Determination, diversification and multipotency of mammalian myogenic cells

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ABSTRACT In amniotes, myogenic commitment appears to be dependent upon signaling from neural tube and dorsal ectoderm, that can be replaced by members of the Wnt family and by Sonic hedgehog. Once committed, myoblasts undergo different fates, in that they can differentiate immediately to form the myotome, or later to give rise to primary and secondary muscle fibers. With fiber maturation, satellite cells are first detected; these cells contribute to fiber growth and regeneration during post-natal life. We will describe recent data, mainly from our laboratory, that suggest a different origin for some of the cells that are incorporated into the muscle fibers during late development. We propose the possibility that these myogenic cells are derived from the vasculature, are multi-potent and become committed to myogenesis by local signaling, when ingressing a differentiating muscle tissue. The implications for fetal and perinatal development of the whole mesoderm will also be discussed.

KEY WORDS: Myogenic determination, myogenic lineages, satellite cells, multipotent progenitors.

Introduction

Differentiation of skeletal muscle is a precocious and crucial step in the development of most metazoan species since it provides the embryo with motility at early stages. Skeletal myogenesis begins shortly after gastrulation, but persists, at least in mammals until the end of post-natal growth and, as a potential for the entire life span of the animal (Hauschka, 1994). Cells of the mesoderm are committed by local signaling to a myogenic fate and, shortly afterwards, begin to synthesize contractile proteins that accumulate in the cytoplasm and self-assemble into sarcomeres. Motility is indeed dependent upon shortening of sarcomeres, which are paracrystalline structures specialized for transforming chemical energy into movement. The advantage of accumulating millions of sarcomeres within a single cytoplasm has led to multinucleation, a different strategy from the coupling of single cells adopted by the heart. Within the highly structured cytoplasm of the multinucleated muscle fiber, mitosis is no longer possible and when experimentally induced by oncogenes it leads to disruption of the spindle and death (mitotic catastrophe). As a consequence, growth of the muscle fiber during fetal and post-natal development depends upon addition of single cells, that must be instructed on when to divide and when to differentiate, by either fusing with pre-existing fibers or among themselves to generate a new fiber. It is therefore obvious that diversification of myogenic cell fate is as crucial as commitment. It allows the production of post-mitotic skeletal muscle during early embryogenesis and at the same time the maintenance of a pool of mitotic progenitors which permits further growth of the tissue as well as regeneration in response to injury.

In this review we will discuss current knowledge on early steps of skeletal myogenesis and possible mechanisms that ensure maintenance of a progenitor pool during later development.

Myogenic commitment: signals from neighboring tissues

Skeletal muscles of the vertebrate body are derived from somites, mesodermal units that segment progressively the paraxial mesoderm in a cranio-caudal succession for an extended period of embryogenesis (Christ and Ordhal, 1994). In newly formed somites, cells located in the dorsal domain, the future
dermomyotome, are specified as myoblasts (and dermal fibroblasts) while cells located in the ventral domain, the sclerotome, will form cartilage and bone. At this stage cells are not yet determined since rotation of the epithelial somite in a dorsal/ventral or medial/lateral direction does not perturb subsequent development, suggesting that signals from the environment determine the identity of cells within the newly formed somite.

Several laboratories have shown that in explants of paraxial mesoderm, axial structures (neural tube/notochord complex) are required to promote myogenesis. Further experiments showed that only precursors of epaxial (back) muscles, located in the dorso-medial domain of newly formed somites, are dependent upon signals from axial structures. In contrast, precursors of hypaxial (limb and body wall) muscles, located in the lateral half of the paraxial mesoderm, do not need the neural tube/notochord complex but rather require a signal from dorsal ectoderm for myogenic commitment (reviewed in Cossu et al., 1996a). It was later shown that cells from medial domain of murine segmental plate, when cultured in the presence of axial structures activate Myf5. In contrast cells from the lateral half of the segmental plate, cultured with their own dorsal ectoderm, will activate MyoD (Cossu et al., 1996b). This suggests that in mammals axial structures activate myogenesis through a Myf5 dependent pathway while dorsal ectoderm acts through a MyoD dependent pathway. The latter is dependent upon previous expression of either Myf5 or Pax3 (Tajbakhsh et al., 1997). Subsequently, the great majority of myogenic cells express both MyoD and Myf5, although with variable intensity. This explains the phenotype of the single MyoD or Myf5 knock-out mice where Myf5 null embryos have initially epaxial muscle defects whereas MyoD null embryos have predominantly hypaxial muscle defects (Rudnicki et al., 1992; Kablar et al., 1997). In both cases but the residual gene is sufficient to support almost normal skeletal muscle development throughout the body.

In the lateral myogenic progenitor cells, MyoD expression and subsequent terminal differentiation is transiently repressed in order to allow migration to the limb and body wall where muscle formation will take place. Although irreversibly committed (as shown by classic transplantation experiments), these myogenic progenitors do not express any member of the MyoD family either in the somite or during migration (Tajbakhsh and Buckingham, 1994). They can be identified by the expression of the transcription factor Pax 3 (Bober et al., 1994) and the receptor tyrosine kinase c-met (Bladt et al., 1998). It is therefore likely that their differentiation is repressed by signals derived from adjacent tissues. Mechanical separation of the lateral plate mesoderm from the paraxial mesoderm in the chick embryo induced expression of MyoD in the lateral half of somites where it is not normally observed. Furthermore it was demonstrated that cells expressing BMP4 could replace this inhibitory activity (Pourquié et al., 1996).

The possible role of Shh and Wnts in the activation of myogenesis

The notochord produces a ventralizing signal that activates Pax1 and specifies a sclerotomal fate: Sonic hedgehog (Shh), normally produced by the notochord can mimic this activity (Fan and T essier-Lavigne, 1994). However Shh is also required to promote myogenesis, and indeed in Shh null embryos, epaxial myogenesis is absent whereas progenitors of hypaxial myogenesis are specified normally (Borycki et al., 1999). On the other hand, the neural tube produces several members of the Wnt family that can activate the myogenic program in the dorsal part of the somite (Stern et al., 1995; Munstenberg et al., 1995).

As discussed above, the onset of MyoD and Myf5 expression is spatially and temporally regulated in mouse embryos. It was therefore interesting to investigate whether the differential activation of Myf5 and MyoD may be promoted by different members of the Wnt family, differentially expressed in neural tube and dorsal ectoderm (Parr et al., 1993). Indeed we observed that the action of the neural tube in activating Myf5 can be replaced by cells expressing Wnt1 while MyoD activation by dorsal ectoderm can be replaced by Wnt7a expressing cells (Tajbakhsh et al., 1998). Wnt7a is expressed in the correct spatio-temporal pattern to be a candidate molecule for this activity. Sonic Hedgehog synergizes with both Wnt1 and Wnt7a, even though it is not expressed in the ectoderm.

With current information at hand, a simple model of signaling activity can be proposed, as shown in Fig. 1. Shh, produced by notochord and floor plate, activates Pax1 and, in conjunction with BMP4 (Murtaugh et al., 1999) chondrogenesis in the future sclerotome. Shh, in conjunction with Wnt1 (and possibly other Wnts) activates myogenesis in the future dermomyotome, via a Myf5 dependent pathway. Different Wnts such as Wnt7a rather activate myogenesis in the lateral domain, probably though a MyoD dependent pathway. This activity is inhibited by BMP4 to prevent premature differentiation; the negative action of BMP4 is counter-
acted, probably through direct protein-protein interactions, by noggin, which is produced by the dorsal neural tube in a Wnt-dependent manner (Hirsinger et al., 1997; Marcelle et al., 1997).

Although the model illustrated in Fig. 1 accommodates our present understanding, the rapid increase in the number of known molecules potentially involved in signaling during embryogenesis suggests that the model we now have is bound to become more elaborate; thus more definitive and stringent experimental evidence will be needed. This will likely include in situ inhibition by specific antibodies (many of which are not yet available) together with detailed analysis of mutant and possibly compound mutant embryos.

From receptors to downstream genes

While BMP and noggin are likely to interact directly, Wnt and Shh act through classic membrane receptors. In the case of Wnts, vertebrate homologues of Drosophila Frizzled are considered as putative receptors (Bhanot et al., 1996) and so far about 10 members of this family have been cloned in different organisms (Wozard and Nusse, 1998; Dierick and Bejsovec, 1999). We studied the expression of eight murine Frizzled (1,3-9) genes during mouse somitogenesis and observed that among those expressed in somites (Fz1, 3, 7, 8, 9), Fz1 is expressed along the medial border, consistent with a possible preferential interaction with Wnt1 from the adjacent dorsal neural tube. On the other hand, Fz7 is expressed in a pattern complementary to Fz1, i.e. along the lateral and caudal edge of newly formed somites, consistent with the possibility of a preferential interaction with Wnt7a. Wnt1 acts through the classical Dishevelled -> GSK3 -> β-catenin pathway; in contrast Wnt7a appears to act through a β-catenin independent pathway (Kengaku et al., 1997) and leads to MyoD rather than to Myf5 activation (Tajbakhsh et al., 1998). It is thus tempting to speculate that Fz1 and Fz7 may mediate the differential response to Wnt1 and Wnt7a and activate different intracellular pathways.

Another possible level of regulation for Wnt signaling may be exerted by the sFRPs (soluble Frizzled Related Proteins) a new class of genes, recently identified in several laboratories (Leys et al., 1997; Wang et al., 1997). These are secreted molecules with a strong homology with the Frizzled extra-cellular domain. Among those examined, only Frzb1 was found to be expressed in the pre-somatic mesoderm and newly formed somites. We recently reported that Frzb1 totally inhibits myogenesis in cultures of pre-somatic mesoderm or newly formed somites, but has no effect on more mature somites or on myogenic cell lines, and thus appears to act differently from intracellular myogenic inhibitors such as Id or Twist. In order to examine the effect of Frzb1 over-expression in vivo, we developed a method based on transient transfection of cells with a Frzb1 expression vector and injection of transfected cells into the placenta of pregnant females before the onset of materno-fetal circulation. Frzb1, secreted by transfected cells, accumulated in the embryo and caused a marked reduction of caudal structures. Myogenesis was strongly reduced and in the most severe cases abolished. Genes downstream of the Wnt signaling pathway such as En1, Noggin and Myf5 were down-regulated but Pax3 and Mox1 were not, excluding a generalized toxic effect (Borello et al., 1999a). The results obtained with this new method are in keeping with the Wnt1-Wnt3a double knockout and corroborate the idea that Wnt signals may act by regulating both myogenic commitment and expansion of committed cells in the mouse mesoderm. Indeed in mouse embryos lacking both Wnt7a and Wnt3a, the medial compartment of the dermomyotome is not formed and the expression of Myf5 is decreased (Ikeya and Takada, 1998) but not abolished, probably because partial activation by Shh has already occurred.

From the data discussed above, it appears that, at least medi ally, Shh and Wnts cooperate to activate myogenesis, but may also instruct diversification between epaxial and hypaxial myogenesis. How this can be achieved in molecular terms is still far from clear. Shh binds to Patched, a receptor that activates an intracellular pathway involving PKA and ultimately leading to activation of several zinc finger proteins, termed Gli. Activated Gli may directly bind to regulatory regions of My5 and MyoD promoters, whose complexity has made these studies difficult. Similarly, Tcf/β-catenin complex, activated by Wnt1 (see below) may directly activate transcription of target genes but also contribute to open the Myf5 locus making it more easily accessible to other transcription factors. It should however be remembered that Shh has been reported to be an important survival factor for paraxial mesoderm (Teillet et al., 1998) and to have mitogenic activity on myoblasts (Duprez et al., 1997). Several Wnts, including Wnt1 and Wnt7a have strong mitogenic and often transforming activity and most likely act as survival factors as well (Wodarz and Nusse, 1999). Thus both molecules have both the potential to activate, directly or
indirectly, transcription of *Myf5* and *MyoD*, and to promote survival and expansion of the committed population. In reality, a combinatorial action of *Shh/Wnt* transcriptional activation, proliferation and survival must account for the final number of differentiated cells in a given structure such as the myotome. This is relevant to the next issue discussed in this review, namely how different fates are chosen within contiguous and probably equivalent cells of the epithelial dermomyotome (Tajbakhsh and Cossu, 1997).

**The generation of myoblast diversity and the origin of different fibers**

Most of the work discussed above on the activation of myogenesis refers to the formation of the myotome, the first patterned array of terminally differentiated, post-mitotic, mononucleated muscle cells (Fig. 2). However we do not know whether commitment of the progenitors of later phases of myogenesis occurs through the same mechanisms and in the same spatio-temporal context. It is important to remember that the activation of the myogenic program in the large majority of mesodermal cells does not directly lead to terminal differentiation. As discussed above for the precursors located in the dorso-lateral region of somites, commitment may occur, but differentiation is repressed until the cells find themselves in the right time and place and, more importantly, after a given number of divisions, so to attain the correct number of myoblasts.

Indeed, only a fraction of myogenic progenitors in the somite terminally differentiate to form the myotome. The remaining are kept in a committed but undifferentiated state, as embryonic and fetal myoblasts that will produce primary and secondary fibers respectively (Kelly and Zachs, 1969) and perhaps satellite cells during later development (Fig. 3). In *Drosophila* lateral inhibition through *Notch* and *Delta* has been shown as the probable mechanism by which adult myogenic progenitors are selected in response to *Wg* signaling (Baylies et al., 1998). It thus appears likely that a similar mechanism may operate in the mammalian somite. Indeed several *Delta* and *Notch* isoforms are expressed in the somites (McGrew and Pourquié 1998) and *Notch* inhibits myogenesis, probably through different intracellular mechanisms (Wilson-Rawls et al., 1999; Nofziger et al., 1999). However direct evidence for a role of Notch in diversifying cell fate in mammalian somites is still missing.

Receptors for growth factors may be pertinent targets for *Notch* signaling. In this context it has been proposed that the dorsal portion of the neural tube inhibits terminal myogenic differentiation through production of growth factors (Baffinger and Stockdale, 1995). Therefore some kind of heterogeneity may be invoked to explain the differential fate of myotomal precursors versus other precursors, similarly to what is observed between embryonic and fetal myoblasts in the developing limb bud (see below). From this point of view it is interesting to note that the neural tube produces various FGFs (Kalcheim and Neufeld, 1990) and the first cells which form the myotome are the only myogenic cells which do not express the FGF receptor FREK (Marcelle et al., 1995). Whether a preselected myogenic population fails to respond to FGF, or local signaling prevents expression of FGF receptors in a homogeneous population is unknown, since the location of myotomal precursors in the dermomyotome is still unknown.

On a teleological basis, this can be explained with the need of maintaining a precursor pool of dividing myogenic cells (to cope with the growing size of the embryo) and at the same time to generate differentiated, post-mitotic skeletal muscle fibers (to allow early movements of the embryo). A possible mechanism to ensure that certain myoblasts will differentiate in an environment that is permissive for proliferation, may be based on the inability of these myoblasts to respond to growth factors and/or to molecules which inhibit differentiation. A few years ago, we proposed a possible mechanism by which TGFβ might influence the process of primary fiber formation in vivo (Fig. 4). Committed myoblasts will proliferate in the presence of mitogens and will differentiate in their absence (Hauschka, 1994). It is therefore conceivable that a gradient of mitogen concentration is established throughout the proximo-distal axis of the growing limb, with the lowest concentration present at the base of the limb, just where primary fibers initially form. Myoblasts will proliferate in the growing distal extremity of the limb bud, where the concentration of mitogens is high (progress zone) and will differentiate first at the base of the limb bud, distant from the source of mitogens. Consistent with this possibility, the number of cells in S phase (BrdU positive) is greatly reduced at the base compared with the progression zone of the limb bud in 10.5 dpc. mouse embryos (Hornbruch and Wolpert, 1970). These observations, however, do not explain why embryonic myoblasts differentiate while fetal ones do not. In as much as embryonic myoblasts are insensitive to TGFβ (Cusella De Angelis et al., 1994), which is abundant in the embryonic limb (Heine et al., 1987), they may differentiate into primary fibers. On the contrary, fetal myoblasts should be prevented from differentiating by TGFβ. It is worth noting that TGFβ is produced by the ectoderm and is
supposed to act in a paracrine fashion on surrounding mesenchymal tissues (Francis-West and Tickle, 1996). Furthermore TGFβ is the only inhibitor of myogenesis which does not stimulate myoblast proliferation (Olson et al., 1986). Thus, fetal myoblasts may enter a quiescent but undifferentiated phase, from which they must subsequently exit and begin a new wave of proliferation to generate the pool of precursor cells necessary to form secondary fibers. This proliferation may well be sustained by peptide growth factors, whose messages are frequently localized within developing muscle fibers in the embryo (Goldfarb, 1996). Indeed co-culture experiments showed that cultured myotubes (and explants of embryonic muscle) release into the medium mitogens which stimulate myoblast proliferation (De Angelis et al., 1992).

The above proposed model has the advantage of reconciling previously unexplained data such as the presence of growth factors in newly formed muscle with the differential effect of TGFβ on embryonic and fetal myoblasts (Cusella De Angelis et al., 1994). However this hypothesis still awaits in vivo functional analysis with dominant positive and negative TGFβ signaling.

A role for PKCθ in myoblast diversification

The model discussed is mainly based upon the selective inhibition of differentiation caused by TGFβ or possibly other growth factors in fetal myoblasts. This may in turn be due to differential expression or activity of molecules involved in the respective transduction pathway. Protein kinase C θ (PKCθ), the predominantly expressed PKC isoform in muscle, is selectively expressed in fetal myoblasts and satellite cells, in vivo and in vitro (Zappelli et al., 1997) and not in embryonic myoblasts. If PKCθ is exogenously expressed in embryonic myoblasts, that are insensitive to TGFβ, it restores the differentiation-inhibitory effect of the molecule. Why differential expression of PKCθ should dictate the differential fate of embryonic versus fetal myoblasts remains, at present, a matter of speculation. However, it is a fact that only a fraction of the total myogenic precursor population differentiates at this time, at the base of the limb bud. All myoblasts in this area are probably exposed to a low concentration of growth factors but to a high concentration of TGFβ. At this time, embryonic myoblasts, which do not express PKCθ and, thus, are insensitive to TGFβ, may differentiate into primary fibers, while fetal myoblasts, which do express PKCθ, should be blocked by TGFβ (Fig. 4). Once primary fibers are formed, they begin to produce growth factors such as FGF and thus promote a new wave of proliferation in fetal cells. The identification of PKCθ as a key component of this pathway permits the design of experiments where expression of this enzyme in vivo, driven by the promoter of genes expressed early in the limb bud, should render all myoblasts sensitive to TGFβ and thus prevent formation of primary fibers. This possibility is currently under investigation.

Satellite cells

Satellite cells are classically defined as quiescent mononucleated cells, located between the sarcolemma and the basal lamina of adult skeletal muscle (Bischoff, 1994). They have been shown to contribute to post-natal growth of muscle fibers, whose nuclei cannot divide. At the end of longitudinal growth, satellite cell become quiescent but can be activated if the existing fibers are damaged or destroyed. In this case they undergo a number of cell divisions producing fusion competent cells, that can either fuse with damaged fibers or form new ones, and other cells that return to quiescence, thus maintaining a progenitor pool. This fact has led to the suggestion that they represent a type of stem cells (Schultz and McCormick, 1994, Miller et al., 1999).

Previous work from this laboratory identified specific features of satellite cells (morphology in vitro, resistance to phorbol esters but susceptibility to TGFβ induced block of differentiation, early expression of acetylcholine receptors and acetyl-cholinesterase) that characterize them as a different class of myogenic cells with respect to embryonic and fetal myoblasts. We also showed that they emerge from the fetal myogenic cell compartment at 16-18 days of development in mouse embryo and at 12-14 weeks in the human embryo (reviewed in Cossu and Molinaro, 1987). In this period intensive myogenesis occurs, leading to a drastic reduction of myogenic mononucleated cells as a consequence of the massive formation of muscle fibers. Since satellite cells do not undergo differentiation at this time, the control of proliferation and differen-

![Fig. 4. Summary of the proposed paracrine control of primary/secondary fiber formation by TGFβ and PKCθ. Embryonic myoblasts, which do not express PKCθ, are insensitive to TGFβ and differentiate in the absence of mitogens. Fetal myoblasts that express PKCθ are inhibited by TGFβ and then resume proliferation in response to growth factors like FGF produced by newly formed primary fibers. When the level of both FGF and TGFβ decrease, fetal myoblasts differentiate into secondary fibers.](image-url)
tiation in these cells must be different, so as to allow the persistence of mononucleated undifferentiated myogenic cells in the post-natal and in the adult muscle. An obvious reason for studying the mechanisms controlling the behavior of these cells is the possibility of manipulating the size of the satellite cell compartment, which would be of paramount importance in developing therapeutic approaches for primary myopathies. Despite their capacity to respond to growth factors and to divide rapidly, satellite cells are able to undergo only a finite number of cell replications. Data from several laboratories have shown that a sharp decrease of the proliferative potential of satellite cells is observed in the early periods after birth, followed by a continuous, slower decline till senility (Schultz and Lipton, 1982). This process is clearly accelerated in the case of primary myopathies where the proliferative potential is rapidly exhausted by the successive phases of degeneration/regeneration, ultimately depleting the progenitor pool.

Are myogenic cells only derived from satellite cells in regenerating muscle?

Satellite cells are the only relatively well-defined myogenic cell in post-natal life. It is currently assumed but not experimentally proved that they represent a single cell type, with a common embryological origin. The origin of satellite cells is presumed to be somitic, but the evidence for this is also not conclusive due to technical difficulties in identifying quail nuclei in chick-quail chimera at the ultrastructural level (Armand et al., 1983). Furthermore, it is surprising that despite a low number of resident, quiescent satellite cells (identified by their location and the expression of M-Cadherin) in adult healthy muscle, hundreds of activated (MyoD positive) satellite cells are seen hours after an injury to the tissue (Grounds et al., 1992). This suggests that cells are recruited to muscle regeneration from additional sources, either locally or systemically.

Indeed, a number of observations had pointed to the unorthodox appearance of muscle cells in a variety of tissues or cell culture systems that were neither myogenic nor derived from somites. For example, spontaneous myogenic differentiation of cells from the brain has been repeatedly documented, but it was only through insertion of the reporter gene LacZ, into the Myf5 locus that it was possible to unequivocally identify Myf5 expressing cells in the nervous system and to show that these cells co-express neural and muscle markers (Tajbakhsh et al., 1994). Hence, even though there is no clue to the physiological significance of these findings, they provided an indication for potential myogenic precursors in sites other than muscle.

Similarly, several laboratories had shown that primary fibroblasts from different organs are able to undergo muscle differentiation at significant frequency when co-cultured with myogenic cells (reviewed in Cossu, 1997).

The first example of this phenomenon was correction by fibroblast-myoblast fusion of the genetic defect of the mdx mouse mutant muscle fibers (Chaudari et al., 1989; Courbin et al., 1989). More recently several laboratories found that dermal fibroblasts, from transgenic animals where a reporter gene is driven by a muscle-specific promoter, can give rise to genetically labeled muscle fibers when injected into skeletal muscle of mdx mice. In vitro myogenesis can also be induced in a mononucleated fibroblast by signals derived from neighboring myogenic cells (Gibson et al., 1995; Salvatori et al., 1995; Breton et al., 1995). It is possible that exposure of a competent mesodermal cell to a myogenic community may recruit it to the myogenic program, much as it happens during early myogenesis both in amphibians and mammals (Gurdon, 1993; Cossu et al., 1995).

A search for donor tissues that may contribute myogenic cells for muscle regeneration identified bone marrow as a possible source. By transplanting genetically-marked bone marrow into immune-deficient mice, we showed that marrow-derived cells can migrate into areas of muscle degeneration, undergo myogenic differentiation, and participate to regeneration of the damaged fibers. Because injury had been induced locally in the Tibialis Anterior, these myogenic progenitors must have reached the site of regeneration via the general circulation (Ferrari et al., 1998).

These results induced us to search for the origin of these circulating myogenic progenitors and to elucidate their possible relationship with resident satellite cells. We observed that the large majority of clones with the typical morphology of mouse adult satellite cells were derived from dorsal aorta and not from somites, the presumed source of all skeletal myogenic cells of the body. In vitro, these aorta-derived myogenic cells express a number of myogenic and endothelial markers that are also expressed by satellite cells. In vivo, aorta-derived myogenic progenitors participate in muscle regeneration and fuse with resident satellite cells (De Angelis et al., 1999). These data suggest that a subset of post-natal satellite cells may be rooted in a vascular lineage. Whether these myogenic vascular cells arise from a primordial pericyte or from endothelial cells proper, as suggested by the expression of endothelial markers, is not currently known. Furthermore these cells may be multipotent since clones of dorsal aorta can give rise to osteoblast-like cells in the presence of BMP-2; indeed multipotentiality is preserved even in adult muscle satellite cells, as proven by the fact that BMP2 can switch them to an osteogenetic fate (Katagiri et al., 1994).

When ingressing a developing muscle anlage, these progenitors should find themselves in a muscle field and thus adopt a satellite cell fate (mimicked by our clonal culture conditions). When the vasculature develops inside a different tissue, these cells may adopt the specific fate of that tissue and contribute to its histogen-esis. The only tissue in which these progenitors may remain easily accessible (because of its loose stroma) may be the bone marrow and this would explain our previous observation (Ferrari et al., 1998). Multipotent mesenchymal cells, capable of producing osteoblasts, chondroblasts, adipocytes and even skeletal muscle have long been known to be present in the bone marrow and are a subject of intense investigation; however bone marrow stromal cells do not express endothelial markers (Caplan, 1991; Prockop, 1997). Whether the cells we describe in embryonic vessels represent the progenitors of multipotent mesenchymal cells, or a separate lineage with at least part of the same developmental potential, remains to be investigated.

Conclusions

As in many other fields of vertebrate developmental biology, information is accumulated through series of experiments that utilize similar approaches. It is almost too obvious to say that analysis of mutant mice will allow the unraveling of the process of commitment, diversification and multipotentiality of mammalian skeletal myogenic cells. Indeed, recent history has shown that only a careful combination of different approaches may be really in-
formative. The complexity of a single mutant phenotype is often such that years of work are required to understand it. For example, after almost ten years since the first report, we still do not know why the Myf5 null mice lack ribs, where the gene is never expressed. In this context, simpler approaches, such as organ cultures that allow experimental manipulation of the tissues, often combined with new available genetic markers, has contributed significantly to unraveling complex biological phenomena such as for example myogenic commitment. The information available now, permits to go back to different mutant embryos and ask whether or not a certain inductive event will occur in the absence of a given gene. In the case of the origin of cells at later stages, both classic chimeric studies and lineage analysis with retroviral or genetic labeling (i.e. the cre-lox system) will allow to define in vivo the fate of progenitors originating from a given structure. Even with the caveat of these systems, it is conceivable that within the next five years we should be able to understand in much greater detail the early steps of mammalian myogenesis.

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