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A Two-Step Mechanism for Myotome Formation in Chick

Short Article

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Summary

The study of the morphogenetic cell movements underlying myotome formation in the chick embryo has led to the emergence of highly controversial models. Here we report a real-time cell lineage analysis of myotome development using electroporation of a GFP reporter in newly formed chick somites. Confocal analysis of cell movements demonstrates that myotome formation involves two sequential steps. In a first phase, incremental myotome growth results from a contribution of myocytes derived solely from the medial border of the dermomyotome. In a second phase, myocytes are produced from all four borders of the dermomyotome. The relative distribution of myocytes demonstrates that the medial and the lateral borders of the somite generate exclusively epaxial and hypaxial muscles. This analysis also identified five myotomal regions, characterized by the origin of the myocytes that constitute them. Together, our results provide a comprehensive model describing the morphogenesis of the early myotome in higher vertebrates.

Introduction

Somites give rise to all the skeletal muscles of the body, with the exception of head muscles (Christ and Ordahl, 1995). Newly formed somites comprise an outer epithelial layer, surrounding a central cavity, the somitocoele. In a few hours, the ventral portion of the somites disaggregates into a ventral mesenchyme, the sclerotome, that gives rise to axial cartilages and bones. The medial wall and the dorsal portion of the somite (i.e., the dermomyotome) remain epithelial. Soon after, the medial wall folds under the dermomyotome, thereby initiating the formation of the primitive skeletal muscle, the myotome. As somites further differentiate, the myotome grows from a supply of cells emanating from the dermomyotome (Christ et al. 1978). Limb and girdle (i.e., appendicular) muscles derive from progenitors located in the lateral dermomyotome that migrate into the limb mesenchyme before undergoing myogenic differentiation. In later embryogenesis, muscle masses separate into epaxial (deep back) muscles, and hypaxial (abdominal and appendicular) muscles. While the dermomyotome is composed of proliferative polarized epithelial cells which express dermomyotome-specific genes, the early myotome comprises postmitotic myocytes which express muscle-specific genes and are observed as mononucleated elongated cells aligned parallel to the embryonic axis. Thus, myotome formation implies a highly coordinated combination of cell migration from the dermomyotome into the myotome, drastic changes in cell shape together with an arrest of the cell cycle and the activation of muscle-specific genes.

The morphogenesis of the myotome has been extensively studied (Kahane and Kalcheim, 1998; Kahane et al., 1998, 2001, 2002; Cinnamon et al., 1999, 2001; Denetclaw et al., 1997, 2001; Denetclaw and Ordahl, 2000; Ordahl et al., 2001; Venters and Ordahl, 2002; reviewed in Brent and Tabin, 2002). However, despite the use of the same animal model—the chick embryo—and similar techniques of investigation—lineage studies using lipophilic fluorescent dyes DiI and DiO—the results lead to two distinct models.

The first model states that the myotome is generated by two permanent stem cell systems, one located at the border of the dermomyotome closest to the neural tube, named the Dorso-Medial Lip (DML), and one in the Ventro-Lateral Lip (VLL). The DML is the main source of the epaxial myotome, while hypaxial muscles derive from the VLL (Denetclaw et al., 1997; Denetclaw and Ordahl, 2000; Venters and Ordahl, 2002). Both borders first generate a thin sheet of muscle fibers. In a second phase, new fibers are deposited in a superficial-to-deep direction; they originate mainly from the DML and the VLL, with a minor contribution from the rostral border (RB) and the caudal border (CB) of the dermomyotome (Denetclaw and Ordahl, 2000). In this model, cells from the DML and the VLL translocate into the myotome, where they directly (i.e., without preliminary migration) elongate to reach the RB and the CB. The myotome expands medio-laterally: older myocytes are displaced laterally as newer ones arise at the DML and the VLL. This mode of myotome growth was named incremental growth. Such a model implies that progenitors within the stem cell population become gradually further apart from their progeny in the myotome, resulting in a "non-coherent" myotomal organization (Denetclaw et al., 2001).

In the second model, the early myotome is believed to be formed by two consecutive waves of cells. The first wave originates from cells present at the medial wall of the epithelial somite. These cells are proposed to constitute a population of postmitotic "pioneer" muscle progenitors that, upon somite dissociation, bend underneath the dermomytome and migrate toward the RB. There, they elongate toward the CB and form the first myocytes (Kahane et al., 1998; Kahane and Kalcheim, 1998). Myocytes derived from pioneer cells were found later in development in both epaxial and hypaxial muscles, leading to the hypothesis that pioneer cells act as

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a scaffold upon which subsequent progenitors organize (Cinnamon et al., 1999; Kahane et al., 2002). These arise from a second wave of cells emanating from all edges of the dermomyotome but entering the myotome only from the RB and the CB (Kahane et al., 1998, 2002; Cinnamon et al., 1999, 2001). Cells from the second wave were observed to elongate among older pioneer myofibers; this mode of myotome formation was thus named intercalating growth (Kahane et al., 1998; Cinnamon et al., 1999). Through this process, the relative position of the progenitors within the dermomyotome is maintained with their progeny in the myotome and defined as "coherent" (Kahane et al., 2002) myotomal growth.

To resolve these discrepancies, it was important to re-address this issue with a lineage analysis technique that would allow the examination of the morphogenetic cell movements underlying myotome formation in real-time and with a high cellular resolution. Here, we have utilized the electroporation of a GFP reporter construct in chick somites (Scaal et al., 2004). By combining this with time-lapse confocal microscopy, we obtained high-resolution images which demonstrate that all borders of the dermomyotome give rise to myocytes. We identified two phases in the formation of the myotome: during a first phase of incremental growth, the myotome expands solely from the translocation of dermomyotome cells through the DML. Once in the myotome, these cells, with no prior migration, elongate to reach the RB and the CB. After this initial period, myocytes from the caudal, the rostral, and the ventro-lateral borders enter the myotome, in a phase that combines incremental growth at the DML and VLL and coherent growth at the RB and the CB. An analysis of the distribution of myocytes from the four borders demonstrates that DML-derived myocytes exclusively colonize the epaxial myotome, while the VLL generates solely hypaxial myotome. Furthermore, we provide evidence for the existence of five myotomal regions, characterized by the origin of the myocytes that constitute them.

Results

The technique of in vivo electroporation allows high-efficiency electroporation of the somitic tissues without disruption of their normal development (Supplemental Figure S1 [http://www.developmentalcell.com/cgi/content/full/6/6/875/DC1]). Here, we have targeted a reporter construct coding for the cytoplasmic form of GFP to the somite borders and examined the morphogenesis of the myotome as development proceeds.

Myocytes Are Generated from All Borders of the Dermomyotome

To investigate whether the DML generates myocytes, we electroporated the medial portion of somites I to V of 25- to 30-somite stage embryos with GFP (to facilitate comparison between experiments, we electroporated interlimb [i.e., thoracolumbar] somites throughout this study). At the time of electroporation, all cells of the epithelial somite are equally susceptible to electroporation and GFP expression is detected as early as 3 hr after manipulation in cells located within the medial wall of the somite (i.e., where putative pioneer cells are located) and directly dorsal to it, in the future DML (Figure 1A and Supplemental Figures S2A and S2C). During the next 2 days of incubation, a continuous flow of myocytes was produced (Figures 1B and 1C) that resulted in a massive labeling of the entire medial half of the myotome. During the time of the experiment (i.e., 48 hr), GFP-labeled cells remained within the DML (arrowheads in Figures 1B and 1C and Figure 3C). This shows that DML cells act as a population of self-regenerating progenitor cells that divide to give rise to both DML cells and myocytes, as previously shown (Denetclaw et al., 2001; Venters and Ordahl, 2002).

To determine whether dermomyotomal cells translocating from the DML directly elongate toward the RB and the CB or first migrate rostrally and only then elongate, we have designed an ex vivo system of embryo culture on solid substrate that allows one to follow in real-time GFP-expressing cells under the confocal microscope (Supplemental Figures 3A–3C). Images taken at different incubation times show a number of progenitors growing bidirectionally to reach the RB and the CB (Figures 1M and 1N). Moreover, we never observed that GFP-positive cells undergo longitudinal migration prior to the formation of myocytes. These data demonstrate that myocytes from the DML elongate directly into the myotome without transient migration toward the rostral somite border.

We then electroporated the lateral portion of the epithelial somites I to V. Three hours after electroporation, GFP-positive cells were specifically located in the lateral portion of the somite (Figure 1D and Supplemental Figure S2B). After 24 hr, GFP-positive cells were observed within the VLL and in the myotome (Figure 1E and Supplemental Figure S2D). Due to the thickness of tissues in the lateral domain, a confocal examination of myocyte elongation could not be performed in live embryos; however, the observation of myocytes that have not yet reached the RB and the CB (red arrowheads in Figure 1E) implies that VLL-derived cells elongate bidirectionally to form full-length myocytes. After 48 hr, numerous GFP-positive myocytes were added from the VLL (Figure 1F). GFP-labeled cells remain within the VLL during the time of the experiment (arrowheads in Figures 1E and 1F and Figure 3G), indicating that the VLL is also composed of self-regenerating progenitor cells.

The rostral and the caudal portion of newly formed somites were electroporated with the GFP-expressing vector. After 8 hr, cells within the RB and the CB specifically expressed GFP (Figures 1G and 1J and sections in Supplemental Figures S2E and S2F). After 28 hr, we observed that many full-sized myocytes had formed from the CB, while fewer arose from the RB (Figures 1H and 1K). Closer examination (Figure 1O) shows that in addition to full-length myocytes, many cells have left the epithelial dermomyotome and entered the myotome; after 48 hr, a considerable number of GFP-positive myocytes have formed from the RB and the CB (Figures 1I and 1L). This indicates that most if not all cells which leave the rostral and caudal epithelial borders of the dermomyotome differentiate into myocytes. As was shown at the VLL and the DML, GFP-positive cells remained in the RB and the CB as long as 24 hr after electroporation (arrowheads in Figures 1H and 1K).

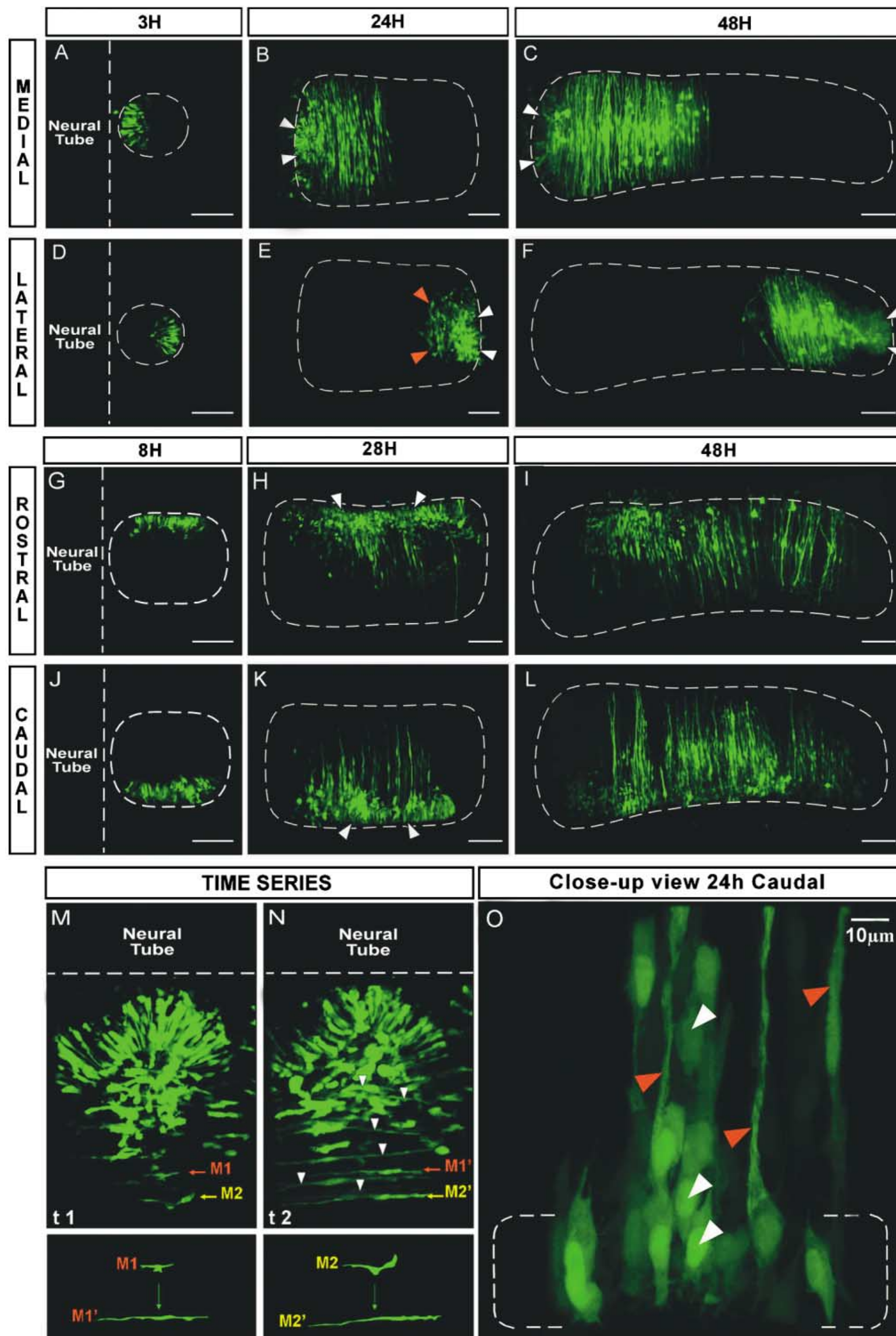

Figure 1. The Four Borders of the Dermomyotome Generate Myocytes

Whole-mount confocal views (image stacks) of fixed (A–L and O) and live (M and N) chick embryos electroporated with GFP in the medial (A–C, M, and N), lateral (D–F), rostral (G–I), or caudal (J–L and O) somite. (A and D) GFP expression 3 hr after electroporation. (B) After 24 hr, many full-sized myocytes arose from the DML; myocytes from the VLL are not yet fully elongated (red arrowhead in [E]). (C and F) GFP-positive myocytes after 48 hr. (M and N) Whole-mount confocal views (image stacks) of an electroporated somite observed at increasing incubation times. (M) GFP-positive, elongating myocytes M1 and M2 are observed in a central location of the somite. A few hours later (N), many elongating and full-sized myocytes are observed (arrowheads); M1 and M2 have grown bidirectionally toward the RB and the CB (M1′ and M2′). Below are schematics depicting the growth of M1 and M2.

(G–L) Embryos electroporated with GFP in the RB (G–I) or the CB (J–L). GFP expression at the RB (G) and the CB (J) after 8 hr. After 28 hr, the CB has generated many nearly full-sized myocytes (K); only few myocytes arose from the RB (H). After 48 hr, both borders have generated many full-sized myocytes (I and L). (O) shows a close-up view of a GFP-labeled caudal dermomyotome border 24 hr after electroporation. The dotted lines delineate the epithelial border; white arrowheads indicate cells leaving the border and entering the myotome. Elongating myocytes are indicated (red arrowheads). Arrowheads in (B), (C), (E), (F), (H), and (K) indicate GFP-positive epithelial progenitor cells remaining within the border of the dermomyotome during the experiment.

Scale bars: (A–L) 100 μm; (O) 10 μm.

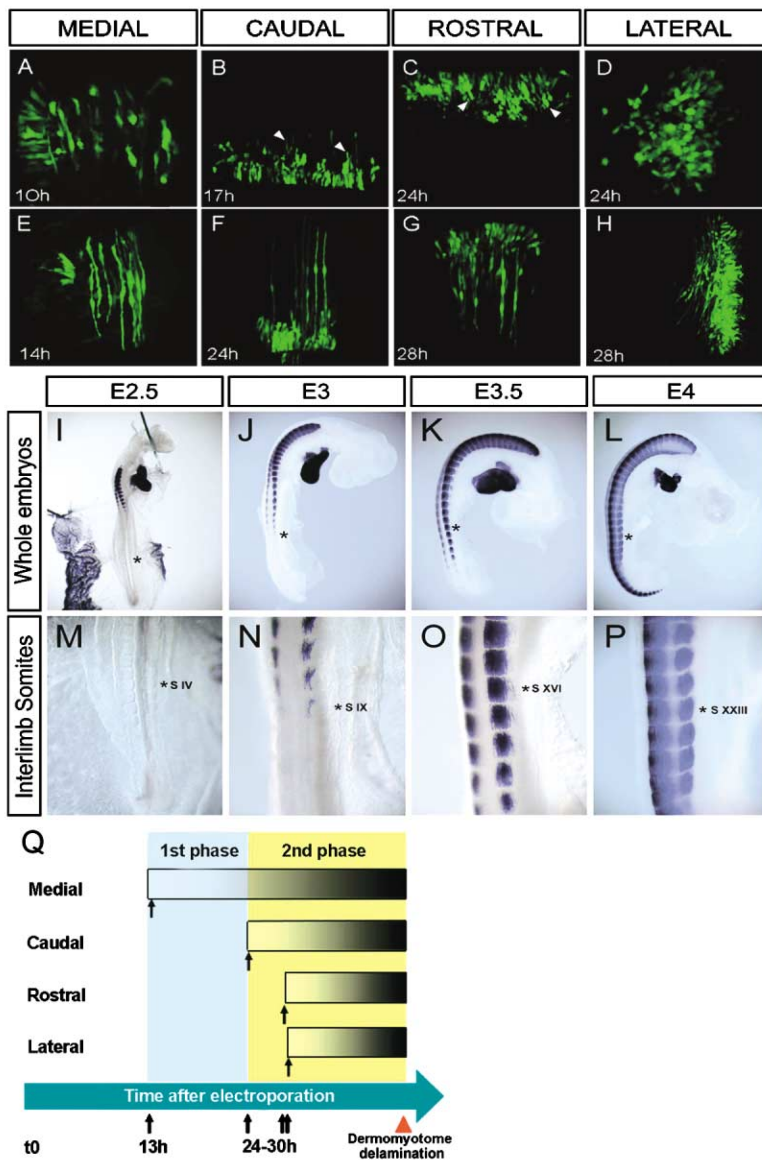

Figure 2. Myotome Forms in Two Sequential Steps

(A–H) Each confocal picture is representative of the results obtained after electroporation of >30 embryos in the medial (A and E), caudal (B and F), rostral (C and G), and lateral (D and H) dermomyotome borders and incubation for the indicated time. 10 hr after medial electroporation (A), GFP-labeled cells have entered the myotome and are aligned in the antero-posterior (AP) axis of the embryo; however, none of these are fully elongated. The first full-sized myocytes are observed 13–14 hr after electroporation (E). 17 hr after CB electroporation (B), GFP-labeled cells enter the myotome (arrowheads). They elongate to reach the opposite border, and full-sized myocytes are observed after 24 hr (F). The RB was delayed by around 4 hr, when compared to the CB (compare [F] and [C] 24 hr after electroporation); full-sized myocytes were observed 28 hr after manipulation (C and G). Finally, the VLL generated full-sized myocytes after 28–30 hr (D and H). (Q) is a schematic that summarizes the dynamics of myocyte formation from each border.

(I)–(P) allow the comparison of the expression pattern of the embryonic form of the Myosin Heavy Chain (whole-mount immunohistochemistry with the monoclonal antibody MF20) to the GFP analysis of myotome formation. (M)–(P) focus on the MF20 staining of an interlimb somite (S IV, i.e., one of the somites electroporated in this study) observed at increasing embryonic ages. At electroporation (E2.5), S IV is epithelial and no myocyte has formed, as shown by the lack of MF20 reactivity (I and M). In an E3 embryo, S IV is located at S IX level; consistent with the GFP lineage analysis, MF20 reactivity is first observed at this level of the embryonic axis (J and N). In an E3.5 embryo, S IV is at S XVI level, an axial level where the GFP lineage analysis demonstrated that full-sized myocytes from the posterior dermomyotome border first appear (K and O). In a 4-day-old embryo, S IV is now at S XXIII level (L and P); myocytes present in the myotome derive from all four borders.

Two Sequential Phases in the Formation of the Myotome

During the course of the experiments described above, we observed that if all borders of the dermomyotome generate myocytes, they initiate this process at different levels of the embryonic axis. To precisely determine the respective timing of myocyte formation from the four borders, embryos were electroporated as above and the first sign of full-length myocyte formation was recorded. This analysis showed that myocytes were first generated from the medial somite 13–14 hr after somite formation (Figures 2A and 2E), corresponding to somite IX (S IX, where S I is the newly formed somite, etc.) in a 3-day-old embryo (E3). In an E3 embryo, S IX is also the first somite that stains positive for the MF20 antibody, which recognizes full-sized myocytes (Figures 2J and 2N), thus confirming the accuracy of our GFP analysis. Full-sized myocytes from the CB first appeared 24 hr after electroporation (E3.5), at S XVI (Figures 2B and 2F). This implies that all myocytes present in somites younger than S XVI in a E3.5 embryo derive solely from the DML (Figures 2K and 2O). The RB generated myocytes next, consistently with a delay of 4–6 hr when compared to the CB (S XVIII; Figures 2C and 2G). Finally, full-sized myocytes from the VLL were observed at S XX (Figures 2D and 2H). These data (summarized in Figure 2Q) demonstrate that during a first phase, myotome expands solely from the translocation of dermomyotome cells through the DML; this translocation takes place concomitantly with a dorsalward growth (i.e., in the direction of the neural tube) of the dermomyotome (Denetclaw and Ordahl, 2000). Through this process, older myocytes are displaced laterally by newer ones arising at the DML, thus resulting in an incremental myotome growth. From S XVI onward,

the myotome is composed of myocytes of mixed origins: DML- and VLL-derived myocytes added to the myotome by incremental growth, while the rostral and caudal border cells contribute to the expansion of the myotome by coherent growth.

Differential Contribution of Each Border of the Dermomyotome to the Formation of Epaxial and Hypaxial Muscles

The electroporation technique results in the extensive labeling of somitic border cells, allowing a global analysis of the distribution of myocytes emanating from the four borders. To analyze the respective contribution of the four somitic borders to the formation of the myotome, we electroporated embryos at E2.5, incubated them for 48 hr, and analyzed, on transverse sections, the distribution of GFP-labeled cells in the myotome. The myotome is composed of an epaxial and a hypaxial domain; in chick embryos, the border between the two domains has been defined by their position relative to the notochord (Figure 3B) and to an indentation of the body wall (Supplemental Figures S1B″ and S1C″; Spörle, 2001; Brent and Tabin, 2002). According to these morphological criteria, we have divided the myotome into an epaxial and a hypaxial domain, which were each arbitrarily subdivided into three regions (a to c, epaxial; d to f, hypaxial; Figure 3B). In each region, the quantity of GFP staining was evaluated.

This analysis demonstrated that DML- and VLL-derived myocytes colonize the medial and the lateral half of the myotome, respectively (Figures 3C and 3G). This is confirmed by whole-mount examination of electroporated embryos (compare Figures 1B and 1C to Figures 1E and 1F). Our data further show that while DML-derived myocytes distribute uniformly within the epaxial domain (Figures 3C and 3D), VLL-derived myocytes are concentrated in the lateral two-thirds of the myotome, leaving a region (d) virtually devoid of VLL-derived myocytes (Figures 3G and 3H, see also Supplemental Figure S1C″).

While whole-mount preparations had shown that myocytes originating from the RB and CB colonize the epaxial and the hypaxial myotome (Figures 1G–1L), transverse sections demonstrated that they are not distributed evenly along the medio-lateral axis of the myotome. Rather, they preferentially colonized a central region of the myotome (regions c and d) where 69 and 76%, respectively, of the overall GFP labeling was observed (Figures 3E, 3F, 3I, and 3J).

Together, these data demonstrate that the contribution of the different borders of the somite to myotome growth is uneven along its medio-lateral axis. They provide evidence for the existence of five myotomal regions, characterized by the origin of myocytes that constitute them. The majority of myocytes in regions a and f are derived from the DML for a and the VLL, respectively; region d is composed of myocytes of rostral and caudal origin; the remaining two regions are formed of myocytes of mixed origins: DML-, rostral-, and caudal-derived for regions b and c; VLL-, rostral-, and caudal-derived for region e.

On transverse sections, we observed that myocytes from the RB and the CB are distributed throughout the entire thickness of the myotome in both the epaxial and hypaxial myotome (Figures 3E and 3I), implying that they intercalate with DML- or VLL-derived myocytes, as suggested previously (Kahane et al., 1998; Cinnamon et al., 1999). This refutes the hypothesis of a stratified myotome, where DML- and VLL-derived myocytes have been proposed to form a primary layer associated with the dermomyotome while rostral- and caudal-derived myocytes would lie deeper, close to the sclerotome (Denetclaw and Ordahl, 2000).

Discussion

Myotome formation is a crucial step of skeletal muscle assembly, during which the organization of muscle cells into dorsal and ventral masses and the orientation of muscle fibers in the antero-posterior axis of the embryo are defined within each segment. The complexity of the morphogenetic movements during this process and the technical challenge that this analysis represents in higher vertebrates has led to divergent interpretations of this dynamic process. The combination of the electroporation of a GFP reporter gene with real-time in vivo confocal examination enabled us to identify single cells within somite borders and to follow them during their translocation into the myotome. In comparison, due to membrane turnover, DiI labeling rapidly results in a particulate labeling of cell membranes which renders the identification of individual cells extremely difficult. With these approaches, we have been able to unequivocally determine the morphogenetic movements underlying early myotome formation in the chick, thereby solving a long-standing controversy in the field (Figure 4).

Myotome Formation: Four Sources, Two Phases, Five Regions

Our results provide the immediate demonstration that the formation of myocytes from the four borders of the dermomyotome is a direct process: cells from the epithelial borders translocate within the myotome and, with no prior migration, elongate in the AP axis of the embryo to form full-sized myocytes. At the DML and VLL, this elongation is bidirectional, while at the RB and CB it is unidirectional.

In addition, our data provide important information on the dynamics of this process. We present a global analysis of the sequence of events during myocyte formation at the four somite borders and their respective contribution to myotome growth. This revealed notable features: the first is that myotome growth can be divided into two phases: (1) an initial phase where myotome expands only in its epaxial domain and from a single source, the medial somite, and (2) a second phase, where the myotome develops in the epaxial and the hypaxial domains through the combined contributions of myocytes from multiple origins (Figure 4A). This subdivision into two distinct phases emphasizes the primordial role that the medial somite, and in particular the DML is playing in the initiation of myotome growth, a notion that was suspected from the morphological studies of somite differentiation (Kaehn et al., 1988; Denetclaw et al., 1997) and from the analyses of the expression

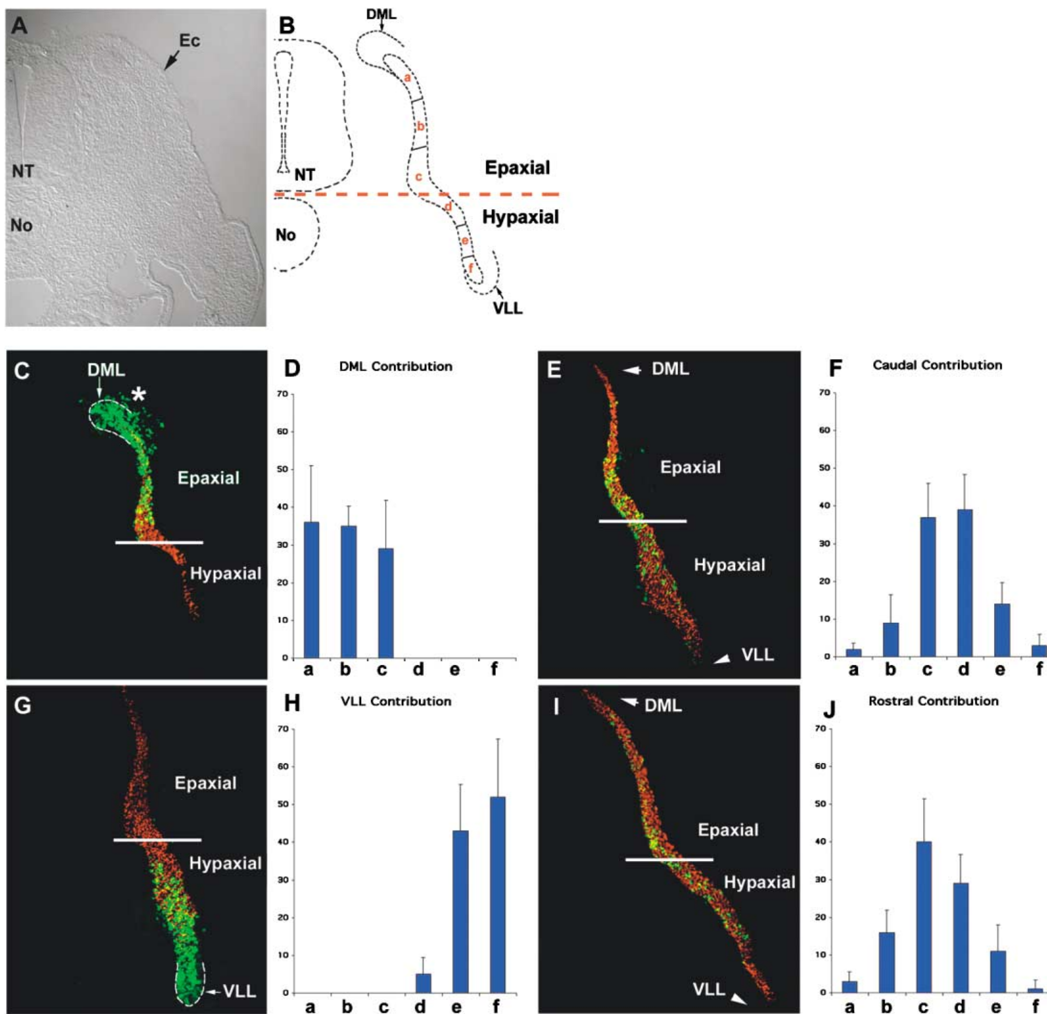

Figure 3. Differential Contribution of the Border of the Dermomyotome to the Formation of Epaxial and Hypaxial Muscles

(A and B) Bright field (A) and schematics (B) of transverse sections through an interlimb somite 2 days after electroporation (at E 4.5). At this stage, only the DML and the VLL remain epithelial. (B) shows the three epaxial and the three hypaxial regions utilized to determine the distribution of GFP-positive cells within the myotome. (C, E, G, and I) Transverse sections representative of the results obtained after medial (C), caudal (E), lateral (G), and rostral (I) electroporation. In red, myotome staining with MF20; in green, GFP-positive cells. (D, F, H, and J) Distribution of GFP-positive cells in each of the myotomal regions. Blue bars represent the average of ten results; standard deviations are indicated. The quantity of GFP staining was counted in each region by determining (with Photoshop) the number of pixels in the GFP/green channel. The asterisk in (C) shows GFP-positive cells from the DML migrating beneath the ectoderm to form dorsal dermis.

patterns of the muscle determination factors Myf5 and MyoD or of the Myosin Heavy Chain (Marcelle et al., 2002, for review, and Figure 2). At present, the relation between the initial and the second phase is unclear. The second phase could depend upon the completion of the initial phase: previously published data do not clarify this point, since the DML was shown to be either necessary and sufficient (Ordahl et al., 2001) or largely dispensable (Kahane et al., 2002) for myotome growth. Alternatively, the myotome formed during the initial phase could serve as an organizing scaffold for myocytes from different origins. Further studies will determine whether or not the first and the second phase are interdependent.

Two days after myotome formation was initiated at the DML, this process is nearly complete. The dermomyotome, including the RB and the CB, has dissociated in its central region and the VLL and DML are soon to de-epithelialize (Figure 4B). Our analysis of the relative contribution of the four borders to myotome formation shows that the medio-lateral axis of the myotome is composed of five regions, characterized by the source of the myocytes that constitute them (Figure 4B). The existence of subdomains in the myotome had been previously proposed, based on the analysis of gene expression patterns in the mouse myotome (Spörle, 2001), and from the study of regulatory regions of the Myf5 gene, which demonstrated that Myf5 transcription in the central myotomal domain is under distinct regulatory control (Hadchouel et al., 2003). From our data, it is tempting to hypothesize that the morphological basis for these molecularly distinct subdomains is the source of the myocytes that compose them.

Finally, our finding that the epaxial and the hypaxial myotomes are composed of mixed populations, some

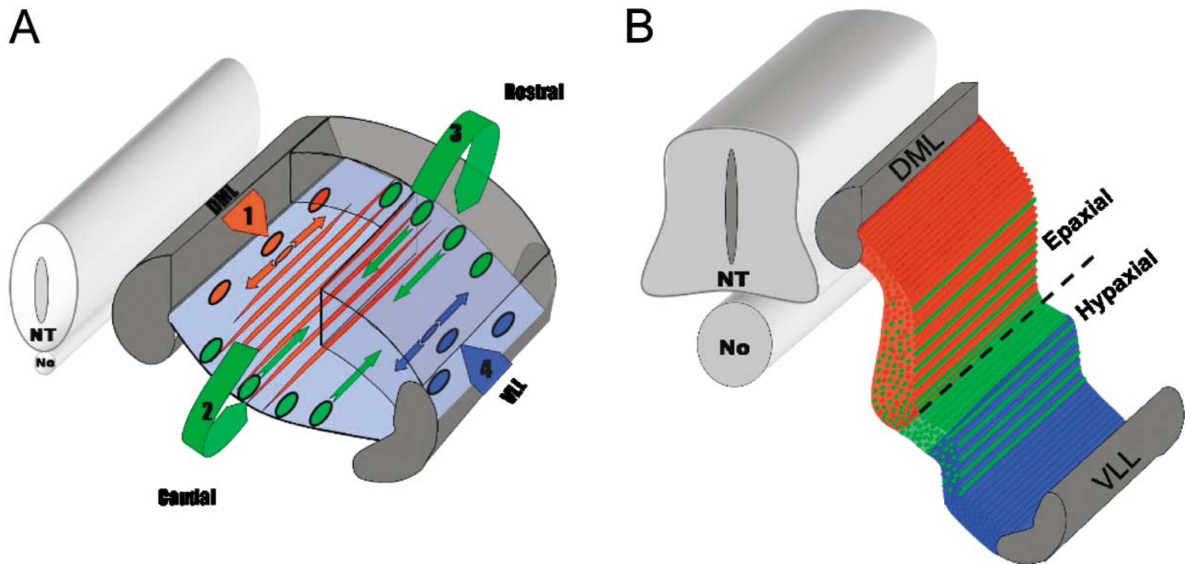

Figure 4. A Model for the Formation of the Early Myotome

(A) Cells present at the borders of the somite contribute to the formation of the early myotome at different rates: first, progenitors emanating from the DML translocate under the dermomyotome and elongate bidirectionally to form myocytes (in red); later, myocytes arise sequentially from the CB, from the RB (in green), and finally from the VLL (in blue).

(B) This results in a primary myotome that is nonhomogeneous with regard to the contribution of the different borders to its formation.

emanating from the DML and the VLL and others from the RB and the CB, has important implications for our understanding of myogenic differentiation in higher vertebrates. Thus, analyses of the factors responsible for epaxial or hypaxial muscle formation should take into consideration not only the signals that activate the myogenic program at the medial or lateral extremities of the somite, as was done in most studies to date, but also at their rostral and caudal edges. This should considerably change the way that early myogenic specification is studied in the future.

Pioneer Cells in the Chick Myotome?

We demonstrate that DML- and VLL-derived myocytes occupy nonoverlapping domains in the myotome. This clearly refutes the hypothesis that cells originating from the medial epithelial somite cross the epaxial-hypaxial border to form a scaffold for further myotome development (Cinnamon et al., 1999; Kahane et al., 2002), but it is in agreement with lineage studies using quail-chick chimeras and DiI labeling techniques (Ordahl and Le Douarin, 1992; Denetclaw et al., 1997; Denetclaw and Ordahl, 2000) and with the analyses of LaacZ clones in mouse, that demonstrated that the epaxial/hypaxial border is set at the time that somites form (Eloy-Trinquet and Nicolas, 2002). Since cells derived from the medial epithelial somite (1) do not serve as a scaffold on which the entire myotome organizes and (2) participate, in a continuous process indistinguishable from the DML cells, in the formation of the epaxial myotome, it is our view that the term "pioneer," which has been employed to characterize their putative specificity, is misleading. In addition, it is confusing in the context of pioneer muscle cells identified in zebrafish.

In conclusion, early myotome formation in the chick results from a complex choreography of cell translocation and myocyte elongation occurring at the epithelial dermomyotome borders. Since the dermomyotome is a temporary structure that disappears through progressive de-epithelialisation, myocyte generation from the borders is a time-limited process. We propose to name "primary myotome" the end-product of the morphogenetic process described here (shown in Figure 4B), encompassing all the postmitotic, mononucleated myocytes generated by the four epithelial borders of the dermomyotome. The mechanisms underlying the next steps of myotome growth, i.e., the emergence of a population of proliferation-competent myoblasts and their fusion to primary myocytes to form multinucleated myofibers, remain to be understood at the molecular and the mechanistic levels.

Experimental Procedures

Electroporation and Confocal Imaging of Somitic Tissues

The electroporation procedure, equipment, and vectors used here have been recently described (Scaal et al., 2004). For observation of tissues at elapsed times, embryos were fixed in formaldehyde, cleared in 90% glycerol/H_2O, and examined with a Zeiss LSM 510 confocal microscope. Image stacks (50–100 μm thick) were treated with Metamorph (Universal Imaging Corp.) and Imaris (Bitplane) image analysis software for image visualization and 3D reconstruction. For time-lapse examination of somite morphogenesis, the embryos placed on a solid substrate (see below) were examined with a Perkin Elmer "Ultra View" confocal head attached to a Leica upright motorized microscope.

Culture of Embryos Explanted on Solid Substrate

The substrate is composed of Agarose 1% supplemented with HEPES-Buffered RPMI (GIBCO-BRL) and 10% Fetal Calf Serum. The embryo and its extraembryonic membranes are transferred, dorsal side up, on the agar. Embryos have been cultured on such substrate for 48 hr without noticeable abnormalities.

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