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Invited Review

Unorthodox myogenesis: possible developmental significance and implications for tissue histogenesis and regeneration

G. Cossu

Department of Histology and Medical Embryology, University of Rome "La Sapienza", Rome, Italy

Summary. During the last few years several reports have described the occurrence of skeletal myogenesis in cells derived from embryonic, fetal and perinatal tissues that usually do not contribute to skeletal muscle in the adult vertebrate body.

After a brief description of current ideas on myogenic determination in higher vertebrates, three examples of this unorthodox myogenesis will be described: 1) the occurrence of myogenesis in chick epiblast cells, cultured in isolation in serum-free medium; 2) the presence of cells endowed with myogenic potential in the embryonic mouse neural tube; and 3) the occurrence of spontaneous or induced myogenesis in mesenchymal cells during fetal and post-natal life.

A possible embryological basis for unorthodox myogenesis, in relation to gastrulation and morphogenetic fields, is then presented. It is also proposed that unorthodox myogenesis may represent a compensatory mechanism for higher vertebrates that have lost much of the regeneration potential of lower vertebrates.

Key words: Myogenesis, Determination, Histogenesis, Differentiation, Myogenic genes

Introduction

Differentiation is commonly defined as a process which leads a cell to express a repertoire of genes specific and characteristic of the tissue where the cell belongs. However we can presently study with some confidence only terminally differentiated cells. In this context it is important to distinguish terminal differentiation from all the previous steps that lead the progeny of a blastomere to progressive diversification from the other cells of the embryo. Indeed, as pointed out by Holtzer many years ago (Holtzer et al., 1973), there is no such cell as an undifferentiated cell: in a given area of an embryo, at a given developmental stage, each cell is already different from cells located in different areas as well as from its ancestors and progeny. Although in most cases fate may still be experimentally changed, cells already express a subset of genes typical, although perhaps not unique, of a particular developmental stage and location. Up to few years ago we knew very few of these genes. With the explosive progress of molecular embryology, new developmental genes are continuously identified and their developmental pattern of expression described. An emerging picture begins to appear where each cell, or rather each group of cells, expresses a unique combination of genes, such as homeogenes and other transcription factors, growth factors and cytokines, extracellular matrix and adhesion molecules, each of which is certainly also expressed in many other places and times. As a result, a progressive specification of position and fate is obtained leading eventually to terminal differentiation. For example a newly formed mesodermal cell (which already expresses mesoderm-specific genes such as *brachiury, noggin*, etc.) may find itself closer to the axial structures (notochord and mesoderm) and thus adopt a paraxial fate, ending up in somites. Later the dorsal part of the somite is specified as dermomyotome and finally a choice between the fibroblastic and the myogenic lineage will be made.

The availability of new molecular markers has allowed us to investigate the expression of specific gene products at the single cell level, during tissue histogenesis and regeneration. Several of these studies have unexpectedly revealed the occurrence of (or the potential for) skeletal muscle differentiation in a significant number of cells belonging to tissues where muscle normally does not form. Furthermore this process has been observed at inappropriate time, either before gastrulation or after the end of organogenesis.

In this review I will briefly discuss current ideas on myogenic determination in mammals, then describe three different examples of unorthodox myogenesis, and then I will discuss possible developmental significance

Offprint requests to: Dr. Guiulio Cossu, Institute of Histology and General Embryology, University of Rome «La Sapienza», 14 Via A. Scarpa, 00161 Italy

in terms of plasticity and regenerative potential of mammalian tissues.

Unorthodox myogenesis is conceptually distinct from trans-differentiation, a phenomenon by which an already differentiated cell can be induced to change the repertoire of gene expressed and to express genes typical of a different tissues. In higher vertebrates, this situation is mainly related to pathology (metaplasia), although trans-differentiation from smooth to skeletal muscle has been recently demonstrated at the single cell level in the post-natal mammalian esophagus (Patapoutian et al., 1995). Trans-differentiation is not discussed in this review.

The origin of myoblasts in mammals

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 (Christ and Ordhal, 1995). Among the cells of the somite, only those which are located 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 this family of muscle specific transcription factors (Rudnicki et al., 1993). 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: Ordhal and Le Douarin, 1991) 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 *Myo D*-dependent pathway. In this case, lateral mesoderm delays the positive effect of the ectoderm suggesting that this tissue produces an inhibitory signal (Cossu et al., 1996a) 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 Sonic hedgehog and Wnts as positive signals (Munstenberg et al., 1995), and BMP4 as a possible inhibitor (reviewed in Cossu et al., 1996b).

Myogenesis as a default process

In apparent contrast with what is exposed above, recent observations suggest a "default" tendency of embryonic cells toward myogenesis, which should be therefore repressed *in vivo* until proper time and place. In contrast with their normal fate, the great majority of cells from the chick epiblast layer, cultured *in vitro*, will undergo myogenesis, even when grown at low clonal density in protein-free medium. Co-culture of the epiblast cells with adjacent tissue will inhibit myogenesis (George-Weinstein et al., 1996). One interpretation of these experiments therefore is that epiblast cells even prior to their entry into the primitive streak and specification as mesoderm, are already programmed for myogenesis and that in the absence of repression exerted by the *in vivo* context, they will differentiate into muscle (Fig. 1). *Notch* has been proposed as a possible mediator of this inhibitory mechanism (Kopan et al., 1994). If this is the case, then the influence of structures surrounding the early somites is not to induce myogenesis but to relieve its repression. By PCR analysis, *MyoD* messenger RNA is detectable in the chick epiblast and in the mouse embryo prior to somitogenesis (Kopan et al., 1994; George-Weinstein et al., 1996). The recent claim that, in Drosophila, wingless is another ligand of *notch* (Couso and Martinez-Arias, 1994), potentially competing with delta, raises the possibility that in vertebrates the Wnts (homologues of wingless) produced by the neural tube or dorsal ectoderm, relieve repression of myogenesis by blocking the *notch* receptor. Even if the receptor for Wnt has been recently identified as *Frizzle* (Bhanot et al., 1996), a protein different from *Notch*, a cross-talk between the two receptors may anyway relieve this repressive action. Whatever the case in molecular terms, the natural tendency of embryonic cells towards myogenesis may explain the peculiar capacity of *MyoD* to activate myogenesis in non muscle cells and should be kept in mind when discussing the unorthodox origin of myogenic cells.

Misplaced cells and lineage infidelity

Spontaneous myogenic differentiation of cells from the brain has been documented a number of times (examples quoted in Tajbakhsh et al., 1994) but it was only through insertion of the reporter gene *LacZ* into the *myf-5* locus that it was possible to unequivocally identify *myf-5* expressing cells in the nervous system and to show that these cells co-express neural and muscle markers

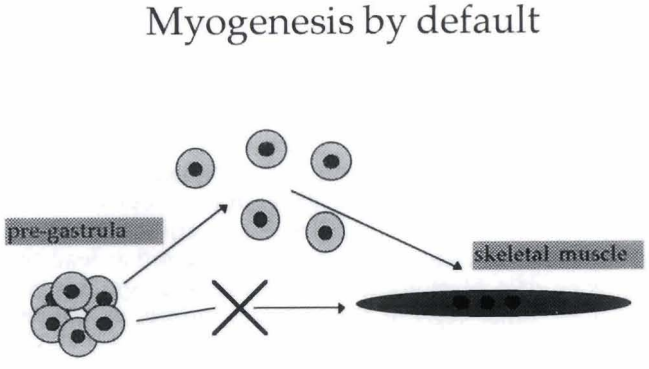

Fig. 1. A schematic representation of the repressive influence of cell-cell interaction on the default tendency to myogenesis of chick pre-gastrula cells.

(Tajbakhsh et al., 1994). A possible explanation for the neural origin of a small population of myoblasts may be found in the process of gastrulation. Fate maps of mammalian gastrulae have shown that the areas predestined to become mesoderm are contiguous and overlapping with areas fated to ectoderm and, moreover these lineages are not separated at the beginning of gastrulation (Lawson et al., 1991). It is conceivable that a few cells, lying at the border between presumptive paraxial mesoderm and neuroectoderm may be trapped into an improper neurogenic field. If this is the case, the presence of ß-galactosidase-positive cells in the neural tube (Fig. 2) suggests that some cells may express *myf-5* in the primitive streak well before somitogenesis. Preliminary observations suggest that this may be the case (Cossu et al., 1996b). If these cells can no longer be converted to a neurogenic fate, they may die or remain quiescent in the ectopic tissue. Expression of *myf-5* in the neural tube *in vivo* would support the possibility that at least a fraction of these cells neither die nor completely convert to muscle, and transient co-expression of neuronal and muscle markers in culture suggests that a conversion to muscle can occur *in vitro*.

Myogenic conversion of mesodermal cells

A different embryological situation is represented by the recently reported myogenic conversion of "fibroblasts" originating from dermis and, to a different extent, other mesodermal tissues. 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). Recently Gibson et al., (1995) found that dermal

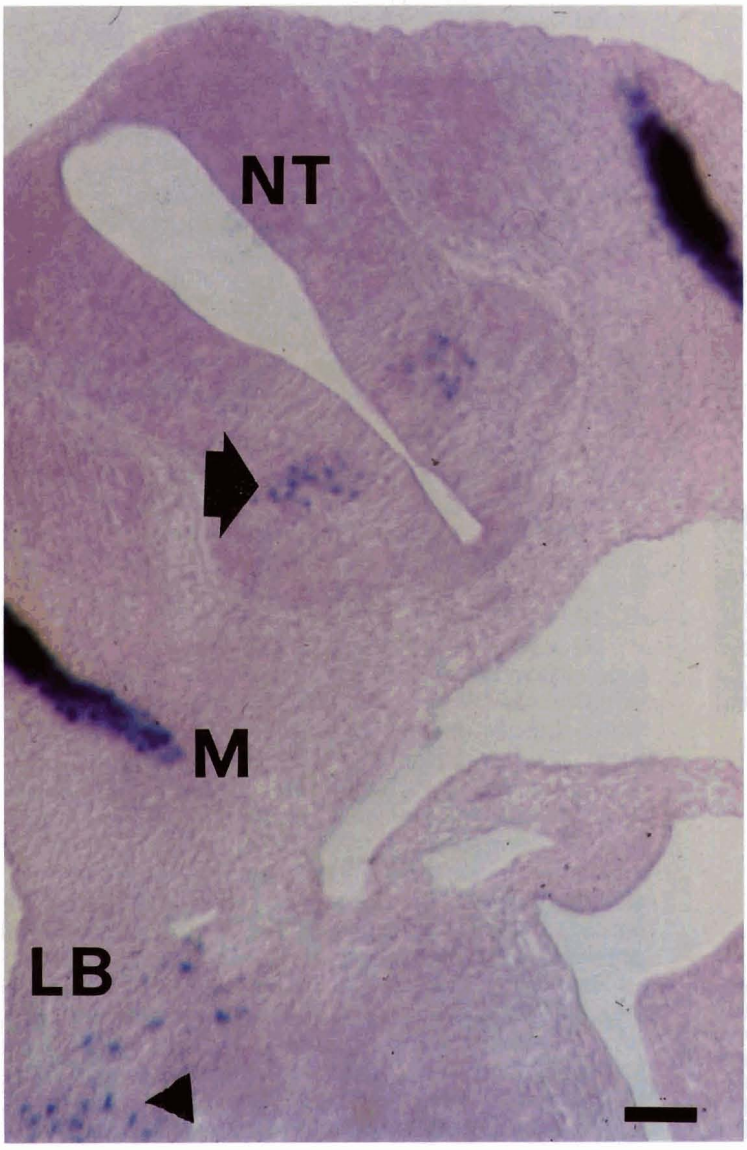

Fig. 2. Transverse section of a 10.5 dpc *myf-5/LacZ* mouse embryo. The arrow shows ß-galactosidase-positive, *myf-5* expressing cells in the neural tube (NT). The majority of ß-galactosidase-positive, *myf-5* expressing cells are located in the myotome (M), while a few *myf-5*-expressing cells are already detectable in the limb bud (LB). Bar: 50μm.

fibroblasts can give rise to muscle fibers when injected into skeletal muscle of mdx mice. These studies showed evidence for the fusion of fibroblasts with myogenic cells. In these cases myogenesis could be activated as 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 would be just a consequence of the acquired myogenic phenotype (Breton et al., 1995; Salvatori et al., 1995).

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 complicated cross-talks between diffusible molecules and specific transcription factors (Davidson, 1993). The recently reported "community effect" for amphibian myogenesis may explain in part how such fields may be established (Gurdon, 1993). While receiving 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 (Cossu et al., 1995). 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 to myogenesis 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 and, furthermore, they receive subthreshold levels of different signals (Fig. 3). These cells may therefore be frozen in a penultimate and possibly bi-potential state. Other examples of lineage switching among mesodermal cells have been reported (Grigoriadis et al., 1988; Katagiri et al., 1994) supporting the idea of mesenchymal cells which maintain a bi-potential or multipotential state. In this state, mesenchymal cells may be induced to adopt a specific pathway of terminal differentiation from signals derived from neighboring differentiating cells, as occurs in a forming muscle field (both *in vitro* and *in vivo* during regeneration). The 10T1/2 cells, which can differentiate into muscle, cartilage and fat, may be the immortalized progeny of one of these cells (Taylor and Jones, 1982).

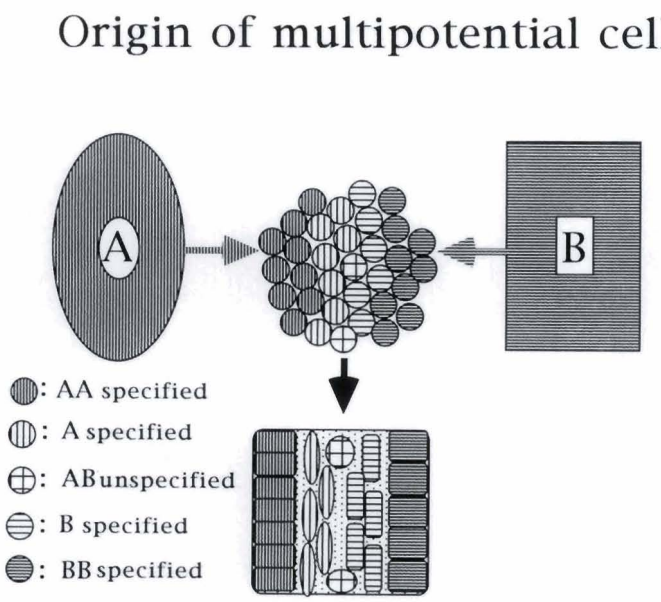

Fig. 3. A simple model showing the possible origin of multipotential cells. A group of cells is subjected to two different inducing signals (**A and B**) emanating from neighboring tissues. Cells closest to these tissues receive a high dose of the signals and are specified as AA or BB. The next layers of cells, receiving lower doses are specified as A and B respectively. Cells which receive subthreshold doses of both A and B signals, are not specified and remain as (AB) multipotential cells.

Conclusions and future perspectives

The examples described above point to the existence of multiple mechanisms by which a cell of the vertebrate body can be forced to adopt a myogenic fate.

Even at the present level of uncertainty, the following possibilities may be proposed:

1) a "default" tendency of pre-gastrula cells, which totally lacks a cellular explanation; it may only be tentatively linked to the so far unique property of myogenic bHLH transcription factors to act dominantly in a foreign cellular environment (Weintraub et al., 1991).

2) a misplacement at, or soon after, gastrulation that leads a cell belonging to an area fated to be paraxial mesoderm to migrate within a different territory and to be trapped in a different tissue. This may explain the frequent and unexplained presence of cell with myogenic potential within the central nervous tissue (Tajbakhsh et al., 1994 and examples therein)

3) a borderline position between two different progenitor fields, which exposes the same cell to subhtreshold levels of two different signaling molecules which specify different fates (i.e. those cells of newly formed somites, lying at the border between future sclerotome and dermomyotome). These cells may not adopt a final fate and thus remain as undifferentiated, possibly multipotential cells. Perhaps it is worth noting that cells which maintain a developmental potency, such as satellite cells or osteoblasts, invariably lie at the border of a tissue, under the basal lamina, and may be experimentally induced to adopt different fates.

Borderline cells might have been exploited during mammalian evolution to compensate for the reduced regenerative capacity of mammalian as compared to lower vertebrate tissues (Muneoka and Sassoon, 1992). In the case of skeletal muscle, regeneration potential may be rapidly exhausted in response to chronic injury,

as occurs in Duchenne Muscular Dystrophy (Partridge, 1991). Surprisingly, in situ analysis of *MyoD* and myogenin expression in regenerating muscle, revealed an unexpectedly high number of positive cells near the area of muscle necrosis (Grounds et al., 1991), far exceeding that of 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.

Hopefully, the increasing understanding of the cellular and molecular control of differentiation will shed further light on the process of unorthodox myogenesis, leading to a better definition in embryological terms. The understanding of this process may bear important implications for ex vivo cell therapy of primary myopathies. The current limitation of this approach lies in the limited number and limited lifespan of myogenic cells which can be obtained from the biopsy of a patient. Fibroblasts from a non damaged site such as skin, may be more easily expanded *in vivo*, transduced with the vector carrying the therapeutic gene, and then converted to myogenesis, before reintroduction in the patient's own muscle tissue. Experiments are in progress to test the feasibility of this approach.

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