ACTIN AND MYOSIN IN A VARIETY OF MYOGENIC AND NON-MYOGENIC CELLS

N. A. Rubinstein, J. C. H. Chi, and H. Holtzer

Department of Anatomy, University of Pennsylvania
School of Medicine, Philadelphia, Penna. 19174

Received January 24,1974

Replicating presumptive myoblasts and bromodeoxyuridine-suppressed myogenic cells, as well as embryonic fibroblasts and chondroblasts, synthesize actin and myosin. The ratio of myosin to actin and the number of myosin light chains synthesized in these cells differ from those in multinucleated myotubes. These findings are discussed in relationship to myogenesis.

Evidence for the presence or absence of contractile proteins in precursor myogenic cells must be re-investigated. Antibodies against chicken skeletal muscle myosin and tropomyosin do not react with extracts of contractile proteins from many kinds of chicken embryonic and mature cells, including presumptive myoblasts (1, 2). The antibodies only react with contractile proteins from definitive, post-mitotic myoblasts, multinucleated myotubes, and mature muscle fibers. These findings have led to the conclusion that only the post-mitotic progeny of replicating presumptive myogenic cells synthesize the definitive contractile proteins characteristic of mature muscle.

Several investigators have reported that myosin or myosin mRNA cannot be detected in cultures of skeletal muscle before the onset of cell fusion (3, 4, 5).

On the other hand, Ishikawa *et al* (6) have demonstrated that many kinds of embryonic and mature cells, including presumptive myoblasts, bind heavy meromyosin, close to their cell surfaces, to form arrowhead complexes characteristic of actin filaments (7). Presumptive myoblasts which incorporated the thymidine analogue

BrdU* also formed arrowhead complexes associated with their cell surfaces; however, these BrdU-suppressed presumptive myoblasts did not form thick or thin filaments for myofibrils. In short, BrdU appears to suppress the formation of actin filaments for myofibrils, but not for the cell surface-associated structures (8).

Bray (9), Adelstein and Conti (10) and others have reported finding actin and myosin in a variety of embryonic and mature cells and have suggested that all cells synthesize these contractile proteins. If this is true, what is the relationship between the contractile proteins in (1) non-muscle cells, (2) precursor myogenic cells, and (3) post-mitotic multinucleated myotubes?

In this paper we demonstrate that presumptive myoblasts and BrdU-suppressed myogenic cells, as well as embryonic fibroblasts and chondroblasts, synthesize myosin heavy and light chains and actin.

Methods:

Breast muscle (11), fibroblasts, and chondroblasts (12) from 11 day old chick embryos were grown as described previously. For BrdU suppression, the muscle cultures were grown continuously in 10 ug/ml BrdU from the time of plating and subcultured after six days. At this time, no multinucleated cells were seen.

Results and Discussion:

Presumptive myoblasts are the replicating precursor cells that give rise to the post-mitotic myoblasts. The presumptive myoblasts do not bind fluorescein-labelled antibody to myosin or tropomyosin, nor do they display thick or thin filaments under the EM. The descendants of the presumptive myoblasts, the myoblasts,

*Abbreviations: BrdU, 5-bromo-2'-deoxyuridine; SDS, sodium dodecyl sulfate.

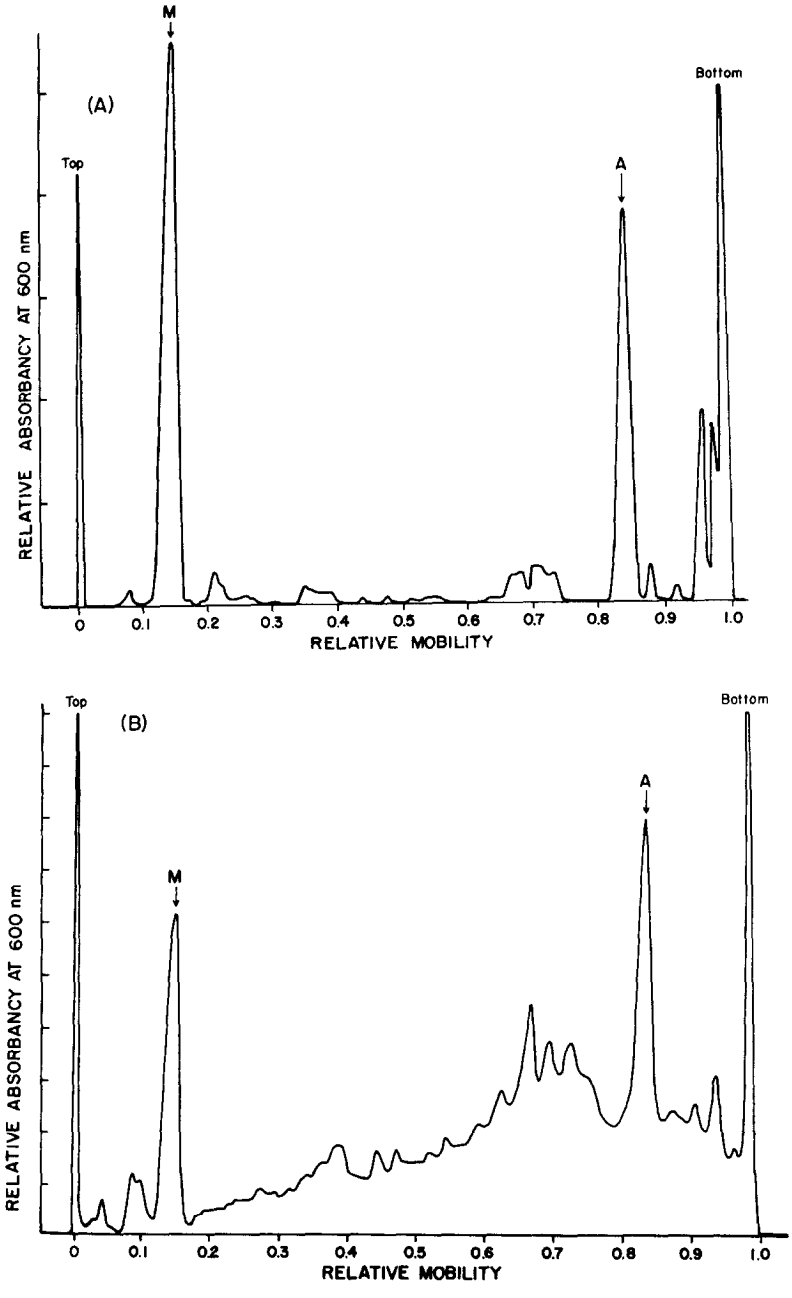

Figure 1. Densitometry tracings of 8% SDS-polyacrylamide gels of actomyosin extracted from myotubes (A) and presumptive myoblasts (B). Breast muscle from 11 day chick embryos was trypsinized, plated at a density of 5 x 10^5/ml, and fed daily. On days 2,3, and 4 the medium contained 1 ug/ml cytosine arabinoside. Presumptive myoblasts were collected from 20 hour old muscle cultures. Actomyosin was isolated by the method of Adelstein et al (22). Crude actomyosin obtained after three precipitations was used. SDS-polyacrylamide gel electrophoresis was used (23) with ethylene diacrylate substituted for BIS-acrylamide. Gels were stained with Coomassie Brilliant Blue R 250, scanned on a Gilford Spectrophotometer. M = myosin. A = actin.

fuse to form multinucleated myotubes which rapidly synthesize and organize actin and myosin into striated myofibrils (1, 2, 8).

During the first 48 hours of a muscle culture, the great majority of cells replicate. While most of these cells are presumptive myoblasts, a small number are probably fibroblasts. Fusion begins around 48 hours and more than 65% of the nuclei in a four day old culture will be in multinucleated myotubes. To obtain almost pure myotubes, cultures were treated with cytosine arabinoside (1 ug/ml) from day two to five. This kills most of the replicating cells (13) and results in cultures with a very high myotube/mononucleated cell ratio.

Figure 1 shows the densitometry tracings of the extracted actomyosin from day six myotubes and from day one presumptive myoblasts. Both the definitive myotubes and the presumptive myoblasts contain proteins of approximately 42,000 and 200,000 molecular weight that correspond to actin and the heavy chain of myosin. In the myotube preparation, the ratio of myosin heavy chain to actin is close to two, similar to that reported for adult skeletal muscle (14). In the presumptive myoblasts, however, the ratio is consistently around 0.9. Densitometry tracings and myosin/actin ratios from fibroblasts, chondroblasts, and BrdU-suppressed myogenic cells are identical to those shown for presumptive myoblasts. Extraction of actin and myosin is not complete; however, solubilization of the residual unextracted actomyosin from all the cell types directly into SDS reveals similar myosin/actin ratios. This indicates that the difference in myosin/actin ratio between the myotubes and the mononucleated cells is not due to differential extraction of the proteins.

Figure 2 shows the comigration of (^{3}H) leucine labelled actomyosin from myotubes and (^{14}C) leucine labelled actomyosin from

Table 1. ATP'ase Activities of Actomyosins Extracted From Myogenic and Non-myogenic Cells

Cell Types	Specific Activities (n mole/mg protein/min)		
	EDTA- K- ATPase	Ca- ATPase	Mg- ATPase
Presumptive Myoblast	21	37	7
Muscle Culture(6 day)	77	209	3
Muscle Culture(6 day) + cytosine arabinoside	79	204	6
BrdU-treated Muscle Culture(6 day)	55	157	27
Muscle Fibroblast	14	33	2
Chondrocyte	41	178	21

ATPase was assayed at 25° in 10 mM imidazole-HCl (pH 7.0), 2 mM ATP, 0.6 M KCl, and either 2 mM EDTA, 10 mM $CaCl_2$, or 5 mM $MgCl_2$ (25). Pi was measured by the method of Fiske and SubbaRow (26), and protein by the method of Lowry (27).

presumptive myoblasts. The peaks coincide exactly. Assuming that the leucine content in presumptive myoblast actin and myosin is similar to that in definitive muscle actin and myosin, the incorporation patterns also reveal a myosin/actin ratio of approximately 0.9. In other experiments, the addition of unlabelled myosin or actin did not change the ratios of the recovered labelled myosin and actin.

Whether the actomyosin is extracted immediately after labelling or after a chase of several days, the ratios remain unchanged. This suggests a mechanism coordinating the synthesis and degradation of these proteins (15).

Table 1 shows that the ATPase activity of the actomyosins from all these cell types is activated by Ca^{++} and inhibited by Mg^{++} at high ionic strength.

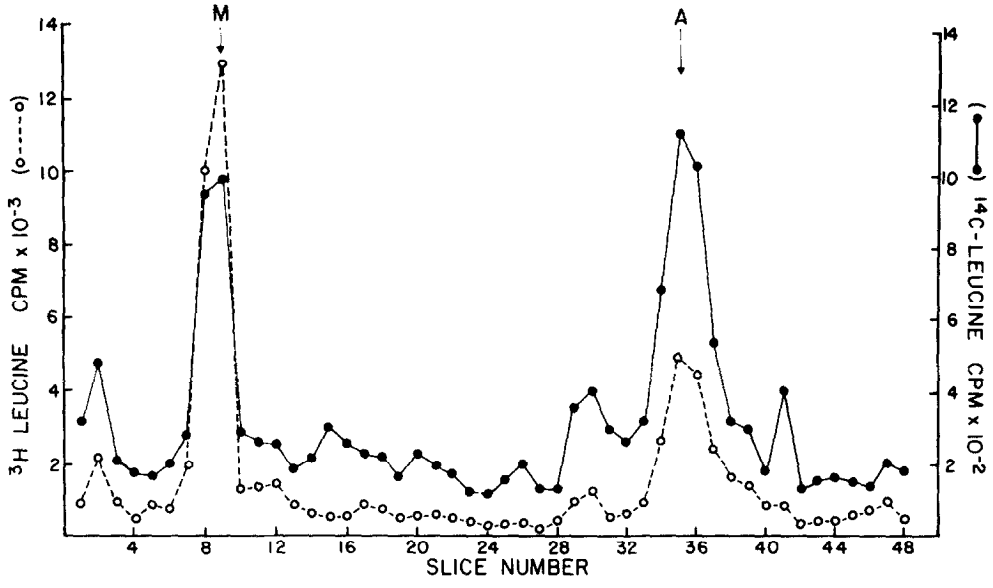

Figure 2. Comigration of myotubes and presumptive myoblast actomyosins. Myotubes were labelled for 24 hours with 10 uCi/plate (^{3}H) leucine (30 - 50 Ci/mmole) and presumptive myoblasts with 3 uCi/plate (^{14}C) leucine (> 270 mCi/mmole) for 24 hours. The plages were rinsed and the cells collected and mixed. The actomyosins from the mixed cells were isolated and the gels prepared as described under Figure 1. They were sliced and counted on a Beckman Liquid Scintillation Counter as described previously (24). M = myosin. A = actin.

If the myosins in these mononucleated cells are identical to those in skeletal muscle, one would expect the same types of myosin light chains. Electrophoresis of actomyosin on 12.5% SDS-polyacrylamide gels reveals that the skeletal muscle contains three types of light chains; both presumptive myoblasts and BrdU-suppressed myogenic cells, derived from the same original breast tissue as the muscle, contain only two. Whether these latter correspond to any of the light chains in muscle myosin has not yet been determined.

These findings should resolve some discrepancies in the literature. For example, the claims that myosin and myosin mRNA synthesis in myogenic cells is initiated only after fusion are untenable. Clearly many kinds of cells including presumptive myoblasts must code for a "myosin-like" mRNA.

The intriguing question now with regard to muscle differentiation is whether or not the myosin and actin synthesized in non-muscle cells, presumptive myoblasts, and BrdU-suppressed myogenic cells are identical to that synthesized in post-mitotic myoblasts and myotubes. In this connection, it is worth stressing that there are, at a minimum, five different light chains just in fast and slow skeletal muscle from the same animal (16, 17). Furthermore, the light meromyosin paracrystals prepared from the breast muscle used in these cultures is different from those prepared from leg slow muscles (18). The finding that antibodies to skeletal myosin did not cross-react with the myosin from smooth muscles and a variety of non-muscle cells (1) demonstrates not only that the myosins from muscle and non-muscle differ, but that even the myosins from different muscles differ in their antigenic properties (See also the demonstration that tropomyosins from different cell types have different properties (19, 20)).

Actin, in contrast, seems to be a very conservative protein. One- and two-dimensional peptide maps reveal no differences among the actins from chicken skeletal muscle, lens epithelium, neurons, or fibroblasts (9). Also, peptide analyses suggest that the actins from widely different organisms are very similar (21).

The presence of actin and myosin in BrdU-suppressed myogenic cells suggests the possibility of two types of genetic control for myosin and actin in myogenic cells: a BrdU-sensitive gene set controlling the myosin and actin destined for myofibrils and a BrdU-insensitive set controlling the myosin and actin found in many cell types.

These findings and those of others (9, 10, 19, 20) lead to the possibility that there exists a ubiquitous cytoplasmic contractile system found in all kinds of cells which involves the

read-out of one genetic program, as well as a fibrillar contractile system found in different kinds of muscle cells which requires the read-out of sub-sets of a different genetic program.

Acknowledgements: Research for this publication was supported by NIH Grant HL-15835 to the Pennsylvania Muscle Institute, by the Muscular Dystrophy Association, and by NSF grant GB-3842x.

References:

1. Holtzer, H. (1970) in O. Schjeide and J. JeVellis, Cell Differentiation, Van Nostrand Reinhold Co., New York, p. 476-503.
2. Holtzer, H., Sanger, J., Ishikawa, H., and Strahs, K. (1973) Cold Spring Harbor Symp. Quant. Biol., 37, 549-566.
3. Yaffe, D., and Dym, H. (1973) Cold Spring Harbor Symp. Quant. Biol., 37, 543-547.
4. Paterson, B., and Strohman, R.C. (1972) Develop. Biol., 29, 113-138.
5. Przybyla, A., Micou-Eastwood, J., and Strohman, R.C. (1973) J. Cell Biol., 59, 274a.
6. Ishikawa, H., Bischoff, R., and Holtzer, H. (1969) J. Cell Biol., 43, 312-328.
7. Huxley, J. (1963) J. Molec. Biol., 7, 281-308.
8. Bischoff, R., and Holtzer, H. (1970) J. Cell Biol., 44, 134-150.
9. Bray, D. (1973) Cold Spring Harbor Symp. Quant. Biol., 37, 567-574.
10. Adelstein, R., and Conti, M. (1973) Cold Spring Harbor Symp. Quant. Biol., 37, 599-605 (1973).
11. Bischoff, R., and Holtzer, H. (1968) J. Cell Biol., 36, 111-127.
12. Abbott, J., and Holtzer, H. (1966) J. Cell Biol., 28, 473-487.
13. Fischbach, G. (1972) Develop. Biol., 28, 407-429.
14. Tregear, R., and Squire, J. (1973) J. Molec. Biol., 77, 279-290.
15. Holtzer, H., Rubinstein, N., Chi, J., Dienstman, S., and Biehl, J., in the press.
16. Lowey, S., and Risby, D. (1971) Nature 234, 81-85.
17. Weeds, A.G., and Pope, B. (1971) Nature 234, 85-89.
18. Sreter, A., Holtzer, S., Gergely, J., and Holtzer, H. (1972) J. Cell Biol., 55, 586-594.
19. Fine, R., Blitz, A., Hitchcock, S., Kaminer, B. (1973) Nature 245, 182-185.
20. Cohen, I., and Cohen, C. (1972) J. Molec. Biol., 68, 383-385.
21. Elzinga, M., and Collins, J. (1973) Cold Spring Harbor Symp. Quant. Biol., 37, 1-7.
22. Adelstein, R., Conti, M., Johnson, G., Pastan, I., and Pollard, R. (1972) Proc. Nat. Acad. Sci. U.S.A., 69, 3693-3697.

23. Weber, K., and Osborn, M. (1969) J. Biol. Chem., 244, 4406-4412.
24. Weintraub, H., Campbell, G., and Holtzer, H. (1972) 70, 337-350.
25. Adelstein, R., Pollard, T., and Kuehl, W. (1971) Proc. Nat. Acad. Sci. U.S.A., 68, 2703-2707.
26. Fiske, C., and SubbaRow, Y. (1925) J. Biol. Chem., 66, 375.
27. Lowry, O., Rosebrough, N., Farr, H., and Randall, R. (1951) J. Biol. Chem., 193. 265-275.