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Characterization of the early development of specific hypaxial muscles from the ventrolateral myotome

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SUMMARY

We have previously found that the myotome is formed by a first wave of pioneer cells generated along the medial epithelial somite and a second wave emanating from the dorsomedial lip (DML), rostral and caudal edges of the dermomyotome (Kahane, N., Cinnamon, Y. and Kalcheim, C. (1998a) *Mech. Dev.* 74, 59-73; Kahane, N., Cinnamon, Y. and Kalcheim, C. (1998b) *Development* 125, 4259-4271). In this study, we have addressed the development and precise fate of the ventrolateral lip (VLL) in non-limb regions of the axis. To this end, fluorescent vital dyes were iontophoretically injected in the center of the VLL and the translocation of labeled cells was followed by confocal microscopy. VLL-derived cells colonized the ventrolateral portion of the myotome. This occurred following an early longitudinal cell translocation along the medial boundary until reaching the rostral or caudal dermomyotome lips from which fibers emerged into the myotome. Thus, the behavior of VLL cells parallels that of their DML counterparts which colonize the opposite, dorsomedial portion of the myotome.

To precisely understand the way the myotome expands, we addressed the early generation of hypaxial intercostal muscles. We found that intercostal muscles were formed by VLL-derived fibers that intermingled with fibers emerging from the ventrolateral aspect of both rostral and caudal edges of the dermomyotome. Notably, hypaxial intercostal muscles also contained pioneer myofibers (first wave) showing for the first time that lateral myotome-derived muscles contain a fundamental component of fibers generated in the medial domain of the somite. In addition, we show that during myotome growth and evolution into muscle, second-wave myofibers progressively intercalate between the pioneer fibers, suggesting a constant mode of myotomal expansion in its dorsomedial to ventrolateral extent. This further suggests that specific hypaxial muscles develop following a consistent ventral expansion of a 'compound myotome' into the somatopleure.

Key words: Avian embryo, Dermomyotome, Dorsomedial lip, MyoD, Myf-5, Myogenin, Myogenesis, Neural tube, Somite, PAX, Pioneer myotomal cells, Sclerotome, Somite, Ventrolateral lip

INTRODUCTION

Skeletal muscles of vertebrate embryos are generated from the metameric somites. The latter structures are initially epithelial, and in response to signals from their local environment, become specified into a dorsal dermomyotome and a ventral sclerotome (Aoyama and Asamoto, 1988; Capdevila et al., 1998; Christ et al., 1992; Dietrich et al., 1997; Fan and Tessier-Lavigne, 1994; Hirsinger et al.,1997; McMahon et al., 1998; Marcelle et al., 1997; Pourquie et al., 1993; Reshef et al., 1998; Spence et al., 1996). While it is generally accepted that the dermomyotome forms muscle and dermis and the sclerotome gives rise to vertebrae and ribs (reviewed by Christ and Ordahl, 1995; and B. Christ, personal communication), new studies suggest that the distal and sternal portions of the ribs derive from specific dermomyotomal regions rather than from the sclerotome (Kato and Aoyama, 1998).

Likewise, the formation of the myotome has lately been subjected to revision. A widely accepted model claims that the myotome is generated by continuous addition of cells from the dorsomedial lip (DML) of the dermomyotome which ingress underneath the dermomyotomal epithelium along the entire length of the somite (Denetclaw et al., 1997). This view has been challenged by recent findings invoking a multiwave nature of myotome formation (Kahane et al., 1998a,b; Kalcheim et al., 1999). According to this alternative model, the DML is not the only domain contributing to myotome development. We found instead that the origin of the primary myotome resides along the medial aspect of the still epithelial somite which is composed of early post-mitotic cells (Kahane et al., 1998a) and which consistently expresses the earliest myogenic genes (Borycki et al., 1997; Ott et al., 1991; Piette et al., 1992; Pownall and Emerson, 1992; Pownall et al., 1996). This medial epithelial layer then bends underneath the nascent dermomyotome reaching the lateral portion of the somite, and its dissociating cells migrate rostrally. Elongation of the pioneer myofibers then occurs in a rostrocaudal direction until a primary myotomal structure is formed.

Subsequent development of the myotome (second wave) is accounted for by progressive cell addition from the dermomyotome lips (Kahane et al., 1998b) whose cells become immediately post-mitotic upon myotome colonization. While a direct elongation of myofibers into the myotome was observed to occur from along the extreme dermomyotome edges (rostral and caudal), cells from the DML were found first to translocate into the above lips and only then contribute to the colonization of the medial portion of the myotome.

These studies left open the question of the migration and fate of cells of the ventrolateral lip (VLL). Grafting of lateral half-epithelial somites from quail donors into chick hosts at limb levels of the axis revealed that the limb musculature was of quail origin and the epaxial muscles were of host type (Ordahl and Le Douarin, 1992). These results suggested that the somites are subdivided into medial and lateral compartments in terms of their fate as epaxial and hypaxial derivatives, respectively. Consistent with this finding, it was subsequently discovered that signals derived from the axial structures (sonic hedgehog, Wnt1) medialize the somite leading to the initial specification of muscle and sclerotome (Cossu et al., 1996; Yamaguchi, 1997 and references therein) and signals from the lateral plate mesoderm (BMP4) initially lateralize the somite (Pourquie et al., 1995, 1996) and in combination with ectodermal cues (Wnt7a) induce a lateral fate such as the formation of the hypaxial muscles (Cossu et al., 1996; Dietrich et al., 1998; Tajbakhsh et al., 1998).

The hypaxial component of the body musculature comprises the limb and the body wall (intercostal and abdominal) muscles. At limb areas of the axis, lateral dermomyotomal cells expressing *PAX3* (Franz et al., 1993; Goulding et al., 1991, 1994; Bober et al., 1994; Williams and Ordahl, 1994), *met* (Epstein et al., 1996; Yang et al., 1996) and the *lbx* genes (Dietrich et al., 1998) dissociate and migrate into the limb anlage formed by the somatopleure to constitute its musculature (Chevallier et al., 1977; Christ et al., 1974a,b, 1977; Grim, 1970; Hayashi and Ozawa, 1995; Jacob et al., 1978). It is often cited in the literature that the body wall muscles also originate from the VLL of the dermomyotome. Nevertheless, several years ago, Christ et al. (1983) proposed that at thoracic and abdominal levels of the axis, where the VLL of the dermomyotomes retain their epithelial conformation, both the lateral dermomyotome and the myotome progress ventrally towards the somatopleure where the two components give rise to the hypaxial muscles. The above observations, however, did not address the mechanism by which this process takes place.

In the present study we have investigated for the first time the precise behavior and fate of the VLL cells at non-limb levels of the axis. We find that these cells continuously colonize the ventrolateral portion of the myotome. Moreover, within this domain, VLL-derived fibers intermingle with fibers emanating from the rostral and caudal lips as part of the second wave of myotome formation. Most notably, the second wave fibers also intermix with preexisting pioneer myofibers, suggesting a compound and highly ordered pattern of lateral myotome formation. Taken together, these and previous results (Kahane et al., 1998a,b; Kalcheim et al., 1998) show that myotomal expansion in the dorsomedial to ventrolateral direction takes place by progressive intercalation along the full extent of the myotome of second-wave myoblasts amongst primary myotomal fibers, rather than by local increments at the dorsal and/or ventral edges. This compound myotome progressively expands into the somatopleure where it gives rise to the hypaxial intercostal muscles.

MATERIALS AND METHODS

Embryos

Fertile quail (*Coturnix coturnix japonica)* eggs from commercial sources were used in this study.

DiI labeling of dermomyotome lip cells and fluorescence imaging

Embryo preparation

Quail embryos were at the 28- to 32-somite stages at the time of dye labeling. Following removal of the vitelline membrane, a unilateral slit was made in the ectoderm over the lateral part of the somites at levels corresponding to the limb bud (somites 17-21), the flank (somites 22-26) or rostral to the limb buds (somites 15-16). A small drop of pancreatin (2% w/v) was then added to assist in the separation of the ectoderm from underlying mesoderm. Enzymatic activity was stopped by newborn calf serum (10% in PBS). The ectoderm completely regenerated over the somites within 1 hour following the labeling procedure.

Dye labeling

Borosilicate tubes with filament (OD=1.0 mm, ID=0.5 mm) were pulled using a vertical puller (Sutter model P-30). Micropipettes were backfilled either with 1, 1′ di-octadecyl-3, 3, 3′, 3′-tetramethylindocarbocyanine perchlorate (DiI, Molecular Probes) or with 3, 3′ di-octadecyl-oxacarbocyanine perchlorate (DiO, Molecular Probes) dissolved in absolute ethanol and further diluted to a final concentration of 0.2% (weight/volume) as previously described (Kahane et al., 1998a,b). Micropipettes were then mounted on a Zeiss micromanipulator. Dye injections were performed by iontophoresis. Current was applied through a Ag/AgCl wire placed in a 2 M LiCl solution immediately before somite injections and a 3 second pulse of 100 nA of current was employed to deliver the dye. Injections were performed under an upright Zeiss Axioscope microscope adapted for holding eggs and equipped with long working distance objectives (LD-Achroplan ×20) and epifluorescence. Embryos were viewed with oblique lighting from a fiber optic light source. Two types of injections were made: (1) to the center of the VLL, (2) to the VLL and the lateral part of the rostral and caudal edges of the dermomyotome. The accuracy of labeling sites was monitored throughout the procedure by observation under a total magnification of ×200 with combined bright-field and epifluorescence optics. Following dye labeling, embryos were further incubated for various lengths of time ranging from time 0 controls to embryonic day 6 (E6). At the end of incubation, embryos were removed from the egg, washed in PBS and fixed in formaldehyde (see below).

Laser scanning confocal microscopy

Fluorescent samples were analyzed using an LSM410 scanning confocal microscope (Zeiss, Jena, Germany) with a He-Ne laser with an excitation wavelength of 543 nm (DiI) and argon laser with an excitation wavelength of 488 (DiO), attached to an Axiovert 135M microscope. Dye-labeled cells were visualized with 10× or 20× oil immersion Plan-Neofluar objectives. The samples were optically screened at 2 μm increments through the Z-axis and sequential images were collected using a Pentium 150 personal computer. The confocal images represent cumulative serial sections that include, in each case, all fluorescently stained cells. Adobe Photoshop (version 4) was used for image processing.

Detection of post-mitotic pioneer cells

Two pulses of [^{3}H]thymidine were applied at 3-hour intervals, starting at E2. By the 27- to 30-somite stage approximately, the embryos were chased with a 100-fold molar excess of cold thymidine until fixation on E6. As shown in Kahane et al. (1998a), this protocol enables visualization of the early post-mitotic cells which retained the label in their nuclei while cells that continued dividing during the chase efficiently diluted the radioactive metabolite and became unlabeled.

Fixation, immunofluorescence and autoradiography

Embryos were fixed in 4% formaldehyde and embedded in paraplast. Serial 7 μm sections were mounted on gelatinized slides. A series of dye-labeled embryos was similarly fixed in 4% formaldehyde, embedded in gelatin and cryostat-sectioned at 15 μm. Immunostaining with desmin antibodies was performed as described in Kahane et al. (1998b). Secondary antibodies were coupled either to FITC or to horseradish peroxydase. Autoradiography after thymidine labeling was performed as previously described (Brill et al., 1995).

RESULTS

Cells of the VLL of the dermomyotome in non-limb areas of the axis contribute to the lateral part of the myotome following a longitudinal translocation into the extreme (rostral and caudal) dermomyotome lips

At limb levels of the axis, the VLL of the epithelial dermomyotome dissociates and its component cells migrate into the limb anlage to form its musculature (see Introduction). However, the exact behavior of VLL cells in specific non-limb regions of the axis where a lip-like structure is known to last for several days, remains unknown. To approach this question, the center of the VLL of somites 16-22, in embryos at the 28 somite stage, was labeled with DiI. Fig. 1 illustrates the fate of fluorescent cells 24 hours later. Labeling of the VLL at the level of the wing (somites 17-21) led to migration of the dye-labeled cells into the limb anlage (Fig. 1A,B, open arrowheads). In contrast, dye labeling of the equivalent region in somites 16 and 22, which are located immediately rostral and caudal to the wing somites, respectively, resulted in the formation of laterally located myofibers spanning the entire rostrocaudal length of the myotome (Fig. 1A,B between arrowheads) and did not significantly contribute to limb cells, as assessed in separate experiments (data not shown). A similar pattern of myotomal fibers resulted from labeling the VLL all along the flank level of the axis (Fig. 2D and data not shown). Thus, while the VLL of the dermomyotome in limb-level somites generates migratory precursors of the limb musculature (see also Introduction), the VLL of the dermomyotome in regions located rostral and caudal to the limb-forming areas contributes to the formation of the myotome.

To further characterize the cellular mechanism by which VLL cells develop into myotomal fibers, DiI injections were made at somitic levels 22 or 23 in 31-32 somite-stage embryos. Labeling was directed to the center of the VLL midway between the rostral and caudal somite extremities (Fig. 2A). Approximately 15 hours following dye labeling, fluorescent cells had spread considerably as discrete spots along the rostrocaudal plane of the VLL (Fig. 2B,C compare cells delimited by dashed line between arrows in B and C with focal spot in A and see also Fig. 1). This occurred in 85% of the labeled somites (n=27) by 15 hours, and in virtually all of the cases by 18, 21 and 24 hours post-labeling (n=15, 19, 24, respectively; Fig. 3).

Myofibers were already visible in the myotomes by 18 hours following labeling of the VLL. In 40% of the somites (n=15) fibers were in the process of elongating into the myotome, thus extending only along a partial length of the myotome (Fig. 2C and Fig. 3). The percentage partial-length fibers increased by 21 hours (58%, n=19) and subsequently decreased by 24 hours (33%, n=24) when more fibers had already reached both somite extremities. The partial-length fibers were always anchored either to the rostral or the caudal edge of the dermomyotome and had only one free end (Fig. 2C, arrowheads point to the myofiber extremities). Unit length myofibers, attached to both rostral and caudal dermomyotome edges, became apparent by 18 hours following dye application in 26.6% of the analyzed somites (n=15). The percentage of somites bearing full-length myofibers progressively increased up to 75% by 24 hours (Figs 2D, 3). As can be seen in Fig. 3, full length and incomplete myofibers were often present in the same somites but in changing proportions as a function of time after labeling. The above data suggest that VLL cells must first reach the rostral or caudal dermomyotome lips prior to elongating into the myotome. Thus, the behavior of VLL cells parallels that of

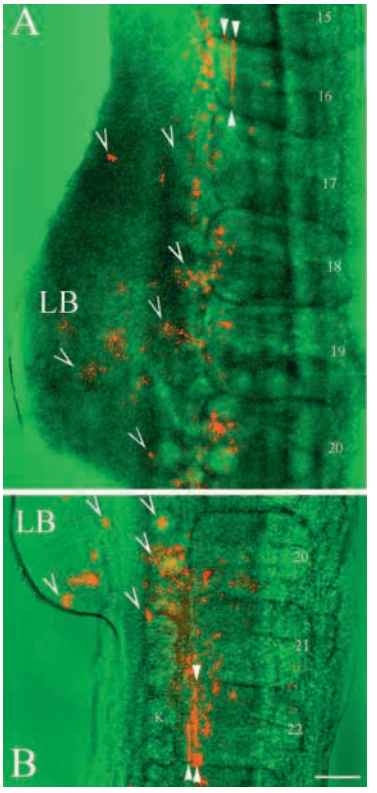

Fig. 1. Differential fate of VLL-derived cells in limb and non-limb levels of the axis. Confocal images taken 24 hours after DiI labeling of the VLLs of somites 16-20 (A) and somites 20-22 (B) in embryos at the 28-somite stage. The fluorescent images are superimposed onto the bright-field images of the embryos. Somite numbers are indicated in the right part of the corresponding segments. Note that labeled cells in somites 16 and 22 mostly formed ventrolaterally located myofibers (between arrowheads). A characteristic rostrocaudal distribution of labeled cells is also seen along the VLL. In contrast, cells labeled in the VLL at the limb level migrate into the limb bud (LB) (open arrowheads) and do not give rise to myotomal fibers. Abbreviation, K; kidney primordium. Bar, 100 μm.

cells of the DML of the dermomyotome (Kahane et al., 1998b), and contrasts with that of the extreme (rostral and caudal) lips which directly deposit myofibers into the longitudinal plane of the myotome (Fig. 4 and Kahane et al., 1998b).

It is also important to stress that myofibers developing from VLL-injected cells colonized the ventrolateral quarter of the myotome remaining close to the VLL (Figs 1, 2C,D, 4). This behavior is opposite to that of the localization of DML-derived myofibers, which contribute to the dorsomedial portion of the myotome (Kahane et al., 1988b). The relatively restricted topographical contributions of both DML cells and of VLL-derived cells which occurs through the extreme lips, are in striking contrast to the behavior of pioneer myofibers which extended along the entire dorsomedial to ventrolateral aspect of a given segment following focal labeling of cells in the medial portion of the epithelial somite (Kahane et al., 1998a, see also Fig. 4).

Formation of the hypaxial intercostal muscles from a ventralward elongation of the myotome

The present results, together with previous data from our laboratory (Kahane et al. 1989a,b) allow us now to propose an updated model of myotome formation (Fig. 4). We have found that a subset of early post-mitotic cells is generated along the medial portion of the epithelial somite, that expresses MyoD, and undergoes a series of morphogenetic movements. The end result is formation of a primary myotomal structure spanning the entire underlayer of the dermomyotome (first wave). Following establishment of this primary structure, cells from the four edges of the dermomyotome contribute to progressive myotome growth (second wave). This is achieved by direct elongation of myofibers into the preexisting structure from along the entire mediolateral extent of the extreme edges of the dermomyotome (rostral and caudal). Cells from the DML and the VLL also contribute to myotome formation, but indirectly through the extreme lips by first translocating into them and then participating in the colonization of the dorsomedial and ventrolateral parts of the myotome, respectively. Most importantly, during myotome growth, cells of the second wave progressively intermingle with fibers of the first wave.

This model raised the question of the mechanism of myotomal growth in the dorsoventral extent, and to this end we have focused on the formation of the hypaxial intercostal muscles. As during epaxial myotome growth, cells from the second wave (extreme lips and DML) continuously intercalate among pioneer myofibers (Kahane et al., 1998b), we asked whether the extensive myotomal migration into the somatopleure takes place by a similar mechanism, i.e; as growth of a compound unit. If this assumption is correct, we expect the myotome-derived intercostal muscles to be composed both of pioneer myofibers and of intercalated fibers of the second wave. Alternatively, if the VLL-derived fibers are added to the ventrolateral myotome simply as ventral increments, only VLL derivatives would be expected to populate the intercostal muscles. Such a result would be consistent with a previously proposed model for myotome growth in the epaxial direction by incremental addition of DML-derived fibers exclusively (Denetclaw et al., 1997).

The hypaxial intercostal muscles partly derive from the primary myotome

To examine whether the hypaxial muscles in the flank region

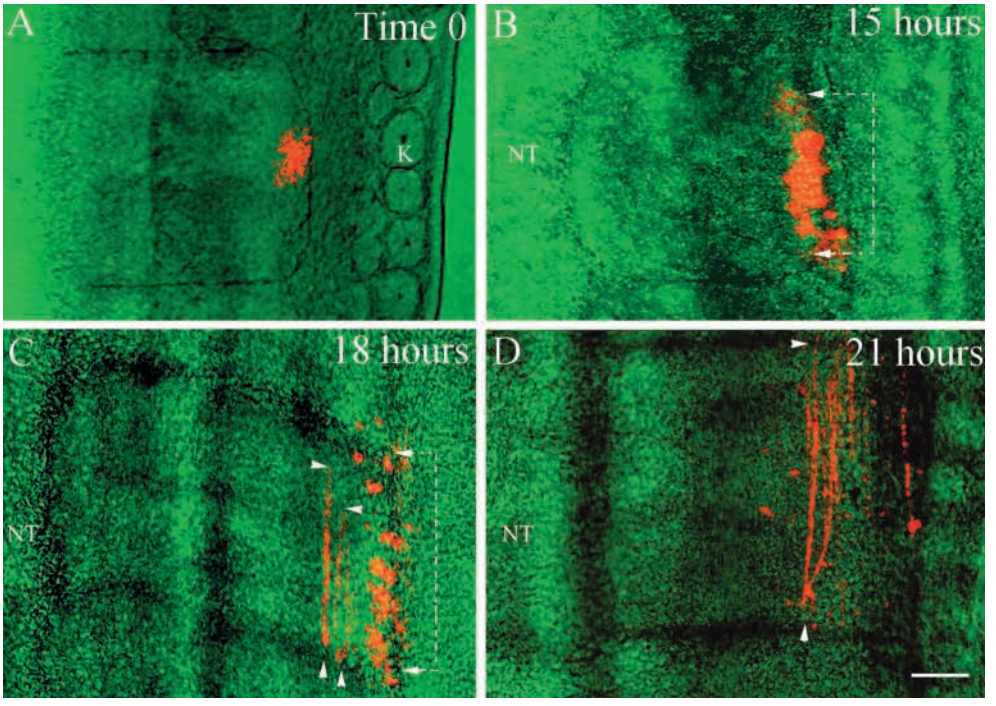

Fig. 2. DiI labeling of the VLL generates ventrolaterally located myofibers following VLL cell relocation to the rostral and caudal edges of the dermomyotome. (A-D) Confocal images depicting a time course of the development of myofibers from VLL cells labeled with DiI in the center of the epithelium. Injections shown were in segments 22-23 of 31- to 32-somite stage embryos. (A) Localized spot of DiI in the center of the VLL immediately following injection. (B) The widespread rostrocaudal distribution of distinct dye-labeled cells along the VLL seen 15 hours post-labeling. This distribution is delimited by the dashed line between arrows. (C) 18 hours following dye labeling a similar rostrocaudal distribution of VLL cells is apparent (dashed line between arrows) and in addition, elongating myofibers are already apparent which have one end attached to the caudal edge of the dermomyotome and one end free in the myotome (arrowheads). (D) 21 hours post-labeling most myofibers have reached both edges of the dermomyotome (marked by arrowheads). To show the myofibers better, this confocal image was integrated over the myotome only, discarding the dermomyotome lips which showed a rostrocaudal distribution of fluorescent cells (not shown). In C and D note that VLL-derived myofibers are located in the ventrolateral domain of the myotome occupying approximately the ventrolateral fourth of a segment. The neural tube (NT) is to the left in all pictures. (see also Fig. 1). Bar, 40 μm.

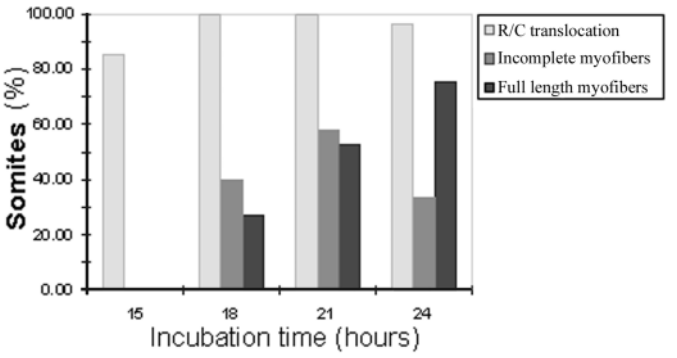

Fig. 3. Quantification of the time course of development of VLL-derived myofibers. Bar graph representing the percentage of dye-labeled somites with rostrocaudal (R/C) distribution of positive cells, with incomplete myofibers anchored to one of the extreme edges, and with full-length myofibers. *n*=27, 15, 19 and 24 somites for 15, 18, 21 and 24 hours of incubation following labeling.

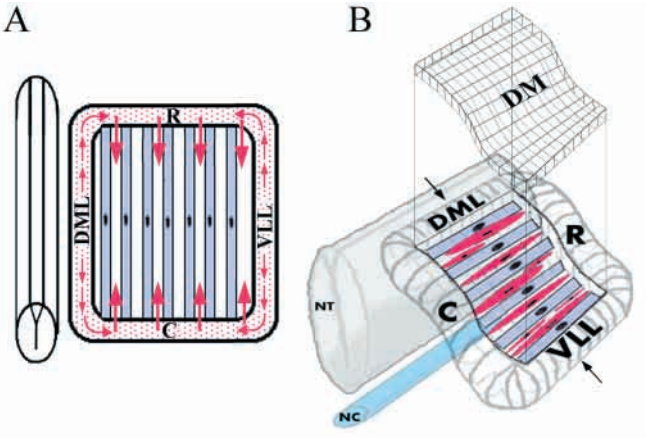

Fig. 4. A two-wave model of myotome development. Schematic model representing the first wave of pioneer myofibers in blue and the second wave in red. (A) The pioneer muscle progenitors originate along the medial portion of epithelial somites and undergo dynamic morphogenetic migrations until they differentiate into myofibers that reach both edges of the dermomyotome and their nuclei become restricted to the center of this structure (Kahane et al., 1989a). From this stage, the major contribution to myotome expansion derives from both the rostral (R) and caudal (C) dermomyotome lips, and to a topographically limited extent also from the dorsomedial (DML) and the ventrolateral (VLL) lips whose cells relocate into the extreme lips prior to generating myotomal fibers (red stippling depicts cell origins, thin red arrows represent movement of DML and VLL cells to the R and C lips, thick red arrows depict myofiber elongation into the myotome). The second wave of cells migrates along the primary myotomal fibers. These younger myotomal cells contribute to myotome expansion dorsoventrally (DML to VLL) and also in the transverse plane (see Kahane et al., 1998b). (B) Three-dimensional view of the myotome and accompanying lips. The center of the dermomyotome (DM) has been lifted up to enable visualization of the underlying myotome. NT, neural tube; NC, notochord.

contain a primary myotomal component, we have pulse-chased quail embryos with thymidine, using a protocol that enabled identification of the pioneer myotomal cells by positive labeling of their nuclei (see Methods, and also Kahane et al., 1998a,b; Kahane and Kalcheim, 1998). Serial analysis of sagittal sections performed at E6, revealed the presence of radiolabeled pioneer nuclei in both hypaxial and epaxial domains of the intercostal muscles (Fig.5) as previously shown for epaxial muscles in additional regions along the axis (Kahane et al., 1998b). In all cases, the labeled fibers were at this stage intermingled with unlabeled fibers (Fig. 5B,C). According to the protocol used, the latter must be myofibers which derived from mitotically active cells during the chase period and had intercalated at a subsequent stage (see below). The presence of pioneer myofibers in the hypaxial domain of intercostal muscles demonstrates that these are generated from the myotome whose core structure is constituted by the early post-mitotic pioneer cells.

The contribution of the second wave of myotome colonization to the formation of hypaxial muscles

The rostral and caudal lips of the dermomyotome were recently shown to generate myotomal myofibers along their entire dorsomedial to ventrolateral extents (Kahane et al., 1998b). We thus tested the hypothesis that the ventrolateral domain of the myotome, formed by cells from the lateral parts of the extreme edges and from the VLL (see Figs 1 and 2), give rise to the hypaxial intercostal muscles. To this end, we labeled 30 somite-stage embryos at somitic levels 23 or 24 with DiI in the lateral halves of both rostral and caudal edges of the dermomyotome and with DiO in the VLL (Fig. 6A). Embryos were further incubated until E5 when the hypaxial domain of the myotome was clearly seen entering the somatopleure (Fig. 6E, insert). Analysis of cryostat sections of the labeled segments revealed in the hypaxial muscle not only the presence of the VLL-derived DiO-positive myofibers but also DiI-labeled myofibers emanating from the rostral and caudal lips (Fig. 6B,C). In addition, fibers bearing either dye were intermingled within the nascent muscle (Fig. 6D), further indicating that prior to elongating into the myotome, intercalation takes place between cells of the second wave generated from the extreme edges and from the VLL. To ascertain that the dye-labeled cells were indeed in the muscle, the same sections were post-stained with desmin antibodies (Fig. 6E-H). As seen in Fig. 6, the dye-labeled cells were a subpopulation of the desmin-positive muscle (compare D with H). Taken together, these results show that the hypaxial muscles of the body wall are formed by a ventralward growth of the compound myotome that includes myofibers from the first and second waves of myotome colonization.

DISCUSSION

In the present study, we have investigated for the first time the precise developmental pattern of VLL cells of the dermomyotome at non-limb regions of the axis. We report first, that VLL cells progressively colonize the ventrolateral portion of the myotome. We also show that this takes place by longitudinal cell translocation along the medial boundary until reaching the rostral or caudal dermomyotome lips from which fibers emerge into the myotome. Second, we have addressed the early generation of hypaxial intercostal muscles from the lateral myotome. We report that these muscles are formed in a complex but highly patterned fashion by cells from the VLL

and the extreme lips which intercalate between each other and among preexisting pioneer myofibers. Thus, hypaxial muscles are generated by lateralward expansion into the somatopleure of the compound myotome that includes two successive waves of myogenic cells. These results are of general significance to the understanding of muscle development as they show that the lateral myotome-derived muscles also contain a primary myotome composed of pioneer fibers originating in the medial aspect of the epithelial somite. They also imply that the myotome expands as a whole by progressive intercalation of second wave myofibers among primary ones (see below).

Results from the present and previous studies (Kahane et al., 1998a,b) provide a more complete picture of myotome development. According to our data, the primary myotome that develops from pioneer cells is likely to provide at least a scaffold for intercalation of cells of the second wave. The latter are generated from all along the rostral and caudal lips of the dermomyotome and symmetrically from the DML and the VLL. Since progenitor cells in these lips continually proliferate they progressively generate fibers that contribute to myotome growth in the dorsoventral direction and in the transverse plane where they initially localize in a medial position with respect to the pioneer fibers. At later stages, pioneer myofibers become increasingly scattered along the dorsoventral extent of the myotome as it grows by virtue of addition of younger cells. In spite of progressive dilution, pioneer fibers still reach dorsal and ventral regions of the epaxial and hypaxial muscles, respectively (Kahane et al., 1998a,b and this study). This suggests that the myotome, comprising all its component waves, expands as a whole in the mediodorsal to ventrolateral extent as opposed to expansion just at the extreme VLL and DML lips. This can be compared to the proportional extension of a spring, by progressive intercalation of second wave cells among myofibers of the primary scaffold (Fig. 7).

A recent model of myotome growth has suggested a medially directed expansion of this structure by incremental ingression of only DML cells (Denetclaw et al., 1997, but see Christ et al., 1978). This interpretation was based on successive injections of DiI and DiO in the same site of the DML which led to an ordered production of fibers, the more lateral being produced the earliest. In agreement with the above data, we also find that the DML contributes to a medial expansion of the myotome (Kahane et al., 1998b). If cells of the DML were the only source of the myotome, then the proposed model suggesting incremental growth in the medial direction would be correct. However, DML cells are neither the only nor the earliest cells to form the myotome. In contrast to these authors who have found only a limited participation of the medial cells of the rostral lip and no contribution at all by cells of the caudal dermomyotomal lip or the VLL (Denetclaw et al., 1997), we report on significant contributions of the entire rostral and caudal dermomyotome lips and also of the VLL, the latter contributing to lateral expansion of the myotome. In addition, in the present and previous studies (Kahane et al., 1998a,b) we showed that progenitors of these four lips progressively integrate by intercalation within the frame of the earlier contribution of the pioneer myofibers, an early subset of cells of which Denetclaw et al. were not aware of, as they started their focal injections only after somite dissociation. Thus, given the multiple sources of myotomal cells and the regulated temporal contributions of the first (pioneer) and second waves, and based on the progressive intercalation of

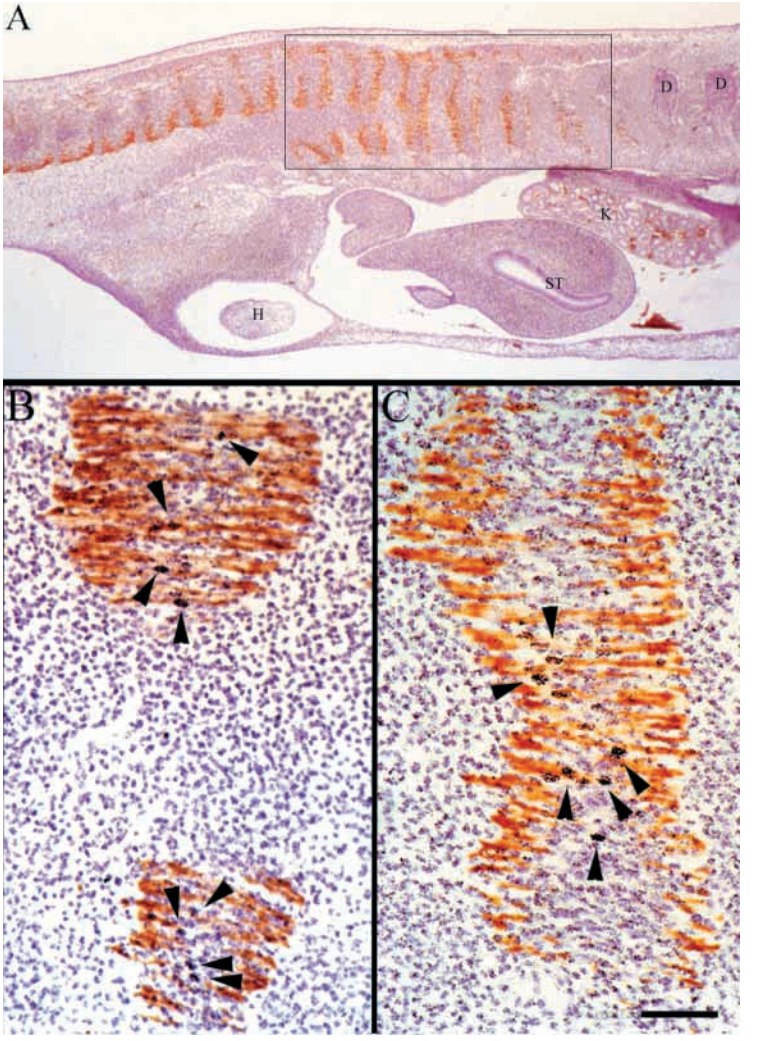

Fig. 5. The presence of early post-mitotic pioneer myofibers in the intercostral muscles. (A) Low magnification sagittal section through an E6 quail embryo pulsed with radiolabeled thymidine as described in Methods. Desmin immunostaining reveals the presence of segmentally arranged muscles including the intercostal muscles (boxed) that contain epaxial (dorsal) and hypaxial (ventral) domains. Rostral is to the left. (B,C) High magnifications showing the presence of thymidine-labeled pioneer nuclei in the intercostal muscles (arrowheads). In B, positive nuclei are observed in both epaxial and hypaxial domains of the muscles. In C, the continuity of the muscle is kept and pioneer nuclei are evident in the center of the structure. The myofibers bearing labeled nuclei are located only along the lateral (external) portion of the muscles (not shown, Kahane et al., 1998b). Therefore, they can be seen only in a few sequential sections. Moreover, due to the curvature of the rib cage, the pioneer nuclei in epaxial and hypaxial domains cannot usually be seen in the same sagittal section. D, dorsal root ganglion, H, heart, K, kidney, St, stomach. Bar, (A) 500 μm; (B,C) 45 μm.

second-wave myoblasts among myofibers of the first wave all along the muscle, including its extremities, we propose here a revised, integrative view of myotome development whereby overall myotome growth occurs in a uniform manner along its dorsoventral extent rather than by local increments. This uniform expansion would correspond to the sum of cell additions from several intermixing sources. First, the rostral and caudal lips, which are progressively added all along the growing structure. Second, local contributions by DML cells, which account for expansion in the medial direction (in agreement with Denetclaw et al., 1997, see Kahane et al., 1998b) and by VLL cells, which participate in growth in the lateral direction (this study).

An outcome of the patterned expansion of the myotome relates to the origin of the hypaxial muscles. We show that the hypaxial muscles that constitute the body wall are generated by a ventralward growth of the myotome. This is because intercostal muscles have a complex origin, similar to that of the epaxial muscles (Kahane et al., 1998a,b), being formed by myogenic precursors deriving from the first and second waves of myotome colonization. The presence of the first wave of pioneer myoblasts in the intercostal muscles was exemplified using a protocol that enabled their positive identification as the earliest post-mitotic cells within the myotome. As previously shown, these early myofibers derive from cells originating in the medial portion of the epithelial somite. Subsequently, they give rise to a primary structure that localizes ventral to the full extent of the dermomyotome reaching both the DML and the VLL of each segment (Kahane et al., 1998a). The observation that hypaxial intercostal muscles contain pioneer myofibers demonstrates that they derive partly from the primary myotome.

We also examined the contribution of the second wave of myoblasts known to derive from the dermomyotome lips (Kahane et al., 1998b), and found that cells of the lateral portions of the extreme dermomyotome lips and of the VLL intermix and further intercalate amongst pioneer myofibers to give rise to the lateral myotome. Christ et al. (1983) suggested, based on observation of somite development at distinct stages, that the hypaxial abdominal muscles derive from the ventral myotome and the VLL of the dermoyotome. Our results now provide direct experimental evidence for this and further demonstrate that the VLL of the dermomyotome progressively incorporates into the lateral myotome on its way into the somatopleure. Notably, the cellular events leading to the formation of the epaxial and hypaxial myotomal domains are likely to be similar, as myofibers directly elongate from the caudal and rostral edges of the dermomyotome in both

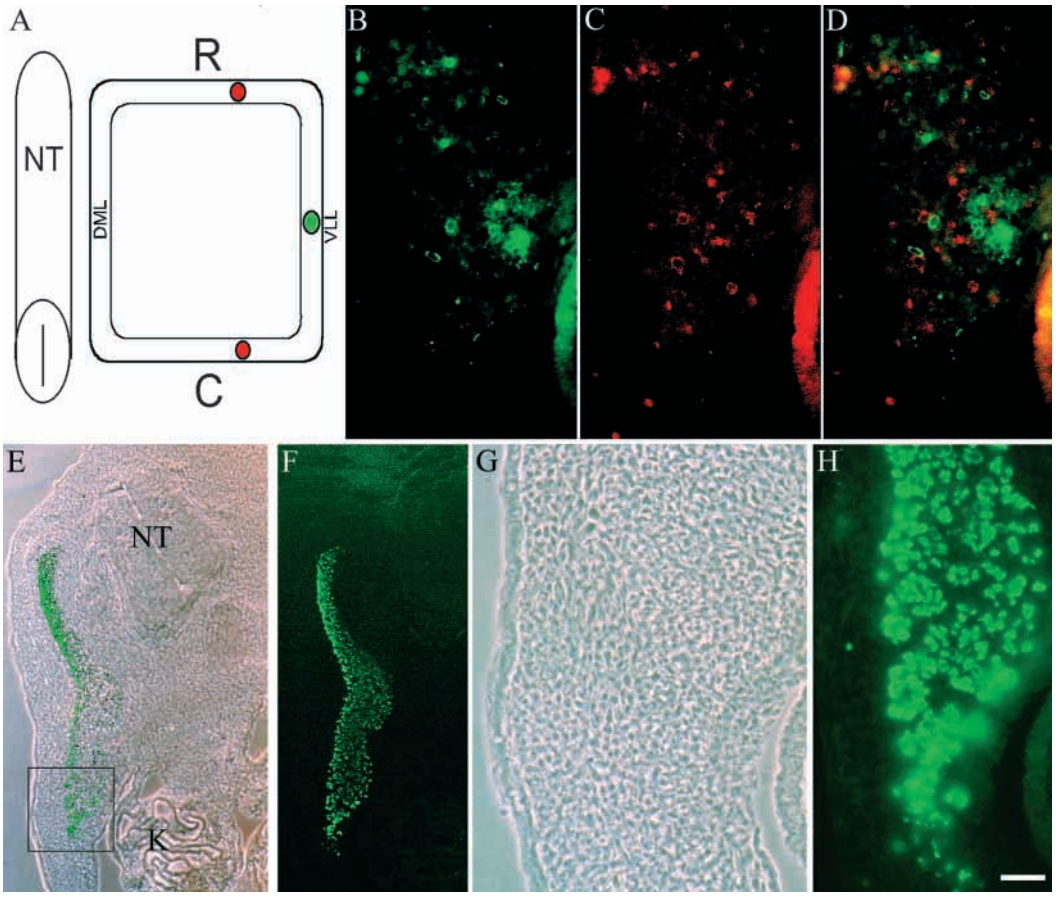

Fig. 6. Hypaxial muscles develop from the ventrolateral myotome which is partly formed by cells of the VLL and the extreme dermomyotome lips. (A) Schematic representation of the labeling procedure. A 30-somite stage embryo was labeled at somitic level 23 with DiO in the VLL (green) and DiI in the lateral part of both rostral and caudal edges of the dermomyotome (red). On E5, embryos were fixed and cryosectioned in the transverse plane. Following localization of the dye-labeled cells (B-D), sections were stained with desmin-specific antibodies to reveal the entire muscle (E-H). B, C and D are high magnification views of the intercostal muscle in the area boxed in E. (B) DiO-labeled cells emanating from the VLL are present in the hypaxial domain of the intercostal muscle. (C) DiI-labeled cells emanating from the rostral and caudal lips are also present in the hypaxial domain of intercostal muscle. (D) B and C were superimposed to show intercalation of fibers having different spatial origins. Note that there is no overlap of DiI and DiO-labeled cells within the desmin-positive muscle. Non-specific fluorescence emanates from erythrocytes and from the neighbouring kidney epithelium. (E,F) Low magnification of the same section post-stained with desmin antibodies (E, fluorescent image superimposed on phase contrast image; F, fluorescent image only). (G,H) Higher magnification of phase contrast and fluorescent images of the hypaxial domain shown in B-D to show that dye-labeled cells are a subset of the desmin-positive fibers. K, kidney primordium; NT, neural tube, Bar, (B,C,D,G,H) 20 μm; (E,F) 80 μm.

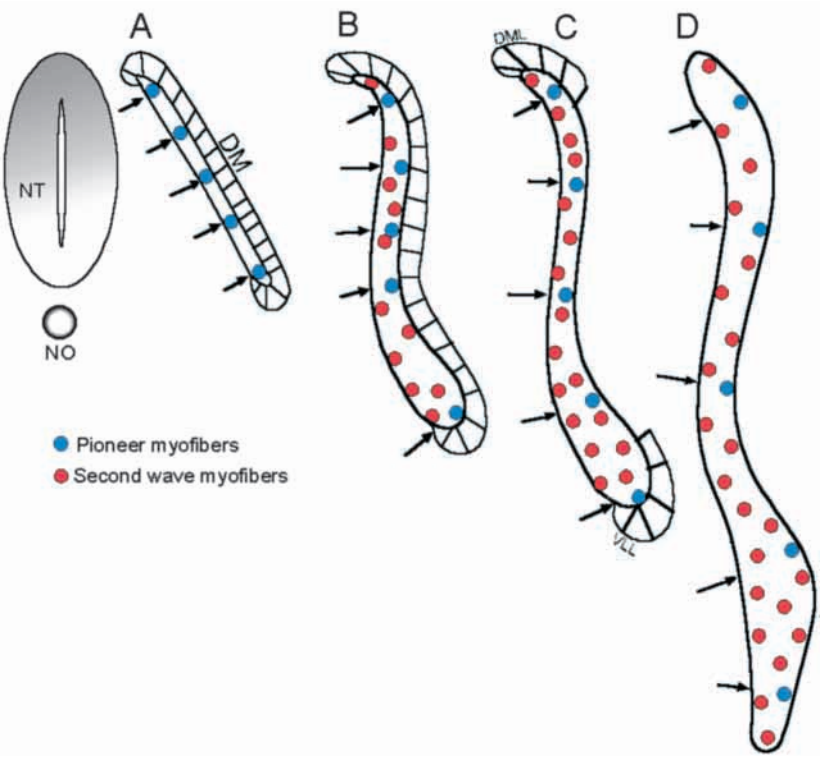

Fig. 7. A model of uniform myotome growth in the dorsoventral extent. Schematic model representing sequential stages of myotome expansion. Transverse sections of primary myotome (A) represented by blue dots close to each other. The dermomyotome (DM) surrounds the young myotome. (B) Myofibers of the second wave (red) initially localize medially to the pioneer fibers. (C) An older stage in myotome development. Note the presence of more fibers of the second wave intercalated among fibers of the first wave whose spacing has now increased. The center of the DM has dissociated into dermis (not shown) while the epithelial DML and VLL still remain. (D) Simplified scheme showing that the myotome has evolved into an intercostal muscle containing an equivalent number of pioneer fibers as the precursor myotomal structure but with a larger number of second wave-myofibers. Arrows point to the increasing spreading of pioneer myofibers along the dorsoventral extent of the muscle at each stage of development. This progressive spreading demonstrates that the compound myotome grows uniformly in the dorsoventral extent. Its dorsomedial region develops into epaxial muscles and the ventromedial region that advances into the somatopleure gives rise to hypaxial muscles.

domains, while DML and VLL cells first translocate into these extreme edges through which they generate muscle fibers (summarized in Fig. 4).

The complex origin and formation of the myotome that generates most epaxial and hypaxial muscles suggests that environmental signals act upon distinct spatiotemporal waves of cells to specify their fate. The pioneer wave of cells which expresses MyoD and Myf5 in avians and Myf5 initially in mice, arises along the medial portion of the epithelial somite and is likely to become progressively specified by the axial structures, the notochord and neural tube via Sonic hedgehog and Wnt signalling (Borycki et al., 1998; Tajbakhsh et al., 1998; reviewed by Currie and Ingham, 1998; see also Kalcheim et al., 1999). Nevertheless, pioneer myofibers do not remain confined to areas adjacent to the midline and extend instead all the way from the medial portion of the somite to its lateral part thus forming the primary myotome. Subsequent waves of cells then progressively intercalate between the myofibers of this primary myotome, and pioneer fibers later remain scattered in both epaxial and hypaxial muscles. These results raise a fundamental question as to the role of the pioneer myofibers in muscle development. Based on the intercalatory nature of myotome growth, we suggest that the pioneers act as a structural scaffold for the guidance of later waves. This hypothesis needs, however, to be put to the test. We will be able to experimentally examine this possibility only when specific and stable means to ablate this early cell population become available. Along this line, deletion of the *Myf5* gene which initially characterizes the early medial cells in the epithelial somite (putative mouse pioneers) indeed leads to a markedly delayed development of both epaxial and hypaxial intercostal muscles (Kablar et al., 1997). This phenotype would be consistent with the pioneers normally being present in both types of muscles and playing perhaps an organizing role. In such a case, it is expected that in the mutants, formation of the primary myotome composed of pioneer fibers is delayed at least until expression of MyoD begins. However, if pioneers totally fail to form and yet muscles organize albeit at later times, this would argue against a role of the pioneer myotome in muscle development. Therefore, a rigorous characterization of the function of the pioneer cells in subsequent muscle development awaits elucidation of selective and stable markers to enable their behavior in normal and experimental embryos to be followed, and selective ablation studies to be carried out.

In contrast to the primary wave of pioneers which is initially specified by medial signals yet develops to span the entire extent of the segment, the second wave of myotome formation consists of a medial domain (formed by cells of the medial part of the extreme edges of the dermomyotome and the DML) and a lateral domain (formed by cells of the lateral part of the extreme edges and the VLL). It is thus reasonable to assume that topographically restricted medial and lateral-derived signals, respectively, account for the specification of these later myotomal cells. Along this line, Shh derived from the floor plate and notochord and Wnt1 from the dorsal neural tube are also able to activate noggin expression in the DML of the dissociated somite (Hirsinger et al., 1997). Noggin was then implicated in patterning the dermomyotome and the myotome by antagonizing neural tube-derived BMP4 (Hirsinger et al., 1997; Marcelle et al., 1997; Reshef et al., 1998). Likewise, specification of the hypaxial domain of the myotome, which in mice initially expresses *MyoD*, was proposed to subserve local signals provided by the ectoderm such as Wnt7a (Cossu et al., 1996; Tajbakhsh et al., 1998). The lateral plate mesoderm in turn inhibits the expression of medial traits (Pourquie et al., 1995) and induces instead an upregulation of *PAX3, lbx and c-met*, markers that characterize myogenic precursors with migratory ability (Dietrich et al., 1998; Pourquie et al., 1995; Epstein et al., 1996; Williams and Ordahl, 1994). Recently, it was reported that lateral signals (mediated in part by

BMP4) have to synergize with dorsal ectodermal signals to induce the formation of hypaxial myogenic cells with migratory characteristics that express the *lbx* gene (Dietrich et al., 1998). Although specification of hypaxial progenitors is likely to be induced by the signals discussed above, our finding that during myotome formation they intermingle with pioneer myofibers that reach lateral areas of the segments, further suggests that following initial specification, the correct spatial patterning of hypaxial myotomal fibers might be influenced by this preexisting structure.

In this and a previous study (Kahane et al., 1998b), we have shown that while both rostral and caudal edges of the dermomyotome directly generate myofibers, cells from the DML and the VLL first translocate into the extreme edges prior to differentiating into myofibers at the medial and lateral extremes of the myotomes. This conclusion is based on the observed time-dependent spreading of dye-labeled cells along both DML and VLL regions prior to the generation of myofibers. Such a longitudinal translocation was not found in the study by Denetclaw et al. (1997) perhaps because embryos were only analyzed after overnight incubation thus missing intermediate stages. Instead, these authors suggested that cells can initiate elongation and differentiation at all active sites simultaneously. If this assumption was correct, then following injections along the DML or VLL, one would expect to find at least some elongating myofibers bearing two free ends on their way to reaching the extreme edges. We were unable to detect such cells. Nor were such cells shown in the study by Denetclaw et al., yet they were drawn in their proposed model. In the many segments labeled in this and our previous study (Kahane et al., 1998b), we could only find partial length myofibers attached to one extreme edge, suggesting that cells first reach one of these edges and then generate fibers. Moreover, the observed cell translocations were always in the plane of the lips and we could never see dye-labeled cells within the myotome unless they already bore a fiber. Thus, we find it unlikely that cells enter the myotome at each point along the entire medial or lateral edges, then generate myofibers in situ, or alternatively, translocate within the myotome itself to either rostral or caudal lips from which they elongate. Based on the above arguments, we propose that cells emanating from both the DML and VLL translocate longitudinally in the plane of, or adjacent to these lips (see below), to one of the extreme edges prior to generating myofibers of the second wave.

The mechanism of longitudinal translocation along the medial and lateral aspects of the dermomyotome is still unknown. Two possibilities can be considered, first, that these cells passively translocate along the medial and lateral edges finally reaching the rostral or caudal lips by virtue of continuous cell proliferation in the epithelia. Alternatively, the DML and VLL epithelia may generate a local migratory subset of myogenic progenitors that moves along these edges until reaching the extreme lips. Various arguments favor the second possibility. First, we have clearly observed that as early as 2-3 hours following dye labeling, dye-positive cells were already found in rostral and/or caudal positions relative to the point of injection (our unpublished data and see Kahane et al., 1998b). As somitic cells divide every 8 hours approximately, this earlier positional shifts might in fact reflect cell movement. Second, preliminary studies in which DML or VLL cells were focally labeled with CM-DiI and their positions recorded as a function of time in serial transverse sections, indicate that some epithelial cells at the curved lips dissociate, localize immediately underneath the lip and move along this area in both rostral and/or caudal directions (our unpublished observations).

The expression pattern of several genes substantiates the idea that the DML and VLL-derived cells move along these edges. The expression of the *lbx* gene reflects axial-specific differences in the lateral domain of the somites being absent from flank regions. In contrast, *PAX3* and *met*, two genes directly implicated in the regulation of myogenic cell dissociation and migration (Goulding et al., 1994; Tremblay et al., 1998; Yang et al., 1996), are also transiently expressed in the VLL and in the DML at axial levels which reveal no apparent long-range cell migration, such as the flank (see for example Dietrich et al., 1997, 1998; Pourquie et al., 1995; Yang et al., 1996; our unpublished observations). This raises the question of the role of *PAX3* and *met* in these sites. It is of interest to note that in contrast to *PAX3* and *met* expression, MyoD is absent in both the DML and VLL for the duration of the second wave of myotome colonization. *MyoD*, but not *PAX3* or *met*, is expressed instead in cells at the rostral and caudal lips of the dermomyotome at the same developmental stages. Altogether, these correlative observations raise the possibility that genes such as *PAX3* and *met* might also be important for longitudinal cell movement along the medial and lateral edges of the dermomyotome. Consistent with this notion, it was recently reported that in Splotch mutant mice lacking *PAX3* gene activity, the dermomyotomes and myotomes fail to elongate medially and laterally, leading to malformations in the entire trunk musculature (Tremblay et al., 1998). Recent evidence, however, argues against a function of *met* in development of epaxial and hypaxial muscles of the body wall as these are apparently normal in $met^{-/-}$ embryos (Dietrich et al., 1999). Moreover, the met ligand, SF/HGF, is apparently absent in flank levels of the lateral plate mesoderm. The possibility that additional ligands bind to and activate met and the actual role of met in regions that show no long-range migration of myogenic precursors, remains to be clarified.

An important issue related to the phenotype of *PAX3* mutants is the total absence of appendicular muscles (Franz, 1993; Franz et al., 1993; Tremblay et al., 1998) which would be expected if the formation of limb musculature depends entirely on migratory population of myoblasts. Surprisingly, the hypaxial body wall muscles existed although they consisted of fewer disorganized fibers (Tremblay et al., 1998). These observations are consistent with the present findings showing that the hypaxial muscles of the body wall originate not only from the VLL of the dermomyotome, which expresses *PAX3*, but have a complex origin that includes primarily the pioneer myofibers and fibers emanating from the rostral and caudal edges of the dermomyotome. The two latter categories of cells are not likely to be affected by lack of *PAX3* activity.

It is interesting to point out in the same context that the rectus abdominis muscle and the distal part of the intercostal muscles were totally absent in Splotch mutants (Tremblay et al., 1998). This would again suggest that the medialmost hypaxial muscles arise exclusively from the migatory, *PAX3*-positive population represented by the VLL of the dermomyotome. So, the possibility cannot be excluded that at later times during ingrowth into the somatopleure, VLL cells become exposed to a significant concentration of lateral plate-derived signals, like their counterparts that are opposite limb regions do at earlier stages. As a consequence of that, VLL cells would not contribute any longer to fibers of the hypaxial

domain of the myotome and instead they dissociate. Being the leading edge of the expanding muscular primordium they give rise to the medialmost localized hypaxial muscles. A similar situation could be envisaged to occur at the DML level. Direct assessment of this fate, however, requires dye labeling just prior to final dissappearance of the epithelial dermomyotome lips, a technically difficult task, at least for the VLL region.

Most interestingly, based on deletion experiments and quail-chick chimerism, Kato and Aoyama (1998) have recently proposed that the rostral and caudal lips of the dermomyotome generate the distal vertebral rib whereas the lateral portion of the dermomyotome differentiates into the medialmost sternal portion of the rib. Based on the observation that the ribs develop later than the myotomes, one can assume that the destiny of the lips of the dermomyotome to become ribs represents a rather late fate of these domains, similar to the above proposed development of the VLL into medial muscles at intercostal and abdominal levels. In any case, it is interesting to point out that at flank regions of the axis, the hypaxial domain of the myotomes including the overlying dermomyotome and its lips grow together as a unit into the somatopleure (see Christ et al., 1983) where the myotome (including all waves) gives rise to intercostal muscles (this study) and the limiting dermomyotome lips later generate the distal portions of the ribs (Kato and Aoyama, 1998) to which these muscles attach.

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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.

Bober, E., Franz, T., Arnold, H.-H., Gruss, P. and Tremblay, P. (1994). *Pax-3* is required for the development of limb muscles: A possible role for the migration of dermomyotomal muscle progenitor cells. *Development* **120**, 603-612.

Borycki, A. G., Strunk, K.E., Savary, R. and Emerson, C.P., Jr. (1997) Distinct signal/response mechanisms regulate pax1 and QmyoD activation in sclerotomal and myotomal lineages of quail somites. *Dev. Biol.* **185**,185-200.

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.

Brill, G., Kahane, N., Carmeli, C., Von Schack, D., Barde, Y.-A. and Kalcheim, C. (1995). Epithelial-mesenchymal conversion of dermatome progenitors requires neural tube-derived signals: characterization of the role of Neurotrophin-3. *Development* **121**, 2583-2594.

Capdevila, J., Tabin, C. and Johnson, R. L. (1998). Control of dorsoventral somite patterning by Wnt-1 and β-Catenin. *Dev. Biol.* **193**,182-194.

Chevallier, A., Kieny, M., Mauger, A. S. and Sengel, P. (1977). Developmental fate of the somitic mesoderm in the chick embryo. In Vertebrate Limb and Somite Morphogenesis (ed. Ede, D. A, Hinchliffe, J. R. and Balls, M.) Cambridge: Cambridge Univ.Press, pp. 421-431.

Christ, B., Brand-Saberi, B., Grim, M. and Wiltig, J. (1992) Local signalling in dermomyotome cell type specification. *Anat. Embryol.* **186**, 505-510.

Christ, B., Jacob, H. J. and Jacob, M. (1974a). Uber den ursprung der flugenmuskulatur. *Experientia* **30**, 1446-1448.

Christ, B., Jacob, H. J. and Jacob, M. (1974b) Experimentelle untersuchungen zur entwicklung der brustwand. *Experientia* **30**, 1449-1451.

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. (1978) On the formation of myotomes in embryos: an experimental and electron microscopic study. *Experientia* **34**, 514-516.

Christ, B. and Ordahl, C. P. (1995). Early stages of chick somite development. *Anat. Embryol. Berl.* **191**, 381-396.

Christ, B., Jacob, M and Jacob, H. J. (1983). On the origin and development of the ventrolateral abdominal muscles in the avian embryo. *Anat. Embryol.* **166**, 87-101.

Currie, P. D and Ingham, P. W. (1998) The generation and interpretation of positional information within the vertebrate myotome. *Mech. Dev.* **73**, 3-21.

Cossu, G., Tajbakhsh, S. and Buckingham, M. (1996). How is myogenesis initiated in the embryo? *Trends Genet.* **12**, 218-223.

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

Dietrich, S., Schubert, F. R. and Lumsden, A. (1997). Control of dorsoventral pattern in the chick paraxial mesoderm. *Development* **124**, 3895-3908.

Dietrich, S., Schubert, F. R., Healy, C., Sharpe, P. T. and Lumsden, A. (1998). Specification of the hypaxial musculature. *Development* **125**, 2235-2249.

Dietrich, S., Abou-Rebyeh, F., Brohman, H., Bladt, F., Sonnenberg-Rietmacher, E., Yamaai, T., Lumsden, A., Brand-Saberi, B,. and Birchmeier, C. (1999). The role of SF/HGF and c-met in the development of skeletal muscle. *Development* **126**, 1621-1629.

Epstein, J. A., Shapiro, D. N., Cheng, J., Lam, P. Y. P. and Maas, R. L. (1996). Pax3 modulates expression of the c-Met receptor during limb muscle development. *Proc. Natl. Acad. Sci. USA* **93**, 4213-4218,

Fan, C.-M. and Tessier-Lavigne, M. (1994). Patterning of mammalian somites by surface ectoderm and notochord: Evidence for sclerotome induction by a hedgehog homolog. *Cell* **79**, 1175-1186.

Franz, T. (1993). The splotch (sp1H) and splotch-delayed (spd) alleles: differential phenotypic effects on neural crest and limb musculature. *Anat. Embryol.* **187**, 371-377.

Franz, T., Kothary, R., Surani, M. A. H. and Grim, M. (1993).The splotch mutation interferes with muscle development in the limbs. *Anat. Embryol.* **187**, 153-160.

Goulding, M. D, Chalepakis, G., Deutch, U., Erselius, J. R. and Gruss, P. (1991). Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. *EMBO J.* **10**, 1135-1147.

Goulding, M., Lumsden, A. and Paquette, A. J. (1994). Regulation of Pax-3 expression in the dermomyotome and its role in muscle development. *Development* **120**, 957-971.

Grim, M. (1970). Differentiation of myoblasts and the relationship between somites and wing bud of chick embryos. *Z. Anat. Entwickl.-Gesch.* **132**, 260-271.

Hayashi, K. and Ozawa, E. (1995). Myogenic cell migration from somites is induced by tissue contact with medial region of the presumptive limb mesoderm in chick embryos. *Development* **121**, 661-669.

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.

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 microscopic study. *Anat. Embryol.* **153**, 179-193.

Kablar, B., Krastel, K., Ying, C., Asakura, A. and Tapscott, S. J. (1997) MyoD and Myf5 differentially regulate the development of limb versus trunk skeletal muscle. *Development* **124**, 4729-4738.

Kahane, N. and Kalcheim, C. (1998). The identification of early post-mitotic cells in distinct embryonic sites and their possible roles in morphogenesis. *Cell Tissue Res.* 74, 59-73.

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

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

Kalcheim, C, Cinnamon, Y. and Kahane, N. (1999) Myotome formation: a multistage process (Special issue on myogenesis) *Cell Tissue Res.* **296**, 161-173.

Kato, N. and Aoyama, H. (1998). Dermomyotomal origin of the ribs as revealed by extirpation and transplantation experiments in chick and quail embryos. *Development* **125**, 3437-3443.

Marcelle, C., Stark, M. R. and Bronner-Fraser, M. (1997) Coordinate actions of BMPs, Wnts, Shh and noggin mediate patterning of the dorsal somite. *Development* **124**, 3955-3963.

McMahon, J. A., Takada, S., Zimmerman, L. B., Fan, C. M., Harland, R. M. and McMahon, A. P. (1998). Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. *Genes Dev.* **12**, 1438-1452.

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

Ott, M. O., Bober, E., Lyons, G., Arnold, H. and Buckingham, M. (1991). Early expression of the myogenic regulatory gene, myf-5, in precursor cells of skeletal muscle in the mouse embryo. *Development* **111**, 1097-1107.

Piette, J., Huchet, M., Duclert, A., Fujisawa Sehara, A. and Changeux, J. P. (1992). Localization of mRNAs coding for CMD1, myogenin and the alpha-subunit of the acetylcholine receptor during skeletal muscle development in the chicken. *Mech. Dev.* **37**, 95-106.

Pourquié, O., Coltey, M., Teillet, M.-A., Ordahl, C. and Le Douarin, N. M. (1993). Control of dorsoventral patterning of somitic derivatives by notochord and floor plate. *Proc. Natl. Acad. Sci. USA* **90**, 5242-5246.

Pourquie, O., Coltey, M., Breant, C. and Le Douarin, N. M. (1995). Control of somite patterning by signals from the lateral plate. *Proc. Natl. Acad. Sci. USA* **92**, 3219-3223.

Pourquie, O., Fan, C. M., Coltey, M., Hirsinger, E, Watanabe, Y, Breant, C., Francis-West, P., Brickell, P, Tessier-Lavigne, M., and Le Douarin, N. M. (1996). Lateral and axial signals involved in avian somite patterning: a role for BMP4. *Cell* **84**, 461-471.

Pownall, M. E. and Emerson, C. P., Jr. (1992). Sequential activation of three myogenic regulatory genes during somite morphogenesis in quail embryos. *Dev. Biol*. **151**, 67-79.

Pownall, M. E., Strunk, K. E. and Emerson, C. P., Jr. (1996). Notochord signals control the transcriptional cascade of myogenic bHLH genes in somites of quail embryos. *Development* **122**, 1475-1488.

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.

Spence, M. S., Yip, J. and Erickson, C. A. (1996). The dorsal neural tube organizes the dermamyotome and induces axial myocytes in the avian embryo. *Development* **122**, 231-241.

Tajbakhsh, S., Borello, U., Vivarelli, E., et al. (1998). Differential activation of Myf5 by different Wnts in explants of mouse paraxial mesoderm and the later activation of myogenesis in the absence of Myf5. *Development* **125**, 4155-4162.

Tremblay, P., Dietrich, S., Mericskay, M., Schubert, F., Li, Z. and Paulin, D. (1998). A crucial role for Pax3 in the development of the hypaxial musculature and the long-range migration of muscle precursors. *Dev. Biol.* **203**, 49-61.

Williams, B. A. and Ordahl, C. P. (1994). *Pax-3* expression in segmental mesoderm marks early stages in myogenic cell specification. *Development* **120**, 785-796.

Yamaguchi, T. P. (1997). New insights into segmentation and patterning during vertebrate somitogenesis. *Curr. Opin. Gen. Dev.* **7**, 513-518.

Yang, X.-M., Vogan, K., Gros, P., and Park, M. (1996) Expression of the met receptor tyrosine kinase in muscle progenitor cells in somites and limbs is absent in Splotch mice. *Development* **122**, 2163-2171.