Int. J. Dev. Biol. 44: 699-706 (2000)

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 metazoa 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 (Hausckha, 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

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0214-6282/2000/$20.00
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Printed in Spain
www.ehu.es/ijdb

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*

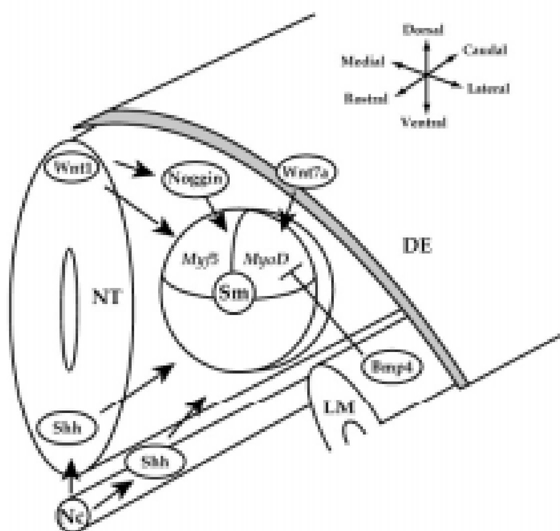

Fig. 1. Model showing influences of signaling molecules on the paraxial mesoderm. *Shh produced by the notochord (Nc) and floor plate acts on the ventral domain of newly formed somites, inducing sclerotome and also on the dorso-medial domain, inducing medial dermo-myotome. Wnt1, produced by dorsal neural tube (NT) acts (with Shh) on the dorso-medial domain of newly formed somites (Sm), where Myf5 expression is soon after observed and epaxial progenitors are specified. Wnt7a, produced by dorsal ectoderm (DE) acts on the dorso-lateral domain, where hypaxial progenitors are specified. BMP4, produced by the lateral mesoderm (LM), prevents MyoD activation and early differentiation in the lateral domain of somites. Its action is counteracted by direct binding of Noggin produced by the dorsal neural tube.*

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 Tessier-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.

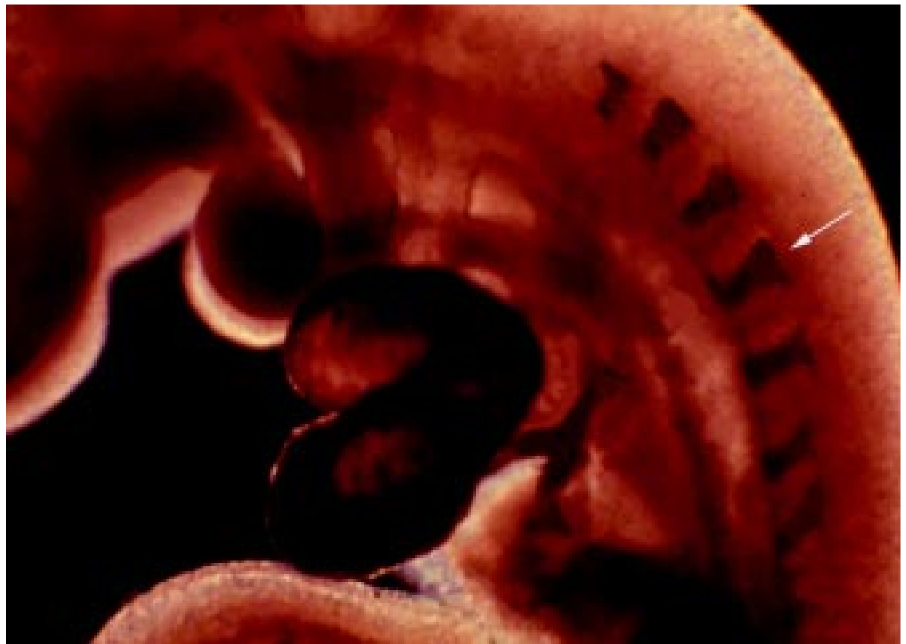

Fig. 2. Newly formed myotomes, revealed by whole-mount immunostaining with an anti-sarcomeric antibody of 9.5 dpc. mouse embryos. *Aligned, myosin positive myocytes (arrow) are regularly patterned in mature somites, and develop progressively following a cranio-caudal sequence. Each unit comprises between 100 and 200 differentiated cells.*

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 Wnt 1 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 classic 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 (Leyns *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 presomitic mesoderm and newly formed somites. We recently reported that *Frzb1* totally inhibits myogenesis in cultures of presomitic 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 *Wnt-1* and *Wnt-3a*, 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 medially, *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 *Wng* 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.

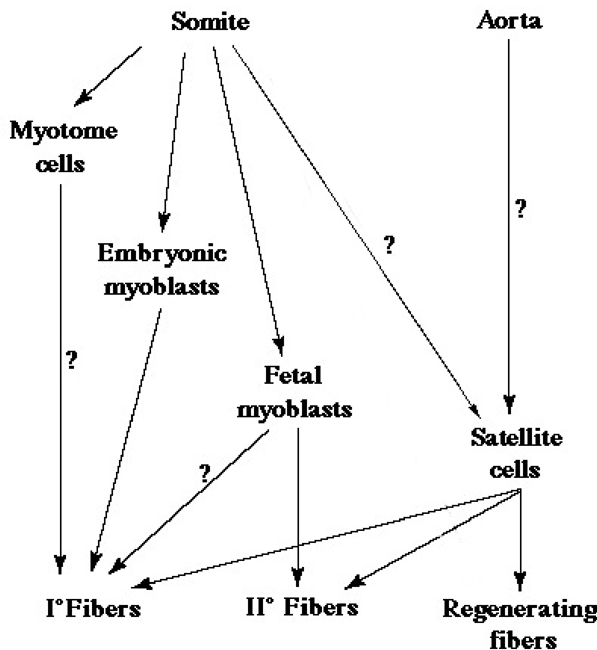

Fig. 3. A simplified model describing myogenic lineages in higher vertebrates. *Early progenitors, mainly deriving from the medial aspect of somites, initially generate the myotome. Other cells of the somite develop as embryonic and fetal (and possibly satellite) cells, responsible for the formation of primary (I°), secondary (II°) and regenerating fibers, respectively.*

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 (Buffinger 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 cells 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-

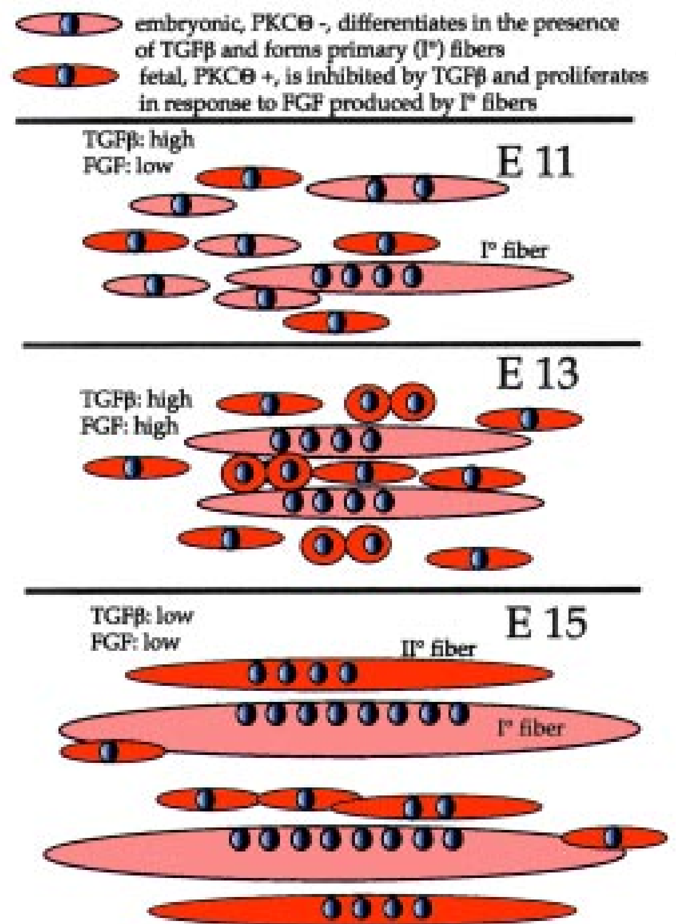

Fig. 4. Summary of the proposed paracrine control of primary/ secondary fiber formation by TGFβ and PKCθ. *Embryonic myoblasts, which do not express PKCθ, are insensitive to TGFβ and differentiate in the absence of mitogens. Fetal myoblasts that express PKCθ are inhibited by TGFβ and then resume proliferation 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.*

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 chimeras 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 as 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 *mdg* 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 *anlagen*, 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 histogenesis. 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 now 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.

Acknowledgements

The work in the Authors' laboratory was supported by grants from Telethon, European Community, HFSP, Fondazione Istituto-Pasteur Cenci Bolognetti, M.U.R.S.T. and A.S.I.

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