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Review

Onto-phylogenetic Aspect of Myotomal Myogenesis in Chordata

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This paper presents an onto- and phylogenetic aspect of myotoamal myogenesis in Chordata. A comparative analysis of early stages of myotomal myogenesis in Chordata indicates that the myogenic process in this phylum underwent evolutionary changes. The first stage of the process is myogenesis leading to development of mononucleate mature muscle cells, the most advanced stage is formation of multinucleate muscle fibres.

Key words: Myotomal myogenesis, Chordata.

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Segmentation of chordate embryos arises during embryonic development through a process that subdivides the paraxial mesoderm into repeating metameric units, somites. Body muscles, i.e. trunk and limb muscles, are entirely derived from the somites, whereas the head muscle develops from three sources: somites (somitomeres), paraxial head mesoderm and pre-chordal mesoderm (NODEN 1983; WACHTER & CHRIST 1992; CHRIST & ORDAHL 1995). Each somite gives rise to a myotomal, sclerotomal and dermatomal population of cells. The myotomal cells differentiate into muscle fibres, whereas the dermatomal and sclerotomal cells produce connective tissue. In lower chordates the myotome is the greatest part of the somite, and its cells differentiate in situ. In birds and mammals the myotome is secondarily formed of cells which have emigrated from the dermatomyotome.

Chordata myotomal myogenesis is complicated both cytologically and genetically. Myotomal muscle differentiation is under the control of a complex signalling pathway from the surrounding tissues, particularly the neural tube and notochord which together induce the transcription of myogenic regulatory factors. Candidate molecules for this complex signalling activity include sonic hedgehog (shh) and Wnt protein as positive signals (XUE & XUE 1996).

Members of two classes of transcription factors play an essential role in virtually every step of skeletal muscle development. The MRF (Myogenic Regulatory Factor) family of basic helix-loop-helix (bHLH) proteins includes MyoD (DAVIS *at al*. 1987), myogenin (WRIGHT *et al.* 1989), myf-5 (BRAUN *et al*. 1989; BRAUN *et al*. 1990) and MRF4 (RHODES & KONIECZNY 1989; HINTERBERGER *et al*. 1991). MRFs contain a highly conserved bHLH segment. Basic region binding to the E-box (CANNTG) present in the promoter and enhancer regions of many muscle-specific genes and HLH domain regulating dimerisation (BUCKINGHAM 1992; OLSON 1992). "Master genes" encoding the MyoD family of myogenic regulatory factors are active only in the skeletal muscle or its precursors during chordate embryogenesis and have a distinct spatio-temporal pattern of expression (OLSON 1990).

Besides the MyoD (MRF) family of myogenic transcription factors, members of the MEF2 (Myogenic Enhancers Factor 2) family of transcription factors play an important role in gene activation during muscle cell differentiation (OLSON *et al.* 1995). Vertebrates have four MEF2 genes which generate proteins containing the highly conserved segment MADS and the adjacent domains. MEF2 proteins bind as either homo-or heterodimers to an A/T rich DNA consensus sequence present in the central region of most muscle-specific genes (ARNOLD & WINTER 1998). These two classes of transcription factors code for skeletal muscle gene activation (ARNOLD & WINTER 1998; OLSON *et al*. 1995; MOLKENTIN & OLSON 1996; BLACK *et al*. 1998).

Under the influence of multiple extracellular signals, multipotent mesoderm precursor cells of the paraxial mesoderm become committed to a skeletal muscle fate. The myoblasts begin to express determination-class muscle regulatory factors. The transcription factors regulate the expression of genes encoding muscle-specific elements (e. g. muscle myosin) and cytoplasmic enzymes (e. g. creatine kinase) as well as proteins required for the establishment of electrophysiological properties of the muscle (e. g. acetylocholin receptors).

Early stages of chordate myotomal myogenesis show its great diversity. This diversity provides valuable material for comparative studies of myogenesis. No comparative analysis of myotomal myogenesis has been carried out to date.

Self-differentiation of myogenic cells in Tunicata

The tail muscles of Appendicularia and ascidian tadpoles are organised in the typical chordate manner (FLOOD 1975). Myogenesis of caudal muscle cells in the larvae of *Diplosoma macdonaldi* and *D. occidentalis* (Ascidiacea) begins when the presumptive muscle cells appear in the caudal rudiment. The presumptive muscle cells multiply through mitosis until each rudiment has a requisite number of cells, populating the entire muscle band. The mononucleate cells arrange in two bilaterally symmetrical bands running the length of the tail on either side of the notochord (SATOH & JEFFERY 1995). Tunicate tail muscles show no metameric structure. Mature mononucleate transversely striated muscle cells are connected by tight junctions (CAVEY & CLONEY 1972).

Muscle cells of ascidian embryos (*Ciona robusta*, *C. intestinalis* and *Holocynthia roretzi*) are derived from the B41 pair of blastomeres at the eight-cell stage as was claimed by CONCLIN (1905) and CROWTHER and WHITTAKER (1983), and also from the b42 and A41 pairs (DENO *et al*. 1984; DENO & SATOH 1984). Presumptive muscle cells of the B-lineage (primary lineage) have extensive potential for self-differentiation under the influence of maternal cytoplasmic determinants that are allocated to specific blastomeres during cleavage (NISHIDA 1997), while those of the A and b lineages (secondary lineage) require probably intercellular interaction during gastrulation (MEEDEL *et al.* 1997; NISHIDA 1990).

Ascidian homologues of the vertebrate myogenic MyoD protein are AMD1 (Ascidian MyoD-related factor) in *H. roretzi* and CiMDF (Ciona intestinalis Muscle Determination Factor) in *C. intestinalis* (SATOH *et al*. 1996). This is only one gene for the myogenic bHLH protein from the ascidian genome (ARAKI *et al*. 1994). MEEDEL *et al*. (1997) showed that two differentially regulated CiMDF transcripts were expressed. Transcript CiMDFa appeared first and was gradually replaced by transcript CiMDFb. The temporal differentiation in the expression of CiMDFa and CiMDFb suggests that proteins may regulate muscle development in the primary and secondary lineages, respectively, and that the transcripts CiMDFa and CiMDFb result from differential processing of primary transcripts. They encode two putative MyoD family proteins demonstrating that ascidians, like-vertebrates, use more than one MyoD family protein during their myogenesis (MEEDEL *et al*. 1997).

Vertebrate *MyoD* family genes are activated only in skeletal muscles and thus the presence of the transcripts in larval tail muscle of *C. imtestinalis* implies a relationship between ascidian muscle and vertebrate skeletal muscle (BONE 1989; MEEDEL *et al*. 1997). The product of this gene binds to the E-box, thus inducing activation of a muscle-specific gene in a manner similar to the induction of the myogenic bHLH protein in vertebrates.

Lately NISHIDA and SAWADA (2001) have indicated that the distribution of mach-1 mRNA in eggs corresponds closely to the distribution of muscle determinant. These results also indicate that macho-1 may be a nuclear protein, which is consistent with its putative function as a transcription factor.

In Acrania the myotomal cells differentiate synchronously

Amphioxus is a key organism for understanding evolution of complex vertebrate myotomal myogenesis. The large medial compartment of each somite is the myotome. All of the myotomal cells differentiate synchronously, becoming striated muscle cells constituting the segmental muscle blocks (HOLLAND *et al*. 1995). The myotome is composed of more than one thousand thin lamellae, stacked together above each other. The lamellae are about 1 μm thick (PEACHY 1961; FLOOD 1968). Throughout life each myotomal muscle cell (lamella) remains mononucleate (HOLLAND *et al*. 1995).

The genome of *Branchiostoma floridae* contains two myogenic bHLH genes *BMD1* and *BMD2* (*Branchiostoma* MyoD). A comparison of the amino acid sequences in the bHLH domain between BMD1, BMD2 and four vertebrate myogenic bHLH gene products, however, showed that neither BMD1 nor BMD2 resembled any of the four genes. These results suggest that the duplication of amphioxus myogenic bHLH gene occurs

independently of that leading to the four myogenic bHLH genes in vertebrates (ARAKI *et al*. 1996).

In Agnatha mononucleate muscle lamellae are present only in ammocoetes

Myogenesis is poorly known in lamprey and hagfish. In lamprey larvae (ammocoetes), as in *Amphioxus,* the myotomes are composed of lamellae stacked horizontally on top of each other, extending along the whole length and width of the myotome. Each muscle lamella has one nucleus (NAKAO 1977; ROVAINEN 1979). In adult lampreys the myotomes are built of slightly flattened multinucleate muscle fibres (FLOOD *et al*. 1977). It is unknown how the mononucleate muscle lamellae develop into multinucleate muscle fibres.

The hagfish is the only other surviving cyclostomate, besides the lamprey. Although these two vertebrates are sometimes considered to be equally primitive, the myotomes of the hagfish are distinctly more advanced than those of the lamprey. The myotomal muscle fibres of the hagfish are nearly cylindrical rather than flat (ROVAINEN 1979).

In Chondrostei the presence of muscle lamellae is not questioned

In "ancient" teleost fishes, such as sturgeon *Acipenser stellatus*, the myotome is built of wedge-shaped, flattened multinuclear lamellae, stacked on top of each other in a dorso-ventral direction. Successive lamellae within the stack have their bases alternating in a medial and lateral direction. The lateral wedges withdraw most of their medial processes and assume the shape of fibres. The medial lamellae split off cylindrical fibres from their medial and possibly from their lateral borders. Most of the lamellae are transformed into irregular cylindrical fibres. Mitoses appear in the dermatomal layer superficial to the myotome. Numerous mitotic and post-mitotic cells migrate from the dermatome through the lateral layer of muscle fibres. Intermediate fibres originate by multiplication, migration and fusion of cells derived from the dermatome. The number of fibres increases by splitting of pre-existing fibres rather than by differentiation *de novo* from the myoblast fusion (FLOOD *et al*. 1987).

During myotomal myogenesis in *Acipenser baeri* the cells of the somite wall elongate, assume a conical shape and fuse to form multinucleated lamellae. This process starts near the notochord and shows a medio-lateral gradient in each somite. The multinucleated lamellae of the white muscle area are oriented perpendicularly to the notochord, while the superficial red fibres remain mononucleated and insert with long processes between the underlying white muscle lamellae. After hatching, the multinucleated lamellae transform into cylindrical muscle fibres. The mechanism behind this process is as yet unknown. The first cells within the intermyotomal fissures appear just before hatching. In the later developmental stages, cells that are structurally similar to those in the intermyotomal fissures are also observed within the myotomes where they insert between the differentiated red and white fibres. These cells may play the role of stem cells for subsequent hyperplastic growth (DACZEWSKA, STOIBER, STEINBACHER in preparation).

In Teleostei the myotomal cells show different developmental potential

1. Myotomal cells have full developmental potential

The zebrafish *Brachydanio rerio* provides a particularly good model for studying early myotomal myogenesis (WATERMAN 1969). DEVOTO *et al.* (1996) characterised two distinct populations of muscle precursors in the segmental plate, adaxial cells adjacent to the notochord and cells lateral to them, which they have named presomitic cells. Adaxial cells migrate radially through the somite. Following migration they form a monolayer of superficial muscle cells (slow muscle fibres). In contrast, lateral presomitic cells remain within the deep portion of the myotome where they become polynuclear through fusion and give rise to fast muscle fibres (VAN RAAMSDONK *et al*. 1974).A subset of adaxial cells does not migrate completely to the lateral surface of the myotome, and instead extends from the notochord to the lateral surface of the somite at the level of the future horizontal myoseptum. These cells are the first muscle fibres to show contractile activity (VAN RAAMSDONK *et al*. 1978; HATTA *et al.* 1991). Cells located on the lateral surface of the myotome remain mononucleate (WATERMAN 1969).The zebrafish does not provide a model for muscle growth since it only reaches a modest ultimate body size of 3-5 cm, and post-larval fibre recruitment is therefore of minor importance in this species (JOHNSTON 1999).

The adaxial cells are committed to becoming myoblasts within a specific slow muscle lineage (DEVOTO *et al.* 1996) under the influence of the glycoprotein Sonic hedgehog (Shh) (LEVIS *et al*. 1999) secreted from the notochord (BLAGDEN *et al.* 1997). Another member of the Shh gene family secreted from notochord also plays a role in the formation of the first muscle cells (CURRIE & INGHAM 1996).

Although the myogenic bHLH proteins are essential for the establishment of muscle cell precursors and their differentiation, much less is known about the process of commitment of mesodermal precursors to the myogenic lineage. MyoD expression first occurs in an early phase, extending from the mid-gastrula to just prior to somite formation. Transcripts are first found only in adaxial cells and the expression zone spreads laterally just prior to somite formation. MyoD expression therefore appears to mark specification of myogenic precursor cells rather than the onset of differentiation. During somite formation, *myogenin* expression follows that of *MyoD* by one to two hours (DEVOTO *et al.* 1996; WEINBERG *et al.* 1996).

Multiple isoforms of MEF2 have also been identified in zebra fish. MEF2D is first activated in presomitic mesoderm, followed by MEF2A and MEF2C (TICHO *et al.* 1996).

In the Teleostei, *Thymallus thymallus*, *Salmo gairdnerii* and *Dicentrarchus labrax* have full developmental potential of myotomal muscles. In *Th. thymallus* the arrangement of the cells in the somite determines their further fates during myogenesis. Two cell populations have been distinguished. First to start differentiating are those cells that build the somite sac wall. These cells elongate centripetally, with their apical parts overlapping. The process of myotube formation starts with fusion of the elongated inter-kinetic myoblasts. Cells which initially were situated in the somite centre remain in the myotome, between the myotubes. In mononucleate cells mitotic figures often occur. A characteristic feature of this stage of myogenesis is the presence of numerous presumptive myogenic cells between the myotubes and at their base (Fig. 1). Mononucleate cells do not fuse with each other, their role consists of fusing with already existing myotubes (MERKEL 1995).

In *S. gairdnerii* mitotic division are observed in the population of presumptive muscle cells containing myofilaments. Myoblasts fuse with each other into multinuclear myotubes. Among them are less differentiated presumptive myoblasts dividing mitotically (Fig. 1). The post-mitotic myoblasts may fuse with the neighbouring myotubes. These cells, found among the muscle fibres, are referred to as myosatellite cells. It is likely that myosatellite cells are precursors of the new differentiating fibres (NAG & NARSALL 1972).

In *D. labrax* myogenic cells become activated in various stages of larval life. In its first half the new fibres of fast muscle are derived from the germinal zone of presumptive myoblasts, lying beneath the red muscle layer. In the second half of larval life, new fibres are produced in the same zone that forms intermediate (or pink) muscle layer. Dorsoventrally the myotome grows throughout larval life, largely by addition of new fibres from germinal zones at the hypo- and epi- adaxial extremites. Towards the end of larval life all these germinal zones become exhausted, but another source of fibres arises as satellite cells, associated with large diameter of presumptive white fibres, is activated to produce new fibres (VEGETTI *et al.* 1990; WATABE 1999).

2. Participation of myotomal cells is insufficient for muscle development

In herring (*Clupea herengus*) embryos several classes of myoblasts were identified. First, a relatively small number of myoblasts (2-6) forms lateral to the notochord in each myotome. These cells elongate to span the whole myotome and give rise to mononucleate muscle fibres. These mononucleate cells correspond to the pioneer cells. A second class of myoblasts gives rise to multinucleated myotubes by the fusion of 2-5 myoblasts. They form the majority of muscle fibres. Prior to hatching another population of myoblasts starts to divide. BrdU labelled cells appear in the myosept between the muscle fibres and on their surface. These latter cells almost certainly correspond to the presumptive satellite cells (JOHNSTON *et al.* 1995).

In rainbow trout (*Oncorhynchus mykiss*), following completion of mitotic divisions, the cells elongate and then fuse to form multinucleate myotubes. The mesenchymal cells derived from muscle precursors are thought to enter the myotomes via the myosepta. These cells migrate deeper into the myotome where they divide mitotically prior to further differentiation and may function as myosatellite cells (STOIBER & SÄNGER 1996). In *O. mykiss* two non-allelic *MyoD* genes: *T MyoD* and *T MyoD2*, exhibit a distinct spatio-temporal pattern of expression that defines discrete cell populations in the developing somite. T MyoD transcripts are detectable within presomites and somite medial cells adjacent to the notochord. Later in development T MyoD expression remains confined to the medial domain of a somite for a long time. By contrast, initial expression of T MyoD2 occurs in somites that have already formed and is limited to the posterior compartment of the somite. However, later the TMyoD2 transcript disappears progressively from the inner part of the myotome. TMyoD and TMyoD2 mRNA are probably transcribed from two distinct genes which were duplicated during the tetraploidization of salmonid genes. The two trout MyoD encoding genes have evolved to become functionally different (RESCAN & GAUVRY 1996; DELALANDE & RESCAN 1999).

The expression of T myogenin is detected first in adaxial cells of the forming somite. Shortly after the formation of the somite, T myogenin expression extends from adaxial cells to the posterior lateral regions of the somite and then progresses towards the anterior region. T MyoD, T MyoD2 and T myogenin are expressed in the developing somite, suggesting that they play distinct roles in the early myogenesis of trout (RESCAN *et al*. 1999). MRF4 has not been reported in fish (WATABE 1999).

In *Rutilus frisii meiolingeri*, *R. rutilus* and *Chalcolburnus chalcoides mento* the myotomal cells are mitotically quiescent. They start to elongate and fuse. Embryonic muscle growth is first mainly due to hypertrophy of the newly established myotube. Before hatching, however, deep muscle growth is aided by superficial addition of cells that originate from the adjacent mesenchymal lining (Fig. 3). These cells may function as myosatellite cells providing the stem cell population for muscle growth (STOIBER & SÄNGER 1996).

In *Cyprinus carpio* , MyoD, myf-5 and MEF2C transcripts are present in the first three somites. However, myogenin and MEF2A transcripts occur at later developmental stages. The level of MyoD, myogenin and MEF2A transcripts declines after hatching and myf-5 gives only a weak signal in older fish. The relatively high levels of MRFs mRNA in juvenile fish probably reflect the recruitment of new muscle fibres from the satellite cell population (KOBIYAMA *et al.* 1998).

Myotomal myogenesis in amphibians

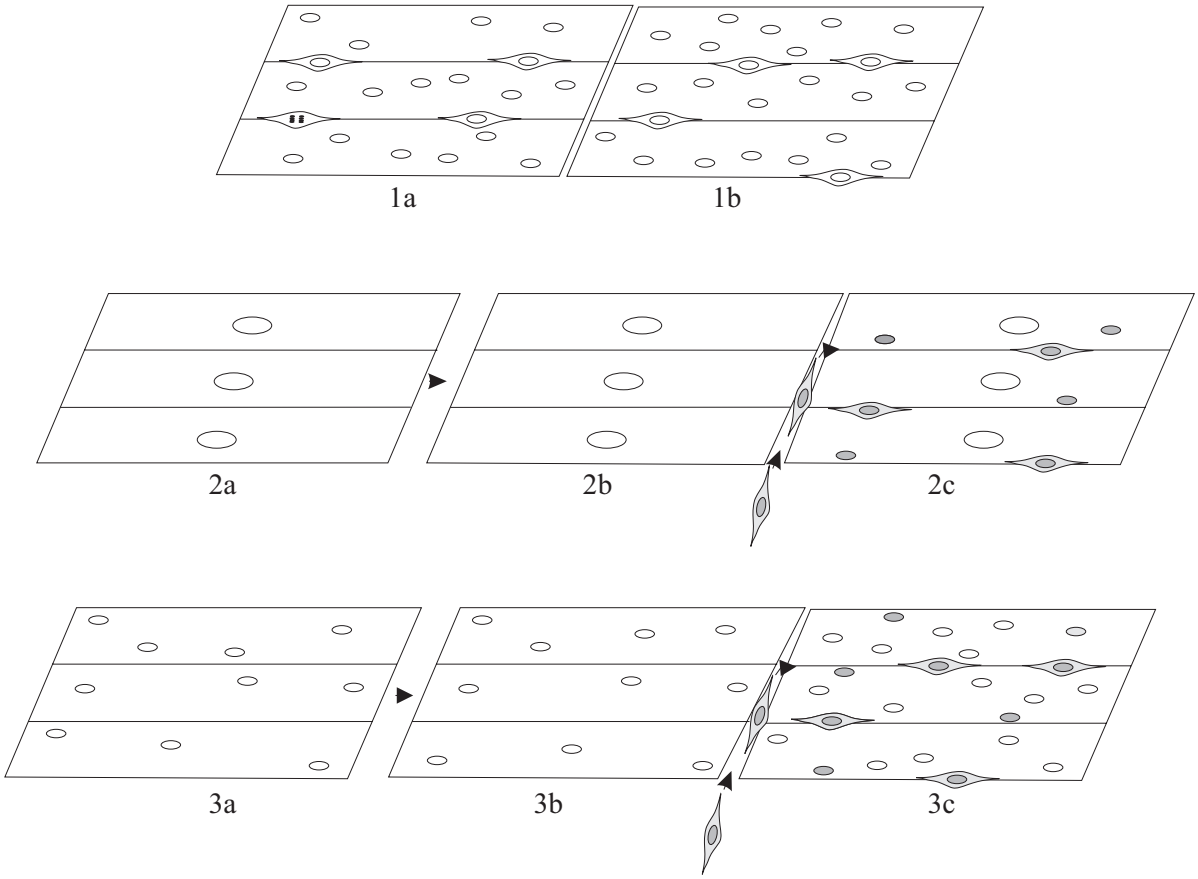
1. Synchronous myogenesis leads to a development of mononucleate myotubes

In *Xenopus laevis* and *Hymenochirus boettgeri* (Pipidae) segmentation is accomplished by rotation of cells through 90^0 around a vertical axis, so that their long axes come to lie parallel to the anterior-posterior axis of the embryo (HAMILTON 1969; KIEŁBÓWNA 1981; YOUN & MALACINSKI 1981; RADICE *et al.*1989; DACZEWSKA 2001).

The elongated myotomal cells of *X. laevis* and *H. boettgeri* span the full length of the myotome and they eventually produce myofibrils and a sarcotubular system (KIEŁBÓWNA 1966; MUNTZ 1975; BLACKSHOW & WARNER 1976; PENG *et al.* 1981; HUONG & HACKADAY 1988; DACZEWSKA 2001) (Fig. 2). In *X. laevis* a single large nucleus is centrally located in the mononucleate myotubes and contains up to an octoploid quantity of DNA. Moreover, it shows a considerable increase in the content of nuclear RNA and histones, proportional to the quantity of DNA (KIEŁBÓWNA 1966; 1971; 1973). In *H. boettgerii* differentiating mononucleate myotubes are initially not accompanied by any other cells. In futher devevelopmental stages, mesenchymal cells appear in the intermyotomal fissure and then in the myotomes. Their role depends on their position. Mesenchymal cells remaining in the intermyotomal fissures differentiate into fibroblasts (collagen fibres were observed next to them), while those that have migrated into myotomes, between myotubes, transform into secondary myoblasts. These cells fuse with the already existing mononucleate myotubes, resulting in an increase in their size and number of nuclei (DACZEWSKA 2001). The mechanism of formation of multinucleate muscle fibres in *X. laevis* is debatable (KIEŁBÓWNA 1966; MUNTZ 1975; BOUDJELIDA & MUNTZ 1987) and requires a re-examination. Preliminary studies indicate that the source of secondary myoblasts are mesenchymal cells fusing with a mononucleate myotube (KIEŁBÓWNA & DACZEWSKA in preparation). In *X. laevis MyoD*-related genes have received attention because of their potentially early position in the mesoderm inductive pathway (HOPWOOD *et al.* 1989). X MyoD protein accumulates in nuclei of the future somite mesoderm from the middle of gastrulation. In neurulae and tail bud embryos it is expressed specifically in the myotomal cells of the somite. The highest levels of MyoD have been found in the presomite mesoderm, suggesting a role of the notochord in induction or maintenance of MyoD expression (HOPWOOD *et al.* 1989; SCALES *et al.* 1990; HARVEY 1992). In *X. laevis myf-5* gene is co-expressed with *MyoD* during development, suggesting that the two genes act together as the primary myogenic switch (HOPWOOD *et al*. 1991).

In *H. boettgeri*, MyoD was detected in the nuclei of nonsegmented mesoderm cells and in the myotomal cells of the forming somite, during their rotation and of the primary myoblasts (DACZEWSKA 2001). In *X. laevis* and *H. boettgeri,* after myogenesis initiation, the MyoD protein is degraded slowly (HOPWOOD *et al*. 1992; HARVEY 1992; DACZEWSKA 2001). Thus a high level of MyoD expression is required for initiation but not for maintenance of myogenesis (HARVEY 1992). It is interesting that MyoD in *H. boettgeri* re-appears at an advanced stage of myogenesis, in the nuclei of mesenchymal cells prior to their fusion with the myotube (DACZEWSKA 2001).

In *Bombina variegata* (Discoglossidae) the elongation of the myoblasts is directed towards the proximal and distal myotome borders (Fig. 2). Differentiating mononucleate myoblasts remain in the inter-kinetic phase. The lack of ^{3}H-thymidine labelled nuclei in myoblasts of the young myotomes and tetraploid quantities of DNA in their nu-



Figs 1-3. Fig. 1 Myogenesis in *Thymallus thymallus*, *Salmo gairdnerii*. 1a, 1b – multinucleate myotubes and mononucleate myoblasts fusing with them. Fig. 2. Myogenesis in *Xenopus laevis*, *Hymenochirus boettgeri*, *Bombina variegata*. 2a – mononucleate myoblasts, 2b – mononucleate myotubes, 2c – mesenchymal cells in the intermyotomal fissure and in myotomes between myotubes. Two classes of myotube nuclei: large nuclei of mesodermal origin and small nuclei of mesenchymal origin. Fig. 3. Myogenesis in *Chlupea herengus*, *Oncorynchus mykiss*, *Rutilus frisii meiolingeri*, *R. rutilus* and *Chalcolburnus chalcoides mento*, *Pelobates fuscus*, *Rana lessonae*, *Triturus vulgaris*. 3a, 3b – multinucleate myotubes, 3c – mesenchymal cells in intermyotomal fissure and in myotomes between myotubes. Multinucleate myotubes with nuclei of mesodermal and mesenchymal origin.

clei indicate that the myoblasts have withdrawn from the cell cycle in the G2/G0 phase. Myofibrils appear in differentiating mononucleate myotubes and grow in number with progressing differentiation. The migration of mesenchymal cells penetrating into the myotomes was traced using ^{3}H-thimidine. The marked cells appeared first in intermyotomal fissures, then in the myotomes. In *B. variegata* polykaryocytes arise as a result of fusion of mesenchymal cells (secondary myoblasts) with mononucleate myotubes. In post-fusion myotubes, two types of nuclei are found, differing in their origin, size and DNA content. The large nucleus of the myotube contains a tetraploid quantity of DNA (4C DNA) whereas the smallest nuclei of the secondary myoblasts contain diploid quantities of DNA. Fusion of secondary myoblasts with myotubes takes place when they are in the G1/G0 phase of the cell cycle (KIEŁBÓWNA & KOŚCIELSKI 1979).

Mesenchymal cells migrating into the myotomes are of sclerotomal and/or dermatomal origin. Their primary feature is the ability to differentiate into connective tissue. They may acquire myogenic potential at a specific developmental stage and in a specific place. In *B. bombina* dermatome and/or sclerotome cells, after leaving the somite, migrate ventralwards. There they enter in contact with somatopleura and start myogenesis of musculus rectus abdominis. Myogenesis starts with a linear arrangement of myoblasts and their fusion (KIEŁBÓWNA 1993). The myogenesis of this muscle follows the model of mesenchymal muscle formation and clearly differs from myotomal myogenesis studied in *B. variegata*.

2. Synchronous myogenesis leads to the development of multinucleate myotubes

In *Pelobates fuscus* (Pelobatidae) the primary myotubes originate from all the myotomal cells and are formed synchronously in each myotome. The number of nuclei per myotube ranges from 3 to 5 (Fig. 3). Striated myofibrils are present in the myotube sarcoplasm. Mesenchymal cells appear

in the myotomes. Spindle-shaped cells adjoin the myotubes. The increase in the number of the fibre nuclei provides direct evidence of the fusion of these cells with the myotomal myotubes. At later developmental stages the spindle-shaped secondary myoblasts form long cords on the surface of the muscle fibres along their long axis. Such an arrangement of the myoblasts precedes their fusion into multinucleate secondary myotubes. In *P. fuscus* the primary muscle fibres develop from the myotomal and mesenchymal cells, but the secondary muscle fibres differentiate only from the mesenchymal myoblasts (KIEŁBÓWNA 1987).

In *Rana lessonae* (Ranidae) myotomal myogenesis follows a course similar to that described for *P. fuscus* (Fig. 3). All the myotomal cells are engaged in myotube formation. The myotubes are a result of fusion (2-3) of myotomal myoblasts. Densitometric measurements of relative DNA content in the nuclei revealed 2C DNA. At later developmental stages mesenchymal cells appear in intemyotomal fissures and then in the myotomes, between the myotubes. These cells fuse with primary myotubes. DNA content in the myotube nuclei is at the 2C level. Myotomal myoblasts and myoblasts of mesenchymal origin prior to the fusion withdraw from the cell cycle at G1/G0 phase (DACZEWSKA & PAŁUCKA 1999).

In *Triturus vulgaris* (Salamandridae) myotomal myogenesis resembles that in *P. fuscus* and *R. lessonae*. Myotomal cells (primary myoblasts) fuse to form 2-3-nucleate myotubes (Fig. 3). At further developmental stages the mesenchymal cells enter the intermyotomal fissure and then they migrate to the myotomes. The cells that remain in the fissure retain fibroblastic potential (they synthesise collagen fibres). Their daughter cells acquire myogenic abilites, their myoblastic potential is evidenced by their ability to fuse with myotubes. The appearance of new nuclei in the myotubes provides indirect evidence of their position with the myotubes. The nuclei of primary myoblasts are located in the axis of the myotube, whereas the nuclei of secondary myoblasts are situated in the sarcoplasm close to the sarcolemma (DACZEWSKA & KIEŁBÓWNA 2000).

In all our studies on amphibian myotomal myogenesis, differentiation of primary myotubes was accompanied by vitellolysis. Myotubes acquire extra nuclei after yolk plate material has been used up. A more detailed analysis of yolk plates during vitellolysis has been done for myotomal myogenesis of *X. laevis* (KIEŁBÓWNA 1975).

Myotomal myogenesis in birds and mammals

In amniota the somite forms as a spherical epithelial ball, derived from the paraxial mesoderm (CHRIST & ORDAHL 1995). Signals from adjacent tissues play an important role in the initial patterning of somite along the dorsoventral and mediolateral axes (ORDAHL & LE DOUARIN 1992; CHRIST *et al.* 1992; DIETRICH *et al.* 1997). Epithelial somites are subdivided into mesenchymal compartments, sclerotome and dermatomyotome with different fates, by signals emanating from the surrounding tissues (CHRIST *et al.* 1992). The dermatomyotome is the primary source of cells for initial formation of the myotome (WILSON-RAWLS *et al.* 1999).

The neural tube and notochord are crucial in patterning myogenic cell lineages during avian and mammalian somite myogenesis. Some studies point to *Wnt* gene family members (STERN *et al.* 1995; TAJBAKHSH & BUCKINGHAM 2000), others suggest that a combination of Wnt signalling and Sonic hedgehog (Shh)is required to initiate myogenesis within the somite (MÜNSTERBERG *et al.* 1995; COSSU *et al.* 1996; BORYCKI *et al*. 1999). The source, identities and action of the signals governing this process remain controversial (see DIETRICH *et al.* 1997). It is unclear how these signals activate transcription of MRF genes.

Cells destined to form skeletal muscle fibres in avian embryos are first identified in spheres of epithelised paraxial somite mesoderm. POWNALL & EMERSON (1992) suggest that the determination of a myogenic cell lineage in quail is a progressive process controlled by the influence of the neural tube on the expression of the *qmf* regulatory genes in newly forming somite. Quail express at least three of the four myogenic regulatory genes that have been described in mammals: *qmf1* homologue *MyoD*, *qmf-2* homologue *myogenin* and *qmf-3* homologue *myf-5*. In quail, *qmf-1* and *qmf-3* are activated sequentially in medially located somite cells, immediately following somite formation. All of these regulatory genes: *qmf-1*, *qmf-2*, *qmf-3,* are expressed in the myotome compartmentalised somites. As the somite matures in an antero-posterior direction, this pattern of expression of the MRF genes is recapitulated and thus appears as waves of expression moving posteriorly.

Most studies dealing with regulation of myogenesis consider the dorsomedial lip of the dermatomyotome to be the initial and major source of progenitors that populate the myotome (PRZYBYLSKI & BLUMBERG 1966; CHRIST & ORDAHL 1995; KAEHN *et al*. 1988). KAHANE *et al.* (1998 a, b) re-evaluated the problem of myotome formation in quail. The origin of the myotome resides in a group of early post-mitotic cells located along the medial aspect of the epithelial somite, prior to dermatomyotome formation. A subset of early post-mitotic progenitors give rise to the primary myotome. These cells lose epithelial conforma-

tion, migrate and then elongate to give rise to the primary longitudinally oriented muscle cells – pioneer cells. The second wave is responsible for myotome expansion. This is achieved by cell addition from both the rostral and caudal lips of the dermatomyotome. Because dermatomyotomal lip progenitors are mitotically active within the somite epithelia, but exit the cell cycle upon myotome colonisation, they can only provide for limited myotome growth, and subsequent waves must take over to ensure further muscle development. Taken together, the data suggest that myotome formation is a multistage process.

In the chicken the first indication of myotome formation appears at the dorsomedial edge of the somite. There is a transition in shape from an oval-pyramidal to an elongated cell. With elongation, the longitudinal axis of the myoblast becomes aligned with the long axis of the embryo. All spindle-shape cells have many thin and thicker myofilaments which aggregate and begin to form the sarcomere (HOLTZER *et al*. 1957; ALLEN & PEPE 1965; STOCKDALE & HOLTZER 1961; PRZYBYLSKI & BLUMBERG 1966). The unique feature of this initial stage of myogenesis is that all events lead to the formation of a functional unit of striated muscle cells. The progression to multinuclearity was evidenced by fusion of mononuclear cells showing a fair degree of differentiation. Subsequently, myotubes increase their number of nuclei by fusion with peripherally located cells which constitute the embryonic stem cell population. The myoblast stem cell population often presents various stages of mitosis (PRZYBYLSKI & BLUMBERG 1966).

According to KAHANE (1998a, b) the pioneer cells which give rise to the primary cells in quail, correspond to myofibril-containing myoblasts in chicken. The cells which populate the myotome in the second stage according to KAHANE *et al.* (1998b) correspond to the stem cell population in chicken, the populations taking part in the myotube growth. Further stages of myotomal myogenesis in birds are unknown.

In mammals specification of somite cell fate depends on epigenetic factors. Several Wnts molecules have the potential both to activate, directly or indirectly, transcription of *Myf-5* and *MyoD* and to promote survival and expansion of the committed population (COSSU & BORELLO 1999). It is important to recognise that signals from the neighbouring tissues do not lead directly to terminal myogenic differentiation at least in the majority of somite cells exposed to the signalling molecules. The cells exposed to Shh and Wnt signals are induced to differentiate terminally into myotomal muscle, while the remaining cells are probably kept in a committed but undifferentiated state. In this state, cells will divide, proliferate and sometimes die until the correct number of myoblasts is attained in the right place and at the right time to produce primary and secondary fibres and satellite cells during later development (COSSU *et al.* 1996; COSSU & BORELLO 1999).

Transcripts of Myf-5 are first detected in epithelial-like cells of the ball-shaped somite and then accumulate in the dermatomyotome, in both rostral and caudal lips prior to myotome formation (OTT *et al.* 1991). The *Myf-5* gene concentrates in the dorsal lip region of the dermatomyotome adjacent to the neural tube and subsequently in the forming myotome (VANTERS *et al.* 1999). *Myogenin* is first detected in the myotome rostral somite. *Myogenin* and *MRF4* appear to be expressed only in cells which are already determined to become muscle. MyoD transcripts are not detectable until much later. MyoD transcription begins, as in myogenin transcription in the multinucleated myotube (SASSOON *et al.* 1989).

Early myogenesis is governed by a complex set of morphological and migratory events which begins with the colonisation of the medial myotome by cells originating from the dorsomedial lip of the dermatomyotome (ORDAHL & LE DOUARIN 1992).

In the mouse, myogenic myotomal precursors appear predominantly within the dorsomedial lip of the dematomyotome (LYONS & BUCKINGHAM 1992; ORDAHL & LE DOUARIN 1992). Then they migrate through the rostral and caudal lips before entering the central myotome and elongating in two directions. Myotomal myocytes (pioneer cells) were found to be mononucleate cells spanning the entire rostrocaudal extent of the myotome, the nuclei of these cells appear large in comparison with the nuclei of the new myocytes which are added at the medial aspect of the myotome (VENTERS *et al.* 1999). The new cells derive from both rostral and caudal dermatomyotomal lips. In cells with more than one nucleus the size of the nuclei often vaied widely. It is possible that this size differential may represent a difference between the large nuclei of the pioneer myocytes and the smaller nuclei of the new myocytes. The results allow for the development of a model of mammalian myogenesis where growth occurs medially by the addition of new cells while mature myocytes are displaced laterally (VENTERS *et al.* 1999; WILSON-RAWLS *et al.* 1999).

Other studies indicate that the entire dermatomyotome epithelium is potentially a source of myogenic progenitor cells (TAJBAKHSH & SPÖRLE 1998).

The migratory routes of muscle progenitor cells from the dermatomyotome epithelium to the formation of the myotome have been described exten-

sively and different models have been proposed. At present, this issue remains unsolved. The further development of mammalian myotomal muscles is unknown.

Concluding Remarks

A comparative analysis of early stages of myotomal myogenesis in *Chordata* indicates that the myogenic process in this phylum follows an evolutionary course. The process starts with myogenesis, which leads to development of mononucleate muscle cells which either constitute the final stage or represent a transitional form.

Mononucleate muscle cells develop in the tail of tunicates. Presumptive muscle cells have extensive potential for self-differentiation under the influence of maternal cytoplasmic determinants (CONCLIN 1905; NISHIDA 1997). In the tunicates there is one gene for myogenic bHLH protein and its two transcripts encode two putative MyoD family proteins (MEEDEL *et al.* 1997).

In amphioxus the myotomal cells differentiate into mononucleate mature muscle lamellae (HOLLAND *et al.* 1995). The genome of amphioxus contains two myogenic bHLH genes, BMD1 and BMD2. ARAKI *et al.* (1996) suggested that the duplication of the amphioxus myogenic bHLH gene occurred independently of that leading to the four myogenic bHLH genes in vertebrates.

In lamprey larvae, as in amphioxus, the myotomes are composed of lamellae (NAKAO 1977; ROVAINEN 1979). In adult lampreys the myotomes are built of slightly flattened multinucleate muscle fibres (FLOOD *et al.* 1977).

Mononucleate muscle cells also differentiate in the myotomes of the zebrafish and herring, but in the neighbourhood of multinucleate fibres. Mononucleate cells, the pioneer cells, are the first ones that display contractile activity (DEVOTO *et al.* 1996; JOHNSTON *et al.* 1995). Differentiation of pioneer cells in the zebrafish is under the influence of sonic hedgehog (shh) (LEWIS *et al*. 1999; BLAGDEN *et al.* 1997). In the zebrafish, *MyoD* expression first occurs prior to somite formation, *myogenin* expression following that of *MyoD* (DEVOTO *et al*. 1996; WEINBERG *et al.* 1996).

Myotomal myogenesis in some amphibians, namely *Xenopus laevis*, *Hymenochirus boettgeri*, *Bombina variegata*, is an exception. In these species all myotomal cells differentiate into mononucleate myotubes, capable of contracting (KIEŁBÓWNA 1966 KIEŁBÓWNA & KOŚCIELSKi 1979; DACZEWSKA 2001). These myotubes provide material for the development of multinucleate myotubes. Expression of the MyoD gene in *X. laevis* manifests itself at early stages of development. The high level of MyoD in presomitic mesoderm suggests a role of the notochord in the induction and maintenance of MyoD expression (HARVEY 1992). In *X. laevis* and *H. boettgeri*, after myogenic initiation, the MyoD protein is degraded slowly (HOPWOOD *et al.* 1992; HARVEY 1992; DACZEWSKA 2001). In *X. laevis* the *myf-5* gene is co-expressed with *MyoD* during development.

A combination of Wnt signalling and Shh are required for initiating myogenesis within the avian and mammalian somite (COSSU *et al*. 1996; BORYCKI *et al*. 1999). Large mononucleate myocytes develop at an early stage of bird and mammal myogenesis.

In quail a subset of post-mitotic progenitors give rise to primary longitudinally oriented muscle cells, pioneer cells (KAHANE 1998 a, b). In the chicken they are probably myoblasts with striated myofibrils developing transversely. Such highly differentiated myoblasts fuse with one another into multinucleate myotubes. In their neighbourhood myogenic cells appear, and these presumably merge with the myotube (ALLEN & PEPE 1995; PRZYBYLSKI & BLUMBERG 1966).

In quail, three genes homologous to MyoD, myogenin and MyF-5 are expressed. All of these regulatory genes are expressed in the compartmentalised somites of the myotome (POWNALL & EMERSON 1992).

In the mouse, myotome formation starts with the release of the dermatomyotome cells. These cells (pioneer cells) elongate, consequently occupying the whole length of the myotome. They are large cells, in the environs of which smaller myogenic cells appear. Small cells can fuse with large myocytes (VENTERS *et al*. 1999; WILSON-RAWLS *et al*. 1999).

Mammals are an exception because in these organisms the myf-5 transcripts appear first, before myogenin, MRF4 and MyoD. Myf-5 transcripts are detectable in somite cells, then in the dermatomyotome. Myogenin and MRF-4 occur in those cells which have already been determined, while MyoD is detected in multinucleate myotubes (SASSOON *et al*. 1989).

Another problem revealed by a comparative analysis of chordate myotomal myogenesis is the synchronous or asynchronous differentiation of myoblasts in each myotome. In a synchronous process all myotomal cells differentiate into mono- or multinucleate muscle cells. In this situation myotomal cells only initiate muscle development. In asynchronous differentiation only a part of the cells become ready to fuse and form multinucleate myotubes. The remaining cells show mitotic abilities. Following completed divisions, these cells

become a source of new myoblasts, fusing with the already existing myotubes. Asynchronously differentiating myotomal cells are capable of independent development into muscle fibres.

Myogenesis in Amphioxus is an example of synchronous differentiation of myotomal cells. Myogenesis in these animals leads to the formation of mononucleate muscle cells (HOLLAND *et al.* 1995).

Likewise, in *B. rerio* myotomal cells differentiate synchronously to result in mono- and multinucleate muscle fibres. Myotomal myogenesis ends at this stage (DEVOTO *et al.* 1996).

In other teleosts, e.g. *Oncorhynchus mykiss*, *Rutilus rutilus*, *R. frissi meidingeri*, *Chalcolburnus chalcoides* and *Clupea harengus* all myotomal cells, having completed mitotic divisions, differentiate synchronously. Mono- and multinucleate myotubes arise as in *C. harengus* and multinucleate myotubes in the remaining fish species. Further development of the myotubes involves mesenchymal cells which penetrate into myotomes via myosepts. These cells fuse with the myotubes leading to hypertrophy of the latter (JOHNSTON *et al*. 1995; STOIBER & SÄNGER 1996).

Myotomal cells of *Thymallus thymallus*, *Salmo gairdnerii* and *Dicentrarchus labrax* differentiate asynchronously and show a full potential of myotomal muscle development. In *Th. thymallus* two cell populations have been distinguished. Cells forming the epithelium of the somite wall are engaged in the formation of multinucleate myotubes in myotomal myogenesis. Cells located in the centre of the somite undergo mitotic divisions in the myotome. These cells are the source of new myoblasts which participate in hypertrophic growth (MERKEL 1995). The situation is similar in *S. gairdnerii.* In the myotome of this species, besides multinucleate myotubes, mitotically dividing cells are present. Post-mitotic myoblasts lead to both hypertrophic and hyperplastic growth of myotubes. In the myotomal myogenesis of *D. labrax*, following formation of multinucleate muscle fibres, presumptive myogenic cells become mitotically active and then differentiate into myoblasts (NAG & NARSAL 1972; VEGETTI *et al*. 1990; WATABE 1999).

Contrary to fishes, in which myotomal cells differentiate synchronously and asynchronously, myotomal cells differentiate only synchronously in amphibians. The synchronous differentiation of all the myotomal cells in *X. laevis*, *H. boettgeri* and *B. variegata* leads to the development of mononucleate myotubes, morphologically and functionally mature, and multinucleate myotubes in *P. fuscus*, *R. lessonae* and *T. vulgaris*. Amphibian myotomal cells only initiate myogenesis of paraxial muscles. Their further development takes place on account of mesenchymal cells which, having immigrated into the myotomes, acquire myogenic abilities. These are secondary myoblasts fusing with myotubes, and also forming secondary myotubes (KIEŁBÓWNA 1966; KIEŁBÓWNA & KOŚCIELSKI 1975; KIEŁBÓWNA 1987; DACZEWSKA & PAŁUCKA 1999; DACZEWSKA & KIEŁBÓWNA 2000; DACZEWSKA 2001).

In amniota dermatomyotome myogenic cells lose epithelial conformation, migrate and populate the myotom. Myotome formation is a multistage process (KAHANE *et al.* 1998 a, b; ORDAHL & LE DOURIN 1992). Also the process of myotomal muscle differentiation occurs gradually (PRZYBYLSKI & BLUMBERG 1966; VENTERS *et al.* 1999)

Myotomal myogenesis in amniota resembles asynchronous myogenesis of the muscles of mesenchymal origin.

A comparative analysis of myotomal myogenesis in Chordata reveals that muscle differentiation is very diverse and the myogenic process follows an evolutionary course.

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