Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7988–7992, October 1990
Developmental Biology

MyoD converts primary dermal fibroblasts, chondroblasts, smooth muscle, and retinal pigmented epithelial cells into striated mononucleated myoblasts and multinucleated myotubes

(myogenesis/myofibrils/desmin/master switch genes)

J. CHOI*, M. L. COSTA†‡, C. S. MERMELSTEIN†‡, C. CHAGAS†, S. HOLTZER‡, AND H. HOLTZER‡§

Departments of *Biochemistry and ‡Anatomy, University of Pennsylvania, Philadelphia, PA 19104; and †Instituto de Biofísica Carlos Chagas Filho, Bloco G, Centro de Ciencias da Saúde, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941 Rio de Janeiro, Brazil

Communicated by Harold Weintraub, June 29, 1990 (received for review June 15, 1990)

ABSTRACT **Shortly after their birth, postmitotic mononucleated myoblasts in myotomes, limb buds, and conventional muscle cultures elongate and assemble a cohort of myofibrillar proteins into definitively striated myofibrils. MyoD induces a number of immortalized and/or transformed nonmuscle cells to express desmin and several myofibrillar proteins and to fuse into myosacs. We now report that MyoD converts normal dermal fibroblasts, chondroblasts, gizzard smooth muscle, and pigmented retinal epithelial cells into elongated postmitotic mononucleated striated myoblasts. The sarcomeric localization of antibodies to desmin, α-actinin, titin, troponin-I, α-actin, myosin heavy chain, and myomesin in these converted myoblasts are indistinguishable from *in vivo* and *in vitro* normal myoblasts. Converted myoblasts fuse into typical anisodiametric multinucleated myotubes that often contract spontaneously. Conversion and subsequent expression of the skeletal myogenic program are autonomous events, occurring in four nonmuscle microenvironments consisting of different combinations of foreign extracellular matrix molecules. Early events associated with conversion by MyoD involve (*i*) withdrawal from the cell cycle, (*ii*) down-regulation of the subverted cell's ongoing differentiation program, and (*iii*) initiation of desmin synthesis in presumptive myoblasts and dramatic redistribution of microtubules and desmin intermediate filaments in postmitotic myoblasts.**

There is an abrupt switch, *in vivo* and *in vitro*, in the differentiation program between normal replicating presumptive myoblasts and their daughters, the mononucleated postmitotic myoblasts (1–3). In early chicken embryos, at a stage when all other cells continue to cycle, myotomal presumptive myoblasts begin to yield postmitotic daughters. Within minutes after their birth, stage 15 myotomal postmitotic myoblasts organize their desmin intermediate filaments and microtubules into bipolar bundles, elongate, and concurrently initiate the synthesis of a cohort of myofibrillar proteins. When only ±4 hr old, they begin to assemble striated myofibrils. The distribution of α-actinin, titin, nebulin, α-actin, troponin-I, tropomyosin, myosin heavy chain (MHC), myosin light chain (MLC), and myomesin in these nascent sarcomeres is similar to that observed in mature muscles (4–7). This invariant sequence involving (*i*) at least two distinct generations of myogenic cells and (*ii*) the developmentally regulated assembly of striated myofibrils in mononucleated myoblasts is also observed in stage 23–24 limb buds and in muscle cultures prepared from day-10 embryos. In the latter systems, assembly into tandem sarcomeres is first evident, not in ±4-hr-, but in 10- to 20-hr-old postmitotic myoblasts (8). This temporal sequence is not observed when L6, L8, L6E9, BC3H1, and other types of immortalized and/or mutagenized myogenic lines are induced to differentiate (9–13).

MyoD induces a number of immortalized and/or transformed cell lines to express several myofibrillar genes and to fuse into multinucleated myosacs (14–16). Other transformed lines such as kidney epithelial cells, HeLa cells, and some hepatoma lines resist conversion. Judging from the published micrographs, MyoD-converted nonmuscle cells, like most myogenic cell lines, do not express the terminal myogenic program fully. They do not, for example, form striated mononucleated myoblasts, nor do they form elongated myotubes rich in striated myofibrils. Determining the intracellular conditions that permit or prevent MyoD from inducing the complete myogenic program in various transformed nonmuscle cells and various normal nonmuscle cells will be useful in probing many aspects of normal and abnormal myogenesis.

Here we describe the conversion by MyoD of four normal phenotypes with decreasing affiliation to the skeletal myogenic lineage: dermal fibroblasts, chondroblasts, gizzard smooth muscle, and retinal pigmented epithelial (RPE) cells. The four types of converted cells faithfully reproduce the temporal sequence characteristic of normal myogenesis *in vivo* and *in vitro*. Postmitotic mononucleated desmin$^+$/myofibrillar protein$^-$ cells appear several days after infection. These elongate, synthesize a cohort of myofibrillar proteins, and assemble them into mature striated myofibrils. Conversion and subsequent expression of the myogenic program are strikingly autonomous events, occurring in four nonmuscle microenvironments. Conversion involves the down-regulation of the original ongoing differentiation programs of the infected cells.

MATERIALS AND METHODS

Cells. Chondroblasts and RPE cells free of contaminating cells (17, 18) were generously donated, respectively, by M. Pacifici and by N. Philp and V. Nachmias from the University of Pennsylvania. Primary cultures of gizzard smooth muscle and quarternary cultures of dermal fibroblasts were prepared and reared as described (19). The cells were plated onto rat collagen-coated Aclar squares at a density of 1.5×10^5 cells per 35-mm dish. Cultures were fed every 2 days with 84% (vol/vol) Dulbecco's modified Eagle's medium, 10% (vol/vol) horse serum, 5% (vol/vol) chicken embryo extract, and 1% penicillin/streptomycin as described (20).

Retroviral Infection. Amphotropic retrovirus containing the MyoD coding region and the control virus were gener-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MHC and MLC, myosin heavy chain and light chain, respectively; RPE, retinal pigmented epithelial.

§To whom all reprint requests should be addressed.

ously donated by H. Weintraub, S. Tapscott, and D. Miller from the Hutchinson Cancer Research Center. Both control and MyoD-containing retrovirus contain the gene for neomycin phosphotransferase, allowing selection of infected cells with the neomycin analogue, G418. Day-2 to -3 cultures were infected by exposure to virus ($5–10 \times 10^5$ colony-forming units) and Polybrene (8 μg/ml) in a final volume of 100 μl. After 8 hr, 1.5 ml of medium was added. Infected cells were selected, using medium containing G418 (0.8 mg/ml) for the next 6 days, followed by medium without G418.

Immunostaining. At various times after infection, the cultures were fixed and double-stained with various combinations of antibodies to desmin, α-actinin, titin, nebulin, α-actin, troponin-I, tropomyosin, MHC, MLC, and myomesin, as described (20).

RESULTS

Assembly of Striated Myofibrils in Myotubes Derived from MyoD-Converted Cells. Cultures of dermal fibroblasts, chondroblasts, gizzard smooth muscle, and RPE cells were infected with control and MyoD amphotropic retroviruses containing the neomycin phosphotransferase gene. After selection in G418, the MyoD-infected cultures were reared in a normal mitogen-rich medium for 2–12 days. On average, the day-8 to -9 postselected cultures contained $3–6 \times 10^6$ cells per dish. Numerous myotubes formed in all types of converted cultures. However, the percent of the total nuclei in myotubes per dish varied greatly from experiment to experiment. In cultures of converted chondroblasts and smooth muscle, the percent of nuclei in myotubes ranged from 5 to 30%. In cultures of converted dermal fibroblasts and RPE cells, the number ranged from 1 to 5%. We cannot explain this variation. No desmin$^+$ or myofibrillar protein$^+$ cells were observed in control cultures.

The myotubes in cultures of converted chondroblasts and dermal fibroblasts were indistinguishable from those prepared from normal day-10 embryonic breast muscles in terms of elongated morphology, number of nuclei, density of myofibrils, and the sarcomeric localization of antibodies to α-actinin, titin, nebulin, α-actin, troponin-I, tropomyosin, MHC, MLC, myomesin, and desmin (Fig. 1). These myotubes contracted spontaneously. Though reasonably normal myotubes emerged in converted smooth muscle cultures, they frequently contained fewer nuclei per myotube and often displayed long irregular pseudopodial extensions. The multinucleated cells that emerged in RPE-converted cultures were the least normal cytologically. They formed poorly adherent myotubes that failed to elongate and frequently retracted from the substrate. Nevertheless these often

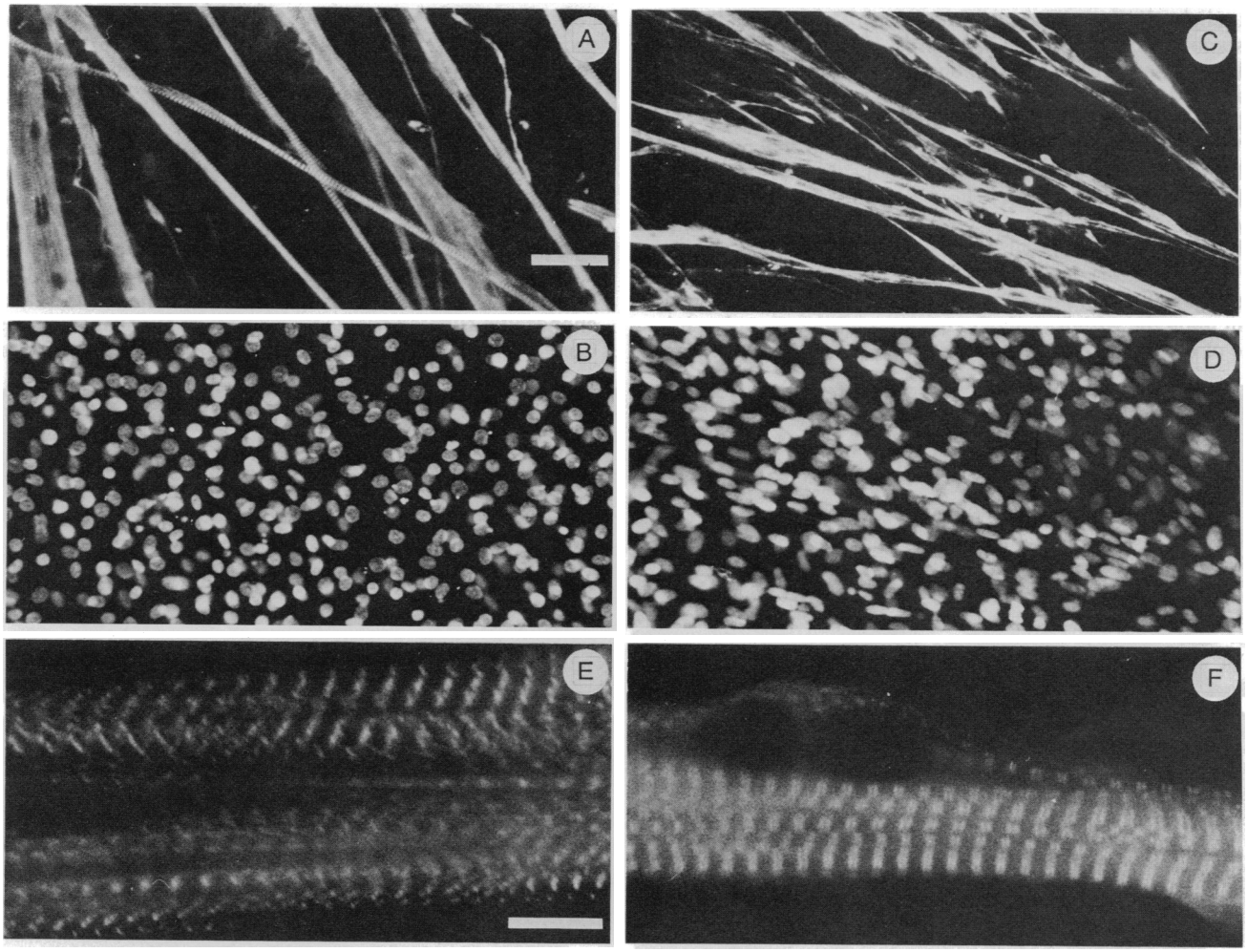

FIG. 1. (*A* and *B*) Postselected day-10 culture of converted dermal fibroblasts stained with anti-MHC and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), respectively. (*C* and *D*) Postselected day-10 culture of converted gizzard smooth muscle cells, stained with anti-MHC and DAPI, respectively. (*A–D*, bar = 50 μm.) (*E* and *F*) High magnification micrographs of two myotubes from a postselected day-10 culture of converted chondroblasts and a converted culture of RPE cells, respectively. The former is stained with anti-myomesin; the latter is stained with anti-titin. Anti-myomesin localizes to the center of each A band; anti-titin localizes to a doublet around each Z band (20). Note the two nuclei in *F*. (*E* and *F*, bar = 10 μm.)

stumpy multinucleated myotubes assembled spontaneously contracting striated myofibrils. Myofibrillar protein$^+$ fragments of detached and moribund myotubes were more common in these than in the other types of converted cultures.

Temporal Sequence in Expression of Desmin and Myofibrillar Proteins. *In vivo* and *in vitro* newborn postmitotic myoblasts are for a brief period desmin$^+$/myofibrillar protein$^-$ (21, 22). Without fusing they begin to elongate and synthesize a cohort of myofibrillar proteins. In all types of day-4 MyoD-infected cultures, the number of mononucleated desmin$^+$/myofibrillar protein$^-$ cells greatly exceeded the number of mononucleated desmin$^+$/myofibrillar protein$^+$ cells (Fig. 2). This ratio was reversed in all older cultures. Double-labeling experiments with a combination of antibodies demonstrated that mononucleated cells positive for MHC invariably were positive for α-actinin, titin, nebulin, α-actin, tropomyosin, MLC, myomesin, and troponin-I. Desmin$^-$/myofibrillar positive$^+$ cells, which are never observed in normal myogenic populations, have not been observed in MyoD-converted cultures. The degree of normality and maturity achieved in many converted myoblasts is illustrated in Fig. 3. Such myoblasts were indistinguishable from those that emerge in (*i*) stage 14–15 chicken myotomes, (*ii*) stage 23–24 limb buds, and (*iii*) cultures prepared from day-10 embryonic breast muscles. The RPE-converted cultures displayed relatively more rounded mononucleated desmin$^+$/myofibrillar protein$^+$ cells than did the other cultures. These rounded cells assembled normal striated myofibrils. However, the rounded cells did not adhere to the nonmuscle extracellular matrix that was secreted by the surrounding nonconverted RPE cells; hence many detached and degenerated.

Withdrawal from the Cell Cycle. Among normal myogenic cells, desmin can be detected in a small subset of replicating cells. [^{3}H]Thymidine is not, however, incorporated into the nuclei of normal cells that bind antibodies to α-actinin, titin, nebulin, α-actin, troponin-I, MHC, MLC, or myomesin (1–7). The obligatory withdrawal from the cell cycle prior to the synthesis of this cohort of myofibrillar proteins is not dependent upon the withdrawal of exogenous mitogens, rather it is one of the main controls that distinguishes normal myogenic cells from virtually all immortalized and/or transformed myogenic cell lines. To determine whether MyoD-converted dermal fibroblasts behave as normal or as immortalized myogenic cells, they were exposed to [^{3}H]thymidine, stained with labeled antibodies to desmin or MHC, and then autoradiographed. The data in Table 1 demonstrate that (*i*) although a small number of desmin$^+$/myofibrillar protein$^-$ cells can be labeled with [^{3}H]thymidine, most such cells have ceased cycling and (*ii*) converted MHC$^+$ cells reared continuously in a mitogen-rich medium do not reenter S phase. These findings demonstrate that the MyoD-converted normal cells withdraw from the cell cycle prior to the accumulation of a cohort of myofibrillar proteins and that these events are independent of the level of exogenous mitogens. With respect to these controls, the converted normal cells differed significantly from L6, L8, L6E9, and BC3H1 cells (9–13).

There is, however, an important difference with respect to the capacity for replication of myogenic cells in primary cultures obtained from day-10 embryonic breast muscles and of myogenic cells generated in MyoD-converted cultures. Between 30 and 50% of all cells in both secondary and tertiary cultures prepared from primaries are postmitotic and myofibrillar protein$^+$ (19, 24). In sharp contrast, less than 0.1% and 0.0% differentiated into muscle in the secondary and tertiary cultures, respectively, prepared from the MyoD-converted cultures. Clearly the capacity for MyoD-converted cells to continue to yield proliferation competent myogenic progeny was very limited compared to some subset of presumptive myoblasts in conventional primary muscle cultures. This difference could be attributed to overexpression of MyoD (14, 25).

Subversion of the Ongoing Differentiation Program of Chondroblasts and RPE Cells by MyoD. To determine the status of the original differentiation program of converted chondroblasts, infected cultures were double-stained with antibodies against chondroitin sulfated proteoglycan or type II collagen chains and with either desmin or MHC. Converted desmin$^+$ or MHC$^+$ cells were never positive for either intracellular chondroitin sulfated proteoglycan or type II collagen chains. Conversely, the numerous cells that stained positively for the

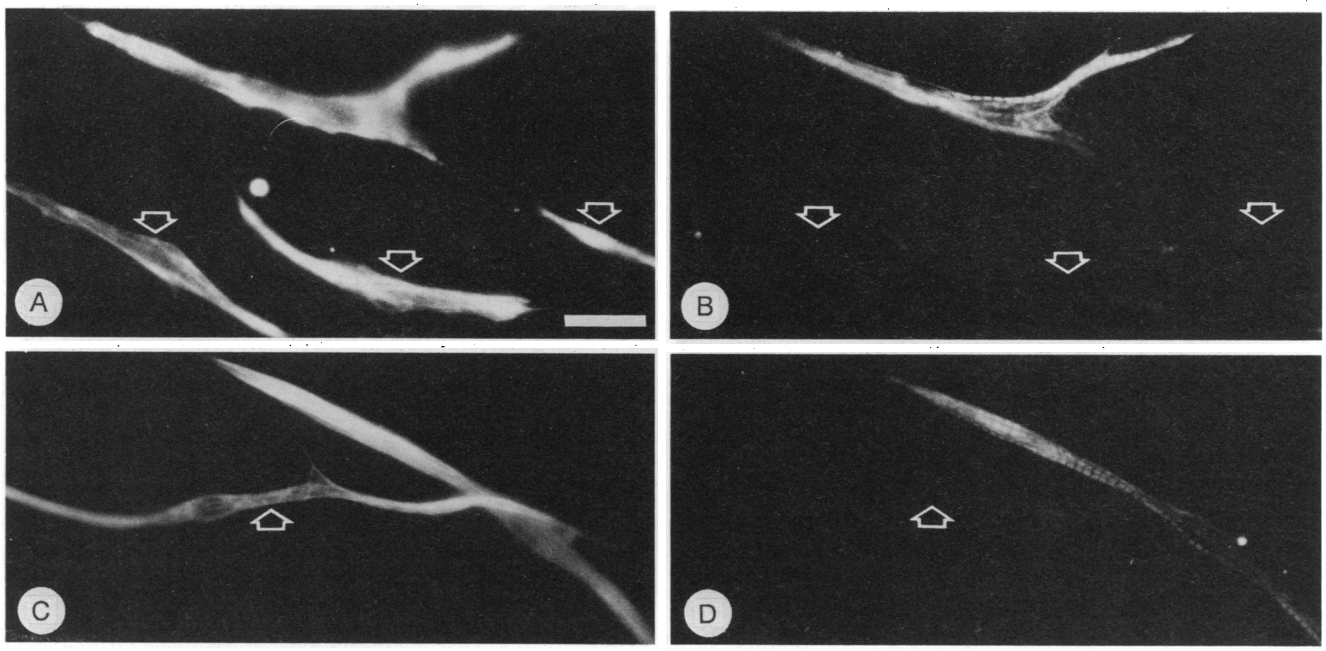

FIG. 2. (*A* and *B*) Double-stained postselected day-4 cultures of converted chondroblasts. (*A*) Anti-desmin (fluorescein). (*B*) Anti-MHC (rhodamine). Arrows point to three elongated desmin$^+$/MHC$^-$ myoblasts. (*A* and *B*, bar = 20 μm.) (*C* and *D*) Double-stained postselected day-4 culture of converted gizzard smooth muscle cells. (*C*) Anti-desmin (rhodamine). (*D*) Anti-MHC (fluorescein). Note the elongated mononucleated desmin$^+$/MHC$^-$ myoblasts. (*C* and *D*, ×530.)

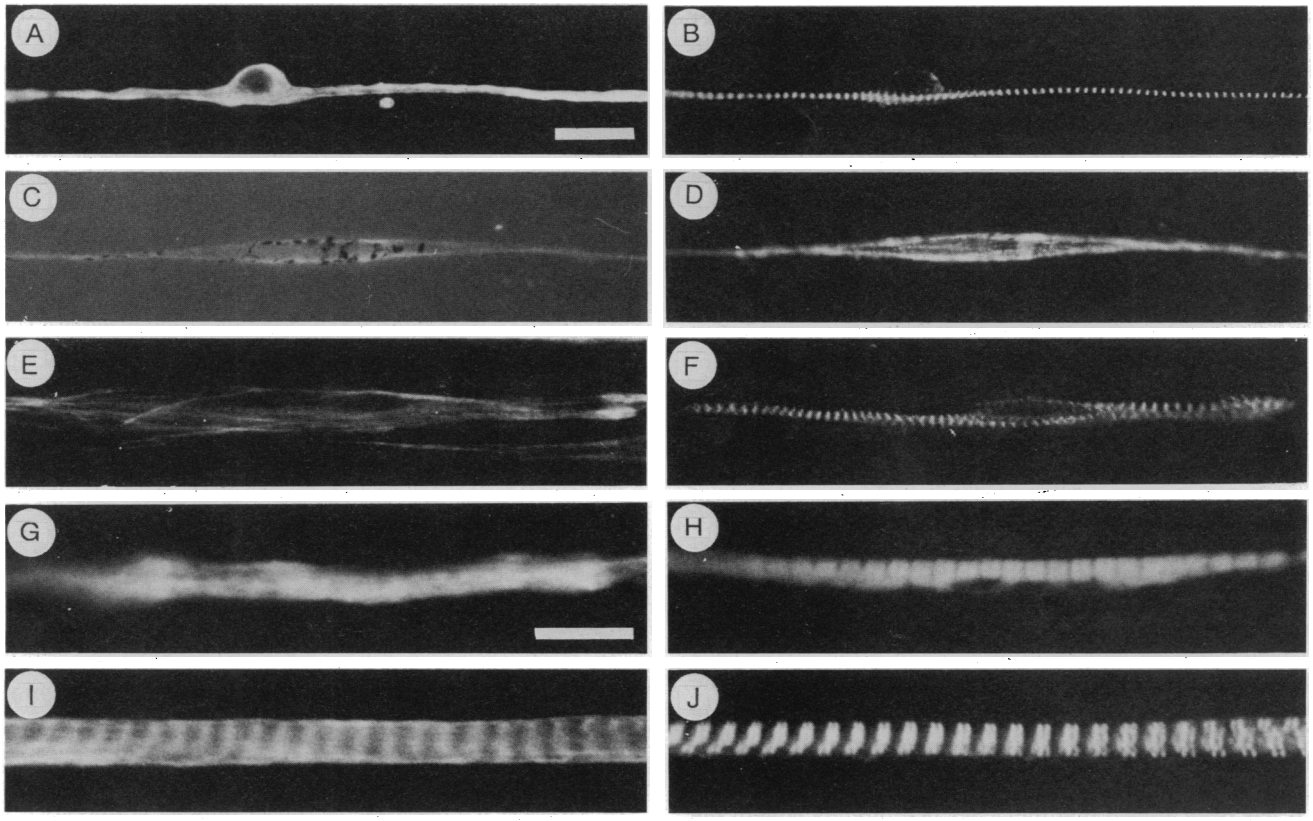

FIG. 3. Representative postmitotic elongated mononucleated myoblasts from different types of converted cultures. (*A* and *B*) Converted dermal fibroblast stained for desmin (fluorescein) and titin (rhodamine), respectively. (*C* and *D*) Converted RPE cell stained for desmin (fluorescein) and for MHC (rhodamine), respectively. (*C*) Combination of bright field and fluorescence microscopy to document the very rare occurrence of melanin granules in a myogenic cell. (*E* and *F*) Converted chondroblast stained for myomesin (rhodamine) and troponin-I (fluorescein), respectively. (*G* and *H*) Converted RPE cell stained for desmin (fluorescein) and MHC (rhodamine), respectively. (*I* and *J*) Converted dermal fibroblast to reveal the localization of desmin (fluorescein) and titin (rhodamine), respectively; note the transverse arrangement of the desmin intermediate filaments, a condition characteristic only of mature myofibrils (23). (*A–F*, bar = 20 μm; *G–J*, bar = 50 μm.)

cartilage-specific molecules in the converted cultures were invariably desmin$^-$ and myofibrillar protein$^-$. Most cells in cultures of chondroblasts infected with the vector carrying the neomycin-resistance gene, but lacking MyoD, were positive for both chondroblast markers; no cells in these cultures were either desmin$^+$ or myofibrillar protein$^+$.

Table 1. Replication in MyoD-converted fibroblasts

Day	[^{3}H]Thymidine$^+$ cells, % of total cells	% of desmin$^+$ cells		
		MHC	[^{3}H]Thymidine$^-$	[^{3}H]Thymidine$^+$
2	23.4	−	56.0	7.0
		+	37.0	0.0
4	9.7	−	3.4	0.7
		+	95.9	0.0
6	3.2	−	10.0	0.0
		+	90.0	0.0
8	0.2	−	7.6	0.0
		+	92.4	0.0

Day-2 postselected cultures of dermal fibroblasts were exposed to [^{3}H]thymidine after growing in normal medium for an additional 2, 4, 6, or 8 days. Cells in each series were exposed to [^{3}H]thymidine for 8 hr and then immediately fixed. They were double-stained with antibodies to desmin and MHC, covered with emulsion, and developed. Values of [^{3}H]thymidine$^+$ cells as a percent of total cells in the dish reflect the overall depression in replication as the cells become confluent (Fig. 1 *B* and *D*). Then 100 desmin$^+$ mononucleated cells from each series were inspected at random. Each desmin$^+$ mononucleated cell was scored as MHC$^+$ or MHC$^-$ and as [^{3}H]thymidine$^+$ or [^{3}H]thymidine$^-$.

At the time of infection all RPE cells display 10–30 melanin granules per cell. As shown in Fig. 3 *C* and *D*, melanin granules have been found in some postmitotic myoblasts as well as in some multinucleated myotubes. It is to be emphasized, however, that melanin granules were extremely rare in myofibrillar protein$^+$ cells. Less than 0.05% of myofibrillar protein$^+$ cells were positive for melanin granules. It is more likely that these rare melanin granules were carried over from the originally infected cells rather than having been actively assembled in the converted cells.

From these data we suggest that irrespective of how MyoD activates the myogenic program in functional chondroblasts and RPE cells conversion rapidly down-regulates expression of their respective ongoing differentiation programs.

DISCUSSION

There are considerable differences between normal myogenic cells and myogenic lines, as well as considerable differences among various myogenic lines. For example, myoblasts in many lines have been reported (*i*) to differentiate biochemically prior to withdrawing from the cell cycle, (*ii*) to down-regulate the synthesis of myofibrillar proteins and reenter the cell cycle when exposed to mitogens, and (*iii*) to become postmitotic as a consequence of, not as a precondition for, fusion into myotubes (9–11). BC3H1 cells although they transcribe several myogenic genes, never withdraw from the cell cycle, always respond to mitogens, never fuse, and do not assemble recognizable myofibrils (12, 13). Some L8 lines, while fusing to form desmin$^+$ myosacs, fail to

assemble recognizable thick or thin filaments, whereas other L8 lines, under the same culture conditions, assemble thick filaments but do not assemble α-actinin/troponin-I/α-actin I–Z–I complexes (unpublished data). Our original expectation was that different normal terminal phenotypes infected by MyoD would behave like one or another myogenic cell line. We also expected that the converted cell's original differentiation program would set constraints on what subfractions of the myogenic program would be expressed. The data instead demonstrate that four terminal phenotypes with various degrees of relatedness to the myogenic lineage respond to MyoD in the same way. Furthermore, this response follows a sequence identical to that observed during normal myogenesis whether in myotomes, limb buds, or conventional cultures (1–8).

From the data presented in Sassoon *et al.* (26), it is likely that, in mouse somites and limb buds, at most, one to two generations separate the first cells with MyoD transcripts from the first postmitotic myoblasts. This correlation of limited replication in $MyoD^+$ cells is also observed in our cultures. In *Xenopus*, however, if all muscle cells are derived from $MyoD^+$ gastrula cells, then clearly such transcripts by themselves do not limit subsequent replication (27). It will be important to learn which type of myogenic cell in the normal myogenic lineage initiates transcription of the MyoD gene and whether these also are the cells that effectively translate the mRNA (3). In this context it is worth mentioning that in preliminary experiments, chicken blastula cells infected with MyoD do not convert into recognizable myogenic cells and do not replicate significantly (unpublished observations).

It has been argued that the genes that directly govern the diversification of cells belong to a different family from those that control polarity, positional information, growth, and morphogenesis. In normal development, cell diversification, it was hypothesized (3–7, 28), depends on the sequential transcription of a small number of "master switch" genes. The finding that MyoD generates myogenic cells independently of surrounding cell adhesion molecules, collagens, proteoglycans, and other tissue-specific extracellular molecules is at least consistent with this view. If converted myogenic cells require specific extracellular molecules to express their respective differentiation programs, they themselves must be the source of such molecules.

On the other hand, the rounded and stunted RPE-converted cells document the importance of appropriate extracellular molecules in providing microenvironments permissive for growth and morphogenesis. The atypical morphology is consistent with the poor adhesion of the emergent converted cells to a matrix secreted by the surrounding nonconverted RPE cells. Alternatively, the poor morphology may reflect an incompatibility between conversion by MyoD and the intracellular milieu of RPE cells. We favor the former explanation because (*i*) converted RPE cells have the same atypical morphology seen with normal myogenic cells plated on inappropriate substrates or fed with inadequate media (29) and (*ii*) early RPE-converted cells prior to the formation of striated myofibrils are similar in appearance to their normal myogenic counterparts.

Unexpectedly, we failed to detect any obvious relationship between the susceptibility to conversion by MyoD and the distance of the four phenotypes to the myogenic lineage. Until more is known about the mechanism of conversion and until possible other master switch genes for other cell lineages are isolated, it is premature to speculate as to the significance of this observation.

We thank Dr. Chris Stoeckert for his thoughtful comments. This work was supported by National Institutes of Health Grants CA-18194, HL-15835 (to the Pennsylvania Muscle Institute), and HL-37675, and 307-TT-0541528 (from the Biomedical Research Support Grant Program); by the Muscular Dystrophy Association; and by the Research Foundation of the University of Pennsylvania. J.C. is a trainee in the National Institutes of Health-funded Medical Scientist Training Program. M.L.C. is supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)-Brasil, Grant 200490/87.7. C.S.M. is supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)-Brasil, Grant 5210/89-2.

1. Okazaki, K. & Holtzer, H. (1966) *Proc. Natl. Acad. Sci. USA* **56,** 1484–1490.
2. Holtzer, H., Sanger, J., Ishikawa, H. & Strahs, K. (1972) *Cold Spring Harbor Symp. Quant. Biol.* **37,** 549–566.
3. Dienstman, S. & Holtzer, H. (1975) *The Cell Cycle and Cell Differentiation*, eds. Reinhert, J. & Holtzer, H. (Springer, New York), pp. 1–25.
4. Holtzer, H., Marshall, J. & Finck, H. (1957) *J. Biophys. Biochem. Cytol.* **3,** 705–723.
5. Holtzer, H. (1978) *Stem Cells and Tissue Homeostasis*, eds. Lord, B., Potten, C. & Cole, R., (Cambridge Univ. Press, Cambridge, U.K.), pp. 1–28.
6. Holtzer, H., Sasse, J., Antin, P. & Pacifici, M. (1986) *Bibl. Anat.* **29,** 109–125.
7. Holtzer, H., Schultheiss, T., DiLullo, C., Choi, J., Costa, M. & Holtzer, S. (1990) *Ann. N.Y. Acad. Sci.*, in press.
8. Hill, C., Duran, S., Lin, K., Weber, K. & Holtzer, H. (1986) *J. Cell Biol.* **103,** 2185–2196.
9. Nadal-Ginard, B. (1978) *Cell* **15,** 855–864.
10. Nguyen, H., Medford, R. & Nadal-Ginard, B. (1983) *Cell* **34,** 281–293.
11. Whalen, R., Bugaisky, L., Butler-Browne, G. & Pinset, C. (1984) in *Developmental Processes in Normal and Diseased Muscle*, eds. Eppenberger, H. & Perriard, J. (Karger, Basel), pp. 155–165.
12. Taubman, M., Smith, C., Izumo, S., Grant, J., Andreadis, A. & Nadal-Ginard, B. (1989) *J. Cell Biol.* **108,** 1799–1806.
13. Schneider, M., Perryman, M., Payne, P., Spizz, R. & Olson, E. (1987) *Mol. Cell. Biol.* **7,** 1973–1977.
14. Davis, R., Weintraub, H. & Lasser, A. (1987) *Cell* **51,** 987–1000.
15. Tapscott, S., Lasser, A., Davis, R. & Weintraub, H. (1989) *Science* **245,** 532–536.
16. Weintraub, H., Tapscott, S., Davis, R., Thayer, M., Adam, M., Lassar, A. & Miller, D. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5434–5438.
17. Pacifici, M., Boettiger, D., Roby, K. & Holtzer, H. (1977) *Cell* **11,** 891–899.
18. Payette, R., Biehl, J., Toyama, Y., Holtzer, S. & Holtzer, H. (1980) *Cancer Res.* **40,** 2465–2474.
19. Yeoh, G. & Holtzer, H. (1977) *Exp. Cell Res.* **104,** 63–78.
20. Schultheiss, T., Lin, A., Lu, M., Murray, J., Weber, K., Masaki, T. & Holtzer, H. (1990) *J. Cell Biol.* **110,** 1159–1172.
21. Dlugosz, A., Tapscott, S. & Holtzer, H. (1983) *Cancer Res.* **43,** 2780–2789.
22. Kaufman, S. & Foster, R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9606–9610.
23. Holtzer, H., Bennett, G., Tapscott, S., Croop, J. & Toyama, Y. (1982) *Cold Spring Harbor Symp. Quant. Biol.* **46,** 317–329.
24. Dienstman, S. & Holtzer, H. (1977) *Exp. Cell Res.* **107,** 355–364.
25. Lin, Z., Deschene, C., Eldrege, J. & Paterson, B. (1989) *Genes Dev.* **3,** 986–996.
26. Sassoon, D., Lyons, G., Wright, W., Lin, V., Lassar, A., Weintraub, H. & Buckingham, M. (1989) *Nature (London)* **341,** 303–306.
27. Hopwood, N., Pluck, A. & Gurdon, J. (1989) *EMBO J.* **8,** 3409–3417.
28. Holtzer, H., Rubinstein, N., Fellini, S., Yeoh, G., Chi, J. & Okayama, M. (1974) *Q. Rev. Biophy.* **8,** 523–557.
29. Nameroff, M. & Holtzer, H. (1969) *Dev. Biol.* **19,** 380–396.