

Paracrine Interactions in the Development of Skeletal Muscle: an Overview

Maria G. Cusella-De Angelis, Giovanni Salvatori, Fabrizio Zappelli, Luciana De Angelis, Giulio Cossu, Mario Molinaro and Marina Bouché

Fondazione Istituto Pasteur-Cenci Bolognetti, Istituto di Istologia ed Embriologia generale, Università di Roma "La Sapienza", Roma, Italy

Abstract

In the previous years much excellent work, carried out mainly on established cell lines, has clarified the molecular mechanisms which control the various differentiation steps, as well as the arrest of proliferation in myoblasts (Buckingham 1994; Lassar et al. 1994; Olson et al. 1995). Although *in vivo* myogenesis, by extrapolating these data, should also be nearly understood in molecular terms, several aspects should also be considered. Recent work from many laboratories have demonstrated that, during skeletal muscle development in higher vertebrates, cellular interactions are essential for the correct formation of the tissue in space and time. These interactions mainly occur between: a) neighbouring tissues and myogenic cells, b) newly formed muscle fibers and the myoblasts which surround them during development. In fact development of skeletal muscle *in vivo* is highly asynchronous, starting soon after somitogenesis and lasting, as a regenerative capacity, for the entire life of the animal (Hauschka, 1994); data from several laboratories have clearly shown, through the years, the existence of different classes of myogenic cells, which show different phenotypes *in vitro*, and possibly *in vivo*, and are probably responsible for the asynchronous differentiation of skeletal muscle (Cossu and Molinaro, 1987; Stockdale and Miller, 1987). This review is centered upon these classes of myoblasts and the signals that they receive from neighboring tissues and/or exchange among themselves.

Key words: myogenic commitment, muscle cells diversification, myoblast differentiation, satellite cells, transforming growth factor β , protein kinase C θ .

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This review will analyze several aspects of skeletal muscle development in higher vertebrates, emphasizing primarily the cellular interactions which occur: 1) between neighboring tissues and myogenic cells, 2) between newly formed muscle fibers and the myoblasts which surround them during development. We and others believe these interactions to be essential for the correct formation of skeletal muscle tissue in space and time. Much excellent work in the previous years, carried out mainly on established myogenic cell lines, has clarified the molecular mechanisms which control the various differentiation steps as well as the arrest of proliferation in myoblasts [5, 38, 57]. This has led to the assumption that, by extrapolating these data, *in vivo* myogenesis should also be nearly understood in molecular terms.

It is well known that development of skeletal muscle *in vivo* is highly asynchronous, starting soon after somi-

togenesis and lasting, as a regenerative capacity, for the entire life of the animal [31]. Although stochastic models can be invoked to explain this asynchrony, data from several laboratories have clearly shown, through the years, the existence of different classes of myogenic cells, which show different phenotypes *in vitro*, and possibly *in vivo* [11, 72]. This review is centered upon these classes of myoblasts and the signals that they receive from neighboring tissues and/or exchange among themselves.

Myogenic commitment: signals and molecules from neighboring tissues

Skeletal myoblasts of the vertebrate body (with the exception of the head) are derived from somites, segmented blocks of paraxial mesoderm which form in a cranio-caudal sequence along the neural tube and the notochord [9]. Among the cells of the somite, only those which are located

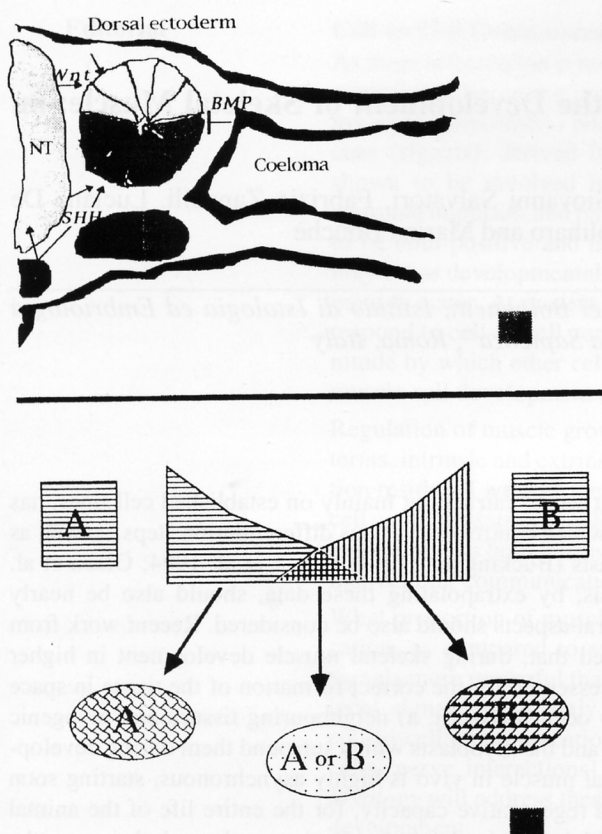


Figure 1. A) Model showing dorsal and ventral influences on the paraxial mesoderm, resulting in the activation of myogenesis through a Wnt-dependent pathway, and of chondrogenesis through a Shh-dependent pathway respectively. The possible localization of a borderline cell (asterisk) is shown. B) Schematic representation of gradients of different inducers, which specify cell towards different fates (A and B) leaving a borderline cells in an unspecified (AB) and bi-potential state.

in the dorsal domain, termed dermomyotome, are specified as myoblasts (and dermal fibroblasts) while cells located in the ventral domain, the sclerotome, will form cartilage and bone. Myogenesis depends upon activation of either *myf-5* or *MyoD*, the two upstream genes of the basic helix-loop-helix family of muscle specific transcription factors [63]. Recent work has shed light on the role of adjacent tissue such as neural tube, notochord and dorsal ectoderm, which appear to activate different myogenic programs in responding mesodermal cells. Specifically the neural tube activates myogenesis in the dorso-medial half of paraxial mesoderm (fated to give rise to epaxial muscles [58]) through a *myf-5* dependent pathway, while the dorsal ectoderm can activate myogenesis in the dorso-lateral precursors of hypaxial muscles (such as limb and body wall) through a *MyoD* dependent pathway [14]. In this case, lateral mesoderm delays the positive effect of the ectoderm suggesting that this tissue produces an inhibitory signal

[13] to maintain the cells in a committed and yet undifferentiated state during the migration to their final destination. Candidate molecules for this complex signaling activity include the secreted proteins *Sonic hedgehog* (*Shh*) and *Wnts* (the mammalian homologue of *wingless*) as positive signals [50], and the *bone morphogenetic factor 4* (*BMP4*) as a possible inhibitor [13] (figure 1). This topic is discussed in greater detail and from a different perspective in the chapter by George-Weinstein et al. mainly focused on chick embryo development [see also 13].

The generation of myoblast diversity and the origin of different fibers

The activation of the myogenic program in mesodermal cells does not directly lead to terminal differentiation. As we have already observed in the case of the precursors located in the dorso-lateral region of somites, commitment may occur, but differentiation is repressed until the cells are positioned, and after a given number of divisions occurs to attain the correct number of myoblasts. In the dorso-medial domain of somites, only a fraction of determined myogenic precursors (myotomal precursors) are induced to terminally differentiate, whereas the remaining cells are kept in a committed but undifferentiated state. Most of these cells, like the precursors of hypaxial muscles, will continue to proliferate to produce primary and secondary fibers [37]. Some of these cells will also give rise to satellite cells (Figure 2). In this context, it has been proposed that the dorsal portion of the neural tube inhibits terminal differentiation through production of growth factors [6]. Therefore, some kind of heterogeneity must be invoked to explain the differential fate of myotomal precursors versus other precursors, similar 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 fibroblast growth factors (FGFs) [40], and the first cells which form the myotome are the only myogenic cells which do not express the FGF receptor FREK [41]. Whether a preselected myogenic population fails to respond to FGF, or local signals prevent expression of FGF receptors in an homogeneous population, is unknown, since the location of myotomal precursors in the dermomyotome has not yet been determined. From a teleological point of view, this can be explained by a need to maintain 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 which 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 long and frustrating search for growth factors which may affect selectively proliferation of fetal versus embryonic cells, failed to yield any possible candidate molecule for such role. In contrast, transforming growth

factor β (TGF β) became an obvious candidate, when it became clear that it can inhibit differentiation of fetal but not of embryonic cells [16]. A few years ago, we proposed a possible mechanism by which TGF β may influence the process of primary fiber formation *in vivo* (Figure 3). This mechanism is based on several assumptions, some of which can be derived from published work, while others had to be verified experimentally. An oversimplified view of the proliferation/differentiation equilibrium of myoblasts in the limb bud assumes that these cells proliferate in the presence and differentiate in the absence of mitogens [10]. Recent *in situ* and organ culture studies have shown that FGF-4 is expressed at very high level in the apical ectodermal ridge (AER) [20, 54], and that FGF-4 can promote limb bud growth in the absence of the AER [55]. 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. Consistent with this possibility (although other mechanisms such as reduction of receptors may be invoked), the number of cells in S phase (BrdU positive) is greatly reduced at the base compared with the progress zone [33] of the limb bud in a 10.5 days post coitum (d.p.c.) mouse embryos. These observations, however, do not explain why embryonic myoblasts differentiate while fetal do not. In as much as embryonic myoblasts are insensitive to TGF β [16, 67], which is abundant in the embryonic limb [32], they may differentiate into primary fibers. In contrast, fetal myoblasts should be prevented from differentiation by TGF β . It is worth noting that TGF β is produced by the ectoderm and is supposed to act in a paracrine fashion on surrounding mesenchymal tissues [39, 47, 60, 66]. Further-

more TGF β is the only inhibitor of myogenesis, so far, which does not stimulate myoblast proliferation [22, 42, 56]. 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 mitogens locally produced by newly formed muscle fibers. The following observations argue in favour of this possibility: 1) peptide growth factors and the relative messages are frequently localized within developing muscles in the embryo [18, 20, 24, 30, 34, 62, 73, 74, 75]; 2) co-culture experiments showed that both cultured myotubes and muscle containing explants of limb buds release mitogens which stimulate myoblast proliferation into the medium [18].

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 TGF β on embryonic and fetal myoblasts [16]. However, to prove this hypothesis, it is necessary to remove TGF β from the limb, and follow muscle histogenesis under these conditions. This is obviously a difficult task, considering that TGF β exists as a family of peptides [43], whose biological activities may vary as function of concentration, localization and half life. Furthermore, TGF β may interact with many other growth factors, as well as with extracellular matrix molecules present in the developing limb. Finally it should be noted that delivery of a soluble molecule to mammalian developing limb *in utero*, still poses relevant technical problems. As a simpler approach to tackle this problem, we developed a limb organ culture system which allows differentiation of embryonic (primary) and fetal (secondary) myotubes to occur on schedule. As expected, addition of TGF β to these cultures prevented appearance of fetal but not of embryonic myotubes. More important, a neutralizing anti-TGF β antibody caused anticipated appearance of fetal myotubes, suggesting that their differentiation may be inhibited by endogenously produced TGF β [16].

The model implies that when secondary myotubes form, the level of myotube-derived mitogens and TGF β should decrease below a threshold necessary to trigger differentiation. In fact, continuous addition of TGF β to the culture prevents the appearance of secondary but not of primary myotubes. On the other hand, addition of serum or FGF to the culture prevents differentiation of both embryonic and fetal myoblasts. Indeed, only a fraction of embryonic myoblasts are inhibited by FGF ([10] and our unpublished observation). This may depend upon the fact that some embryonic myoblasts are already committed when they were explanted *in vitro* or, alternatively, that there are differences among embryonic myoblasts in their response to FGF [70]. The level of FGF is known to decrease in the fetus [71] and our preliminary data suggest a dramatic decrease of the endogenous level of TGF β in mouse limbs

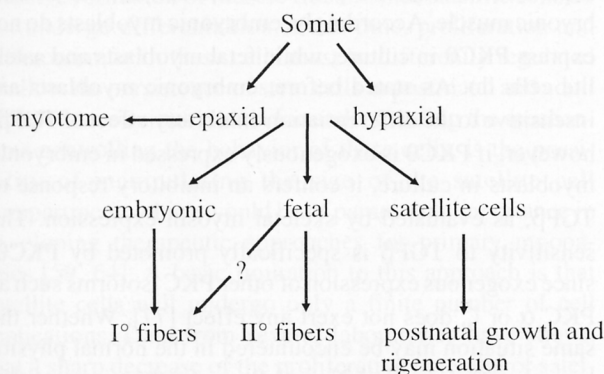


Figure 2: A simplified model describing myogenic lineages in higher vertebrates: only epaxial precursors, deriving from the medial aspect of somites, generate the myotome. Both epaxial and hypaxial precursors, which derive from the lateral aspect of somites, give rise to embryonic, fetal and satellite cells, responsible for the formation of primary (I°), secondary (II°) and regenerating fibers, respectively.

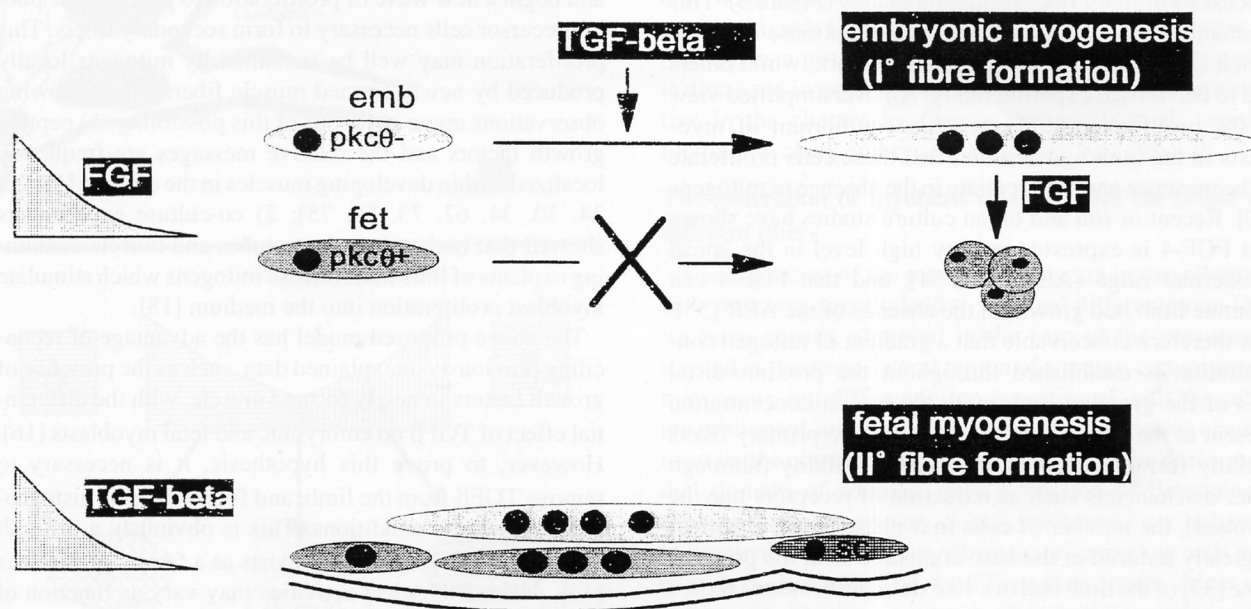
PARACRINE CONTROL OF *IN VIVO* MYOGENESIS

Figure 3. Summary of the proposed paracrine control of primary/secondary fiber formation by TGFβ, through a PKCθ-mediated mechanism. For details, see text.

between 15 and 17 d.p.c., coincident with secondary fibers formation. Work is in progress to clarify these events.

Paracrine control of myoblast proliferation and differentiation

The model outlined above is mainly based upon the selective inhibition of differentiation caused by exposure of fetal myoblasts to TGFβ. If the model is correct, one expects that differential expression of molecules involved in the transduction of extracellular signals may contribute to the different phenotypes. Very little information is available regarding the TGFβ induced signaling events. Most studies have been conducted in cells where TGFβ interferes with cell proliferation. However, in muscle cells, TGFβ inhibits differentiation without interfering with cell growth, suggesting that TGFβ may be able to activate different intracellular signaling mechanisms, depending upon the status or type of cells. The first insight on the molecular basis of the TGFβ signaling events in myogenic cells comes from the observation that the predominantly expressed protein kinase C (PKC) isoform in muscle, PKCθ, is selectively expressed in fetal myoblasts and satellite cells, *in vivo* and *in vitro* [77]. PKC constitutes a family of serine/threonine protein kinases, often involved in different cellular processes, such as growth and differentiation. To date eleven different isoforms have been identified and cloned, and, although no substrate specificity

among the different isoforms has been described, the informations available suggest specific roles of each of them in different systems [3, 19, 48, 51].

Among the PKC isoforms tested, PKCθ is the only one expressed in fetal and post-natal muscle but not in embryonic muscle. Accordingly, embryonic myoblasts do not express PKCθ in culture, while fetal myoblasts and satellite cells do. As stated before, embryonic myoblasts are insensitive to the differentiation-inhibitory effect of TGFβ; however, if PKCθ is exogenously expressed in embryonic myoblasts in culture, it confers an inhibitory response to TGFβ, as evaluated by skeletal myosin expression. The sensitivity to TGFβ is specifically promoted by PKCθ, since exogenous expression of other PKC isoforms such as PKC α or ζ, does not exert any effect [77]. Whether the same situation may be encountered in the normal physiological state is unclear. Nonetheless, our data indicate that the TGFβ signaling pathways are specifically mediated by PKCθ in myogenic cells. Why differential expression of PKCθ should dictate the differential fate of embryonic versus fetal myoblasts remains a matter of speculation. It is a fact, however, that only a fraction of the total myogenic precursor population differentiate, at the embryonic period of development, in the base of the limb bud. All myoblasts in this area are probably exposed to a low growth factor concentration but to a high concentration of TGFβ. At this

time, embryonic myoblasts do not express PKC θ , thus they are insensitive to TGF β and may differentiate into primary fibers, whereas differentiation of fetal myoblasts, which express PKC θ , is prevented by TGF β (Figure 3). Once primary fibers are formed, they begin to produce growth factors such as FGF [20, 30] and, thus, promote a new wave of proliferation in fetal cells. The identification of PKC θ 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 provide each cell with PKC θ . If the prediction is correct, all myogenic cells should become sensitive to TGF β , and primary fibers should not be formed in the mouse developing limbs. This possibility is currently under investigation.

Satellite cells and the paracrine control of their function

Satellite cells are classically defined as quiescent mononucleated cells, located between the sarcolemma and the basal lamina [44]. Satellite cells exhibit specific features that distinguish them from embryonic and fetal myoblasts. Previous work from this laboratory identified specific markers of satellite cells [11]. Furthermore, Megeney et al. [45] recently showed that in MyoD deficient mice regeneration is reduced, suggesting that this class of myogenic cells is more dependent upon this factor than other classes of myogenic cells.

Satellite cells emerge from the fetal myogenic cell compartment at 16-18 days of development in mouse embryo and at 12-14 weeks in human embryo. 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, proliferation and differentiation in these cells must be differently regulated to allow the persistence of stem cells in post-natal and adult muscle. An obvious reason for understanding 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 [59, 64]. A basic limitation to this approach is that satellite cells will 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 and slower decline until senility [68, 69]. The reason for this phenomenon is unclear but three general mechanisms have been proposed, which are not mutually exclusive: 1) satellite cells continue to divide during post-natal life and use their finite number of divisions *in vivo*; 2) satellite cells may express an autonomous program that reduces their ability to replicate during post-natal life; this mechanism suggests that a progressive desensitization to specific hormones/growth factors is responsible for the finite number of cell cycles; 3) alternatively it may be postulated that myogenic cell proliferation

is under paracrine control which declines during post-natal life.

Several reports indicate that satellite cells in culture are mitotically activated by addition to the medium of growth factors such as fibroblast growth factor, platelet derived growth factor, insulin growth factor, adrenocorticotropin and melanocyte stimulating hormones, leukemia inhibiting factor, colony stimulating factor granulocyte/macrophage, arginine vasopressine, hepatocyte growth factor [18, 21, 52, 76]. The physiological role of these factors is supported by the findings that some of these factors are also secreted by developing and regenerating muscle fibers [18, 28]. To test the first hypothesis, satellite cells, cloned in low serum containing medium, were challenged with these growth factors, in order to force their limited proliferation potential. However, even high concentration of the mitogens were ineffective, suggesting that either cells have become completely insensitive to these growth factors or that other mechanisms may be involved [46].

Previous results from this and other laboratories have shown that differentiated myotubes secrete factors stimulating replication of undifferentiated myoblasts. Furthermore, it is well known that regenerating skeletal muscle fibers secrete factors which activate satellite cells replication [1, 18, 35]. This evidence supports the hypothesis that differentiating or regenerating muscle fibers may exert a paracrine stimulation of undifferentiated myogenic cells replication. The decline of this regulatory mechanism in satellite cells replication during the post-natal life, could be responsible for the reduction in satellite cell proliferation. To address this problem, satellite cells were isolated from mouse at different ages, and grown in culture in the presence of conditioned medium from myogenic and non myogenic cells. The result of this experiment showed that conditioned medium of differentiated myotubes from new born mouse may revert the progressive decrease of replication potential of satellite cells, occurring through the post-natal life of the animal. Other cell types were ineffective in producing this effect. A similar experimental approach was exploited to investigate whether muscle fibers *in vivo* exert the same stimulatory action on satellite cells replication. To this purpose, muscle fibers from young mouse, whose satellite cells have high mitogenic potential, were extracted in saline buffer, and the extract was added to the culture medium of satellite cells from animals of different age. These cells, which normally exhibit a progressive decrease with age of their mitogenic potential, were induced to revert this property, and to produce clones of larger size as those from younger animals. On the other hand, extracts from aged muscle were ineffective [46]. These data strongly suggest that a reduced concentration of mitogenic signals, operating through a paracrine mechanism within the muscle fiber, might be responsible for the progressive loss of the satellite cell replication capability, through the life span of the animal.

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

Recent evidence, from several laboratories, have shown that primary fibroblasts from different organs, and 10T1/2 mesenchymal cells as well, are able to undergo muscle differentiation, at significant frequency, when cocultured with myogenic cells. The first example of this phenomenon was the correction of the genetic defect of the *mdg* mouse mutant muscle fibers, by fibroblast-myoblast fusion [8, 15]. Recently Gibson et al. [23] found that dermal fibroblasts can give rise to muscle fibers when injected into skeletal muscle of *mdx* mice. These studies show evidence for the fusion of fibroblasts with myogenic cells. In these cases myogenesis could be activated as it occurs in heterokaryons, where the fibroblast nucleus is exposed to muscle transcription factors. However, we and others reported that myogenesis can also be induced in a mononucleated fibroblast, by signals derived from neighboring myogenic cells: in this case fusion is just a consequence of the acquired myogenic phenotype [4, 65].

It remains to be explained why these mesodermal cells require a "muscle field" to undergo myogenesis and have not already undergone spontaneous differentiation. In vertebrates, organs or parts of them are established starting from "progenitor fields", which are dependent upon complex cross-talks between diffusible molecules and specific transcription factors [17]. The recently reported "community effect" (for amphibian myogenesis) may explain, in part, how such fields may be established [27]. In addition to inductive signals, embryonic cells must check that they are surrounded by similar cells in order to be committed to a given fate. We have recently observed the existence of a "community effect" for mammalian myogenesis as well [12]. In the examples of myogenic conversion reported here, however, cells come from tissues that have already completed morphogenesis and, therefore, we may imagine a recruitment depending on the occurrence of cells which are not uniquely determined, such as those cells whose precursors lay at the border of a "progenitor field". Since any field must, by definition, possess sharp boundaries, those cells which find themselves at the border of a "progenitor field", may escape from the "community", since they are not completely surrounded by sister cells; furthermore, they receive subthreshold levels of different signals. These cells may, therefore, be "frozen" in a penultimate and possibly bi-potential state (figure 1B). Other examples of lineage switching among mesodermal cells have been reported [25, 36], supporting the idea of mesenchymal cells which maintain a bi-potential or multipotential state [7]. In this state, mesenchymal cells may be induced to adopt a specific pathway of terminal differentiation from signals derived from neighboring differentiating cells, as it occurs in a forming muscle field (both *in vitro* and *in vivo* during regeneration). 10T1/2 cells may well be the immortalized progeny of one of these cells. This situation,

which is conceptually different from the plasticity of the already differentiated state [2], might have been exploited during mammalian evolution to compensate for the reduced regenerative capacity of mammalian, as compared to lower vertebrate tissues [49]. In the case of skeletal muscle, regeneration potential may be rapidly exhausted in response to chronic injury, such it occurs in Duchenne Muscular Dystrophy [59]. Surprisingly, *in situ* analysis of MyoD and myogenin expression in regenerating muscle revealed an unpredicted high number of positive cells near the area of muscle necrosis [26], far exceeding that of the expected resident satellite cells. These MyoD expressing cells might derive from satellite cells of neighboring muscles, which have rapidly migrated into the damaged area, but may, as well, represent recruitment into myogenesis of resident mesodermal cells, which appear as fibroblasts, but may maintain a myogenic potential.

In addition to its relevance to mammalian myogenesis, myogenic conversion may be exploited for practical purposes: rescue of dysgenic skeletal muscle is impaired by the low number of satellite cells which can be transduced *ex vivo* (especially if coming from an already diseased muscle tissue). Fibroblasts from a more accessible and less damaged site, such as the dermis, could be expanded, transduced, and then converted to myogenesis *in vitro*. The final number of transduced autologous myogenic cells may be, therefore, increased one or more orders of magnitude.

Concluding Remarks

Muscle development is a multistep process, based on the heterogeneity of the myogenic cell lineage. The mechanisms leading to the sorting out of the different myogenic compartments begin to be understood and appear as a cascade of cell interactions between inducers and competent cells. Factors secreted by paracrine mechanisms, their receptors on the induced cells and the signal transduction mechanisms activating lineage specific genes, are the components of a complex interacting system leading to the expression of the terminally differentiated muscle phenotype. An accurate balance between positive and negative inducers appears to be operative in the early stages of muscle determination, when signals derive mostly from neighboring tissues. Later, the asynchronous differentiation of embryonic and fetal muscle fibers may depend on the inability of embryonic myoblasts to respond to signals inhibiting their differentiation. Work from many laboratories has identified TGF β as a specific inhibitor of fetal myoblast differentiation. Recent evidences, discussed here, indicate that PKC θ is involved in the transduction pathway generated by this growth factor, and that the lack of PKC θ expression in embryonic myoblasts may explain the insensitivity of these cells to the inhibitory action of TGF β . The mechanisms leading to developmental differences of the transduction pathways among myogenic precursor cells are presently unclear, and represent a task for future investigations.

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Address correspondence to:

M. Bouché, Istituto di Istologia ed Embriologia generale, Università di Roma "La Sapienza", via A. Scarpa 14, 00161 Roma, Italy.

References

- [1] Bischoff R: A satellite cell mitogen from crushed adult muscle. *Dev Biol* 1986; 115: 140-147.
- [2] Blau HM: Differentiation requires continuous active control. *Annu Rev Biochem* 1992; 61: 1213-1230.
- [3] Bouché M, Zappelli F, Polimeni M, Adamo S, Wetsel WC, Senni MI, Molinaro M: Rapid activation and down-regulation of protein kinase C α in 12-O-tetradecanoylphorbol-13-acetate-induced differentiation of rhabdomyosarcoma cells. *Cell Growth Differ* 1995; 6: 845-852.
- [4] Breton M, Li ZL, Paulin P, Harris JA, Rieger F, Pincon-Raimond M, Garcia L: Myotube driven recruitment of myogenic cells during *in vitro* myogenesis. *Dev Dynam* 1995; 202: 126-136.
- [5] Buckingham M: Muscle: the regulation of myogenesis. *Curr Opin Gen Dev* 1994; 4: 745-751.
- [6] Buffinger N, Stockdale FE: Myogenic specification of somites is mediated by diffusible factors. *Dev Biol* 1995; 169: 96-108.
- [7] Caplan A: Mesenchymal stem cells. *J Orthopaed Res* 1991; 9: 641-650.
- [8] Chaudari N, Delay R, Beam KG: Restoration of normal function in genetically defective myotubes by spontaneous fusion with fibroblasts. *Nature* 1989; 341: 445-447.
- [9] Christ B, Ordhal CP: Early stages of chick somite development. *Anat Embryol* 1994; 191: 381-396.
- [10] Clegg CH, Linkhart TA, Olwin BB, Hauschka SD: Growth factor control of skeletal muscle differentiation: commitment to terminal differentiation occurs in G1 phase and is repressed by fibroblast growth factor. *J Cell Biol* 1987; 105: 949-956.
- [11] Cossu G, Molinaro M: Cell heterogeneity in the myogenic lineage. *Curr Top Dev Biol* 1987; 23: 185-208.
- [12] Cossu G, Kelly R, Di Donna S, Vivarelli E, Buckingham M: Myoblast differentiation during mammalian somitogenesis is dependent upon a community effect. *Proc Natl Acad Sci USA* 1995; 92: 2254-2258.
- [13] Cossu G, Tajbakhsh S, M Buckingham M: Myogenic specification in mammals. *Trends Genet* 1996a; 12: 218-223.
- [14] Cossu G, Kelly R, Tajbakhsh S, Di Donna S, Vivarelli E, M Buckingham M: Activation of different myogenic pathways: myf-5 is induced by the neural tube and MyoD by the dorsal ectoderm in mouse paraxial mesoderm. *Development* 1996b; 122: 429-437.
- [15] Courbin P, Koenig J, Ressouches A, Beam KG, Powell JA: Rescue of excitation-contraction coupling in disgenic muscle by addition of fibroblasts *in vitro*. *Neuron* 1989; 2: 1341-1350.
- [16] Cusella-De Angelis MG, Molinari S, Ledonne A, Coletta M, Vivarelli E, Bouché M, Molinaro M, Ferrari S, Cossu G: Differential response of embryonic and fetal myoblasts to TGF β : a possible regulatory mechanism of skeletal muscle histogenesis. *Development* 1994; 120: 925-933.
- [17] Davidson EH: Later embryogenesis: regulatory circuitry in morphogenetic fields. *Development* 1993; 118: 665-690.
- [18] De Angelis L, Cusella-De Angelis MG, Bouché M, Vivarelli E, Boitani C, Molinaro M, Cossu G: ACTH-like peptides in postimplantation mouse embryos: a possible role in myoblast proliferation and muscle histogenesis. *Dev Biol* 1992; 151: 446-458.
- [19] Dekker LV, Parker PJ: Protein kinase C - a question of specificity. *Trends Biochem Sci* 1994; 19: 73-77.
- [20] Drucker BJ, Golfarb M: Murine FGF-4 gene expression is spatially restricted within embryonic skeletal muscle and other tissues. *Mech Dev* 1993; 40: 155-163.
- [21] Florini JR, Ewton DZ, Magri KA: Hormones, growth factors, and myogenic differentiation. *Annu Rev Physiol* 1991; 53: 201-216.
- [22] Florini JR, Roberts AB, Ewton DZ, Falen SL, Flinders KC, Sporn MB: Transforming growth factor-beta. A very potent inhibitor of myoblast-differentiation, identical to the differentiation inhibitor secreted by buffalo rat liver cells. *J Biol Chem* 1986; 261: 16509-16513.
- [23] Gibson AJ, Karasinski J, Relvas J, Moss J, Sherratt TG, Strong PN, Watt DJ: Dermal fibroblasts convert to a myogenic lineage in mdx mouse muscle. *J Cell Sci* 1995; 106: 207-214.
- [24] Gonzales A, Buscaglia M, Ong M, Baird A: Distribution of basic fibroblast growth factor in the 18-day rat fetus: localization in the basement membranes of diverse tissues. *J Cell Biol* 1990; 110: 753-765.
- [25] Grigoriadis AE, Heersche JNM, Aubin JE: Differentiation of muscle, fat, cartilage and bone from

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- progenitor cells present in a bone marrow derived clonal cell population: effect of dexamethasone. *J Cell Biol* 1988; 106: 2139-2151.
- [26] Grounds MD, Garrett KL, Lai MC, Wright WE, Beilharz MW: Identification of skeletal muscle precursor cells *in vivo* by use of MyoD1 and myogenin probes. *Cell Tissue Res* 1991; 267: 99-104.
- [27] Gurdon JB: Community effect and related phenomena in development. *Cell* 1993; 75: 501-506.
- [28] Hannon K, Kudla AJ, McAvoy MJ, Clase KL, Olwin BB: Differentially expressed fibroblast growth factors regulate skeletal muscle development through autocrine and paracrine mechanisms. *J Cell Biol* 1996; 132: 1151-1159.
- [29] Harris AJ, Duxson MJ, Fitzsimons RB, Rieger F: Myonuclear birthdates distinguish the origins of primary and secondary myotubes in embryonic mammalian skeletal muscle. *Development* 1989; 107: 771-784.
- [30] Haub O, Goldfarb M: Expression of the fibroblast growth factor-5 gene in the mouse embryo. *Development* 1991; 112: 397-406.
- [31] Hauschka SD: The embryonic origin of skeletal muscle, in *The scientific basis of myology*. Academic Press, 1994, pp. 3-72.
- [32] Heine UI, Munoz EF, Flandrs KC, Ellingsworth LR, Lam H-YP, Thompson NL, Roberts AB, Sporn MB: Role of transforming growth factor in development of the mouse embryo. *J Cell Biol* 1987; 105: 2861-2876.
- [33] Hornbruch A, Wolpert L: Cell division and morphogenesis of the chick limb. *Nature* 1970; 226: 764-776.
- [34] Joseph-Silverstein J, Consigli SA, Lyser KM, Ver Pault C: Basic Fibroblast Growth Factor in the chick embryo: immunolocalization to striated muscle cells and their precursors. *J Cell Biol* 1989; 108: 2459-2466.
- [35] Kardami E, Spector D, Sthroman RC: Myogenic growth factor present in skeletal muscle is purified by heparin affinity chromatography. *Proc Nat Acad USA* 1985; 82: 8044-8047.
- [36] Katagiri T, Yamaguchi A, Komaki M, Abe E, Takahashi N, Ikeda T, Rosen V, Wozney JM, Fujisawa-Sehara A, Suda T: Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage. *J Cell Biol* 1994; 127: 1755-1766.
- [37] Kelly AM, Zachs S: The histogenesis of rat intercostal muscle. *J Cell Biol* 1969; 42: 154-169.
- [38] Lassar AB, Skapek SX, Novitch B: Regulatory mechanisms that coordinate skeletal muscle differentiation and cell cycle withdrawal. *Curr Opin Cell Biol* 1994; 6: 788-794.
- [39] Lehnert SA, Akhurst RJ: Embryonic expression pattern of TGF β type-1 RNA suggests both paracrine and autocrine mechanisms of action. *Development* 1988; 104: 263-273.
- [40] Lumdsen A: The cellular basis of segmentation in the developing hindbrain. *Trends Neurosci* 1990; 13: 329-335.
- [41] Marcelle C, Wolf J, Bonner-Fraser M: The *in vivo* expression of the FGF receptor FREK mRNA in avian myoblasts suggests a role in muscle growth and differentiation. *Dev Biol* 1995; 172: 100-114.
- [42] Massagué J, Cheifetz S, Endo T, Nadal-Ginard B: Type β transforming growth factor is an inhibitor of myogenic differentiation. *Proc Nat Acad Sci USA* 1986; 83: 8206-8210.
- [43] Massagué J: The transforming growth factor β family. *Annu Rev Cell Biol* 1990; 6: 597-641.
- [44] Mauro A: Satellite cells of skeletal muscle fibers. *J Biophys Biochem Cytol* 1961; 9: 493-495.
- [45] Megeney LA, Kablar B, Garret K, Anderson JE, Rudnicki MA: MyoD is required for myogenic stem cell function in adult skeletal muscle. *Gene Dev* 1996; 10: 1173-1183.
- [46] Mezzogiorno A, Coletta M, Zani BM, Cossu G, Molinaro M: Paracrine stimulation of senescent satellite cell proliferation by factors released by muscle or myotubes from young mice. *Mech Ageing Dev* 1993; 70: 35-44.
- [47] Millan FA, Denhez F, Kondaiah P, Akhurst RJ: Embryonic gene expression pattern of TGF β 1, β 2, and β 3 suggest different developmental function *in vivo*. *Development* 1991; 111: 131-144.
- [48] Mischak H, Pierce JH, Goodnight JA, Kazanietz MG, Blumberg PM, Mushinski JF: Phorbol ester-induced myeloid differentiation is mediated by protein kinase C- α and - δ and not by protein kinase C- β II, - ϵ , - ζ , - η . *J Biol Chem* 1993; 268: 20110-20115.
- [49] Muneoka K, Sassoon D: Molecular aspects of regeneration in developing vertebrate limbs. *Dev Biol* 1992; 152: 37-49.
- [50] Münsterberg AE, Kitajewski J, Bumcrot DA, McMahon AP, Lassar AB: Combinatorial signaling by Sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite. *Gene Dev* 1995; 9: 2911-2922.
- [51] Murray NR, Baumgardner GP, Burns DJ, Fields AP: Protein Kinase C isotypes in human erythroleukemia (K562) cell proliferation and differentiation. *J Biol Chem* 1993; 268: 15847-15853.
- [52] Nervi C, Benedetti L, Minasi A, Molinaro M, Adamo S: Arginine-vasopressin induces differentiation of skeletal myogenic cells and up-regulation

- of myogenin and myf-5. *Cell growth Differ* 1995; 6: 81-89.
- [53] Nishizuka Y: Studies and perspectives of protein kinase C. *Science* 1986; 233: 305-312.
- [54] Niswander L, Martin GL: FGF-4 expression during gastrulation, myogenesis, limb and tooth development in the mouse. *Development* 1992; 114: 755-768.
- [55] Niswander L, Martin GR: FGF-4 and BMP-2 have opposite effects on limb growth. *Nature* 1993; 361: 68-71.
- [56] Olson EN, Stenberg E, Hu JS, Spizz G, Wilcox C: Regulation of myogenic differentiation by the type β transforming growth factor. *J Cell Biol* 1986; 103: 1799-1855.
- [57] Olson EN, Perry M, Schulz RA: Regulation of muscle differentiation by the MEF2 family of MADS box transcription factors. *Dev Biol* 1995; 172: 2-14.
- [58] Ordahl CP, LeDouarin NM: Two myogenic cell lineages within the developing somite. *Development* 1992; 114: 339-353.
- [59] Partridge TA: Myoblast transfer: a possible therapy for inherited myopathies? *Muscle Nerve* 1991; 14: 197-179.
- [60] Pelton RW, Nomura S, Moses HL, Hogan BLM: Expression of transforming growth factor β 2 RNA during murine embryogenesis. *Development* 1989; 106: 759-767.
- [61] Porqu   O, Fan CM, Coltey Hirsinger E, Watanabe YM, Breant C, Francis-West P, Brickell P, Tessier-Lavigne M, Le Douarin N: Lateral and axial signals involved in avian somite patterning: a role for BMP4. *Cell* 1996; 84: 461-471.
- [62] Ralphs JR, Wylie L, Hill DJ: Distribution of insulin like growth factor peptides in the developing chick embryo. *Development* 1990; 109: 51-58.
- [63] Rudnicki MA, Schnegelsberg PNJ, Stead RH, Braun T, Arnold HH, Jaenisch R: MyoD or Myf-5 is required for the formation of skeletal muscle. *Cell* 1993; 75: 1351-1359.
- [64] Salvatori G, Ferrari G, Mezzogiorno A, Servidei S, Coletta M, Tonali P, Giavazzi R, Cossu G, Mavilio F: Retroviral vector-mediated gene transfer into human primary myogenic cells leads to expression in muscle fibers *in vivo*. *Hum Gene Ther* 1993; 4: 713-723.
- [65] Salvatori G, Lattanzi L, Coletta M, Aguanno S, Vivarelli E, Kelly R, Ferrari G, Harris AJ, Mavilio F, Molinaro M, Cossu G: Myogenic conversion of mammalian fibroblast induced by differentiating muscle cells. *J Cell Sci* 1995; 108: 2733-2739.
- [66] Schmid P, Cox D, Bilbe G, Maier R, McMaster GK: Differential expression of TGF β 1, β 2 and β 3 genes during mouse embryogenesis. *Development* 1991; 111: 117-130.
- [67] Schofield JN, Wolpert L: Effect of TGF β 1, TGF β 2 and bFGF on chick cartilage and muscle cell differentiation. *Exp Cell Res* 1990; 191: 144-148.
- [68] Schultz E: Satellite cell proliferative compartments in growing skeletal muscles. *Dev Biol* 1996; 175: 84-94.
- [69] Schultz E, Lipton GH: Skeletal muscle satellite cell: changes in proliferation potential as a function of age. *Mech Ageing Dev* 1982; 13: 377-383.
- [70] Seed J, Hauschka SD: Clonal analysis of vertebrate myogenesis. VIII. Fibroblast growth factor (FGF)-dependent and FGF-independent muscle colony types during chick wing development. *Dev Biol* 1988; 128: 40-49.
- [71] Seed J, Olwin BB, Hauschka SD: Fibroblast Growth Factor levels in the whole embryo and limb bud during chick development. *Dev Biol* 1988; 128: 50-57.
- [72] Stockdale FE, Miller JB: The cellular basis of myosin heavy chain heterogeneity during development of avian skeletal muscle. *Dev Biol* 1987; 123: 1-9.
- [73] Stylianopoulos F, Efstratiadis A, Herbert J: Pattern of the insulin-like growth factor II gene expression during rat embryogenesis. *Development* 1988; 103: 497-506.
- [74] Tollfsen SE, Sadow JL, Rotwein P: Coordinated expression of insulin-like growth factor II and its receptor during muscle differentiation. *Proc Natl Acad Sci USA*, 1989; 86: 1543-1547.
- [75] Wilkinson D, Sangita B, McMahon AP: Expression pattern of the FGF-related proto-oncogene int-2 suggest multiple roles in fetal development. *Development* 1989; 105: 131-136.
- [76] Yablonka-Reuveni Z, Seifert RA: Proliferation of chicken myoblasts is regulated by specific isoforms of platelet-derived growth factor: evidence for differences between myoblasts from mid and late stages of embryogenesis. *Dev Biol* 1993; 156: 307-318.
- [77] Zappelli F, Willems D, Osada S, Ohno S, Wetsel WC, Molinaro M, Cossu G, Bouch   M: The inhibition of differentiation caused by TGF β in fetal myoblasts is dependent upon selective expression of PKC θ . A possible molecular basis for myoblast diversification during limb histogenesis. *Dev Biol (in press)*.