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
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 Fgfl8 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-Saberi 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 is being expressed in all the cells of the segmental plate and later on in the dermomyotome, becomes up-regulated in the lateral part of the 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 premuscular 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...
Molecules associated with cadherins, the catenins (reviewed by George-Weinstein, 1981; Krenn et al., 1991). 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 adhesive-ness 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 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 andautopodial 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; Kiency 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 TGFβ2, Eya1 and Eya2 (Xu et al., 1997), follistatin (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 Hurle et al. (1980). 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, IGFs, 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 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 group (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 TGFβ 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 knock-out mice develop muscles 2-3 times the size of wild-type mice which is essentially the opposite to what is observed in follistatin 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 hyper-trophy 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 myoblasts 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 myotgens 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 Yablonska-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 endothelium 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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