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Myogenic conversion of mammalian fibroblasts induced by differentiating muscle cells

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SUMMARY

Somite-derived skeletal myoblasts are supposed to be the sole source of muscle fibre nuclei during pre- and post-natal development, but evidence is accumulating for unorthodox contributions to muscle fibre nuclei from other cell types. For example, in tissue culture, fibroblasts can fuse with dysgenic myoblasts and restore correct membrane function.

We report here the results of a series of experiments investigating this phenomenon and its possible mechanism. 10T1/2 cells, infected with a replication defective retrovirus encoding the bacterial enzyme β-galactosidase, fused to form β-galactosidase positive, differentiated myotubes when cocultured with differentiating uninfected C2C12 or primary myogenic cells, but this did not occur when they were cocultured with other cells such as 3T3 fibroblasts or PC12 pheochromocytoma cells. Myogenic conversion ranged from 1 to 10% of the 10T1/2 cell population and required close cell interaction between the different cells types: it was not induced by conditioned medium or extracellular matrix deposited by C2C12 cells. Myogenic conversion was also observed in vivo, after injection of similarly infected 10T1/2 cells into regenerating muscle. Conversion was seen also after coculture of uninfected 10T1/2 cells with primary chick myoblasts, thus demonstrating that it was not dependent upon viral infection and that there is no species or class barrier in this phenomenon. Primary fibroblasts, isolated from different organs of transgenic mice carrying a *Lac Z* marker under the control of a muscle-specific promoter, restricting β-galactosidase expression to striated muscle cells, also underwent myogenic conversion, when cocultured with C2C12 myoblasts. The efficiency of this conversion varied with their embryological origin, being common in cells with a dorsal mesoderm lineage but rare in cells of ventral mesoderm origin. These experiments demonstrate that myogenic conversion is a true embryological feature of mammalian mesodermal cells. Conversion of mononucleated cells was also observed, showing that fusion is not a pre-requisite for myogenic differentiation and may indeed be a consequence of differentiation induced by short-range local signalling. We conclude that a proportion of adult cells of mesodermal origin may conserve a bi- or multi-potential state of determination throughout the life of an animal, enhancing the regenerative capacity of the tissues in which they reside.

Key words: myogenesis, myoblast differentiation, mesoderm lineage

INTRODUCTION

Skeletal muscles of the vertebrate body are derived from the dorsal portion of somites, the dermomyotome (Buckingham, 1992; Wachtler and Christ, 1992). As far as we know, the dermomyotome gives rise to two main cell types, namely myoblasts and fibroblasts. Myoblasts are the precursors of skeletal muscle fibres, express myogenic determination factors such as MyoD and, upon removal of growth factors, differentiate into multinucleated muscle fibres (Weintraub et al., 1991; Olson, 1992; Emerson, 1993). Fibroblasts, in contrast, are far less characterised cells, which only express markers common to most mesodermal cells, such as collagens and vimentin (Holtzer, 1978). They colonise the dermal layer of the skin (at least in the dorsal region of the body) and, probably, the stromal component of dorsal muscles. Fibroblasts, defined as above, are found in virtually every organ of the body, but their origin and their possible differentiative potentials are at present largely unknown (Caplan, 1991). Fibroblasts can be immortalised by a variety of means and many different fibroblast cell lines are currently available: among these are 10T1/2 cells which are capable of differentiating into colonies of muscle, cartilage or fat cells after exposure to 5′-aza-cytidine (Taylor and Jones, 1979). Because of this feature, they have been instrumental in the identification of muscle regulatory genes (Davis et al., 1987). While 10T1/2 cells can be easily converted

to muscle by over-expression of these genes, spontaneous myogenic conversion is not observed.

Normal fibroblasts, however, have been shown to fuse with dysgenic mdg myoblasts and to restore normal expression of the membrane calcium channel lacking in this mutant (Chaudari et al., 1989; Courbin et al., 1989). This was suggested to be the consequence of infrequent random fusion after which the fibroblast nucleus would be exposed to muscle transcription factors as it occurs in heterokaryons (Blau et al., 1985).

Here we report that 10T1/2 cells, as well as primary fibroblasts from different organs, will undergo muscle differentiation at significant frequency when cocultured with myogenic cells. This myogenic conversion also occurs in vivo, requires close cell-cell interaction but not necessarily cell fusion and is related to the embryological origin of fibroblasts.

MATERIALS AND METHODS

Transgenic mouse lines

The *MLC3F-nlacZ* construct contains 2 kb of the mouse *fast myosin light chain 3 (MLC3F)* promoter with 1 kb of sequence downstream of the transcription initiation site and a 3′ muscle-specific enhancer from this gene, fused to a *nlacZ*-SV40 polyA sequence. In 2 independent transgenic lines the *nlacZ* reporter gene is strongly expressed in skeletal muscle from 9 days of development (Kelly et al., 1995), and in all differentiated skeletal muscle cells in culture (Cossu et al., 1995). Heterozygous transgenic males were crossed with CD1 outbred female mice. The day a vaginal plug was seen was taken as 0.5 days post coitus (d.p.c.).

Cell cultures

Cells were grown in growth medium (GM) consisting of DMEM medium (Gibco) supplemented with 10% foetal calf serum (Flow), 300 mM β-mercaptoethanol and 50 mg/ml gentamycin, or in differentiation medium (DM), i.e. DMEM supplemented with 2% horse serum from Flow, and 50 mg/ml gentamycin).

10T1/2 and 3T3 fibroblasts, C2C12 myogenic cells and PC12 pheocromocytoma cells were grown as described (Cossu et al., 1995). Primary avian foetal myoblasts were prepared from breast muscle of 11 day old chick embryo by digestion with 0.05% trypsin as described (Ferrari et al., 1990). Murine satellite cells were prepared from the hind limb muscles of 2 month old C57BL/6J mice as described (Salvatori et al., 1993).

10T1/2 fibroblasts or C2C12 myogenic cells were infected with LBSN recombinant retrovirus, which expresses *n-LacZ* under the control of Moloney Leukaemia Virus Long Terminal repeat, essentially as described (Salvatori et al., 1993). Briefly, growing cells were cultured with supernatant from AM12 packaging cells for two days and selected in G418 (0.7 mg/ml) for a week. After this time all cells expressed β-galactosidase (β-gal) reporter gene in their cytoplasm.

Primary fibroblasts were isolated from *MC3LF-nlacZ* transgenic embryos, ranging in age from 15 to 17 d.p.c. and 10 day old mice. The forelimbs of the embryos were stained for β-gal activity and β-gal positive embryos were pooled. Newborn transgenic mice were identified after β-gal staining of the distal tip of the tail. The brain, the heart, the dermis, the lungs and the skeletal muscle of foetal and 10 day old mice were finely minced with scissors and then digested with 0.1% collagenase-0.1% dispase for various times at 37°C, washed in complete medium and then gently pipetted to obtain a single cell suspension. Primary cultures were grown in GM until cells reached confluence and then shifted to DM to induce differentiation of possible myogenic cells present in the culture. The cells were then sub-cultured one or three times, depending on the presence of myoblasts in the tissue of origin, until no more β-gal positive, differentiated muscle cells could be observed after 3 days in DM. At this time, 10^5 fibroblasts from these cultures were mixed with the same number (unless otherwise specified) of 3T3 fibroblasts, of C2C12 myogenic cells or of primary satellite cells cultured from non-transgenic mice. The cultures were grown for two days in GM, then shifted to DM for two additional days. At the times indicated, cultures were fixed and stained for β-gal activity and incubated with different antibodies.

In order to inhibit DNA synthesis, cultures were treated for 2 hours with 5 mg/ml mitomycin C, washed 5 times and then used for coculture experiments. To examine the effect of conditioned medium on myogenic conversion of 10T1/2, we used a coculture assay previously described (Salvatori et al., 1993). Briefly, 60 mm dishes were marked on the underside to define three areas: (i) a 10 mm-wide external ring where 10^5 C2C12 myoblasts were plated in 0.5 ml of medium; (ii) a 10 mm-wide internal ring where no medium was added; (iii) a 20 mm diameter central area where 10^5 10T1/2 were plated in 0.5 ml of medium. Care was taken not to mix media in the center and in the peripheral areas of the dish for the first 6-8 hours of culture until all cells had attached to the dish. At this time, the dish was flawed with another ml of medium, so that 10T1/2 cells were continuously exposed to the conditioned medium of myoblasts without contact between these two cells type. After 2 days in GM, the cultures were shifted to DM for 2 additional days and then analysed for the presence of β-gal positive, differentiated muscle cells in the centre of the dish. To verify whether myogenic conversion may be induced by extracellular or surface molecules synthesised by differentiating C2C12 myoblasts, cultures were extracted with 1 M urea in PBS for 30 minutes at 4°C. The urea extract was dialysed against several changes of DMEM, supplemented with 2% horse serum and antibiotics, and added to cultures of LBSN-infected 10T1/2 which had been grown in GM for the previous 2 days. Alternatively, sister cultures of C2C12 myoblasts were treated with 0.05% Nonidet-P40 in PBS for 30 minutes at 4°C. After this time the dishes were washed 5 times with DM and then LBSN-infected 10T1/2 were added in DM and scored for differentiation after 2 and 4 days.

Immunocytochemistry

The following antibodies were used in this study:

(1) MF20, a monoclonal antibody which recognizes all sarcomeric myosins (Bader et al., 1982) donated by D. Fischman.

(2) A rabbit antibody against sarcomeric proteins which was produced in our laboratory (Tajbakhsh et al., 1994).

(3) An anti-MyoD polyclonal antibody, donated by S. Alema (Hasty et al., 1993).

(4) F5D, an anti-myogenin monoclonal antibody, donated by W. Wright (Cusella de Angelis et al., 1992).

(5) 3.2.B5. A monoclonal antibody, produced in our laboratory, which reacts with a 170 kDa nuclear protein present in all avian cells tested (chick, quail and duck). The antibody does not react with cells from mammals or other classes of vertebrates (E. Vivarelli et al., unpublished).

Cultured cells fixed with fresh 4% paraformaldehyde and washed 3× in 1% bovine serum albumin (from Sigma) in phosphate buffered saline (BSA/PBS) were incubated overnight at 4°C with different polyclonal and monoclonal antibodies; after incubation, samples were washed 3× in 1% BSA/PBS and incubated with a fluorescein-conjugated goat anti-rabbit Ig and with a rhodamine-conjugated goat anti-mouse Ig for 1 hour at room temperature (both second antibodies, from Cappel, were used at 1:30 dilution). After 3 final washes, culture dishes were mounted in 75% glycerol/PBS (pH 8), and observed under an epifluorescence Zeiss Axiophot microscope.

Cultures and muscles were stained for β-gal activity as described (Tajbakhsh et al., 1994).

Implantation of LBSN infected cells into host muscles

LBSN infected, β-gal positive 10T1/2 fibroblasts or C2C12 myogenic cells were trypsinized, pelleted, resuspended in DMEM and injected into regenerating quadriceps of C3H mice which had received an injection of 50 ml of 10^{-5} M cardiotoxin (Latoxan) 24 hours earlier. Approximately 10^6 cells were injected in a volume of 20 ml and the

muscles were analysed in whole mount after 1, 2 or 3 weeks. After staining, the muscles were cryostat sectioned and β-gal positive areas selected for histological examination.

RESULTS

Myogenic conversion of 10T1/2 fibroblasts

As a first approach to study the possibility of myogenic conversion of fibroblasts, we decided to use retroviral infected 10T1/2 fibroblasts, to ensure that an inheritable, cell autonomous marker would label only this cell population. 10T1/2 cells were infected with the replication defective retroviral vector LBSN, derived from MoMLV, that expresses the *LacZ* reporter gene under the transcriptional control of the LTR. 10^5 infected cells were cocultured with the same number of uninfected C2C12 myogenic cells or primary satellite cells for 24 hours in GM. Differentiation was then induced by shifting the culture to DM. After 48 hours several β-gal positive myotubes appeared, often in small clusters (Fig. 1), suggesting that 10T1/2 cells had fused with C2C12 myogenic (or satellite) cells or with themselves. These 10T1/2-containing myotubes expressed all the muscle markers analysed, namely myosin heavy chains, desmin, troponin T, myogenin and MyoD (data not shown). Because the virus is replication incompetent, infected cells do not release infectious particles and therefore C2C12 myoblasts could not be infected. When similarly infected 10T1/2 cells were cocultured with other uninfected cell types such as NIH-3T3 fibroblasts or PC12 pheochromocytoma cells, no β-gal expressing myotubes were observed (data not shown), thus indicating that myogenic conversion of infected cells only occurred in the presence of myogenic cells.

In order to investigate whether conversion of 10T1/2 cells would also occur during myogenesis in vivo, we injected 10^5 LBSN-infected 10T1/2 cells into the regenerating gastrocnemius of C3H mice. Fig. 2A shows that, while the majority of β-gal expressing cells was localised in the connective tissue surrounding newly formed muscle fibres, occasional β-gal positive fibres could be seen, demonstrating fusion of infected 10T1/2 cells. As controls, we injected similarly infected C2C12 cells, the great majority of which fused into regenerating fibres (Fig. 2B).

Myogenic conversion of primary fibroblasts

10T1/2 cells efficiently differentiate into muscle cells upon treatment with 5′-aza-cytidine (Taylor and Jones, 1982) or upon transfection with members of the MyoD family of HLH transcription factors (Weintraub et al., 1991). It was therefore relevant to establish whether myogenic conversion upon coculture with myogenic cells was a unique feature of 10T1/2 cells or may be exhibited by primary non-immortalised fibroblasts. To answer this question we assayed for myogenic conversion fibroblasts isolated from different foetal or early post-natal organs of transgenic mice which carry the *LacZ* reporter gene with a nuclear localisation signal under the transcriptional control of the *MLC3F* promoter. In these mice all skeletal muscles, together with a subset of cardiac muscle, express the reporter gene from the onset of differentiation throughout the life of the animal (Kelly et al., 1995). Cells were cultured from brain, liver, lung, skin, heart and skeletal muscle of these mice. Each of these organs is composed of a variety of cell types and, in the case of dermis, myogenic cells may be present. However, after repeated subculture in serum rich medium, the only cells which continued to grow had a typical fibroblast-like morphology and expressed no muscle specific markers when shifted to culture conditions (DM) permissive for differentiation. To see whether this population of 'fibroblast-like' cells retained a myogenic potential, we cocultured these cells either with C2C12 or with 3T3 cells. Myogenic conversion was detected by nuclear staining for β-gal activity. This allowed easy identification of a few β-gal positive nuclei among a large number of cells. Cells expressing β-gal in their nuclei were then stained for the expression of sarcomeric myosin heavy chains to confirm that terminal differentiation had occurred. The number of positive cells was scored in two independent experiments in which negative controls (i.e. the same cells cocultured with 3T3 cells contained no β-gal positive cells) Fig. 3 shows an example of these cultures derived from the dermis. Fig. 3A,C shows a mononucleated, myosin positive myocyte with a β-gal positive nucleus (arrowhead) together with a myotube showing at least two β-gal positive nuclei. Fig. 3B,D shows an hybrid myotube with one β-gal positive and two β-gal negative nuclei (arrows). It is important to note that the presence of mononucleated, differentiated

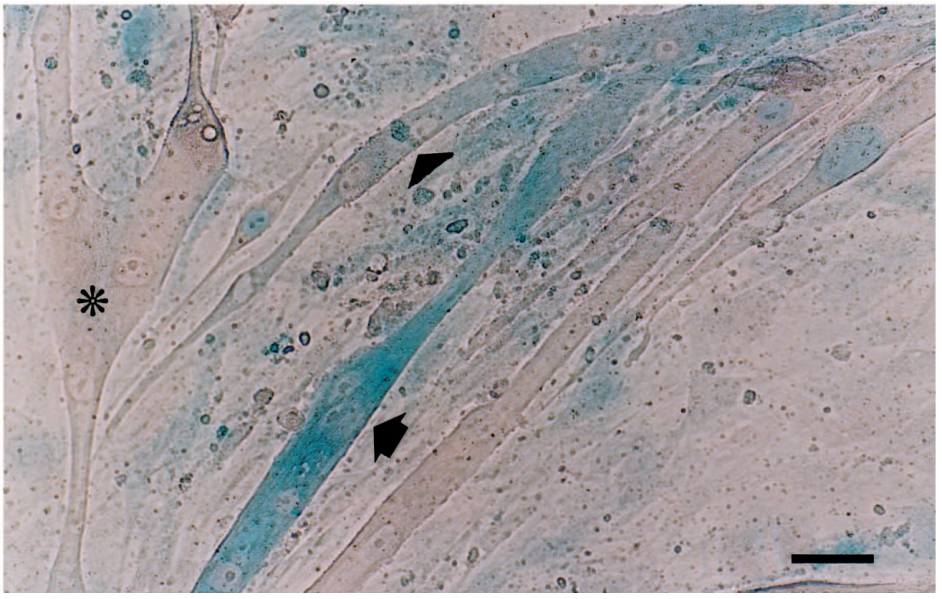

Fig. 1. Example of β-gal positive myotubes present in cocultures of LBSN-infected 10T1/2 and C2C12 cells. Note the coexistence of strongly positive (arrow) with weakly positive (arrowhead) and negative (asterisk) myotubes which suggest the occurrence of fusion among different proportions of infected and uninfected cells. Bar, 15 μm.

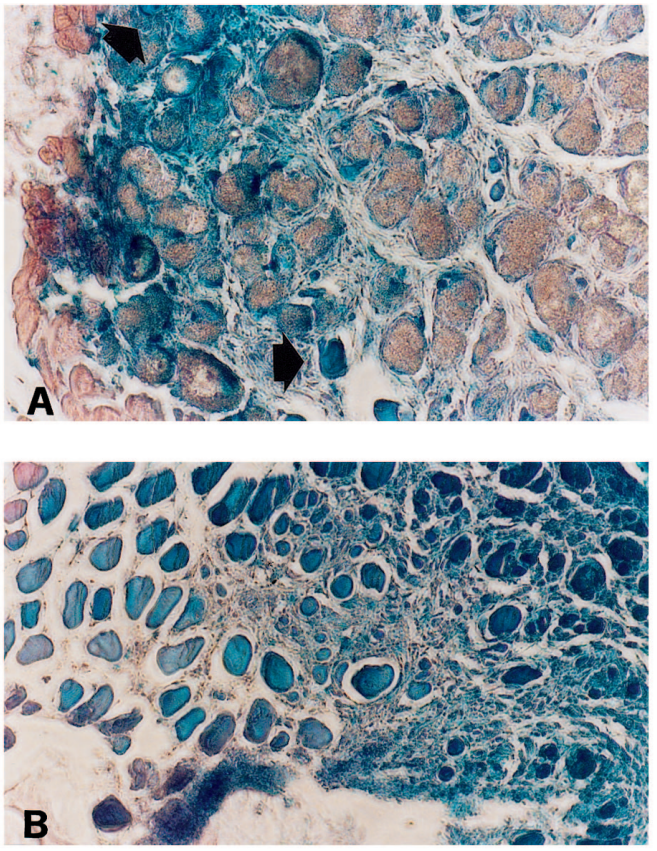

Fig. 2. Transverse cryostat sections of the regenerating gastrocnemius of C3H mice 7 days after the injection of 10^6 LBSN-infected 10T1/2 (A) or C2C12 (B) cells, stained for β-gal activity. Note that in A, the majority of β-gal positive cells are localized outside the fibers. However, few β-gal positive fibers are indicated by arrows. In B, the majority of regenerated fibers are β-gal positive. Bar, 50 μm.

myocytes implies that myogenic conversion can occur independently of cell fusion. A quantitative analysis of the data relative to the different fibroblasts is reported in Table 1, which shows that myogenic conversion occurred at relatively high frequency in foetal as well as in post-natal fibroblasts from brain, skin and skeletal muscle. In contrast myogenic conversion did not occur at significant levels in fibroblasts from liver, lung or heart.

In all the experiments described so far, at least one of the cell populations was represented by an established cell line, whose membrane features may be altered in such a way as to favour the short-range interactions (see below) which are necessary for myogenic conversion. We therefore performed one experiment where dermal fibroblasts from *MLC3F-nLacZ* mice were cocultured with primary satellite cells from non-transgenic mice. Also in this case differentiated β-gal positive appeared after 4 days of coculture in similar, if not slightly higher proportion, than in the experiments reported above (data not shown).

The nature of myogenic conversion

To characterise the possible basis of myogenic conversion in fibroblasts, we investigated whether this phenomenon required membrane contact, or was mediated by medium released molecules, either soluble or attached to the underlying extracellular matrix. To this purpose we exposed LBSN-infected 10T1/2 cells to: (1) medium conditioned by differentiating C2C12 cells; (2) material extracted from the surface of the same cells with 1 M urea; (3) substrate-attached material obtained after detergent solubilization of C2C12 cells. We observed no case of fusion or differentiation of 10T1/2 cells (data not shown), making it unlikely that a stable secreted differentiation signal is involved. If rather close cell to cell interaction is required for myogenic conversion to occur then, the relative proportion of inducing and induced cells should influence the frequency of myogenic conversion. In fact an excess of 10T1/2 cells resulted in a progressive reduction of conversion (expressed as percentage of cocultured 10T1/2 cells). Increasing the proportion of inducing C2C12 cells did not affect the proportion of 10T1/2 cells which underwent myogenic conversion (Fig. 4).

We next examined whether myogenic conversion was dependent upon DNA synthesis. To test this possibility we cocultured mitomycin C-treated or control 10T1/2 with mitomycin C-treated or control C2C12 in any possible combination. Conversion occurred at similar frequencies whether or not one or both cell types had been treated with the drug thus showing that DNA replication is required neither for the inducing nor for the induced cells (data not shown).

In order to test whether myogenic conversion is species-restricted, and also to verify whether it may be enhanced by some metabolic alteration induced by viral infection, we cocultured uninfected 10T1/2 cells with primary chick foetal myoblasts. After fusion had occurred, we double stained the culture with a monoclonal antibody, that recognises avian but not mammalian nuclei and with a polyclonal antibody which recognises sarcomeric myosin heavy and light chains. The arrow in Fig. 5 shows a large mouse nucleus (showing typical mouse staining with Hoechst in Fig. 5C) near to several smaller chick nuclei within a multinucleated myotube, stained by the anti-sarcomeric myosin antibody (Fig. 5A). Careful examination reveals few dots of antigen recognised by the monoclonal antibody (Fig. 5B) present in the mouse nucleus. This is due to the fact that the chick antigen enters the mouse nucleus, confirming that the chick and mouse nuclei are indeed within the same cytoplasm. The nuclei of mouse mononucleated cells are unstained. Thus fusion occurs among 10T1/2 cells and primary chick myogenic cells, with a frequency of 1-6% of the fused nuclei.

DISCUSSION

The idea that muscle fibres originate from somites and connective tissue from somatopleural mesoderm, has been widely accepted since the classic quail-chick transplant experiments of Christ et al. (1977) and Chevallier et al. (1977). Thus it comes as a surprise to find that mesenchymal cells (many of which are of non somitic origin) may be induced to express a myogenic phenotype. The first example of this phenomenon was correction by fibroblast-myoblast fusion of the genetic defect of the mdg mouse mutant muscle fibres (Chaudari et al., 1989; Courbin et al., 1989). Recently Gibson et al. (1995) found that dermal fibroblasts can give rise to muscle fibres 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, the data reported here show that myogenesis can also be induced in a mononucleated fibroblast by signals derived from neigh-

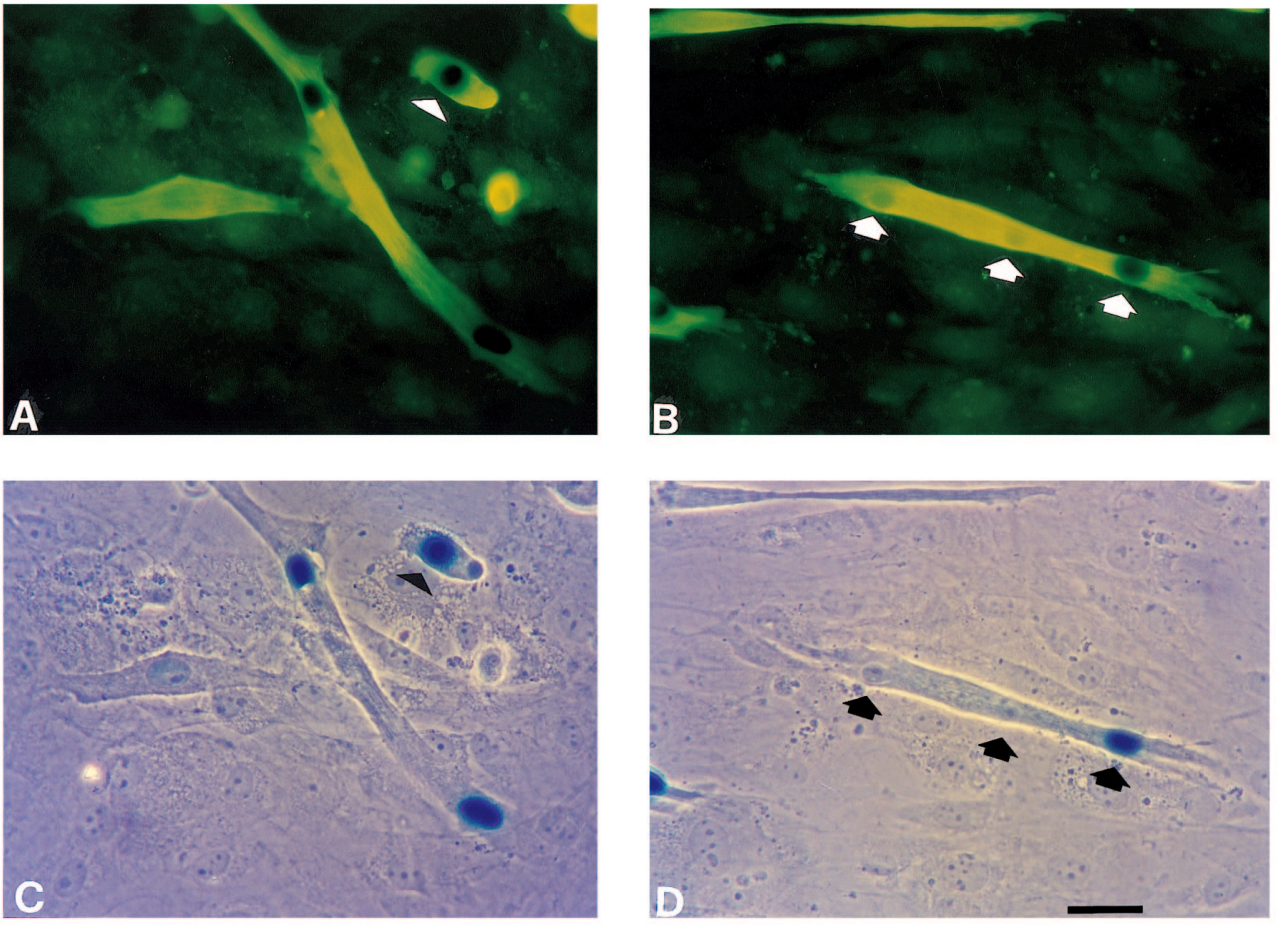

Fig. 3. Immunofluorescence analysis of a 4 day old coculture of tertiary dermal fibroblasts from 10 day old MC3LF-*nLacZ* transgenic mice with C2C12 myogenic cells, stained with anti-sarcomeric MF2O monoclonal antibody (revealed by FITC-conjugated anti mouse IG) (A,B,) and for β-gal activity (C,D). Note that both multinucleated converted myotubes (some containing β-gal positive and negative nuclei, shown by arrows) and monunucleated converted myocytes (arrowhead) can be observed. Bar, 10 μm.

bouring myogenic cells: in this case fusion would be just a consequence of the acquired myogenic phenotype. It is important to note that the two mechanisms are not mutually exclusive.

In addition, the experiments reported here extend these previous observations and point to a possible mechanism. We use a variety of independent approaches to confirm the phenomenon: (1) 10T1/2 cells, infected with a replication defective retrovirus encoding β-gal, can form β-gal positive myotubes when cocultured with uninfected C2C12 or primary myoblasts but not with other cells; (2) this phenomenon also occurs in regenerating normal muscle in vivo; (3) uninfected 10T1/2 fuse with chick myoblasts, thus showing that conversion does not depend upon viral infection and that there is no class barrier in this phenomenon; (4) myogenic conversion also occurs in primary fibroblasts from brain, skin and skeletal muscle but not from liver, lung or heart. This point needs to be discussed in detail since it appears that embryonic origin may influence the occurrence of myogenic conversion. Dermal fibroblasts are derived from the dermatome and epaxial muscles almost certainly have a connective tissue derived from the dermatome. Hypaxial and limb muscles are likely to contain a mixture of fibroblasts from somitic and somatopleural origin. Fibroblasts associated with the viscera are most likely derived from the somatopleura. The origin of mesodermal cells of the brain is unclear (De Vitry et al., 1994), either by ingrowth from outer connective layers or following blood vessel invasion of the neuroectoderm. Spontaneous myogenic differentiation of cells from the brain has been documented a number of times, even though it still lacks a clear explanation (discussed in detail in Tajbakhsh et al., 1994). Thus it appears that dorsal but not ventral mesoderm (in addition to the neuroectoderm) may contain cells with a residual myogenic potential. This would be in agreement with the natural origin of skeletal muscle in vertebrates, but does not explain why these mesodermal cells require a 'muscle field' to undergo myogenesis and have not already undergone spontaneous differentiation. During *Drosophila* embryogenesis, the recruitment of mesodermal cells to myogenesis by founder cells is a well documented phenomenon (Bate, 1990). In vertebrates, 'progenitor fields' must be established by complicated cross-talks between diffusible molecules and specific transcription factors (Davidson, 1993). The recently reported 'community effect' for amphibian myogenesis (Gurdon, 1993) may explain in part how such fields may be established (and limited: Kato and Gurdon, 1994). 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 depending on the

Table 1. Percentage of myogenic conversion in fibroblasts from different organs

Source	Fetal (19 dpc)	Neonatal (10 dpn)
Brain	5.5	3.5
Liver	<0.1	<0.1
Dermis	4.7	3.1
Skeletal muscle	2.4	1.7
Heart	0.7	0.3
Lung	0.3	<0.1

Fibroblasts were prepared from different organs of MC3LF-*nlacZ* transgenic mice as detailed in Materials and Methods. Secondary (brain, liver, heart and lung) or tertiary (skeletal muscle and dermis) fibroblasts were used for the experiments, after checking that no cells with β-gal positive nuclei were present in the cell population. 10^5 fibroblasts were mixed with an equal number of C2C12 myogenic cells and cocultured for 2 days in a high serum medium and for 2 additional days in differentiation medium. The cultures were then stained for β-galactosidase activity and the number of β-gal positive nuclei was recorded (both in multi and in mononucleated cells) in two separate 60 mm dishes. β-gal positive nuclei are expressed as percentage of half of the total nuclei (assuming that the two populations had proliferated at a similar rate during the growth period); β-gal positive nuclei in mononucleated cells ranged between 10 and 25% of total β-gal positive nuclei. Negative controls were carried out by plating the same number of fibroblasts from the same suspension together with 3T3 fibroblasts. Only experiments whose controls contained no β-gal positive cells (in a 60 mm dish) were analysed.

occurrence of cells which are not uniquely determined. For examples these may be cells whose precursors have been laid at the border of a 'progenitor field' (and thus have been exposed to different signals) and frozen in a penultimate and possibly bi-potential state. Other examples of lineage switching among mesodermal cells have been documented (Grigoriadis et al., 1988; Katagiri et al., 1994) supporting the idea of mesenchymal cells which maintain, at least in vitro 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 neighbouring differentiating cells, as it occurs in a forming muscle field (both in vitro and in vivo during regeneration). 10T1/2 may well be the immortalised progeny of one of these cells. This situation, which is conceptually different from the plasticity of the already differentiated state (Blau, 1992), 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 such as occurs in Duchenne Muscular Dystrophy (Partridge, 1991). Surprisingly, in situ analysis of MyoD and myogenin expression in

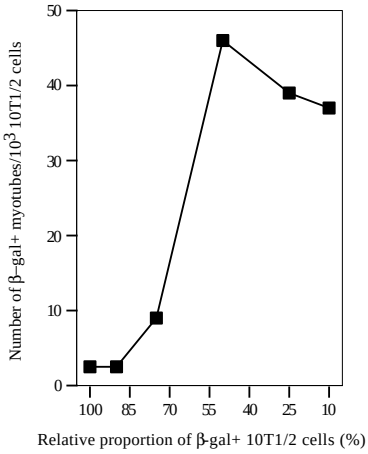

Fig. 4. Appearance of β-gal positive myotubes (cells containing more than two nuclei) as a function of the ratio between LBSN-infected, β-gal positive 10T1/2 and uninfected C2C12 myoblasts in 5 day old cocultures. The number of β-gal positive myotubes was calculated after scoring four 60 mm dishes in two separate experiments and expressing the resulting number per 10^3 β-gal positive cells plated.

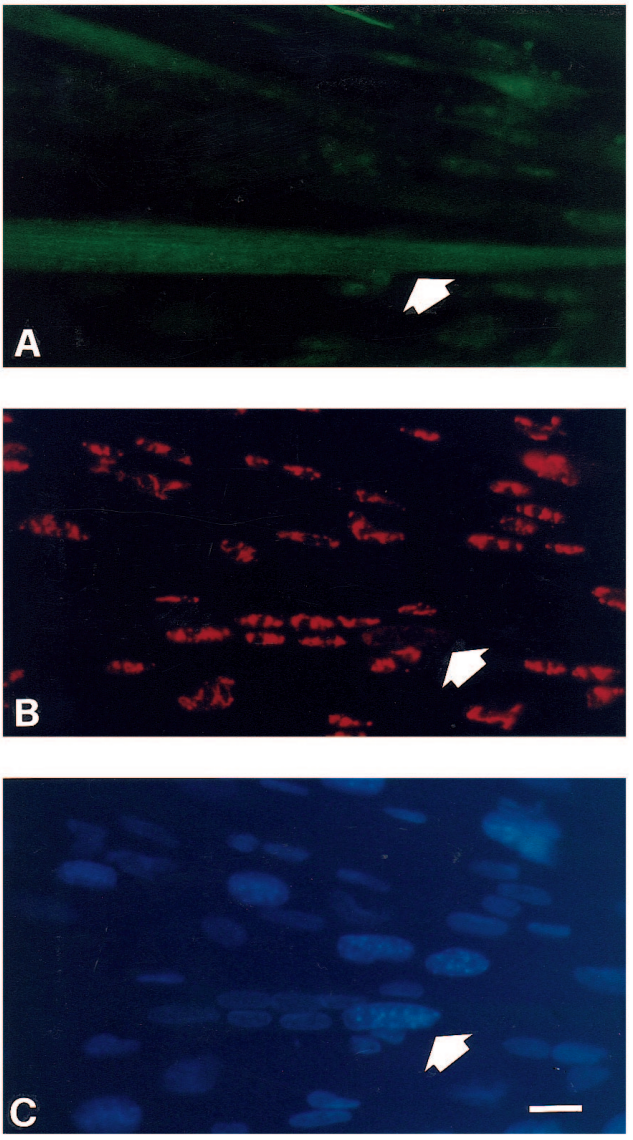

Fig. 5. Double-immunofluorescence of a 5 day old coculture of 10T1/2 with primary chick fetal myotubes, stained with a polyclonal anti-sarcomeric antibody, followed by FITC-conjugated goat anti-rabbit IG, (A) and with the 3.2.B5 monoclonal anti-avian nuclei antibody, followed by TRITC-conjugated goat anti-mouse IG (B). Nuclei are stained with Hoechst (C). Note that only one mouse nucleus (indicated by an arrow) is present in a myotube containing other chick nuclei. Traces of antigen in this nucleus reveal that the chick antigen, synthesized in the myotube cytoplasm, has begun to enter the mouse nucleus. Nuclei of monunucleated mouse cells are completely negative. Bar, 10 μm.

regenerating muscle, revealed an unpredicted 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 neighbouring 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.

Our initial studies on the possible mechanisms of myogenic conversion, point to membrane events as crucial steps: since close contact among cells is required, we can hypothesize that there are signals released through surface molecules such as cadherins (Takeichi, 1991) or CAMs (Walsh and Doherty, 1991), or communications via gap junctions, or micro-fusion events which allow enough cytoplasm exchange to activate endogenous MyoD. These possibilities are currently being investigated.

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. Preliminary experiments with human foetal fibroblasts suggest that myogenic conversion may also be observed in man.

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