Evolutionary history of vertebrate appendicular muscle

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Summary
The evolutionary history of muscle development in the paired fins of teleost fish and the limbs of tetrapod vertebrates is still, to a large extent, uncertain. There has been a consensus, however, that in the vertebrate clade the ancestral mechanism of fin and limb muscle development involves the extension of epithelial tissues from the somite into the fin/limb bud. This mechanism has been documented in chondrichthyan, dipnoan, chondrostean and teleost fishes. It has also been assumed that in amniotes, in contrast, individual progenitor cells of muscles migrate from the somites into the limb buds. Neyt et al.[1] now present the exciting finding that in zebrafishes this presumably derived mechanism involving individual cell migration, is present. They conclude, based on data on sharks, zebrafishes, chickens, quails and mice that the derived mechanism was present in the sarcopterygians. This conclusion, however, may be premature in the light of further data available in the literature, which show a highly mosaic distribution of this character in the vertebrate clade. Furthermore, a developmental mode exists that is intermediate between the supposed ancestral and derived modes in teleosts, reptiles and possibly amphibians. *BioEssays* 23:383–387, 2001. © 2001 John Wiley & Sons, Inc.

Controversy over the developmental origin of fin and limb muscles

The developmental origin of fin and limb muscles in vertebrates has been a subject of controversy for more than 100 years. Only for the chondrichthyan sharks, has the explanation appeared straightforward from the beginning. In 1884, Dohrn[2] found that extensions of the trunk myotomes (embryonal muscle segments) protruded into the limb buds and subsequently developed without de-epithelialization into fin musculature. These findings have since been confirmed,[1,3,4] and the mechanism has now been documented in sharks, rays, sturgeons, lungfishes and some teleost species (e.g. for elasmobranchs in *Torpedo*, *Mustelus*[3] and *Scyliorhynus canicula*,[1] for chondrostei in *Acipenser sturio*,[5] for lungfishes *Ceratodus*;[6] for teleosts in *Salmo salar*,[7] *Exocoetus volicans*,[8] *Salmo gairdneri*[9,10] and *Esox lucius*).[9]

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For vertebrate groups other than fishes opinion has been divided for a long time. Many authors in the first half of the twentieth century assumed that limb and fin muscles originated from mesenchymal cells of the lateral plate mesoderm, at least in the case of amphibian limbs and teleost forefins (e.g. Refs. 7, 11–16). They assumed that bone and muscle precursor cells migrated together to the fin or limb bud. Others pleaded for a somitic origin of the muscles in amphibians.[17,18] An important source of confusion was the lack of a reliable marking technique for tracing migrating mesenchymal cells; in consequence, the presence of migrating cells was often missed. A second source of confusion arose from the experimental transplantation of limb buds to other parts of the flank area. The limb bud was seen as a self-organizing unit that could develop on its own, especially in amphibians. It was not realized, however, that limb buds could recruit cells from somites other than those in the proximity of the limb buds. Adding to the confusion was the finding that extirpation of all somites in the neighbourhood of the prospective limbs did not lead to a disturbance of muscle development in the limb.[17,18] These results caused many scientists to believe that the mesenchymal cells of the limb bud were all derived from the lateral plate mesoderm. The availability of reliable marking techniques in the seventies allowed the important discovery that, in chickens and mice, individual mesenchymal cells of the somites migrate to the limb bud soon after its emergence and subsequently develop into leg and wing musculature.[19–21]

The consensus view in textbooks now is that fin and limb musculature in all vertebrates originates from the somites (dorsal segmented divisions of the paraxial mesoderm), either by extension of epithelial somitic myotomes (fishes) or by the de-epithelialization of the dermamyotome of the somite into individual mesenchymal cells and the migration of these mesenchymal cells to the limb bud (amniotes) (Fig. 1A,B).

Migration of individual muscle progenitor cells from the somites to the fin bud in zebrafishes

Neyt et al.[1] now provide exciting new information on the development of the fin of zebrafishes (*Brachydanio rerio*) that alters our view on the evolutionary history of limb muscle development in vertebrates. They show that the presumably derived mechanism of individually migrating muscle progenitor cells is present in zebrafishes. They marked individual

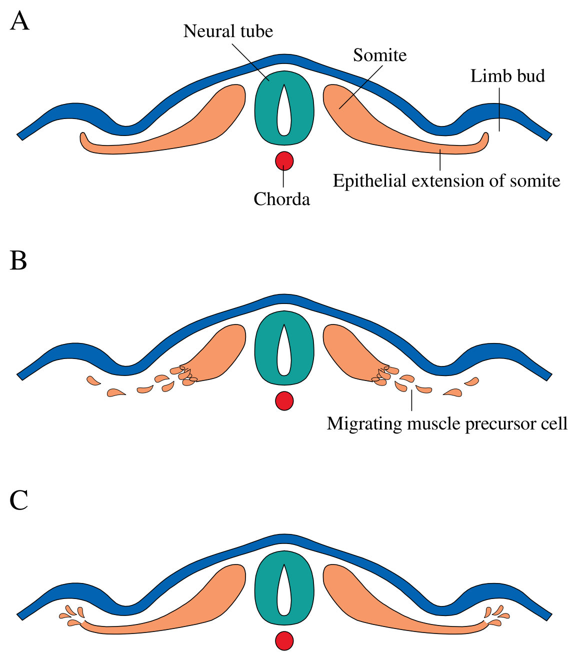

Figure 1. Schematic representation of limb muscle development **A:** by epithelial extension of the somitic myotome to the limb bud (as in sharks), **B:** by de-epithelialization of muscle precursor cells in the somitic myotome followed by migration of the individual mesenchymal cells to the limb bud (as in birds and mammals) and **C:** a stage intermediate between **A** and **B** in which there is epithelial extension to the limb bud followed by de-epithelialization of the distal tips of the somitic myotome and migration of the mesenchymal cells further into the limb bud (as in most teleosts), see also Fig. 2.

somite cells and followed the migration of these cells to the forefin bud. In addition, they found expression of the myogenic transcription factor MyoD only in the fin buds and not in cells between the somite and the fin bud. Based on data on sharks, zebrafishes, chickens, quails and mice, Neyt et al.[1] conclude that the supposed derived mechanism is ancestral for the sarcopterygians. This conclusion cannot yet be drawn, however, on the basis of this information alone. Information is necessary on intermediate groups.

Phylogenetic information on the developmental mechanism of limb muscle formation in vertebrates

Fishes

As mentioned above, the primitive developmental mechanism of epithelial extensions of the somite to the limb bud has been reported in diverse groups of fishes. Significantly, both for lungfishes and the primitive actinopterygian sturgeons, the primitive developmental mode has been reported. Lungfishes are more closely related to tetrapods than teleosts. In teleosts, both mechanisms are present: (1) migration of individual cells from the somite has been shown in zebrafish[1], and (2) the presence of epithelial extensions has been documented for salmons, pikes and flying fishes [7–10] (Fig. 2). Harrison[7] claims that the mechanism of epithelial extension only occurs in the pelvic fins in *Salmo salar*, and no such extensions could be found for pectoral ones. The same pattern has been described by Wiedersheim[22] for the chondrostean *Acipenser sturio.* Mollier,[5] however, showed epithelial extensions for both pectoral and pelvic fin in *Acipenser sturio* and both Corning[9] and Grimm[10] for the pectoral fin of *Salmo gairdneri*.

There is an important difference between the mechanism of epithelial extension of the myotome in teleosts and in elasmobranchs. In teleosts, the muscle progenitor cells dissociate from the epithelium upon arrival in the limb bud[8–10] (Fig. 1C and 2). The limb bud at that stage consists only of

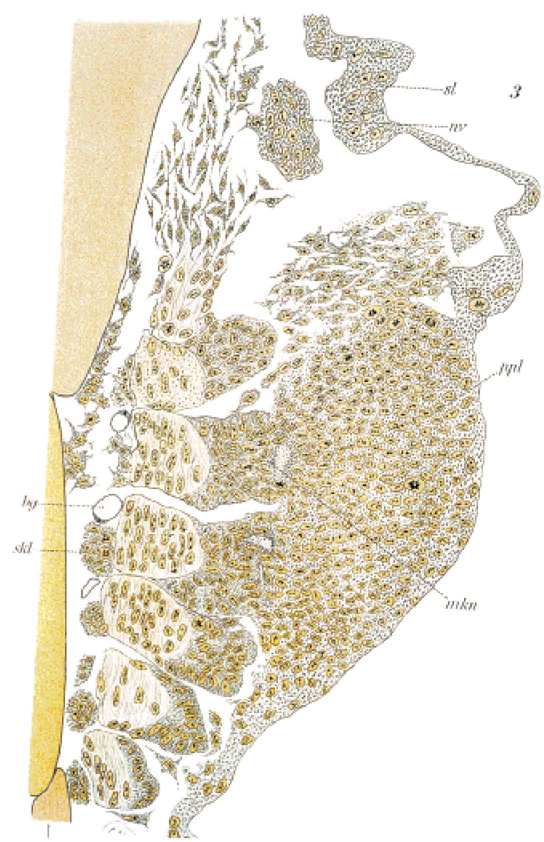

Figure 2. Horizontal section of an *Exocoetus volitans* embryo at the stage of 28–30 somites (330 × ; mkn, epithelial extension of the somite (Muskelknospe); skl, sclerotome; ppl, forelimb bud (Pectoralplatte); bg, blood vessel (Blutgefäsz); nv, nervus vagus; sk, sclerotome; from Derjugin, 1908.

mesenchymal cells. Presumably the mesenchymal muscle precursor cells then migrate into the limb bud. In contrast, in elasmobranchs, the muscle progenitor cells do not become mesenchymal, but differentiate directly into muscle fibers[1–4] (Fig.1A). The mechanism of epithelial extension in these teleosts is, thus, intermediate between that of elasmobranchs and zebrafishes where there is no epithelial extension. There are, thus, at least two developmental modes for the fin musculature in teleosts and both differ from the elasmobranch mechanism: (1) epithelial extension followed by de-epithelialization and migration of mesenchymal cells in most studied teleostean species and probably in lungfishes[6] and (2) de-epithelialization in the somite followed by migration of mesenchymal cells in at least the pectoral fin of zebrafishes. Neyt et al.[1] did not investigate the muscle development of the pelvic fin in zebrafishes. As the pelvic fin develops much later than the pectoral fin, when somites are no longer present, development may involve epithelial extension of the myotome.

Amphibians

In amphibians, the situation is confusing. Epithelial extensions were described in the hindlimbs of the urodelan *Rana sylvatica*, *Ambystoma punctatum* and *Bufo* by Field.[17] Rylkoff[18] supports Field in his idea that the myotome supplies the muscle cells and observes that the border between limb bud and myotome is never sharply delineated (unscharfen Übergang). However, although in Field's figure the myotome is depicted in the proximity of the limb bud, no epithelial extension reaching the limb bud can be observed. In addition, Harrison[23] could find no such epithelial extensions in *Amblystoma punctatum*. Ryke[24] observed in *Xenopus laevis* that "practically from the whole latero-ventral portions of the larval myotome, cells are proliferated and migrate in the direction of the limb bud", suggesting migration of individual cells of the larval myotome to the limb bud. Williamson et al.[25] also assume a myotomal origin of the limb bud muscles and they cite Hinchliffe and Johnson[26] for evidence. Hinchliffe and Johnson[26] do not explicitly state this. On limb muscle development in newts, they cite a study of Romer,[27] but this study is on the lizard *Lacerta* and does not provide the required information for newts that is suggested by Williamson et al.[25] However, Williamson et al.[25] themselves have found indirect support for the migration of individual cells. They show the presence of cytotactin proteins in fibrils of the extracellular matrix that run from the myotome to the limb bud rudiment. Cytotactin proteins are known to be associated with cell migration and differentiation as they are expressed along the pathway of migrating neural crest cells.

Combining the observations of Ryke,[24] Rylkoff[18] and Williamson et al.,[25] it therefore seems likely that individual mesenchymal cells originating from the larval myotome migrate to the limb bud in *Xenopus*. The migration appears to occur early in the development of the limb bud as Nicolas et al.[27] have found that the myogenic transcription factors Myf-5 and MyoD are expressed very soon after the formation of the limb bud. The muscle cells do not originate directly from the somites, because the development of limb buds occurs late in amphibians when the somites are no longer present and especially late in anurans with their extreme metamorphosis. The migrating cells appear to be secondarily derived from the somites via the epithelial larval trunk myotome.

Reptiles

Paradoxically, in all reptiles studied, the presumed ancestral mechanism of epithelial extensions of the somites has been described (Fig. 1). The cells of the epithelial myotome dissociate into mesenchymal cells in the limb bud before differentiating into muscle fibers, as in many teleosts. Reptiles are the most widely studied vertebrate class with respect to limb muscle development and there are reports on 13 species of lizards, turtles, tortoises and snakes (e.g. Corning[29] on *Lacerta agilis*, *L. viridis* and *Natrix natrix*, Rahmani[30] on *Ophisaurus apodus*, Vasse and Pieau[31] on *Testudo graeca*, Vasse[32] on *Emys orbicularis*; Raynaud et al.[33] on *Dermochelys coriacea*, *Anguis fragilis*, *Podarcis sicula* and several *Natrix* species). In snakes and slow worms, limb buds only form rudimentary limbs, but even these limb buds receive somitic extensions which then start to necrotize upon arrival in the limb bud.[30,33]

Birds and mammals

In birds and mammals, the evidence for de-epithelialization in the dermamyotome of the somite and migration of individual muscle progenitor cells to the limb and wing buds is well documented but, as mentioned, only based on data for chickens, quails, mice (e.g. Refs. 19–21) Interestingly, in chickens and mice, the precursor cells for the tongue also migrate as mesenchymal cells and in mice this is also the case for the precursor cells of the diaphragm muscles (e.g. Refs 34,35).

A scenario for the evolution of limb muscle development

Based on these data, gradual evolutionary change seems plausible from epithelial extension without de-epithelialization and migration, as found in elasmobranchs (Fig. 1A), to the advanced mode of de-epithelialization in the somite and migration of individual precursor cells from the somite to the limb as found in birds, mammals and zebrafishes. All these mechanisms involve the dissociation of epithelium into individual mesenchymal cells followed by migration of mesenchymal cells. De-epithelialization of the myotome and short-distance migration, as is observed in reptiles, most of the studied teleosts and probably lungfishes, evolved first in combination with epithelial extension. (Fig. 1C). (In reptiles, the epithelial extension sometimes ends at the limb bud and sometimes reaches further into the limb bud before the de-epithelialization.) In amphibians, the epithelial extension of the myotome to the trunk appears, at least in *Xenopus*, to be followed during metamorphosis by de-epithelialization of larval trunk myotome that is close to the limb bud and migration of the dissociated mesenchymal cells to the limb bud. In the final evolutionary step, in zebrafishes, birds and mammals, de-epithelialization of the muscle precursor cells happens early in embryogenesis in the lateral part of the somitic dermamyotome, followed by migration of the individual cells to the limb bud (Fig. 1B). There is, thus, no epithelial extension of the myotome in this case. The somite lies very close to the limb bud, so that the actual distance of migration from the somite to the limb bud is small.

The molecular pathway for de-epithelialization and migration of mesenchymal cells in chickens has to a large extent been unravelled, (e.g. Refs. 34–36). It is important to find out whether, as seems plausible, similar molecular pathways are involved in the de-epithelialization and migration of the other vertebrate groups.

Possible phylogenetic scenarios for the evolution of migration of individual muscle precursor cells from the somite to the limb bud

The distribution of the presumed ancestral vertebrate mechanism of epithelial extension in limb and fin muscle development is, thus, highly mosaic over vertebrate groups. It has been observed in elasmobranchs, dipnoi, chondrostei, teleostei, reptiles and possibly amphibians. The derived mechanism of individual cell migration without epithelial extension has been found in teleosts, birds, mammals and possibly, amphibians. Chondrostei and dipnoi are closer to tetrapods than teleosts. A possible scenario is, therefore, that the derived developmental mode was acquired several times independently, in teleosts, in the ancestors of birds and mammals and possibly in amphibians. Alternatively, it is possible that the derived mode only developed once and that chondrostei, lungfishes, certain teleostei, reptiles and possibly amphians reverted to the primitive mode.

It is possible that it is easier to revert to the primitive method than to repeatedly evolve the mechanism of migration of individual cells.The step from de-epithelialization of cells of the myotome followed by migration of these cells into the limb bud, as documented in reptiles and some teleosts, to de-epithelialization of cells in the somitic myotomal part followed by further migration, as documented in zebrafishes, birds and mammals, however, may not be a major evolutionary step. Further studies, especially on sarcopteryians and amphibians, but also on more actinopterygian fishes (including the pelvic fin of zebrafishes!) and more species of birds and mammals are urgently needed to further solve the mystery of the evolutionary history of limb and fin muscle development. Studies on the molecular pathways in fin and limb development will allow a rapid unraveling of this long-standing mystery.

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