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Four sarcomeric myosin heavy chain genes are expressed by human fetal skeletal muscle cells differentiating in culture

Rebecca Feghali,[1] Ilene Karsch-Mizrachi,[1] Leslie A. Leinwand,[1] and D. Stave Kohtz[2]

[1] Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York, and
[2] Department of Pathology, Mount Sinai School of Medicine, New York, New York

Expression of four sarcomeric myosin heavy chain (MHC) genes was examined in continuously passaged human fetal (18–22 week) skeletal myoblasts and in myoblasts induced to differentiate by low mitogen medium. Although embryonic MHC mRNA predominated at all time points following induction, three additional MHC genes were expressed at lower levels. These consisted of perinatal, slow, and fast skeletal MHC genes. Temporal regulation of MHC gene expression was observed. In myoblasts and early induced cultures, embryonic and fast transcripts were detected, accompanied in later induced cultures by the accumulation of perinatal and slow MHC transcripts. In situ hybridization analysis of uninduced cells revealed that sarcomeric MHC transcripts originated from a small population of spontaneously fused multinucleated cells. Taken together, these observations demonstrate that human fetal myoblasts induced to differentiate in culture execute a developmental program that includes temporally regulated expression of four distinct sarcomeric MHC genes.

Skeletal muscle is generated by the differentiation and fusion of skeletal myoblasts. Differentiation of skeletal myoblasts is accompanied by mitotic arrest and de novo synthesis of the sarcomeric and membrane polypeptides required for contractile activity. Concurrently, the cells morphologically transform into large syncytia by fusion, then mature into contractile fibers. Skeletal muscle fiber diversity results from differential expression of multiple isoforms of sarcomeric proteins. The different isoforms are generated either by differential expression of several genes (e.g., myosin heavy chain, Gauthier and Lowey, 1977; Gauthier et al., 1982; Weydert et al., 1985), or by differential splicing of single genes (e.g., troponin T, Breitbart and Nadal-Ginard, 1987). Sarcomeric protein isoform expression varies with the developmental stage of the muscle fiber (discussed below), its anatomic location (Gauthier and Lowey, 1977), and its workload (reviewed by Jolesz and Sreter, 1981). In addition, extrinsic factors, such as innervation (Butler-Browne et al., 1982; Gambke et al., 1983) and hormones (Butler-Browne et al., 1984; Whalen et al., 1985; Mahdavi et al., 1987) can influence the pattern of fiber types and isoform distribution observed in skeletal muscle.

The standard histochemical classification of fast and slow fiber types is based on the ATPase

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Correspondence: D. Stave Kohtz, Department of Pathology (1194), Mount Sinai School of Medicine, 1 Gustave Levy Place, New York, NY 10029 Tel (212) 241-9169 Fax (212) 534-7491

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activity of the myosin heavy chain molecule (Barany, 1967; Wagner and Giniger, 1981; Lowey et al., 1983; Reiser et al., 1985). Sarcomeric myosin heavy chain isoforms in different fiber types can also be classified on the basis of reaction to specific monoclonal antibodies, relative mobility in low density nondenaturing gels, and hybridization with isoform-specific probes (reviewed by Schafer et al., 1987). As myofibers mature in vivo, embryonic, perinatal, and adult isoforms of myosin heavy chain can be distinguished. This nomenclature refers to the developmental stage at which each isoform is first observed.

Immunochemical studies have shown that an established clonal murine skeletal myoblast cell line (C2C12) has the intrinsic potential to differentiate into myotubes first expressing embryonic and subsequently perinatal isoforms of sarcomeric MHC (Silberstein et al., 1986). Observation of sarcomeric MHC isoform protein expression in this cell line using isoform-specific monoclonal antibodies revealed attenuated expression of the earlier embryonic isoform accompanying accentuated expression of the later perinatal isoforms in maturing myotubes. Two other clonal murine myoblast cell lines (C2C7 and T984) have been shown to differentiate into muscle fibers expressing first embryonic and perinatal MHC mRNAs, and subsequently adult fast MHC transcripts (Weydert et al., 1987). Previous studies of sarcomeric MHC expression in differentiating human myogenic cultures have been restricted to the detection of total MHC mRNA (Gunning et al., 1987) or to the detection of adult fast and slow isoforms (Wade et al., 1990). In this study, the intrinsic potential of continuously passaged human fetal myoblasts to differentiate into muscle fibers expressing a temporally regulated developmental program of sarcomeric MHC genes is evaluated. The results of Northern blots and polymerase chain reaction (PCR) analyses presented here using probes specific for embryonic, perinatal, slow (β), and fast MHC isoforms revealed that the MHC genes in differentiating human myoblasts follow temporally distinct programs of expression.

Materials and methods

Generation of human fetal skeletal muscle cultures

Samples of 19–22 week fetal thigh muscle were collected at autopsy from anonymous donors. Muscle tissue was minced into 1 mm fragments, and washed with Hank's balanced salt solution lacking Ca^{2+} and Mg^{2+} (HBSS; Gibco) buffered at pH 7.4 with 20 mM HEPES. The tissue fragments were pre-digested with 100 μg/ml collagenase (Gibco) in HBSS (supplemented with 50 μM $CaCl_2$) for 20 minutes (37°). The suspended fragments were then decanted, and digested twice with 25 μg/ml trypsin (Gibco) for 30 minutes (37°). The released cells from each trypsin digestion were decanted and immediately suspended in Dulbecco's modified Eagle's medium (DMEM) containing 20% fetal bovine serum (HyClone). The cells were then washed twice with DMEM containing 20% fetal bovine serum, and plated on standard tissue culture plasticware (Corning) in high mitogen medium (DMEM supplemented with 20% fetal bovine serum, 1% bovine embryo extract [Gibco], 50 ng/ml basic fibroblast growth factor [Collaborative Research], 25 ng/ml multiplication stimulating activity [Collaborative Research], MEM non-essential amino acids [Gibco], MEM vitamins [Gibco], 1 mM sodium pyruvate, and 100 μg/ml gentamicin). This medium was chosen by the following criteria: bFGF and MSA have been shown previously to promote the mitogenesis of skeletal myoblasts (Linkhart et al., 1981; Ewton and Florini, 1981); serum promotes the mitogenesis and adhesion of skeletal myoblasts; bovine embryo extract reduces the number of feedings required for continuous growth of the cells, either by stabilizing MSA and bFGF, and/or by providing a source of transforming growth factor β, an inhibitor of skeletal myoblast differentiation. The concentrations of bFGF and MSA used to grow the myoblasts were initially derived from previously published dose response curves (Linkhart et al., 1981; Ewton and Florini, 1980, respectively), and found to be sufficient for long-term growth of myoblasts. Myoblasts were induced to differentiate by culturing in DMEM supplemented with 4% dialyzed donor horse serum, MEM non-essential amino acids, MEM vitamins, 1 mM sodium pyruvate, and 100 μg/ml gentamicin.

Preparation of RNA and Northern blotting

Total cellular RNA was prepared from cell cultures by guanidinium/isothiocyanate denaturation and cesium chloride density centrifugation as described previously (Chirgwin et al., 1979). RNA was resolved by formaldehyde agarose gel

electrophoresis (as described by Maniatis et al., 1982), transferred and bound to GeneScreen (New England Nuclear) as described by the manufacturer. Probes were ^{32}P-labeled with T4 polynucleotide kinase. Sequences and hybridization conditions for isoform-specific probes are given below.

Polymerase chain reaction assays

MHC isoform mRNA expression was characterized by PCR analysis of cDNA synthesized from RNAs isolated during a time course both with and without thyroid hormone. One microgram of RNA from each time point was reverse-transcribed using 100 picomoles of random hexamers (Boehringer Mannheim) as the primers in 500 mM KCl, 200 mM Tris-Cl (pH 8.4), 25 mM $MgCl_2$, 1 mg/ml bovine serum albumin, RNAsin, 1 mM of each dNTP, and 50 U AMV reverse transcriptase (Life Sciences) at 42°. The reverse transcriptase was inactivated at 95° for 5 minutes. A consensus region for all four isoforms was used to generate the sense oligonucleotide (5′-CAGGACACCAGCGCCCA-3′). Two different oligonucleotides were generated to the same 3′ region to generate the antisense oligonucleotides. One is identical for the embryonic, perinatal, and fast isoforms (5′-TCCTCGGCCTCCTCCAGC-3′); the other matches the slow isoform (5′-TCCTCTGCCTCATCCAGCTC-3′). Sixty nanograms or the upstream and downstream primers and 1 U of Taq polymerase (Perkin-Elmer Cetus) were added to the reaction. Samples were denatured at 92°, annealed at 52°, and extended at 74° for 1 minute each for 30 cycles. The products were separated on an 0.8% agarose gel and transferred to the GeneScreen membrane. The filters were hybridized with radiolabeled oligonucleotide probes specific for each of the 4 MHC isoforms. The sequence of the embryonic-specific oligonucleotide is: 5′-CGTCAGCTCCTTGACCCTCCG-3′; the perinatal is: 5′-TACGTTTCTGTTCATTTTCA-3′; the slow (β) is: 5′-CGCTCGCTCTTCCTCATGC-3′; the fast is: 5′-AGCTAGATTGGTGTTGG-3′. GeneScreen membranes were prehybridized and hybridized in 7% sodium dodecyl sulfate (SDS), 10X Denhardt's, 20 mM $NaPO_4$, 5X SSC, and 150 μg/ml heat-denatured salmon sperm DNA. Probe was added to the hybridization mix at a concentration of 2.0×10^6 cpm/ml. The embryonic-specific probe was hybridized at 54°, the perinatal-specific at 52°, the slow-specific at 52°, and the fast-specific at 50°. The filters were washed 1 hour in 3X SSC, 20 mM $NaPO_4$, 5% SDS and 10X Denhardt's. The filters were then washed for another hour in 1X SSC and 1% SDS at their respective hybridization temperatures.

Protein blotting

Culture monolayers were washed twice with normal saline before being immersed directly in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (100 mM Tris [pH 6.8], 2% SDS, .25% phenol red, 5% β-mercaptoethanol, 10% glycerol). After the monolayer dissolved, the samples were removed and heated to 100° for 10 minutes. Samples were electrophoresed through 7% SDS polyacrylamide gels, transferred to nitrocellulose, and blotted with monoclonal antibody MF-20 as described previously for other mAbs (Kohtz et al., 1987). Monoclonal antibody MF-20 (Bader et al., 1982) was generated by growing hybridoma cells in pristane-primed nude mice and harvesting Ascites fluid.

In situ hybridization

In situ hybridization on tissue culture cells was carried out according to the conditions outlined by Singer et al. (1986). Following the procedures described by Promega, ^{35}S-α-UTP labeled RNA probes were synthesized. Human fetal skeletal muscle cells were propagated and allowed to differentiate on collagen- or gelatin-coated glass coverslips. At 24–48 hour intervals after induction, coverslips were removed from medium and washed gently with Hank's Balanced Salt Solution (Gibco), then fixed in 4% paraformaldehyde in PBS and 5 mM $MgCl_2$ (pH 7.4) for 15 minutes. The coverslips were washed in PBS with 5 mM $MgCl_2$, and stored at 4° in 70% ethanol. Cells were rehydrated in PBS with 5 mM $MgCl_2$, then incubated in 200 mM tris (pH 7.4), 100 mM glycine for 10 minutes to quench free aldehydes. Before hybridization, the cells were heated to 65° for 10 minutes in 50% deionized formamide, 2X SSC. The samples were hybridized in a mixture containing 20 ng single strand RNA probe, 20 μg yeast tRNA, 20 μg salmon sperm DNA, 50% formamide, 2X SSC, 0.2% BSA, 10% dextran sulfate and vanadyl-sulfate ribonucleotide complex RNase inhibitor (BRL) for 4 hours at 37°. The samples were washed first in 50% formamide,

2X SSC, then in 50% formamide 1X SSC for 30 minutes at 37°. The cells were then washed at room temperature for 30 minutes with several changes of 1X SSC. The coverslips were then dehydrated with graded changes of ethanol. The slides were dipped in Kodak NTB-2 emulsion diluted 1:1 with water, air dried, and kept in a light-proof container for 5 days at 4°. The slides were developed with Kodak D19 for 7 minutes, rinsed with water for 1 minute, fixed for 3 minutes, then stained with hematoxylin and eosin, and mounted in Permount. The slides were viewed under dark field microscopy.

Results

Characterization of continuously passaged cultures of human fetal skeletal myoblasts

Human fetal skeletal myoblasts (HUSK cells) were derived from thigh muscle of autopsy specimens (19–22 weeks p.c.). HUSK cells were cultured in high mitogen medium (DMEM supplemented with 20% fetal bovine serum, 1% bovine or chick embryo extract, 50 ng/ml basic fibroblast growth factor [bFGF] and 25 ng/ml multiplication stimulating activity [MSA]) and induced to differentiate in low mitogen medium (DMEM supplemented with 4% horse serum). Phase microscopy revealed that the proliferating cells were spindle-shaped and fibroblastic, and in a single microscopic field of myoblasts several cells were observed in mitosis (Fig. 1A). At 70–90% confluence, the cells were transferred to low mitogen medium, and newly formed syncytia (early myotubes) appeared within 24 hours. Early myotubes (1–3 days post induction) were flat and square-shaped, and contained numerous nuclei irregularly arranged in the center of the syncytium (Fig. 1B). Later (3–7 days post induction) thinner, more refractile myofibers were observed, and these often displayed spontaneous contractions (Fig. 1C and D).

The expression of sarcomeric MHC protein is an important biochemical marker of myogenic differentiation, and thyroid hormone has been shown to regulate MHC genes positively and negatively in a fiber-dependent manner (Izumo

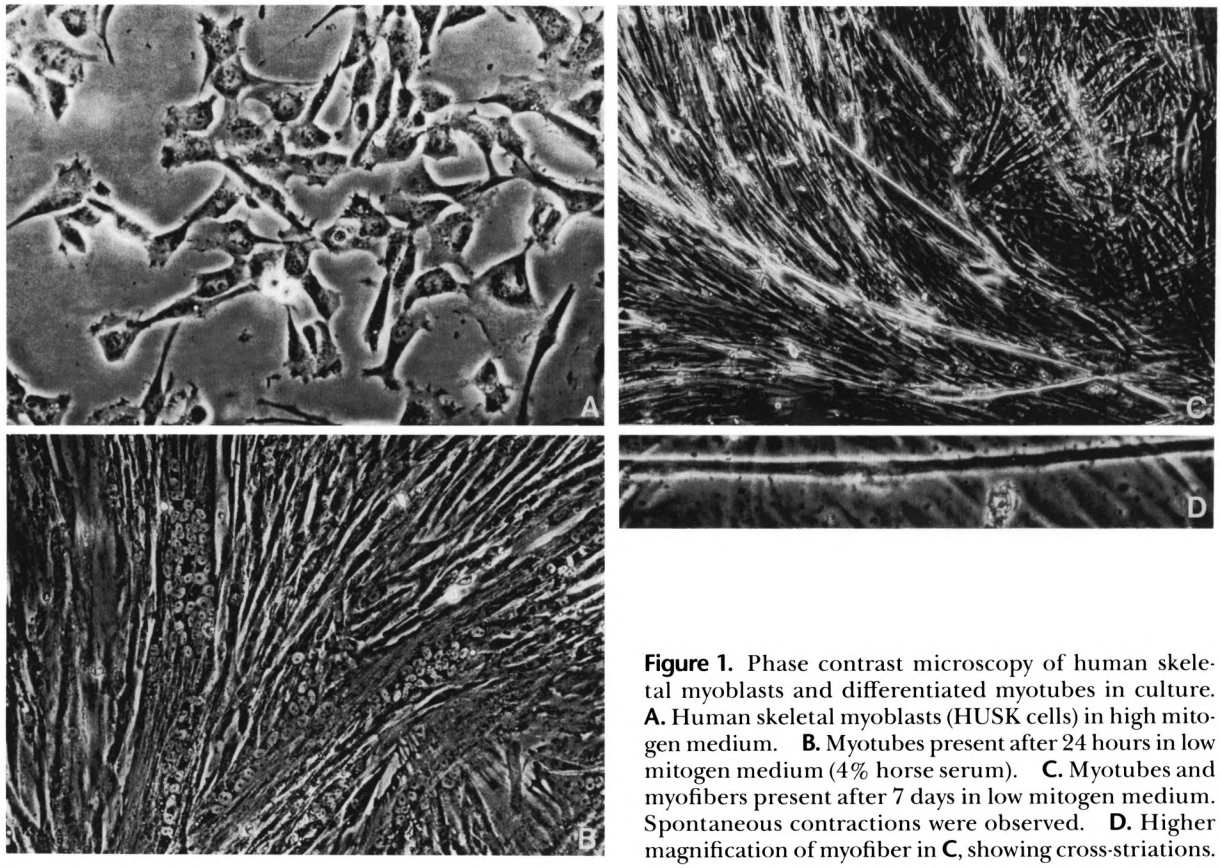

Figure 1. Phase contrast microscopy of human skeletal myoblasts and differentiated myotubes in culture. **A.** Human skeletal myoblasts (HUSK cells) in high mitogen medium. **B.** Myotubes present after 24 hours in low mitogen medium (4% horse serum). **C.** Myotubes and myofibers present after 7 days in low mitogen medium. Spontaneous contractions were observed. **D.** Higher magnification of myofiber in **C**, showing cross-striations.

et al., 1985). To characterize the expression of sarcomeric MHC protein and its dependence on the presence of thyroid hormone in this culture system, HUSK cells were induced to differentiate in low mitogen medium containing dialyzed horse serum (thyroid hormone-free), homogenized at various times post-induction, and immunoblotted with monoclonal antibody MF-20 (anti-sarcomeric myosin heavy chain; Bader et al., 1982). Cultures were performed in duplicate, and one flask at each time point was supplemented with 1 μg/ml thyroid hormone (T4; a 20-fold increase over basal level). Sarcomeric myosin heavy chain protein expression was observed 12 hours after the cells were induced to differentiate, reached peak levels 4 days post induction, then decreased between 6 to 8 days post-induction (Fig. 2). The reduced level of MHC protein observed in older cultures correlates with detachment and atrophy of some myotubes. The addition of thyroid hormone attenuated the accumulation of MHC slightly between 1 and 2 days post induction, but the MHC level was equivalent by 4 days post induction (Fig. 2). Except for this minor effect, the addition of thyroid hormone to the differentiation medium produced negligible changes in total sarcomeric MHC expression of differentiating HUSK cells (see below as well).

Transcription of four isoforms of sarcomeric MHC by human fetal myoblasts differentiating in culture

The availability of gene-specific nucleic acid probes provided an excellent means of characterizing the type and timing of MHC gene expression in HUSK muscle cell cultures. Total cellular RNA was prepared from parallel flasks of differentiated HUSK cells cultured in the presence or absence of T4 at various times post-induction. Samples were analyzed by Northern blot for the presence of embryonic, perinatal, and slow MHC isoform mRNAs. Embryonic MHC was the most abundant form observed at all time points. Perinatal MHC (Feghali and Leinwand, 1989) was observed at lower levels, while slow (β) MHC was observed at the lowest levels (Fig. 3). Accumulation of embryonic MHC was first detected by Northern blot 12 hours post-induction, while perinatal and slow MHC expression were first observed 24 hours post-induction. The highest levels of all three isoforms (as determined by Northern blot analysis) were observed 4 days post-induction, although embryonic MHC transcripts were detected at significant levels at most points post-induction. In contrast to PCR amplified preparations and in situ hybridization analyses (below), sarco-

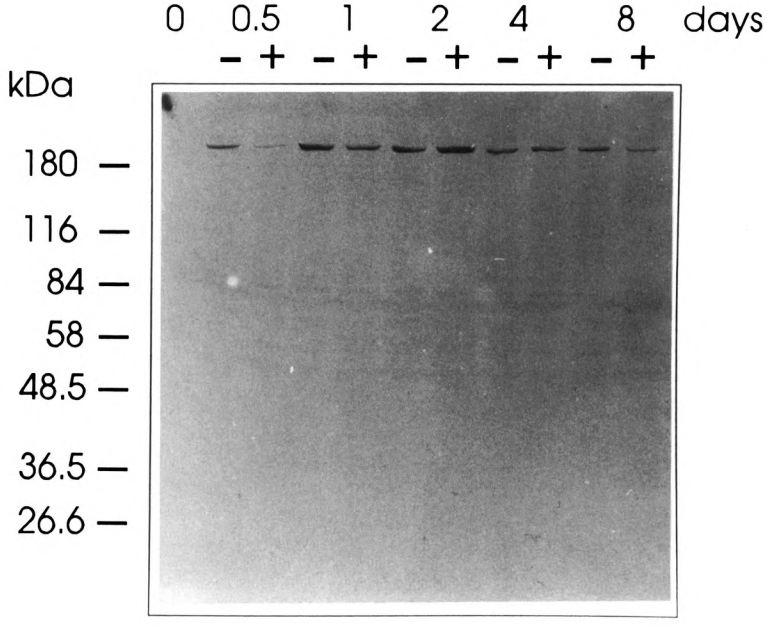

Figure 2. Sarcomeric MHC protein expression in differentiating human skeletal myoblasts. Cultures were grown in high mitogen medium (0 days), then induced to differentiate in low mitogen medium lacking (−) or supplemented with (+) 500 ng/ml thyroid hormone. At the indicated times post-induction, the cultures were dissolved in SDS-PAGE sample buffer, and Western blotted with monoclonal antibody MF-20 (anti-sarcomeric myosin heavy chain).

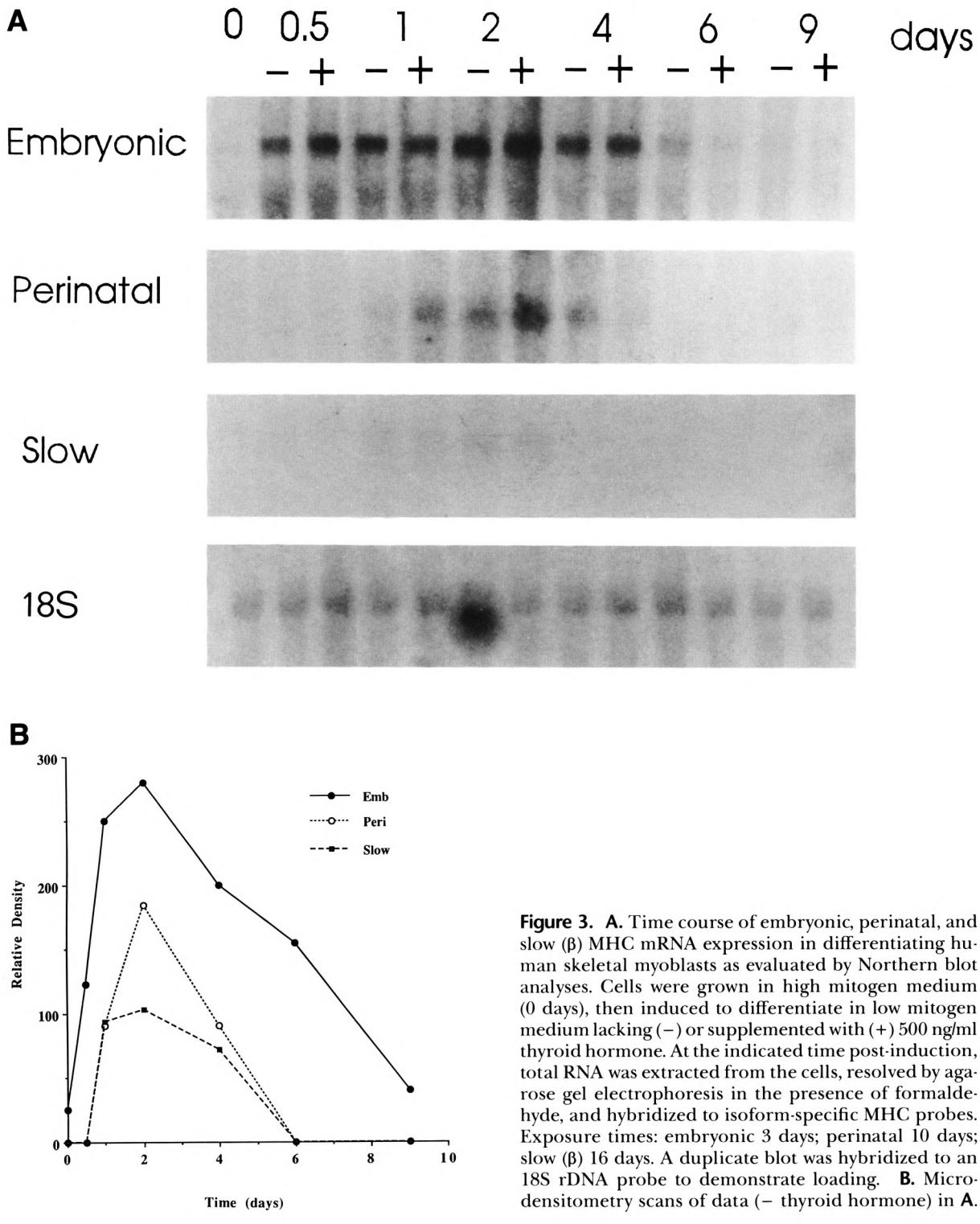

Figure 3. A. Time course of embryonic, perinatal, and slow (β) MHC mRNA expression in differentiating human skeletal myoblasts as evaluated by Northern blot analyses. Cells were grown in high mitogen medium (0 days), then induced to differentiate in low mitogen medium lacking (−) or supplemented with (+) 500 ng/ml thyroid hormone. At the indicated time post-induction, total RNA was extracted from the cells, resolved by agarose gel electrophoresis in the presence of formaldehyde, and hybridized to isoform-specific MHC probes. Exposure times: embryonic 3 days; perinatal 10 days; slow (β) 16 days. A duplicate blot was hybridized to an 18S rDNA probe to demonstrate loading. **B.** Microdensitometry scans of data (− thyroid hormone) in **A**.

meric MHC mRNAs were not detected in uninduced cultures of myoblasts by Northern blot analysis. The addition of thyroid hormone to the differentiation medium (DMEM + 4% dialyzed horse serum) had no obvious effect on the quantitative or qualitative accumulation of sarcomeric MHC mRNA.

Polymerase chain reactions based on oligo-

nucleotides representing conserved regions of all known sarcomeric MHCs (see Materials and Methods) were used to amplify cDNA reverse transcribed from mRNA. Isoform-specific oligonucleotide probes were then used to distinguish the presence of embryonic, perinatal, slow, and fast isoforms by hybridization. Embryonic MHC mRNA was detected in uninduced cultures, while perinatal MHC mRNA was not detected until 1 day post-induction (Fig. 4). Transcripts of an additional MHC isoform observed in fetal and adult fast fibers (Karsch-Mizrachi and Leinwand, unpublished observations) were also detected by PCR in uninduced cells and in differentiated cultures. Slow MHC mRNA was detected in uninduced and induced cultures. Although an exceptionally strong signal for slow MHC was detected at 4 days post-induction (+ thyroid hormone), the significance of this datum is not clear, as these assays were not performed under quantitative conditions. While no quantitative conclusions about mRNA levels can be drawn from these PCR data, it can be concluded that appearance of perinatal MHC RNA is delayed relative to the appearance of the other three MHC mRNAs.

In situ hybridization analysis was used to identify the cell population(s) in the uninduced cultures that express embryonic MHC. RNA probes (^{35}S-labeled) corresponding to unique 3′ untranslated regions of the embryonic and perinatal MHC genes were hybridized to myoblast culture that had been maintained in high mitogen medium. Figure 5A demonstrates that expression of the embryonic MHC gene in uninduced cultures is restricted to spontaneously formed multinucleated syncitia. Examination of a large number of cells revealed that virtu-

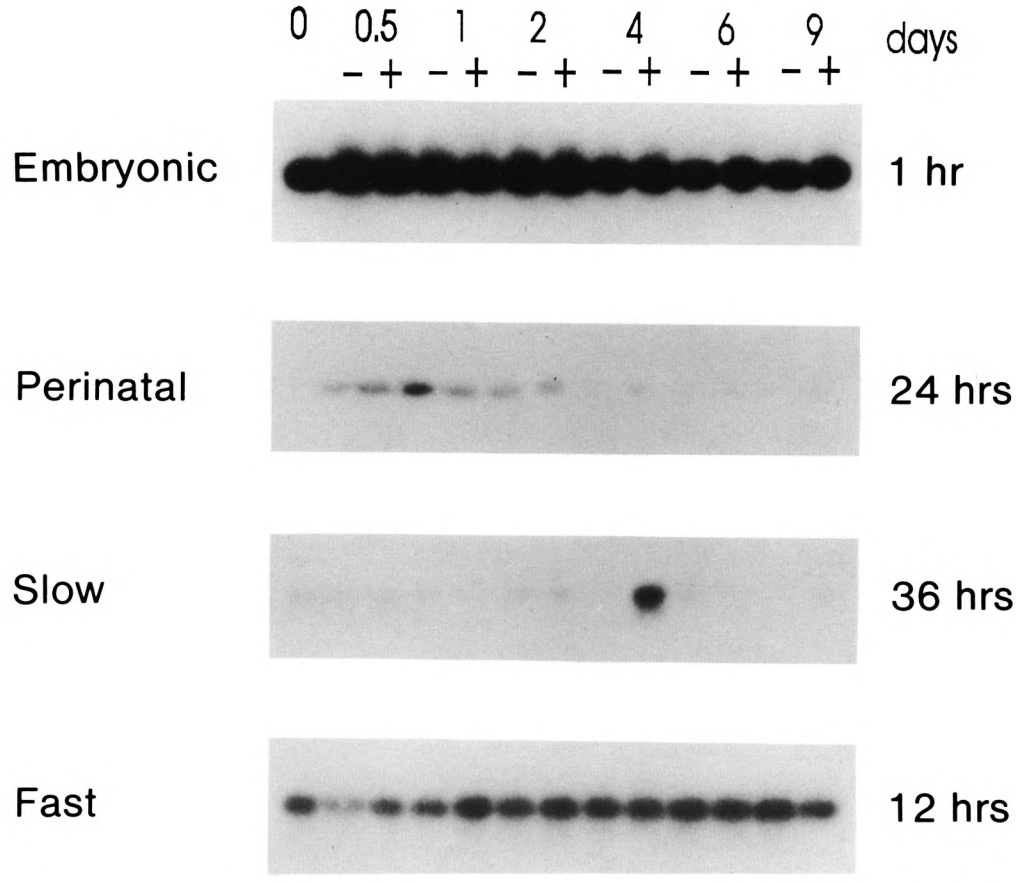

Figure 4. Embryonic, perinatal, and adult fast MHC mRNA expression in differentiating human skeletal myoblasts evaluated after polymerase chain reaction amplification. Cells were cultured, and RNA was generated as outlined in Figure 3. Polymerase chain reaction products were analyzed by hybridization to isoform-specific MHC probes. Exposure times of the autoradiographs are indicated on the right.

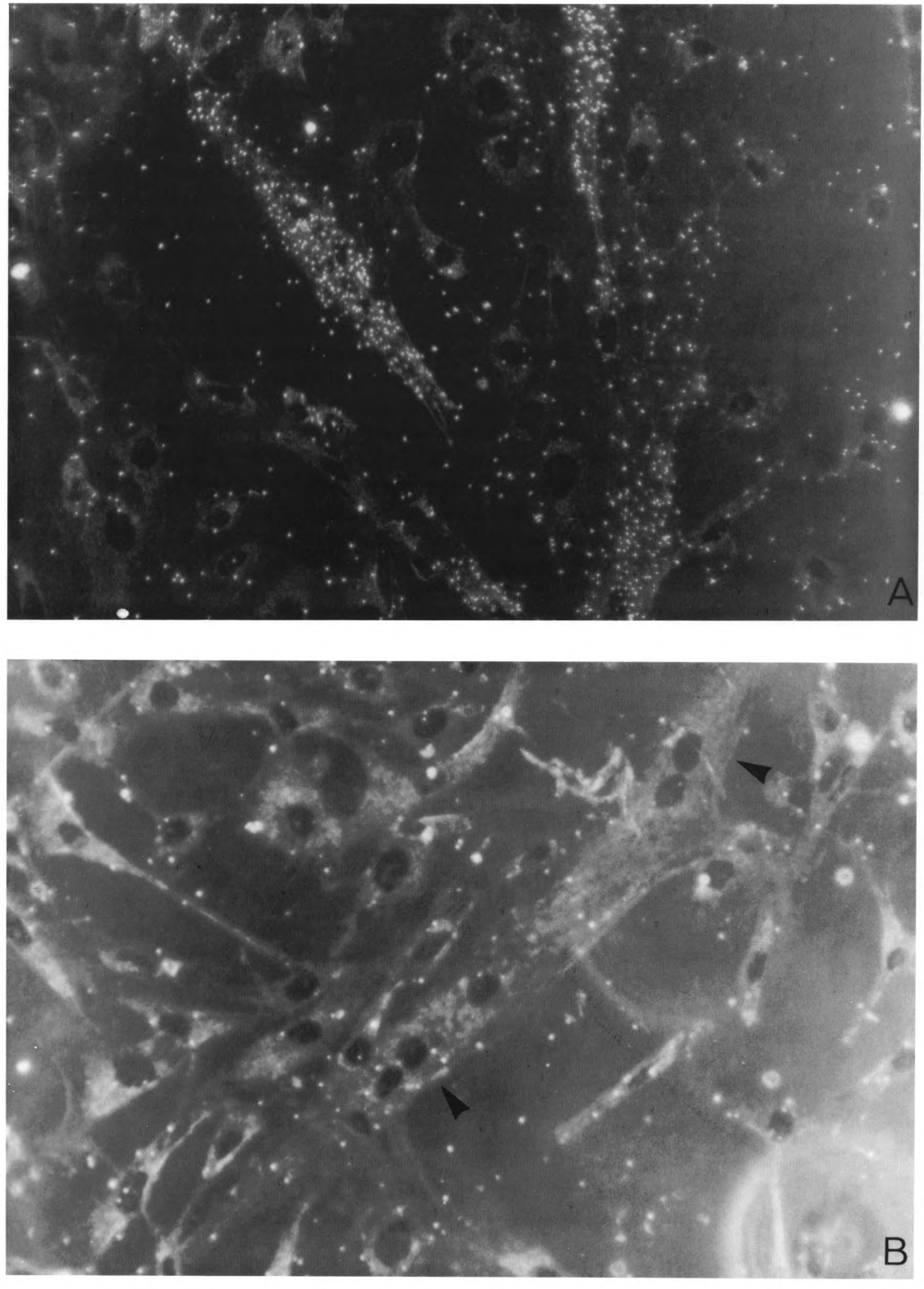

Figure 5. Embryonic and perinatal MHC in situ hybridization analyses of human fetal muscle cell cultures. Gene-specific ^{35}S-labeled RNA probes were hybridized to high confluence human fetal myoblast cultures (in high mitogen medium) and exposed to photographic emulsion. **A** and **B** show dark field microscopy of hematoxylin/eosin stained fields. The cells in **A** were hybridized to a embryonic MHC probe, while those in **B** were hybridized to a perinatal MHC probe. The arrows indicate spontaneously fused multinucleated cells.

ally all multinucleated syncitia were expressing embryonic MHC transcripts. Expression of the perinatal gene is not seen in a similar population of cells (Fig. 5B), where there are obvious multinucleated syncitia (identified by arrows) not binding the perinatal probe. Although it is not apparent from this experiment whether these syncytia are actively translating the embryonic MHC transcripts, it is clear that they do not mature in high mitogen medium into myotubes expressing the perinatal MHC gene. Fast MHC expression was also detected in spontaneously formed multinucleated syncitia of uninduced cultures and not detected in myoblasts (data not shown). Slow MHC expression was not detected by in situ hybridization in these cultures.

Discussion

Studies in avian systems have revealed that different lineages of myoblasts can be directly associated with the generation of specific fiber types. Three types of myoblasts have been isolated from avian embryos, as defined immunologically by their progeny myofibers: fast, fast/slow, and slow (Miller and Stockdale, 1986a,b). The relative abundance of each myoblast type in cultures derived from embryonic tissue can be summarized as fast > fast/slow >> slow. In developing fetal muscle, these myoblast populations are superceded by a fourth independent population of fast myoblasts (Miller et al., 1985; Miller and Stockdale, 1986a,b), resulting in the generation of cultures containing mainly fast and some fast/slow myoblasts. A clonal murine myoblast cell line (C2C12) has allowed immunochemical demonstration in culture of the development of myofibers expressing perinatal MHC from those expressing embryonic MHC (Silberstein et al., 1986). Studies using C2C12 cells differentiating in culture suggest that a distinct transition occurs in myofibers that results in attentuated expression of embryonic MHC and subsequent enhanced expression of perinatal MHC. In another study using different clonal murine myoblast cell lines (C2/7 and T984), accumulation of fast IIb MHC transcripts was not observed in myotubes 48 hours post-induction, but was observed 6 days post-induction. Embryonic MHC transcripts were observed at equivalent levels at both time points, indicating that accumulation of adult IIb MHC isoforms followed temporally the accumulation of embryonic isoforms (Weydert et al., 1987).

In this report, the time course of sarcomeric MHC mRNA accumulation is described for the differentiation of continuously passaged cultures of human fetal skeletal myoblasts. Isoform expression was evaluated by Northern blot analysis using isoform-specific probes and blot analysis of sarcomeric MHC transcripts amplified by polymerase chain reaction (PCR). Embryonic MHC mRNA was the predominant form that accumulated in the differentiating cells at all time points. At lower levels, accumulation of transcripts for perinatal, slow (β), and an isoform of fast MHC (distinct from previously characterized embryonic and perinatal isoforms) was also detected. Studies of the temporal pattern of sarcomeric MHC transcript accumulation showed that expression of genes for embryonic, perinatal, and a fast isoform of MHC is regulated independently in HUSK cells, while expression of the slow (β) MHC gene parallels that of the perinatal MHC gene, albeit at a lower level. Embryonic MHC transcripts were the most abundant at virtually all time points, and attenuation of transcripts for one isoform did not accompany enhanced expression of another. Instead, expression of embryonic, perinatal, and slow (β) MHC genes all reached their peak levels 2 to 4 days post-induction. This suggests that attenuation of embryonic MHC gene expression in favor of later isoforms (as is observed during fetal and neonatal development) may require maturation factors extrinsic to this culture system.

The experiments presented here do not consider the distribution of multiple MHC transcripts within individual cells. In particular, it will be important in future experiments to determine whether fast and slow isoforms are expressed in distinct myotubes, and whether coexpression of embryonic, perinatal, and adult isoforms occurs in either fast or slow myofibers.

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References

D. Bader, T. Masaki, and D. Fischman (1982), J Cell Biol 95, 763–770.

M. Barany (1967), J Gen Physiol 50 suppl., 197–218.

R. Breitbart and B. Nadal-Ginard (1987), Cell 49, 793–803.

G. Butler-Browne, L. Bugaisky, S. Cuenoud, K. Schwartz, and R. Whalen (1982), Nature 299, 830–833.

G. Butler-Browne, D. Herlicoviez, and R. Whalen (1984), FEBS Lett 166, 71–75.

J. Chirgwin, A. Przybala, R. MacDonald, and W. Rutter (1979), Biochemistry 18, 5294–5299.

D. Ewton and J. Florini (1980), Endocrinology 106, 577–583.

R. Feghali and L. Leinwand (1989), J Cell Biol 108, 1791–1797.

B. Gambke, G. Lyons, J. Haselgrove, A. Kelly, and N. Rubinstein (1983), FEBS Lett 156, 335–339.

G. Gauthier and S. Lowey (1977), J Cell Biol 74, 760–779.

G. Gauthier, S. Lowey, P. Benfield, and A. Hobbs (1982), J Cell Biol 92, 471–484.

P. Gunning, E. Hardeman, R. Wade, P. Ponte, W. Bains, H. Blau, and L. Kedes (1987), Mol Cell Biol 7, 4100–4114.

S. Izumo, B. Nadal-Ginard, and V. Mahdavi (1985), Science 231, 597–600.

F. Jolesz and F. Sreter (1981), Annu Rev Physiol 43, 531–552.

I. Karsch-Mizrachi, M. Travis, H. Blau, and L. Leinwand (1989), Nucl Acids Res 17, 6167–6179.

D. Kohtz, V. Georgieva-Hanson, J. Kohtz, W. Schook, and S. Puszkin (1987), J Cell Biol 104, 897–903.

T. Linkhart, C. Clegg, and S. Hauschka (1981), Dev Biol 86, 19–30.

S. Lowey, P. Benfield, D. Leblanc, and G. Waller (1983), J Musc Res Cell Motil 4, 717–726.

V. Mahdavi, S. Izumo, and B. Nadal-Ginard (1987), Circ Res 60, 804–814.

J. Miller and F. Stockdale (1986a), Proc Natl Acad Sci USA 83, 3860–3864.

J. Miller and F. Stockdale (1986b), J Cell Biol 103, 2197–2208.

J. Miller, M. Crow, and F. Stockdale (1985), J Cell Biol 101, 1643–1650.

P. Reiser, R. Moss, G. Giulian, and M. Greaser (1985), J Biol Chem 260, 14403–14405.

T. Maniatis, E. Fritsch, and J. Sambrook (1982), Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

D. Schafer, J. Miller, and F. Stockdale (1987), Cell 48, 659–670.

L. Silberstein, S. Webster, M. Travis, and H. Blau (1986), Cell 46, 1075–1081.

R. Singer, J. Lawrence, and C. Villnave (1986), Biotechniques 4, 230–250.

R. Wade, C. Sutherland, G. Reinhold, L. Kedes, E. Hardeman, and P. Gunning (1990), Dev Biol 142, 270–282.

P. Wagner and E. Giniger (1981), Nature 292, 560–562.

A. Weydert, P. Daubas, E. Lazaridis, P. Barton, I. Garner, D. Leader, F. Bonhomme, J. Catalan, D. Simon, J. Guenet, F. Gros, and M. Buckingham (1985), Proc Natl Acad Sci USA 82, 7183–7187.

A. Weydert, P. Barton, A. Harris, C. Pinset, and M. Buckingham (1987), Cell 49, 121–129.

R. Whalen, M. Toutant, G. Butler-Browne, and S. Watkins (1985), J Cell Biol 101, 603–609.