

Limb muscle development

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ABSTRACT Skeletal muscle precursors for the limbs originate from the epithelial layer of the somites, the dermomyotomes. We summarize the steps of limb muscle development from the specification of precursor cells in the dermomyotome, the directed migration of these cells to and within the limb buds to muscle growth and differentiation. All steps are controlled by local signaling between embryonic structures. In dermomyotome development, signals from the neural tube, the ectoderm and the intermediate and lateral mesoderm result in a medio-lateral patterning. Only the lateral portions of the dermomyotomes give rise to muscle precursor cells destined to enter the limb buds. As a prerequisite for migration, precursor cells have to de-epithelialize as a result of interactions between SF/HGF and its receptor c-met. Precursor cells adopt a mesenchymal morphology without losing their myogenic specification. This is achieved by the expression of the transcription factors Pax3, Pax7 and myf5. During migration, premature differentiation has to be kept at bay to enable motility and proliferation. After having reached their target sites, the dorsal and ventral myogenic zones, myogenesis is initiated by the activation of the muscle determination factors MyoD, myogenin and MRF4. Finally, we briefly summarize the process of muscle hypertrophy and regeneration during which aspects of developmental processes are reinitiated.

KEY WORDS: *muscle development, limb bud, hypaxial muscle, migrating precursor cells, signaling molecules*

Introduction

About thirty years ago, it has been experimentally evidenced that muscles of the limbs and the ventral body wall originate from the somites (Christ *et al.*, 1974a, b; Christ *et al.*, 1977; Chevallier *et al.*, 1977). At limb levels, the lateral dermomyotomal edges de-epithelialize and individual mesenchymal muscle precursor cells migrate into the somatopleural mesoderm of the limb anlagen where they proliferate, differentiate and eventually form individual muscles. The muscles become attached to tendons that have originated from somatopleural cells like the muscular connective tissue (reviewed by Christ and Ordahl, 1995).

In recent years, many genes and signaling molecules have been identified that are involved in the process of somite maturation and compartmentalization, delamination of muscle precursor cells from the lateral edge of the dermomyotomes, and control of muscle precursor cell migration, proliferation and differentiation (reviewed by Brand-Saberi and Christ, 1999). It is the aim of this review to summarize the current knowledge of limb myogenesis and to pose questions where problems are being still unsolved.

Patterning of Dermomyotome and Muscle Precursor Cell Origin

All muscle cells produced by the somites take their origin from the dermomyotome, an epithelial structure, located in the dorsal part of the somite between neural tube and somatopleural mesoderm beneath the surface ectoderm. The formation of the dermomyotome requires signals from the ectoderm (Christ *et al.*, 1972; Schmidt *et al.*, 1998) and is a result of the dorsoventral patterning of the somite. This process is controlled by antagonistic actions of ventralizing and dorsalizing signals originating from adjacent tissues and traversing the extracellular matrix (ECM) that surrounds the somite (Christ and Ordahl, 1995). The notochord and the floor plate of the neural tube provide ventralizing signals, including Sonic hedgehog (Shh), Noggin and FGFs that control somite polarization and sclerotome development (Fan and Tessier-Lavigne, 1994; Johnson *et al.*, 1994; McMahon *et al.*, 1998; Borycki and Emerson, 2000). Sclerotome formation is characterized by the expression of Pax1 and Pax9 and an epithelio-mesenchymal transition of cells in the ventral half of the somite (Deutsch *et al.*, 1988; Brand-Saberi *et al.*, 1993; Pourquié *et al.*, 1993). The dorsalizing signals that induce and maintain the dermomyotome

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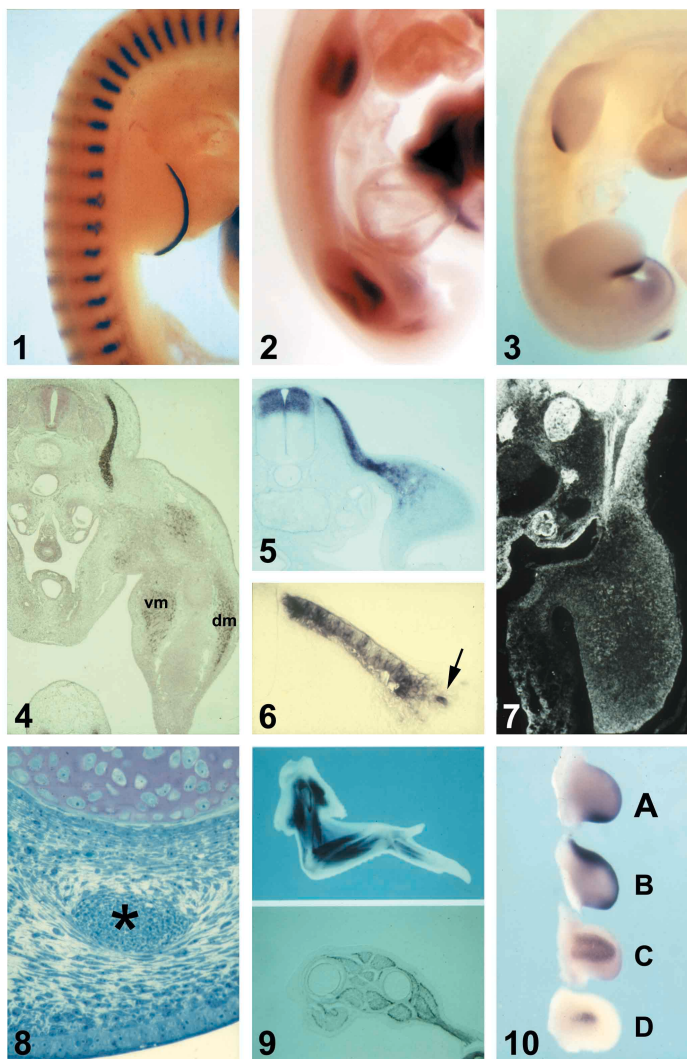


Fig. 1. Development of tissue pattern in the limb. (1) HH-stage 22 chick embryo co-stained for expression of *Fgf8* (blue) and *MyoD* (red). Note the overlapping, but not identical transcript distribution of *Fgf8* and *MyoD* in the myotomes (Courtesy of Daniel Stolte, Freiburg). (2) *Lbx1* expression in HH-stage 21 chick embryo. Dorsal and ventral myogenic zones of the limb buds are *Lbx1*-positive. The dorsal myogenic zone has started to downregulate *Lbx-1* expression. (3) HH-stage 23 chick embryo; Sonic hedgehog (*Shh*) in situ hybridization. (4) Transverse section of a 5-day chick embryo stained for desmin with an anti-desmin antibody; vm, ventral muscle mass; dm, dorsal muscle mass. (5) Transverse section of a HH-stage 18 chick embryo stained for the expression of *Pax3*. Note the *Pax3*-positive muscle precursor cells migrating from the lateral dermomyotome into the limb bud. (6) Dermomyotome of a 3-day chick embryo stained for expression of follistatin. Arrow: myogenic precursors leaving the lateral dermomyotome edge (Courtesy of Dr. Ketan Patel, London). (7) Transverse section of HH-stage 24 embryo at limb bud level; antibody staining against *N-cadherin*. *N-cadherin* is present on migrating myogenic cells and to a lesser extent, in the stationary mesenchyme. It is furthermore present in the axial organs, axons and mesonephric tissues. (8) Transverse section through a toe of a 10-day chick embryo showing a tendon of the flexor digitorum muscle (asterisk). (9) Wing of a 10-day chick embryo stained for desmin with an anti-desmin antibody. Note the muscle pattern that has developed from the dorsal and ventral muscle masses. (10) Wing buds of HH-stage 22 embryos. Stained for expression of *BMP2* (A), *BMP4* (B), *Pax3* (C), and *MyoD* (D). (Courtesy of Dr. Helge Amthor, Freiburg).

have been identified to be mediated by Wnt proteins and can be visualized by the expression of *Pax3*, *Pax7* and *myf5* (Goulding *et al.*, 1994; Kiefer and Hauschka, 2001). Fan *et al.* (1997) have shown that cells expressing *Wnt1*, *Wnt3a*, *Wnt4* and *Wnt6* can induce and maintain the expression of the dermomyotomal markers *Pax3*, *Pax7*, and *Sim1* in tissue culture. The expression of dermomyotomal markers can be enhanced and expanded by an ectopic expression of Wnt proteins (Capdevila *et al.*, 1998; Wagner *et al.*, 2000).

In addition to the dorsoventral compartmentalization of the somite resulting in the formation of dermomyotome and sclerotome, mediolateral compartments are formed in the somite. This results in a patterning of the dermomyotome of which the medial part gives rise to epaxial muscle and dermis of the back, whereas the lateral part subsequently yields hypaxial muscle. The border between the two myogenic lineages divides the epithelial somite into a medial and a lateral half (Ordahl and Le Douarin, 1992). In an early thoracic dermomyotome, it is the medial third of this epithelial layer that consists of epaxial muscle precursors and prospective dermis cells whereas the lateral two thirds form hypaxial muscle (Huang and Christ, 2000). Epaxial muscle precursor cells express *MyoD* and muscle proteins and differentiate within the epaxial myotome, a second epithelial layer to which cells are added by growth from the dorsomedial dermomyotome lip (Fig. 1.1 and 1.4; Denetclaw *et al.*, 1997; Ordahl *et al.*, 2001).

Like dorso-ventral patterning, the medio-lateral compartments of the early somite develop as a result of balance a medializing and lateralizing signals (Ordahl and Le Douarin, 1992; Christ and Ordahl, 1995). Interestingly, some of the signals involves in dorso-ventral patterning also play a role in medio-lateral patterning. Epaxial myogenesis requires signals from the axial structures. After ablation of both the neural tube and notochord, epaxial muscle does not develop (Christ, 1970; Rong *et al.*, 1992). Signals from notochord and neural tube are required to turn on and maintain *MyoD* expression in the epaxial myotome of avian embryos *in vivo* and *in vitro* (Pownall *et al.*, 1996; Dietrich *et al.*, 1997; Münsterberg and Lassar, 1995; Stern *et al.*, 1995; Buffinger and Stockdale, 1994). The notochord signal required for epaxial myogenesis has been identified to be Sonic hedgehog (*Shh*) (Fan *et al.*, 1995). After ablation of either the entire or the dorsal neural tube prior to somite formation leaving the notochord in place, *MyoD* expression is only transiently expressed in the epaxial myotome indicating that at least a second signal is required for the maintenance of epaxial muscle development (Christ *et al.*, 1992; Spence *et al.*, 1996; Dietrich *et al.*, 1997; Bober *et al.*, 1994). It has been shown that *Wnt1*, *Wnt3a* and *Wnt4* are expressed in the dorsal half of the neural tube at the time when epaxial myogenesis is initiated. In mouse embryos lacking both *Wnt1* and *Wnt3a*, the medial part of the dermomyotome is not formed (Ikeya and Takada, 1998).

Hypaxial muscle originates from the lateral part of the dermomyotome (Fig. 1.5, 1.6 and 1.7). At interlimb level, the lateral dermomyotome develops a hypaxial myotome. The lateral parts of the dermomyotome and myotome grow as epithelial "muscle buds" into the somatopleura and give rise to thoracic and abdominal muscles (Christ *et al.*, 1983). At limb levels, the lateral dermomyotome desintegrates to release individually migrating muscle precursor cells that invade the limb buds where they continue to proliferate and later on differentiate (Fig. 1.5, 1.6 and 1.7; reviewed by Brand-Saberi and Christ, 1999). The mechanisms

of hypaxial muscle cell specification are not well understood to date. It has been shown that the surface ectoderm is required to form the hypaxial dermomyotome (Schmidt *et al.*, 2001). Wnt proteins have been suggested to be the ectodermal signals (Roelink, 1996; Dietrich *et al.*, 1998). Wnt7a and to lesser extent Wnt4 and Wnt5a can activate myogenesis in mouse paraxial mesoderm explants (Tajbakhsh *et al.*, 1998). Cauthen *et al.* (2001) have recently shown that *Wnt6* is uniformly expressed in the surface ectoderm immediately adjacent to the *Pax3* expression domain in the dorsal somite just at the stage of hypaxial muscle precursor cell migration.

Another signaling molecule involved in hypaxial muscle development is BMP4 which has been shown to induce lateral characteristics of the dermomyotome. Such characteristics are the expression of *Sim1*, (Pourquié *et al.*, 1995, 1996), *Lbx1* (Jagla *et al.*, 1995; Dietrich *et al.*, 1998) and *c-met* (Bladt *et al.*, 1995). It has been concluded that Wnt proteins and BMP4 act in concert to specify hypaxial muscle precursor cells (Dietrich *et al.*, 1998).

It is, however, not quite clear if the specification of hypaxial muscle precursor cells does actually take place within the dermomyotome because lateral dermomyotomal cells give rise to different cell types such as endothelial cells of blood vessels and lymphatics and even cartilage cells forming the scapula blade (Wilting *et al.*, 1995; Wilting *et al.*, 1997; Wilting *et al.*, 2000; Huang *et al.*, 2000). Lateral dermomyotomal cells expressed in addition to *Pax3*, *Pax7* and *myf-5*, are specific cellular markers including VEGF receptor *Quek1*, the homeobox gene *Meis2* and *EphA4* (Eichmann *et al.*, 1993; Cecconi *et al.*, 1997; Schmidt *et al.*, 2001). In any case, muscle precursor cells that have already invaded the limb buds are found to be determined to form muscle (Mauger and Kieny, 1980; Wachtler *et al.*, 1981; Williams and Ordahl, 2000). To date it is suggested that signals from the axial structures do not influence hypaxial myogenesis. Stolte *et al.* (2002a,b), however, have been able to show that the expression of *Fgf8* even in the hypaxial myotome depends on Shh produced by the axial structures meaning that there does exist an influence of notochord and neural tube on muscle cell development in the hypaxial domain.

Migration of Limb Muscle Precursor Cells

Prerequisite of the migration of muscle precursor cells into the limb buds is the delamination of cells. It has been shown that de-epithelialization and subsequent migration of dermomyotomal cells can be induced ectopically by grafting of proximal limb bud mesoderm to the flank level (Hayashi and Ozawa, 1995). The underlying molecular mechanism is an interaction between the transmembrane tyrosine kinase receptor *c-met* expressed by the dermomyotome cells and its ligand scatter factor/hepatocyte growth factor (SF/HGF) that is produced by somatopleural cells of the limb buds (Bladt *et al.*, 1995).

Targeting of the genes for either the ligand or the receptor results in the absence of muscle in the limbs. An ectopic application of exogenous SF/HGF leads to de-epithelialization of the dermomyotomal edges even at interlimb level (Fig. 2.11 and 2.13; Brand-Saber *et al.*, 1996; Heymann *et al.*, 1996). The resulting phenotype of *c-met* and *SF/HGF* knockout mice resembles the phenotype of a naturally occurring mutation in the *Pax3* gene called *splotch* (Franz *et al.*, 1993; Bober *et al.*, 1994). *Pax3* initially expressed in all the cells of the segmental plate and later on in the dermomyotome, becomes up-regulated in the lateral part of the

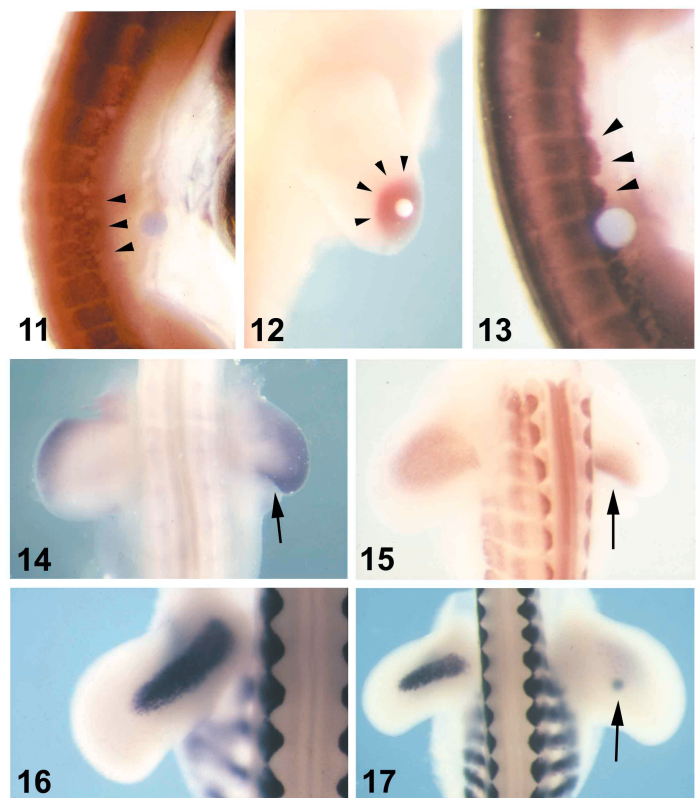


Fig. 2. Signaling in limb muscle development. (11) Grafting of a bead soaked in SF/HGF at interlimb level. The lateral dermomyotomes start to de-epithelialize (arrowheads). Pax3 in situ hybridization (courtesy of Alexander Bonafede, Freiburg). (12) Experimental induction of SF/HGF (arrowheads) by an FGF2-bead in the limb bud after removal of the AER. AER removal leads to loss of SF/HGF expression and can be rescued by FGF2 (*). (13) Grafting of a bead soaked in FGF2 at interlimb level. Pax3 positive cells are released from the lateral dermomyotomes (arrowheads) and migrate as individual cells into the flank, due to ectopic induction of SF/HGF. (14) Ectopic expression of SF/HGF at the posterior limb bud margin (arrow) as a result of ZPA removal. Normal expression on control side to the left (*). (15) As a result of ectopic expression of SF/HGF, the Pax3 positive muscle precursor cells accumulate at the posterior margin of the limb bud (arrow; *). (16) Wing bud of a HH-stage 25 chick embryo after MyoD in situ hybridization. (17) Effect of ectopic Shh on wing muscle development after 24 h following insertion of the Shh bead. MyoD is downregulated due to prolonged proliferation (courtesy of Dr. Helge Amthor, Freiburg). (*, courtesy of Dr. Martin Scaal, Freiburg).

dermomyotome (Williams and Ordahl, 1994) and has been shown to regulate the expression of *c-met* (Epstein *et al.*, 1996; Yang *et al.*, 1996; Tajbakhsh *et al.*, 1997). The *c-met* promoter contains a Pax3 binding site, and Pax3 can drive reporter gene expression from the *c-met* promoter *in vitro*. Therefore, it can be concluded that Pax3 controls the release of migrating muscle precursors *in vivo* by activating *c-met*. Pax3, Pax7, and myf5 are being expressed in the migratory muscle precursor cells and in the proliferating cells of limb pre-muscular masses (Fig. 2.15; Williams and Ordahl, 1994; Kiefer and Hauschka, 2001; Swartz *et al.*, 2001).

A gene expressed exclusively in the lateral dermomyotomes at sites of muscle precursor cell detachment and in migratory muscle precursors, is the homeobox gene *Lbx1* (Fig. 1.2; Jagla *et al.*, 1995; Dietrich *et al.*, 1998). *Lbx1* expression depends on Pax3, because *Lbx1* expression is absent in *Splotch* mice

(Mennerich *et al.*, 1999; Dietrich *et al.*, 1999). *Lbx1* expression is maintained during muscle precursor cell migration and is down-regulated shortly after muscle-specific gene expression is initiated in the limb. In mice that lack *Lbx1*, muscle precursor cells form and delaminate from the lateral edges of the dermomyotomes at limb levels but do not move into the limb buds and do not settle at the sites of future dorsal and ventral muscle masses (Fig. 1.2; Schäfer and Braun, 1999; Brohmann *et al.*, 2000; Gross *et al.*, 2000). *Lbx1* is suggested to determine lineage-specific properties of migrating myogenic precursors and to be essential for the recognition of cues that guide these cells and maintain their migratory potential.

It has been shown that the cell adhesion molecule N-cadherin is involved in the migration and homing of muscle precursor cells as well as in the differentiation of myoblasts (Brand-Saberi *et al.*, 1996b; George-Weinstein *et al.*, 1997). The cadherin-mediated adhesiveness of cells can be modulated by phosphorylation of intracellular molecules associated with cadherins, the catenins (reviewed by Gumbiner 1995; Birchmeier *et al.*, 1996; Huber *et al.*, 1996). *N-cadherin* is strongly expressed in the dermomyotome, the migrating muscle precursors, and more moderately expressed by stationary somatopleural cells in the myogenic zones and progress zone (Fig. 1.7). After *in vivo* injection of antibodies and Fab-fragments against the homophilic binding site of N-cadherin into the wing bud mesoderm, aggregates of myoblasts are found in the myogenic zone due to immobilization. It is concluded that the invasion and homing to the dorsal and ventral myogenic zones where the premuscular masses are formed depends on homophilic interactions between the migrating cells and the stationary somatopleural cells by means of N-cadherin (Brand-Saberi *et al.*, 1996b).

There are further prerequisites for the migration of muscle precursor cells. Fibronectin has to be available for migration through the intercellular spaces (Brand-Saberi *et al.*, 1993). The intercellular spaces have to be large enough, which *in vivo* is achieved by differing concentrations of hyaluronic acid (Kosher *et al.*, 1981; Krenn *et al.*, 1991).

As mentioned earlier, the *EphA4* receptor tyrosine kinase is strongly expressed in the lateral part of the dermomyotomes at the level where migratory muscle precursors detach (Schmidt *et al.*, 2001). Recently, it has been suggested that the interaction of EphA4 that is expressed in the migratory cells and its ligand ephrin-A5 guides the cells to their appropriate territories in the limb, disallowing entry into abnormal regions (Swartz *et al.*, 2001).

Muscle precursor cells have to migrate distally in the limbs as long as the limb anlage grows by distal apposition. They, however, never reach the most distal region of the growing limb bud, the so-called progress zone (Brand *et al.*, 1985).

SF/HGF has been found to be continually expressed during of limb bud outgrowth (Fig. 2.12 and 2.14). Here, it increases the motility of myogenic precursor cells probably by modulation of N-cadherin-mediated adhesiveness. At the same time SF/HGF maintains their undifferentiated state during migration (Scaal *et al.*, 1999).

Muscle Growth and Differentiation

Myogenic precursors that have invaded the limb mesenchyme aggregate and differentiate into dorsal and ventral premuscular masses (Christ *et al.*, 1977). With the growth of the limb buds the

muscular masses are becoming subdivided in a proximo-distal direction into stylopodial, zeugopodial and autopodial dorsal and ventral muscle masses revealing a differentiation gradient from proximal to distal. Each muscle mass splits up to eventually form individual, anatomically distinct muscles. Quail-chick chimeras have given evidence that the muscle pattern is not autonomously pre-specified within the somitic muscle precursor cells, but appears to be determined by the somatopleural mesoderm of the limb bud (Christ and Jacob, 1980; Grim und Wachtler, 1991). Yet it is not clear which of the specific components of the somatopleural mesoderm is the source of the patterning information. This is also true for tendons which develop autonomously and can even be formed in the absence of muscles (Fig. 1.8; Shellswell and Wolpert, 1977; Jacob and Christ, 1980; Kieny and Chevallier, 1979; Brown *et al.*, 1999; Kardon, 1999). The later steps of correct muscle-tendon patterning and the maintenance of tendons have been found to depend on reciprocal interactions between muscle and tendon (Kardon, 1999). Distal tendons differ from more proximal tendons in their molecular identity. They express the transcription factors *Six1* and *Six2* (Oliver *et al.*, 1995) and the receptor *EphA4* (Patel *et al.*, 1996) while proximal tendons do not. Other tendon-specific markers are TGF β 2, *Eya1* and *Eya2* (Xu *et al.*, 1997), *folliculin* (D'Sousa and Patel, 1999) and *scleraxis* (Schweitzer *et al.*, 2001). Tenascin is first detected in HH-stage 26 wing. It has been shown that tendon progenitors are induced by ectodermal signals and that the progenitor cell fate can be repressed by BMP signaling. The close relationship between ectoderm and early tendon anlage was earlier shown by Blechschmidt (1961) and Hurler *et al.* (1990). The endogenous expression of noggin within the condensing cartilage is suggested to contribute to the induction of distal tendons (Schweitzer *et al.*, 2001). Yamamoto *et al.* (1998) have shown that *Hoxa-11* and *Hoxa-13* which in the migrating muscle precursors are under the control of the limb mesenchyme and polarizing signals are involved in muscle and tendon patterning in the limb bud.

The premuscular masses in the limbs consist of two parts: a superficial layer of *Pax3* and *myf5* expressing proliferating muscle precursor cells and a deep layer in which differentiating myoblasts express *MyoD* and muscle proteins (Figs. 1.9, 1.10 and 2.16). Embryonic muscle growth is the result of a balance between proliferation and differentiation (Patel *et al.*, 2002). An increased proliferation rate of muscle precursors results in a stimulation of muscle growth, and an enlargement of muscle size (Füchtbauer, 2002). This can be achieved by FGFs, IGFI, BMPs and by a combination of BMPs and follistatin whereas Noggin, an antagonist of BMP signaling, reduces muscle mass (Amthor *et al.*, 1999; 2002; Floss *et al.*, 1997; Hannon *et al.*, 1996; Barton-Davis *et al.*, 1998). *Twist* represses muscle development by competition with MRFs for E-proteins and acts through active repression of muscle specific genes and by inhibiting the trans-activation by Mef2 (Spicer *et al.*, 1996; Hebrok *et al.*, 1997; Füchtbauer, 2002). In this way, *twist* that is expressed in the limb buds has been suggested to be an important regulator of muscle growth and differentiation (Füchtbauer, 1995; Stoetzel *et al.*, 1995; Füchtbauer, 2002). Delfini *et al.* (2000) have shown that after experimental activation of the Notch pathway, muscle precursor cells continue to express *Myf5* and *Pax3* whereas *MyoD* is down-regulated, resulting in inhibition of terminal muscle differentiation. SF/HGF keeps muscle precursor cells in a proliferating and undifferentiated state (Scaal *et al.*, 1999). On the other hand, BMPs at high concentration

induces apoptosis and muscle loss (Amthor *et al.*, 1998). The loss of FGF Receptor 1 (FGFR1) signaling accelerates muscle differentiation resulting in a reduction of muscle size (Itoh *et al.*, 1996; Flanagan-Steet *et al.*, 2000). It has been shown that FGFs delay the onset of differentiation of myoblasts obtained from day 4-12 chick wing buds while at the same time there is a subset of myoblasts derived from HH-stage 23-27 embryos that requires FGF for myogenic differentiation. It seems that the early population of limb myoblasts contains discrete subclasses of cells that are FGF independent. At later stages only FGF independent myoblasts persist. This issue of growth factor influence on limb myogenesis has been most extensively analyzed by the Hauschka (Clegg *et al.*, 1987; Pirskanen *et al.*, 2000; Seed and Hauschka, 1988; Templeton and Hauschka, 1992) and the Olwin group (DeHamer *et al.*, 1994; Flanagan-Steet *et al.*, 2000; Hannon *et al.*, 1996; Olwin *et al.*, 1994a; Olwin *et al.*, 1994b).

More recently myostatin, a member of the TGF β superfamily, has been proposed as a regulator of myogenesis. It is expressed at early and late stages of myogenesis and regulates the amount of skeletal muscle cells (McPherron *et al.*, 1997). *Myostatin* knockout mice develop muscles 2-3 times the size of wild-type mice which is essentially the opposite to what is observed in *folliculin* knockout mice (Matzuk *et al.*, 1995). Krüger *et al.* (2001) have recently shown that Shh acts as a survival and proliferation factor for hypaxial muscles corroborating the observation that ectopic expression of Shh in chicken limb buds induces muscle by hypertrophy probably via up-regulation of BMP expression (Figs. 1.3 and 2.17; Duprez *et al.*, 1998; Amthor *et al.*, 1998).

All anatomic muscles of adult vertebrates have their origins in several waves of muscle fiber formation as development proceeds (Stockdale, 1997). The primary fibers form during embryonic development and lay down the anlagen of all future muscles. The amount of muscle mass formed from the primary myoblasts is extremely small. Their role may be to define the type, shape and location of a muscle. Primary fibers traverse the muscle anlage from tendon to tendon and become innervated at multiple endplates prior to formation of secondary fibers (Duxson and Usson, 1989; Duxson and Sheard, 1995). Secondary fibers form and insert on the surface of primary fibers beginning near the sites of innervation of primary fibers and initially do not traverse the entire length of a muscle. The secondary fibers increase rapidly in number and nucleation and separate from the primary fibers.

All muscle fibers form by fusion of myoblasts with one another (Stockdale and Holtzer, 1961). The myoblasts can be subdivided in three categories: embryonic myoblasts, fetal myoblasts and satellite cells (Stockdale, 1992, 1997). They form fibers which can be identified by the expression of specific isoforms of myosin heavy chain (MyHC) and number of nuclei (Miller and Stockdale, 1986; Ontell *et al.*, 1993). Embryonic, fetal and adult myoblasts in the limb have their origins in the migratory population of muscle precursors (Christ *et al.*, 1977).

Embryonic myoblasts in the limb buds express *MyoD* and begin to fuse into small primary fibers, a process that requires surface molecules which mediate heterophilic and homophilic cell-cell recognition such as N-CAM, N-cadherin and M-cadherin (reviewed by Arnold and Braun, 2000). In birds, myoblasts of fetal and adult characteristics replace the embryonic myoblasts until the end of the first week. It is still an open question, if the precursors of embryonic myoblasts are also precursors of fetal and adult myo-

blasts or whether there are separate migratory populations for each within the somite.

During embryonic development, and before functional innervation, a highly stereotopic pattern of slow- and fast contracting primary muscle fibers is established within individual muscles of the limbs, from distinct populations of myoblasts (Nikovits *et al.*, 2001). The fibers can be distinguished by distinct morphological and biochemical properties (Seed and Hauschka, 1984; Stockdale, 1992) and classified by different specific isoforms of the myosin heavy chain (MyHC) (Miller and Stockdale, 1992; DiMario and Stockdale, 1997). Nikovits *et al.* (2001) have concluded that an intrinsic commitment to either a fast or a slow fiber-type lineage occurs in myogenic precursors while still within the somite. This is in accordance with data of fiber type specification in zebrafish (Blagden *et al.*, 1997; Currie and Junghans, 1996; Du *et al.*, 1997). Cann *et al.*, 1999 was able to show that in explant cultures of avian somites exogenous Shh leads to a marked expansion of the slow fiber population. It has been suggested that selective amplification of committed myoblasts to form fast- or slow fibers occurs in response to proliferative signals that originate in the limb stroma (Nikovits *et al.*, 2001).

In a recent paper Bren-Mattison and Olwin (2002) have presented a model according to which Shh represses differentiation of posterior myoblasts in the ventral muscle mass, allowing mitogens present in the limb bud mesenchyme to stimulate their proliferation, while absence of Shh induces precocious differentiation of early myoblasts fated to express slow MyHC. The precocious differentiation depletes the pool of proliferating myoblasts and ultimately results in less muscle due to loss of slow MyHC fibers.

Regulator Genes that control Limb Myogenesis

The MRF genes *MyoD*, *Myf5*, *Myogenin* and *MRF4* encode bHLH transcription factors that are expressed in myogenic progenitor cells and are essential for determination and differentiation of muscle cells (Figs. 1.1, 1.10 and 2.16; Ott *et al.*, 1991; Pownall and Emerson, 1992; Sassoon, 1993). Overexpression of the respective genes, especially *MyoD* and *Myf5*, can convert many different cell types to a myogenic fate (Choi *et al.*, 1990; Weintraub, 1993). *MyoD* and *Myf5* are upstream of *Myogenin* and *MRF4* and *MyoD* and *Myf5* double knockout mice do not form muscle (Rudnicki *et al.*, 1993). MRFs activate muscle specific genes by binding to their promoters. *Myogenin* and *MRF4* are activated later in myoblasts at the onset of differentiation (Borycki *et al.*, 1997). *Pax3* expressed in the dermomyotome is essential for the migration of muscle precursor cells. Mouse embryos that are double mutant for *Myf5* and *Pax3* lack all body muscles (Tajbakhsh *et al.*, 1997) indicating that *Pax3* has function in the upstream regulation of *MyoD*. It has, however, to be kept in mind that non-somatic muscle precursors for head muscle do neither express *Pax3*, nor the highly related gene *Pax7* (Mansouri *et al.*, 1996). Head muscle develops normally in *splotch/myf5* double mutant mice (Tajbakhsh *et al.*, 1997). Members of the bHLH-transcription factors also interact with the second type of myogenic regulators. MEF2 (myocyte specific enhancer factor-2) belongs to the MADS family of transcription factors (Cserjesi and Olson, 1995). *Mef2* expression is required in addition to members of the bHLH family since it enhances and stabilizes their expression (Molkentin and Olson, 1996; Brand-Saberi and Christ, 1999).

Muscle Hypertrophy and Regeneration

Hypertrophy of muscle in the postnatal period in most vertebrates is under the control of mechanical stress that is transduced by a number of signaling molecules. It can be identified by an increase in the number of nuclei in the fibers, increase in the number of satellite cells, and hypertrophy of the fibers (Kadi and Thornell, 2000). IGF-I, IGF-II are among the signaling molecules and are produced by muscle fibers themselves (Gerrard *et al.*, 1998). Transgenic mice that overexpress *IGF-I* demonstrate a marked hypertrophy of muscle fibers and targeted disruption of IGF-I expression in the mouse leads to a reduction of fiber size and muscle hypoplasia (Coleman *et al.*, 1995; Fournier and Lewis, 2000). As mentioned earlier, myostatin is involved in the control of muscle mass. Zhu *et al.* (2000) have shown that mice with a disruption in the *myostatin* gene exhibit a significant increase in muscle fiber size.

Muscle regeneration requires activation of the mononuclear satellite cells or myogenic differentiation of bone marrow-derived hematopoietic stem cells (reviewed by Patel *et al.*, 2002; Parrish, 1996; Bittner *et al.*, 1999; Ferrari *et al.*, 1998). In a reciprocal study, Jackson *et al.* (1999) have shown that mononucleated cells from muscle have the capacity to reconstitute the hematopoietic system of irradiated mice. The c-met receptor is present on satellite cells in normal muscle tissue (Tatsumi *et al.*, 1998). Another gene that has been identified to be expressed in satellite cells is *Pax7* (Seale *et al.*, 2000). A number of growth factors have been found to be involved in the activation of satellite cell proliferation and *MyoD* expression (reviewed by Grounds and Yablonka-Reuveni, 1993) while HGF/SF inhibits muscle regeneration (Lefaucheur and Sebille, 1995). Floss *et al.* (1997) have shown that FGF-6 is a critical component to stimulate, attract, or activate satellite cells for differentiation.

New and unexpected sources of myogenic progenitor cells have recently been described not only to be the bone marrow mesenchyme (Ferrari *et al.*, 1998; Bittner *et al.*, 1999) but also the limb-bud mesenchyme of muscleless knock-out mice c-met^{-/-} and Pax3^{-/-} (Bailey *et al.*, 2001) and from the embryonic dorsal aorta (De Angelis *et al.*, 1999) suggesting that muscle progenitor cells could be endothelia-derived. In this connection it is interesting to note that in the somites, myogenic and angioblastic cells exist side by side and endothelia of the aorta and the limb bud are at least partially of somitic origin (Wilting *et al.*, 1995; Brand-Saberi *et al.*, 1995). Finally, a new population of muscle progenitor cells has been described to reside in adult muscle itself as a so-called "muscle-derived side population" (Gussoni *et al.*, 1999).

Concluding Remarks

Limb muscle in the adult accounts for more than half of the entire skeletal muscle mass. This is in marked contrast to the situation in the early embryo. To understand the process involved in limb myogenesis we can study the origin of its precursor cells and their behaviour as they invade and populate the limb bud mesenchyme. Our insights have become more detailed as we have identified new regulatory molecules. Correlating the process of muscle development with the signals involved in limb bud patterning can be expected to refine our knowledge further. As in other fields of research, questions of quantity control will have to be addressed after having understood some of the questions concerning the

quality control. It is still unknown in how far early and late muscle populations and satellite cells are connected, or when these diverge as separate lineages. Answers to these questions can be expected from clonal studies of myogenic cells in the limb bud. It can be expected that the study of myogenic stem cells will further enhance the insights into limb muscle development.

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