGF, and TGF-beta1 interact synergistically to promote somite myogenesis in vitro. *Dev. Biol.* **224**, 189-203.

Pourquié, O. and Tam, P. P. (2001). A nomenclature for prospective somites and phases of cyclic gene expression in the presomitic mesoderm. *Dev. Cell* **1**, 619-620.

Quinn, L. S., Nameroff, M. and Holtzer, H. (1984). Age-dependent changes in myogenic precursor cell compartment sizes. Evidence for the existence of a stem cell. *Exp. Cell Res.* **154**, 65-82.

Quinn, L. S., Holtzer, H. and Nameroff, M. (1985). Generation of chick skeletal muscle cells in groups of 16 from stem cells. *Nature* **313**, 692-694.

Reshef, R., Maroto, M. and Lassar, A. B. (1998). Regulation of dorsal somitic cell fates: BMPs and Noggin control the timing and pattern of myogenic regulator expression. *Genes Dev.* **12**, 290-303.

Stern, H. M. and Hauschka, S. D. (1995). Neural tube and notochord promote in vitro myogenesis in single somite explants. *Dev. Biol.* **167**, 87-103.

Stern, H. M., Lin-Jones, J. and Hauschka, S. D. (1997). Synergistic interactions between bFGF and a TGF-beta family member may mediate myogenic signals from the neural tube. *Development* **124**, 3511-3523.

Tajbakhsh, S., Rocancourt, D., Cossu, G. and Buckingham, M. (1997). Redefining the genetic hierarchies controlling skeletal myogenesis: Pax- 3 and Myf-5 act upstream of MyoD. *Cell* **89**, 127-138.

Tajbakhsh, S., Borello, U., Vivarelli, E., Kelly, R., Papkoff, J., Duprez, D., Buckingham, M. and Cossu, G. (1998). Differential activation of myf5 and MyoD by different Wnts in explants of mouse paraxial mesoderm and the later activation of myogenesis in the absence of myf5. *Development* **125**, 4155-4162.

Teillet, M., Watanabe, Y., Jeffs, P., Duprez, D., Lapointe, F. and le Douarin, N. M. (1998). Sonic hedgehog is required for survival of both myogenic and chondrogenic somitic lineages. *Development* **125**, 2019-2030.

Trainor, P. A., Tan, S. S. and Tam, P. P. (1994). Cranial paraxial mesoderm: regionalisation of cell fate and impact on craniofacial development in mouse embryos. *Development* **120**, 2397-2408.

Venters, S. J., Thorsteinsdottir, S. and Duxson, M. J. (1999). Early development of the myotome in the mouse. *Dev. Dyn.* **216**, 219-232.

Wachtler, F., Christ, B. and Jacob, H. J. (1982). Grafting experiments on determination and migratory behaviour of presomitic, somitic and somatopleural cells in avian embryos. *Anat. Embryol.* **164**, 369-378.

Wagner, J., Schmidt, C., Nikowits, W., Jr and Christ, B. (2000). Compartmentalization of the somite and myogenesis in chick embryos are influenced by wnt expression. *Dev. Biol.* **228**, 86-94.

Williams, B. A. and Ordahl, C. P. (1997). Emergence of determined myotome precursor cells in the somite. *Development* **124**, 4983-4997.

Williams, B. A. and Ordahl, C. P. (2000). Fate restriction in limb muscle precursor cells precedes high-level expression of MyoD family member genes. *Development* **127**, 2523-2536.