Generality of vertebrate developmental patterns: evidence for a dermomyotome in fish

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SUMMARY The somitic compartment that gives rise to trunk muscle and dermis in amniotes is an epithelial sheet on the external surface of the somite, and is known as the dermomyotome. However, despite its central role in the development of the trunk and limbs, the evolutionary history of the dermomyotome and its role in nonamniotes is poorly understood. We have tested whether a tissue with the morphological and molecular characteristics of a dermomyotome exists in nonamniotes. We show that representatives of the agnathans and of all major clades of gnathostomes each have a layer of cells on the surface of the somite, external to the embryonic myotome. These external cells do not show any signs of terminal myogenic or dermogenic differentiation. Moreover, in the embryos of bony fishes as diverse as sturgeons (Chondrostei) and zebrafish (Teleostei) this layer of cells expresses the *pax3* and *pax7* genes that mark myogenic precursors. Some of the *pax7*-expressing cells also express the differentiation-promoting myogenic regulatory factor Myogenin and appear to enter into the myotome. We therefore suggest that the dermomyotome is an ancient and conserved structure that evolved prior to the last common ancestor of all vertebrates. The identification of a dermomyotome in fish makes it possible to apply the powerful cellular and genetic approaches available in zebrafish to the understanding of this key developmental structure.

INTRODUCTION

Rapid movement is a characteristic of animal life that became possible with the evolution of striated muscle. As the deuterostome lineage gave rise to vertebrates, most striated muscle became anchored to the newly evolved endoskeleton, innervated by central motor neurons and responsible for voluntary movement. In vertebrates, most skeletal muscle begins to develop during maturation of the somites. The somites themselves are transient embryonic structures located in pairs on either side of the axial organs. They arise from the rostrocaudally progressing segmentation of the trunk paraxial mesoderm. The somites of most vertebrates form as blocks of tissue with an epithelial coating (epithelial somite) and a space in the center, the somitocoel, which may contain a loose meshwork of cells. During amniote somite maturation, a ventral part of the epithelial somite delaminates into the mesenchymal sclerotome, whereas dorsal cells retain their epithelial nature. This dorsal portion is referred to as the dermomyotome and, as well as being the origin of trunk and limb skeletal muscle, is also the source of other mesodermal tissues in the amniote body, including dermis and vascular endothelia. Thus, the dermomyotome is a multipotent tissue important for the development of skin, blood vessels, and the striated muscles responsible for voluntary movement (Buckingham et al. 2003; Olivera-Martinez et al. 2004; Scaal and Christ 2004).

In amniotes, the four edges of the dermomyotome (the dorsomedial, ventrolateral, rostral, and caudal dermomyotome lips) are the main sources of early myogenic cells that translocate underneath the dermomyotome epithelium and lead to growth of the myotome (the first skeletal muscle fibers in the body). At these lips, the dermomyotome epithelium curls inward, cells leave the dermomyotome, enter the myotome and elongate into muscle fibers. New muscle fibers are added progressively at the edges of the myotome until the dermomyotome is fully de-epithelialized (Denetclaw and Ordahl 2000; Kahane et al. 2001; Ben-Yair et al. 2003). Cells

from the ventrolateral lip also delaminate and migrate later-ally to yield muscle in the limb buds and ventral body wall. Thus, the dermomyotome is the source of myogenic cells in the trunk and limbs of amniotes (see Tajbakhsh 2003).

The dermomyotome also contributes to other tissues. Studies of dermis development in chick and mouse indicate that in both, dermal progenitors de-epithelialize from distinct dermomyotome regions, particularly the central sheet, to form the subectodermal mesenchyme before differentiating into distinct areas of the dorsal dermis (Ben-Yair and Kalcheim 2005). Angiogenic cells from the dermomyotome form the walls of blood vessels and lymphatic vessels in the dermis, the body wall and the limb buds of amniote embryos (Kardon et al. 2002; He et al. 2003). Myogenic precursors responsible for post-natal growth and muscle repair also de-rive from the somite, presumably from the dermomyotome (Armand et al. 1983). It is unclear when during development dermomyotome cells become restricted to the generation of one of these fates (Huang and Christ 2000; Scaal and Christ 2004).

The above model for development of dermomyotome into myotome, dermis, and other cell types has largely been elucidated in a few "model" amniotes. In many teleosts, my-ogenic differentiation begins at much earlier stages of devel-opment, relative to its commencement in amniotes. Myogenic regulatory factors (MRFs: *myf5*, *myoD,* or *myogenin*) and myosin heavy chain genes are expressed prior to segmenta-tion in cells adjacent to the notochord, called adaxial cells (Weinberg et al. 1996; Kobiyama et al. 1998; Coutelle et al. 2001; Rescan 2001). In zebrafish (*Danio rerio*), around the time of incorporation into a somite, adaxial cells begin to differentiate into elongated muscle fibers, dependent on midline-derived Hedgehog signaling. During this elongation, adaxial cells move laterally to form a monolayer of mono-nucleated embryonic slow muscle fibers located superficially on the somite (Devoto et al. 1996). Following the movement of slow muscle precursors to the surface of the somite, deeper cells differentiate as fast muscle fibers (Blagden et al. 1997; Henry and Amacher 2004). Similar movement of adaxial cells probably occurs in other teleosts, based on histological and gene expression studies (e.g., pearlfish and trout, Stoiber et al. 1998; Rescan et al. 2001). The development of a primary myotome prior to the establishment of a dermomyotome in amniotes may be analogous to the development of adaxial cells in teleosts (Kalcheim et al. 1999).

After this embryonic phase of myogenesis, several phases of muscle growth have been defined in teleosts. During strat-ified hyperplasia, new muscle fibers form either solely at the dorsal and ventral extremes of the myotome, or in addition in a layer between the superficial muscle layer and the deeper fast fibers (Rowlerson and Veggetti 2001). Subsequently, mo-saic hyperplasia is distributed throughout the myotome sug-gesting that myogenic precursors are resident within the

tissue, as is the case with amniote satellite cells (Rowlerson and Veggetti 2001). The distinctions between embryonic, stratified, and mosaic hyperplasia suggest that there may be several distinct mechanisms for developing muscle fibers in teleosts. The distinct mechanisms of *myf5* induction in nu-merous regions of the mouse somite suggest that amniotes also have several modes of somitic myogenesis analogous to stratified hyperplasia (Hadchouel et al. 2003). Moreover, the classical description of secondary fiber formation in amniotes is analogous to mosaic hyperplasia (Kelly and Rubinstein 1980). Thus, fish myogenesis has numerous similarities to that in amniotes.

The role of a dermomyotome in nonamniote myotome and dermis development has been generally ignored in recent years, simply because it has been taken for granted that a dermomyotome (sensu amniotes) does not occur in these an-imals (Hollway and Currie 2003; but see also Kaestner 1892; Stickney et al. 2000; Sporle 2001). Modern work on zebrafish has occasionally used the term dermomyotome to refer to the nonsclerotome compartment of the somite (Nornes et al. 1996; Schvarzstein et al. 1999), but there has been no evidence suggesting a layer of myogenic cells external to the embryonic myotome homologous to the amniote dermomyotome. We combine new research with a critical re-evaluation of pub-lished work to test whether the dermomyotome is conserved through all vertebrates, including teleosts. We first outline briefly the evolutionary origins of the dermomyotome in ver-tebrates, and then explore the evolution and function of the dermomyotome in nonamniote vertebrates. We discuss evi-dence that cells previously called "external cells" in teleosts have the morphological and molecular characteristics of a dermomyotome. We suggest that study of the teleost der-momyotome may shed light on the cellular and genetic basis for the development of dermomyotome form. Finally, we discuss the implications of the inclusion of nontetrapod ver-tebrates for understanding the evolution of dermomyotome function in vertebrates.

DAWN OF THE DERMOMYOTOME

The primary ancestral function of mesoderm was likely myo-genesis because skeletal, cardiac, and smooth muscles are major derivatives of mesoderm in both protostomes and deu-terostomes. In most metazoans, there are two (or more) phases of myogenesis, with myogenesis during larval growth occurring from cells "set aside" during earlier embryonic de-velopment: two phases of myogenesis have been proposed as a basal bilaterian character (Peterson et al. 1997). It is unclear whether the earliest chordate larvae had a large myogenic precursor population. Extant urochordate larvae have unseg-mented tail mesoderm that is primarily one type of skeletal muscle flanking a stiffened rod-like notochord, and urochor-

dates are small with little significant muscle growth prior to metamorphosis (Jeffery and Swalla 1997). During the evolution of chordates, a segmented mesoderm yielded somite-derived cartilage and tendon cells in addition to muscle. Thus, the somites became specialized for production of the musculoskeletal system of trunk and tail.

The life history and size of the earliest chordates is unknown; however, it is clear that muscle growth became quite significant in chordates. In cephalochordates and vertebrates, muscle growth persists beyond embryogenesis, suggesting that some myogenic cells must have been reserved for later myogenesis. In extant segmented cephalochordates like amphioxus, two kinds of muscle form in the anterior segments: slow muscle fibers are located superficially and fast muscle fibers are located deep in the myotome, just as in teleosts (Lacalli and Kelly 1999). Although extant cephalochordates may have diverged considerably from the main vertebrate lineage, the fact that early myogenesis is similar in cephalochordates and teleosts suggests that the common ancestor already had rather sophisticated myogenesis. Like amphioxus, vertebrates form several early populations of myotomal muscle fibers (reviewed in Bone 1978) and muscle growth and repair continues throughout life. This raises the possibility that in all chordates, the somites contain not only precursors to early embryonic myogenesis, but also populations of myogenic stem cells underlying later muscle growth.

Agnathans from the Lower Cambrian reveal that early vertebrates could be large, requiring significant post-embryonic muscle growth (Shu et al. 1999). In contrast to cephalochordates, where no late myogenic cells have been described, cells similar to the dermomyotome may be present in the lamprey, a representative of agnathan vertebrates (Fig. 1). A lamprey has stacks of muscle lamella similar to those in amphioxus (Maurer 1906). The 26-day lamprey tail also has a distinct layer of "undifferentiated" cells on the external surface of the myotome underlying the ectoderm (Fig. 2A, from Nakao 1977). These "external cells" are sparsely distributed, forming an extremely thin layer of cells on the surface of the myotome. These cells are not muscle, as revealed by their lack of myofibrils; we suggest that they are multipotent precursor cells.

To address whether undifferentiated cells external to the embryonic myotome are evolutionarily widespread, we examined embryos of the skate *Raja erinacea*, an elasmobranch example of the chondrichthyan branch of gnathostome diversity (Fig. 1). We used a pan-myosin antibody to label the primary myotome and found that there is a layer of somite cells external to the primary myotome (Fig. 2B). This external cell layer is curled inward at its dorsomedial and ventrolateral edges (Fig. 2C), forming structures strikingly similar to the dorsomedial and ventrolateral lips of the dermomyotome in amniotes (Fig. 2E). Available molecular markers do not allow us to determine if the cells between the external cell layer and the epidermis are neural crest cells or the beginning of the skate dermis.

In addition to amniotes, embryos of other sarcopterygians have external cells (Sunier 1911). In embryos of the African clawed frog, *Xenopus laevis*, Hamilton (1969) described a "dermatome" as a thickened epithelial veil hanging over the myotome. The thickness of the *Xenopus* external cell layer varies according to the stage of development and the anterior–posterior position in the embryo; it can be a structure morphologically similar to the cuboidal epithelial monolayer in amniotes and skates, or a very thin squamous epithelium more similar to that seen in lampreys (Fig. 2D, Grimaldi et al. 2004). At dorsomedial and ventrolateral extremes this layer expresses myogenic markers as it curls inward in a manner similar to amniote dermomyotome and skate (Grimaldi et al. 2004). Thin external cells have also been described in lungfish, a nontetrapod representative of the sarcopterygians (Maurer 1906). Thus, representatives of the agnathan vertebrates, chondrichthyans, and sarcopterygians all have a layer of undifferentiated cells external to the embryonic myotome. In the amniotes, this external cell layer is the dermomyotome. The simplest interpretation of the similar position, morphology, and lack of myosin labeling is that a dermomyotome epithelium is a shared, ancestral vertebrate characteristic. This begs the question: what happened to the dermomyotome in actinopterygians, the sister group to the sarcopterygians (Fig. 1)?

EXTERNAL CELLS IN RAY-FINNED FISHES: TOPOLOGICAL DERMOMYOTOME

To test whether the external cell layer is a primitive characteristic in actinopterygians, we have examined sturgeon (*Acipenser ruthens*), which, as a chondrostean, exemplifies a clade rooted close to the base of the modern actinopterygian radiation. Sturgeons, like most teleosts, have an embryonic myotome consisting of distinguishable superficial (presumed slow) and deep (presumed fast) fibers (Bone 1978; Flood and Kryvi 1982; Flood et al. 1987). On the external surface of this embryonic myotome is a thin layer of cells very similar to the external cells of lamprey as discussed above and anurans (Fig. 3A, compare with Fig. 2, A and D; Grimaldi et al. 2004). These external cells do not show any characters of differentiated muscle visible in the electron microscope, and are not labeled by pan-myosin immunolabeling (P. Steinbacher and W. Stoiber, unpublished).

External cells are present not only in nonteleost actinopterygians. A transient population of undifferentiated external cells has been found in diverse teleosts (summarized in Fig. 1), including herring (*Clupea harengus*, Johnston 1993), eel (*Muraena* sp., Fig. 3B, modified from Sunier 1911), pacu (*Piaractus mesopotamicus*, Dal Pai-Silva et al. 2003), sea bass (*Dicentrarchus labrax*, Veggetti et al. 1990) and sea bream (*Sparus aurata*, Ramirez-Zarzosa et al. 1995; Patruno et al. 1998). External cells have been most thoroughly characterized
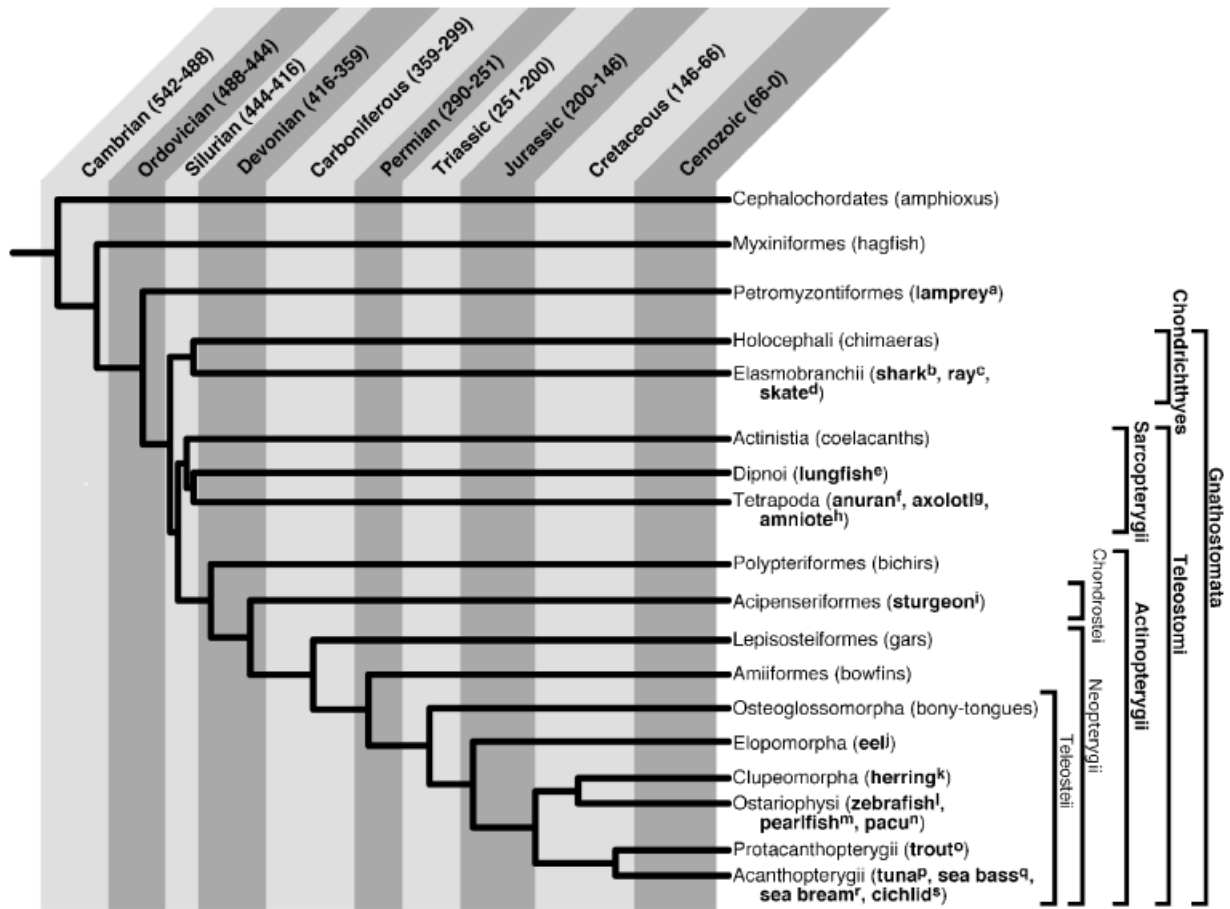
Fig. 1. Phylogenetic relationships among selected groups of extant vertebrates. Cladogram is based on Helfman et al. (1997) with some modifications based on recent analysis of the evolutionary relationships between Petromyzontiformes, Myxiniformes, and the Gnathostomata (Furlong and Holland 2002; Takezaki et al. 2004). The time of divergence between groups is approximately indicated (Stiassny et al. 2004). In embryos of all the animals listed in bold there is evidence of a layer of undifferentiated cells external to the embryonic myotome. Selected references for each are as follows: [a]Maurer (1894), Nakao (1977), see Fig. 2A; [b]Kaestner (1892), Maurer (1906); [c]Kaestner (1892), Maurer (1894), Sunier (1911); [d]Sunier (1911), see Fig. 2, B and C; [e]Maurer (1906), Sunier (1911); [f]Maurer (1894); [g]Tajbakhsh 2003, see Fig. 2E; [h]Maurer (1894), Grimaldi et al. (2004), see Fig. 2D; [i]Maurer 1894, see Figs. 3A and 4A; [j]Sunier (1911), see Fig. 3B; [k]Johnston (1993); [l]Waterman (1969), see Fig. 4, B–D, and F; [m]Stoiber et al. (1998), Fig. 3C; [n]Dal Pai et al. (2000); [o]Maurer (1894), Vialleton (1902), see Fig. 3, E and F; [p]See Fig. 3D; [q]Veggetti et al. (1990); [r]Ramirez-Zarzosa et al. (1995), Lopez-Albors et al. (1998); [s]See Fig. 4E.

in zebrafish, where they are present from the end of segmentation to at least the newly hatched larva (Waterman 1969). In electron micrographs, external cells are extremely flattened cells on the external surface of the primary myotome. They do not show any characteristics of immature muscle fibers, such as myofibrils or clusters of filaments and ribosomes. External cells overlap each other and there may be specialized junctions between adjacent external cells (Waterman 1969). We have re-examined zebrafish external cells by electron microscopy and confirmed Waterman's characterization (Groves et al. 2005 and W. Stoiber, unpublished). Although described previously, external cells in these fish species have not been recognized as homologous to the dermomyotome of tetrapods.

Zebrafish are highly derived cyprinid teleosts, raising the possibility that the flattened external cells in this species are not an ancestral characteristic, but an evolutionary novelty. Another cyprinid, the pearlfish (*Rutilus frisii meidingeri*) has flattened external cells similar to those of zebrafish shortly after the end of segmentation. Pearlfish external cells form an apparently squamous epithelial layer closely apposed to the primary myotome but clearly separated from the epidermis (Fig. 3C). Thus, both cyprinids for which there is reliable histological information have a thin layer of external cells outside of the embryonic myotome. It is not clear how many of the over 2000 other cyprinid species are similar.

Flattened external cells are not unique to cyprinids. The very distantly related yellow fin tuna is a scombroid perco-morph teleost that attains a large adult size. Like sturgeon, eel and the cyprinids, tuna embryos have a continuous layer of extremely thin cells on the external surface of superficial
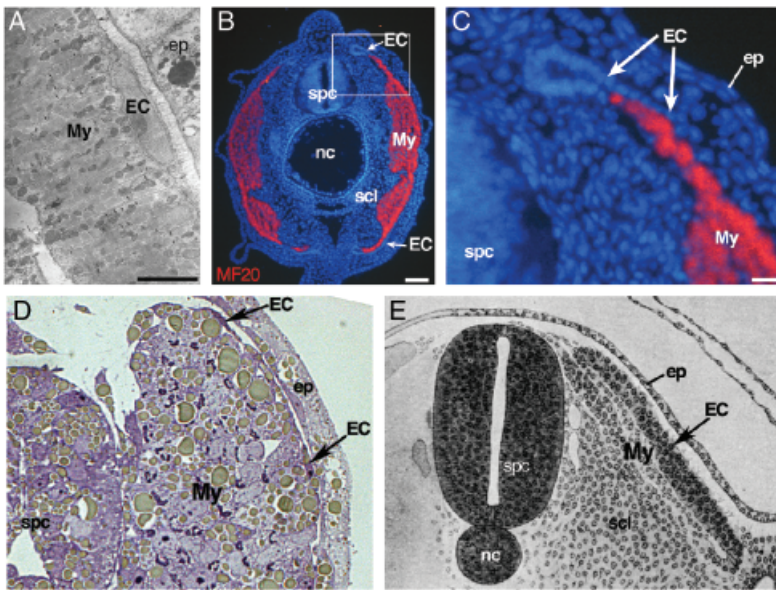
Fig. 2. Representative agnathan, chondrichthyan, and sarcopterygian embryos each have a layer of undifferentiated cells external to the Myotome (My). (A) ECs in a lamprey, *Lampetra japonica*. Electron micrograph of a transverse section of a midtrunk My of a 5-day lamprey (modified from Nakao 1977). The My is composed of horizontal muscle lamellae, bounded on the lateral surface by undifferentiated EC which are separated from the ep by a large extracellular space containing basal lamina (thick arrow). Scale bar = 5 μm. (B) ECs in a skate *Raja erinacea*: transverse section of the anterior tail of an embryo. The My is immunolabeled with the MF20 myosin antibody (red), and nuclei with Hoechst 33258 (blue). An apparent epithelial cell layer external to the My does not express myosin at this time. Scale bar = 100 μm. (C) High-magnification view of boxed area in (B). The EC form structures similar to the dorsomedial and ventrolateral lips of the amniote dermomyotome. Scale bar = 25 μm. (D) ECs in *Xenopus* sp. Transverse section of somite 8 in a stage 35 tadpole (modified from Grimaldi et al. 2004). External to the My is a thin layer of ECs. (E) "ECs" in a chick, *Gallus gallus*: drawing of transverse section through the trunk of an embryo (modified from Lillie 1919). The chick somite begins as an epithelial sphere and then de-epithelializes as it becomes subdivided into scl, My, and an epithelial layer external to the My. This epithelial layer was called the dermatome by early investigators, but is now known as the dermomyotome; we have labeled it "ECs" to emphasize its similarity to other vertebrates. EC, external cells; My, myotome; ep, epidermis; scl, sclerotome; spc, spinal cord; nc, notochord.
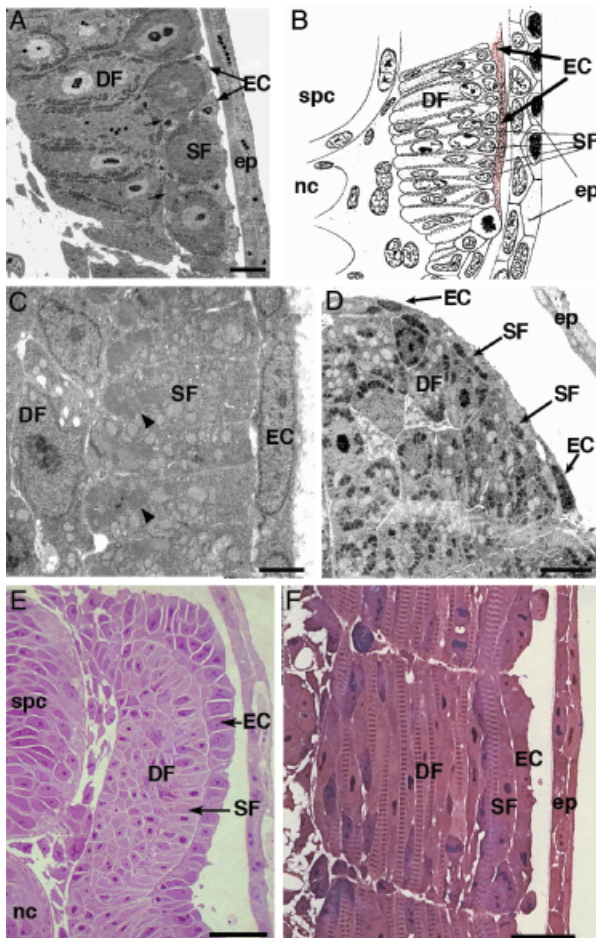

Fig. 3. Actinopterygian embryos have a layer of undifferentiated cells external to the myotome. All species examined have two layers of differentiated muscle fibers: superficial (SF) and deep (DF). (A) ECs in the sturgeon *Acipenser ruthenus*. Semi-thin transverse section posterior to the anal vent of an embryo at hatching (at least 60 somites). External to the SF is a layer of thin ECs. Scale bar = 2 μm. (B) ECs in an eel *Muraena* sp. Transverse section of 5-day-old embryo; drawing modified from Sunier 1911. A layer of ECs (pale red) is clearly attached to the myotome and separated from the epidermis (EP). (C) ECs in the pearlfish *Rutilus frisii meidingeri*. Electron micrograph of a segment of the dorsolateral surface of the myotome in a transverse section of a 40 somite stage embryo. ECs form a very thin squamous epithelium and show no evidence of myogenic differentiation. Scale bar = 2 μm. (D) ECs in the yellowfin tuna *Thunnus albacares*. Transverse section of an epaxial quadrant from an embryo at hatching (21 h post-fertilization). Some flattened ECs are present at the lateral surface of the myotome. SF are very small in size and arranged in a discontinuous layer. Scale bar = 5 μm. (E) ECs in trout *Salmo trutta*. Semi-thin transverse section just posterior to the anal vent of an eyed stage trout embryo (55–60 somites). ECs lie over the SF as in other actinopterygians. However, the cells form an apparent cuboidal epithelium more similar to the external layer in skate (Fig. 1B) and chick (Fig. 1C). Scale bar = 25 μm. (F) ECs in trout are not elongated and do not show any indications of myogenic differentiation. Semi-thin horizontal section of an eyed stage trout (55–60 somites), just posterior to the anal vent. Superficial and DF have clear myofibril striations. No myofibrils or any other signs of differentiation are found in ECs. Scale bar = 25 μm. EC, external cells; DF, deep fibers; SF, superficial fibers; scl, sclerotome; spc, spinal cord; nc, notochord; ep, epidermis.

muscle fibers, similar to that in some stages of *Xenopus* (Fig. 2D, Grimaldi et al. 2004). On the other hand, the trout external cell layer is a robust, apparently cuboidal epithelium (Fig. 3, E and F), much more similar to that in the chondrichthyan skate (Fig. 2, B and C) and sarcopterygian chick (Fig. 2E). Cichlids, illustrated by *Astatotilapia burtoni*, a labroid percomorph teleost, also have a robust external cell layer (see Fig. 4E). Nevertheless, the embryonic myotome of these teleosts is similar to that in cyprinids, with a superficial layer of small diameter (presumably slow) fibers and a deeper mass of larger diameter (presumably fast) fibers that grow by stratified hyperplasia (E. G. Adiarte and S. H. Devoto, unpublished, Rescan et al. 2001; Rowlerson and Veggetti 2001). Thus, it is probably a general characteristic of teleosts to have an apparently myogenic external cell layer, but the thickness of this layer is not conserved phylogenetically.

In summary, representatives of all extant vertebrate taxa have a layer of nonmuscle cells on the external surface of the embryonic myotome (Fig. 1). The thickness of this external cell layer varies considerably between species and developmental stages, and fails to correlate with evolutionary relationships or adult body size.

MOLECULAR CHARACTERISTICS OF THE EXTERNAL CELL LAYER

If the external cell layer in nonamniotes and the dermomyotome of amniotes are homologous, they should express similar sets of genes. Fortunately, teleosts, and especially zebrafish, are now open to a level of molecular genetic and manipulative embryology comparable with that in amniotes. As discussed above, neither external cells nor dermomyotome cells express myogenic differentiation markers such as myosin. Myogenic precursors in the amniote dermomyotome express either or both the *pax3* and *pax7* genes, each of which is required for normal myogenesis (Pownall et al. 2002). Pax3 is required for migration of myogenic precursors into the limb bud, and Pax7 is required for the normal development of post-natal myogenic precursors (Seale et al. 2000; Oustanina et al. 2004). Recently, the external cell layer of *Xenopus* was shown to express *pax3* throughout most of its dorso-ventral extent, and MRFs Myf5 and MyoD near the dorsal and ventral tips of the myotome and elsewhere (Grimaldi et al. 2004). Thus, in the amphibian *Xenopus*, external cells are likely to include myogenic precursors. The lamprey also expresses Pax3/7 in cells on the external layer of the embryonic myotome, with particularly strong expression near the dorsal and ventral tips, suggesting that external cells include myogenic precursors in agnathans (Kusakabe and Kuratani 2005). In early diverging, ancestral actinopterygians such as the sturgeon, a Pax7 antibody labels nuclei in flattened cells on the external surface of the somite, suggesting that sturgeon

external cells include myogenic precursors (Fig. 4A). Sturgeon has in addition some Pax7-positive nuclei deeper in the myotome, on the surface of differentiating cells. Whether these cells are similar to myogenic precursors of amniote satellite cells (Seale et al. 2000) or have another fate remains to be determined. In zebrafish, *pax3* and *pax7* mRNAs are expressed sequentially in the somites (Seo et al. 1998). The Pax7 antibody strongly labels the nuclei of cells on the surface of the somite (Fig. 4B). Some of these nuclei are flattened round nuclei over the external surface of the central part of the somite. *Pax3* mRNA is also expressed in cells external to the superficial monolayer of the myotome, but still apparently within the somite (Fig. 4, C and D and Groves et al. 2005). *Pax3* mRNA is expressed prior to *pax7* mRNA (Seo et al. 1998 and data not shown); this may in part explain why there appear to be more *pax3*-positive cells. The morphology and distribution of these *pax3*- and *pax7*-expressing cells are similar to the external cells, suggesting that external cells in zebrafish include myogenic precursors. As in *Xenopus* and amniotes, the *pax3* and *pax7* expressing cells cover the entire dorsoventral extent of the embryonic myotome. Other Pax7 positive cells have elongated nuclei within the horizontal or transverse septa, which may be within migrating external cells.

We examined the expression of Myf5 and Myogenin to identify specified myogenic precursors. In a cichlid, *Astatotilapia* sp., Myf5 is expressed most strongly in cells at the dorsal and ventral tips of the myotome, subjacent to the external cell layer (Fig. 4E), In zebrafish, Myogenin is expressed in cells at the dorsal and ventral tips of the myotome, but also in cells in the lateral third of the myotome. Some of the most lateral cells expressing Myogenin also express Pax7, suggesting that Pax7-expressing external cells are myogenic (Fig. 4F).

Dermal precursors begin to express collagen genes whereas they are still part of the dermomyotome. In both *Xenopus* and trout, *col1a1* is expressed in dermis and also in external cells, suggesting that external cells share with the amniote dermomyotome the property of including dermal precursors (Grimaldi et al. 2004; Rescan et al. 2005). Similarly, in zebrafish, cells within the somite but external to the superficial slow muscle layer express *col1a2* (Le Guellec et al. 2004; Sire and Akimenko 2004).

In conclusion, our analysis of Pax3, Pax7, Myf5, and Myogenin expression suggests that the external cell layer includes myogenic precursors, whereas the expression of collagen suggests that the external cell layer includes dermal precursors. Gene expression analysis supports a homology between amniote dermomyotome and teleost external cells.

CONSERVATION OF THE DERMOMYOTOME

We propose that the dermomyotome is an evolutionarily ancient structure that is conserved in all vertebrates. Represent-

atives of all vertebrate taxa have a layer of undifferentiated cells external to the embryonic myotome. This external cell layer is a robust cuboidal epithelium in at least one representative of the three major gnathostome clades: chondrichthyans (skate), sarcopterygians (chick), and actinopterygians (trout). We have also shown that actinopterygians share with tetrapods the expression of Pax3 and Pax7 in the external dermomyotomal cell layer. Finally, we have shown that MRF genes are expressed in some of these cells. Below we discuss the practical and evolutionary issues that are raised by the realization that the dermomyotome evolved prior to the radiation of the vertebrates, and has been conserved in teleosts.
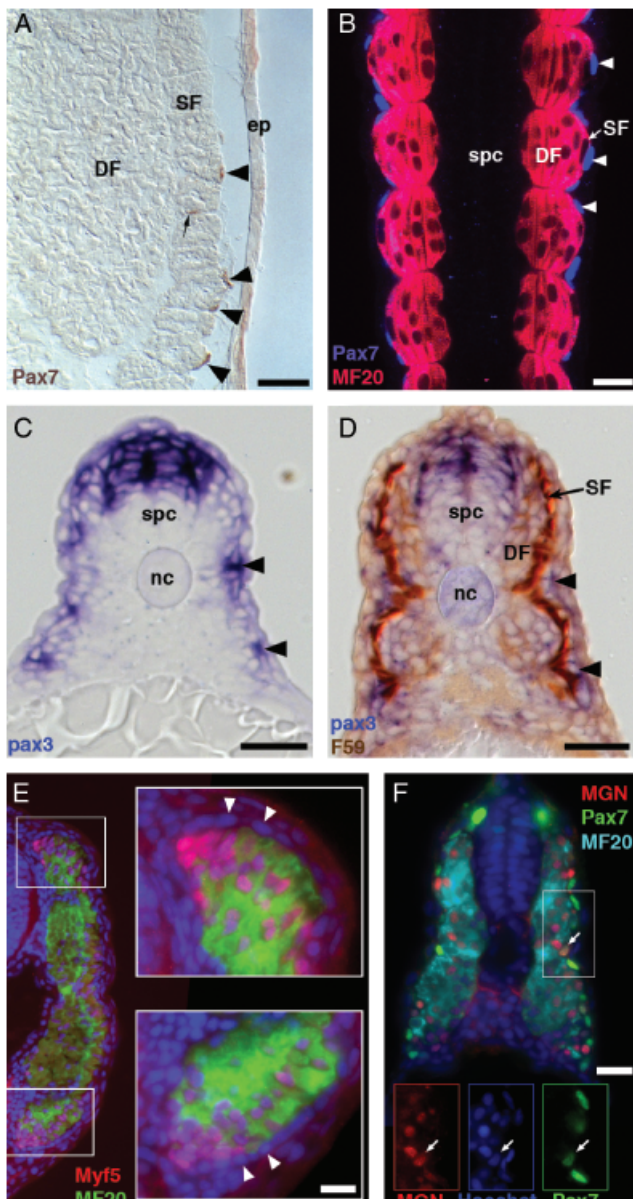
Dermomyotome and myogenesis

The basic cellular mechanism that generates the primary, embryonic myotome is quite similar in all vertebrates that have been examined. In several well-characterized species, including some teleosts, amniotes, and amphibians, myotome formation has been described as a multistage process. Paraxial mesoderm cells closest to the dorsal midline are the first cells to express MRFs; in quail, *Xenopus*, and zebrafish, *myf5* is expressed in the medial cells of the still fully epithelial somite (Pownall et al. 2002). These medial cells probably give rise to the primary myotome (a sheet of mononucleated fibers). This primary myotome is immediately expanded as other cells that were initially not the most medial somitic cells differentiate into muscle fibers. In amniotes, myotome expansion is achieved by the immigration of myogenic cells from the dermomyotome lips into the myotome. The presence of a morphological dermomyotome expressing known myogenic genes

Fig. 4. Cells external to the embryonic myotome express myogenic *pax* genes in actinopterygians (A) Sturgeon, *Acipenser ruthenus*, larva, 14 days post-hatching. Pax7-positive cells (*arrows*) external to, and inserted between, the superficial dorsal myotome fibers in near the anal vent, revealed with a Pax7 antibody (brown, DSHB). Note that the stained cells are at positions similar to those of the external cells in Fig. 3A. Scale bar, 25 µm. (B) Pax7-positive, myosin-negative cells have the same distribution as external cells in zebrafish (*Danio rerio*). Zebrafish embryos at the end of segmentation (24 h) were double labeled with Pax7 antibody (blue, arrowhead) and a myosin antibody (MF20, red) and optically sectioned in the horizontal plane by confocal microscopy. Pax7-positive nuclei are external to the superficial muscle fibers of the myotome, which are distinguished from the multinucleated DF by being mononucleated. Scale bar, 25 µm. (C) Pax3 is expressed in somitic cells on the external surface of the zebrafish myotome (arrowheads). Embryos at the end of the segmentation period (24 h) were labeled for Pax3 (Seo et al. 1998) in whole mount by in situ hybridization and then sectioned transversely. Pax3 is also expressed in the dorsal neural tube. Scale bar, 25 µm. (D) Pax3-expressing somitic cells (purple, arrowheads) in zebrafish are external to the myotome and do not express the superficial muscle marker slow myosin (F59 antibody, brown). Scale bar, 25 µm. (E) Myf5-expressing cells are subjacent to the external cell layer in cichlid embryos. Cichlid (*Astatotilapia burtoni*) embryos at the early pectoral fin bud stage were fixed, and transverse sections from the tail were labeled with antibodies to myosin (MF20, green) and to myf5 (Santa Cruz Biotechnology, Santa Cruz, CA; green), nuclei were counterstained with Hoechst 33258 (blue). External cells form a continuous layer surrounding the MF20-labeled myotome. Myf5 is expressed in myotomal cells subjacent to the dorsomedial and ventral tips of the external layer. Compare with Fig. 2, B, C, and E. Scale bar, 50 µm; inset, 10 µm. (F) Pax7 and Myogenin are coexpressed in zebrafish. Zebrafish embryos at the end of segmentation (24 h) were sectioned and labeled with antibodies against Myogenin (Santa Cruz Biotechnology, green), Pax7, and myosin (MF20, cyan). Myogenin is expressed in cells in the lateral portion of the myotome, the most lateral of which co-express Myogenin and Pax7. Scale bar, 25 µm. SF, superficial fibers; DF, deep fibers; spc, spinal cord; nc, notochord; ep, epidermis.

in all vertebrates strongly argues that at least some cellular and molecular mechanisms of myotome expansion are also conserved. We suggest that in all vertebrates, myogenic precursors express *pax3* and *pax7* as external cells on the surface of the embryonic myotome, and down-regulate these genes as they begin to express MRF genes and move into the myotome.

The cellular embryology of the dermomyotome remains incompletely understood in any organism. In amniotes, dermomyotome origin is generally described as a default that occurs if sclerotome is not induced, but in aquatic vertebrates, where sclerotome develops late, this is not a viable mechanism and the primary choice appears to be dermomyotome versus myotome. In teleosts, it is not clear if the myogenic precursor cells that migrate from the somite to the fin buds are derived from the dermomyotome, as limb bud myogenic precursors are in amniotes (Neyt et al. 2000). Outstanding questions about the dermomyotome in amniotes may be profitably addressed in teleosts. Basic aspects of dermomyotomal cell lineage and cell movements are poorly understood. For example, it is unknown whether the dermomyotome derives from one type of epithelial somite cell or multiple types. Are there any differences in cell fate between dorsal cells in the anterior of the somite and dorsal cells in the posterior of the somite? Are *pax3* and *pax7* expressed in the same dermomyotomal cells? If not, do these transcription factors identify distinct types of myogenic precursor cells? In addition, there is considerable controversy over whether cells from the dermomyotome lips all move directly into the myotome, or whether some first migrate to the rostral or caudal edge of the dermomyotome before entering the myotome (Denetclaw and Ordahl 2000; Ben-Yair et al. 2003). Finally, there is controversy over whether the earliest myotome develops from cells that were never part of the dermomyotome (Kahane et al. 2001; Gros et al. 2004). The small and rapidly developing dermomyotome of zebrafish makes these questions relatively simple to address. The same detailed lineage and time-lapse analyses that have been so fruitful in understanding segmentation and early embryonic myotome specification should be informative for understanding dermomyotome cell lineage and cell behavior. Zebrafish genetic and cell biological approaches provide a new means of unraveling the molecular basis for the initial specification of dermomyotome and its subsequent differentiation into muscle fibers.

Dermomyotome and dermis formation

Within the amniotes, the dorsal dermis is derived from the dermomyotome (Scaal and Christ 2004). In both birds (chick) and mammals (mouse) dermal progenitors have been shown to de-epithelialize from the dermomyotome regions including the dorso-medial lip. Moreover, single cells in dermomyotome can contribute to both dermis and later myotomal myogenesis

in chick (Ben-Yair and Kalcheim 2005). These progenitors first merge into the subectodermal mesenchyme before becoming part of distinct areas of the dermis (Olivera-Martinez et al. 2004; Scaal and Christ 2004). As mentioned above, collagen is expressed in cells on the external surface of the teleost myotome, underlying the epidermis (Le Guellec et al. 2004; Sire and Akimenko 2004; Rescan et al. 2005). Cell lineage labeling can directly test whether these cells are derived from the dermomyotome.

Dermomyotome and vascular formation

The dermomyotome contributes to blood vessels in amniotes. In the avian somite, angioblasts from the dermomyotome migrate into the dorsal dermis and also to the ventrolateral body wall and the limbs (Wilting et al. 1995; He et al. 2003). Clonal analysis in the chick has shown that angioblasts and myoblasts originate, at least in part, from the same precursor cells in the dermomyotome (Kardon et al. 2002). Somitic cells in zebrafish can be converted to a vascular fate by over-expression of a single gene, *scl* (Gering et al. 2003). Whether external cells in teleosts generate vascular endothelial cells during normal development can be readily determined by the types of cell lineage experiments discussed above.

Evolutionary considerations

The dermomyotome is a transient structure in all species, of variable thickness, becoming apparent shortly after somite formation, and disappearing during the early growth period. The thickness of the dermomyotome does not correlate with evolutionary relationships between species, nor does it correlate with ultimate body size or morphology of the adult. Further characterization of the early development of external cells and their subsequent differentiation may indicate whether the variation in thickness simply reflects differences in the relative developmental stage at which we and others have looked for the dermomyotome and/or has functional implications.

There are several possible reasons for dermomyotome conservation. First, other cells of the embryo may depend on the epithelial cell layer for their normal development. These may include neural crest or the primordium of the lateral line, both of which likely migrate on the external surface of the somite in all vertebrates. Second, an epithelial external layer may provide a mechanical constraining force for the embryo prior to the development of more mature connective tissue and epidermis. This mechanical role may be required to shape the myotome. Third, some aspect of myogenic cell differentiation or morphogenesis may require cell–cell interactions that are only possible in an epithelium. Finally, the epithelium may provide a simple and adaptable mechanism for sequestering stem cells capable of contributing to later muscle growth. One or more of these factors may be a developmental

constraint that has maintained an epithelial external cell layer in all vertebrates.

No sign of the dermomyotome has been reported in cephalochordates, raising the question of whether its first appearance coincides with the vertebrate radiation. If the dermomyotome is an ancient and conserved structure that evolved in the last common ancestor of all extant vertebrates, it may have a similar level of significance for developmental evolution as, for example, that already attributed to the neural crest (though clearly in different ways). Although the importance of the dermomyotome has long been accepted in the amniotes, the idea is new for other vertebrates including teleosts.

Acknowledgments

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