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Differences among myosins synthesized in non-myogenic cells, presumptive myoblasts, and myoblasts

(myogenesis/polymorphic molecules/immunofluorescence/myosin heavy and light chains/microfilaments)

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ABSTRACT Myosins synthesized in non-myogenic cells and replicating presumptive myoblasts differ from those synthesized in postmitotic mononucleated myoblasts and myotubes. Myoblasts and myotubes synthesize the definitive light chains, MLC_1 and MLC_2. These light chains display different molecular weights in sodium dodecyl sulfate–polyacrylamide gels from the fibroblast light chains FLC_1 and FLC_2 synthesized in non-myogenic cells and presumptive myoblasts. There are immunological differences between the myosin heavy chains synthesized in myoblasts and myotubes and those synthesized in non-myogenic cells and presumptive myoblasts. Fluorescein-labeled antibodies against skeletal light meromyosin are bound only along the lateral edges of emerging and definitive A-bands. This antibody to light meromyosin is not bound to the outside of, or the microfilaments subtending, the plasma membrane in non-myogenic cells or in myoblasts or in myotubes.

These findings suggest that: (*1*) non-myogenic cells and replicating presumptive myoblasts synthesize similar myosin heavy and light chains; (*2*) replicating presumptive myoblasts synthesize a different set of myosins from those synthesized by their postmitotic daughters, the myoblasts; (*3*) the myosins associated with the plasma membranes of non-myogenic and myogenic cells are products of structural genes distinct from those coding for the myosins for skeletal myofibrils.

The penultimate and ultimate compartments of the myogenic lineage consist of presumptive myoblasts and myoblasts, respectively. The metabolic repertoires of replicating presumptive myoblasts are strikingly different from those of their daughters, the postmitotic, mononucleated myoblasts. Myoblasts are not readily, if ever, dislodged from their postmitotic state. Myoblasts, but not presumptive myoblasts, possess the singular capacity (*1*) to form myotubes by fusing with other myoblasts and (*2*) to organize myosin and actin monomers into interdigitating thick and thin filaments (1, 2).

There are conflicting reports as to whether these two types of myogenic cells synthesize myosin. Yaffe and coworkers (3, 4) have reported that presumptive myoblasts transcribe, but do not translate, myosin mRNAs, and that the myosin mRNAs stored in replicating presumptive myoblasts are translated only after the cells have fused to form myotubes. Prives and Paterson (5) claim that some event uniquely coupled to fusion is obligatory for the translation of the contractile proteins. Strohman and coworkers (6, 7) conclude that no myosin-like molecules are synthesized in mononucleated myogenic cells, and that myosin mRNAs are transcribed and translated only in myotubes. Heywood and co-workers (8) suggest that replicating presumptive myoblasts lack an initiation factor for the translation of myosin mRNAs and that this factor first appears in myotubes.

Abbreviations: Ara-C, cytosine arabinonucleoside; EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; BrdUrd, bromodeoxyuridine; MLC, myosin light chain; FLC, fibroblast light chain; A-LMM, antibody against light meromyosin.

In contrast, it has been reported that both presumptive myoblasts and myoblasts synthesize myosin molecules, but that the myosins differ in the two types of myogenic cells (9, 11, 15). Evidence that the myosin heavy chains extracted from presumptive myoblasts differ from those in myoblasts is based on the failure of the former to precipitate antibody against skeletal myosin either in Ouchterlony immunodiffusion tests or histochemically, using the fluorescein-labeled antibody technique (1, 2, 9, 10).

This report stresses the abrupt shift in the kinds of myosin heavy and light chains synthesized as replicating presumptive myoblasts divide and yield daughter postmitotic, mononucleated myoblasts. Myosin light chains synthesized by presumptive myoblasts are more similar to the myosin light chains synthesized by non-myogenic cells (11, 12) than to the light chains synthesized in myoblasts and myotubes. Antibodies against skeletal light meromyosin are bound exclusively to the lateral borders of emerging and definitive A-bands in myoblasts and myotubes. The labeled antibody, however, is not bound to the plasmalemma of either presumptive myoblasts or myoblasts. These findings suggest that myosins associated with the plasmalemma of both non-myogenic and myogenic cells may be the product of structural genes distinct from those coding for the myosins in skeletal myofibrils. This switch in the kinds of myosins synthesized in mother presumptive myoblasts versus daughter myoblasts is consistent with the notion of a quantal cell cycle separating cells in different compartments of the myogenic lineage (2, 9, 11, 15).

MATERIALS AND METHODS

Cultured Cells. Standard 6-day muscle cultures were prepared by trypsinizing the breast muscles of 10-day chick embryos (13). During the first 2 days these cultures consist largely of presumptive myoblasts, and lesser numbers of myoblasts, fibroblasts, and mesenchyme cells (14). Significant numbers of multinucleated myotubes first appear in these standard cultures on day 3. Cultures consisting almost exclusively of pure multinucleated myotubes were prepared by adding cytosine arabinonucleoside (Ara-C, 1 μg/ml) to kill replicating cells (11). Cultures of pure presumptive myoblasts are currently impossible to prepare; however, it is known that during the first 30 hr of culture over 70% of the mononucleated cells in the standard cultures are presumptive myoblasts (15). Cultures of pure fibroblasts and pure chondroblasts were prepared as described in Abbott *et al.* (14). Cultures greatly enriched for mononucleated, post-mitotic myoblasts were prepared by adding medium containing 1.75 mM ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) (6) and Ara-C (1 μg/ml). Under these conditions the mononucleated myogenic cells are

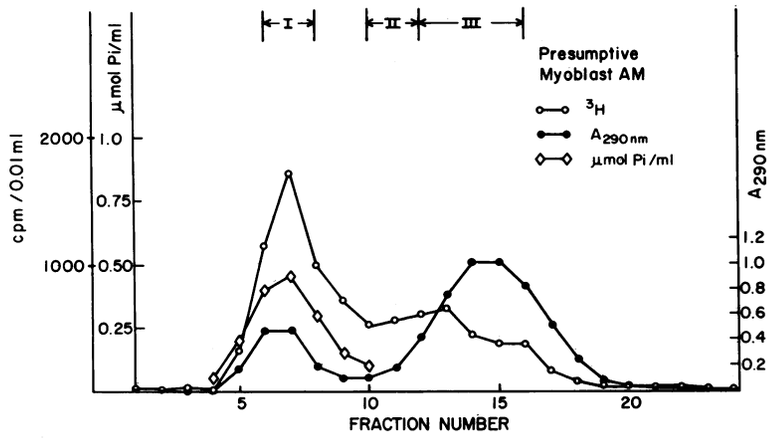

FIG. 1. Chromatographic purification of myosin isolated from presumptive myoblast cultures. A 1.0 × 32 cm Bio-Gel A-15m column equilibrated with column buffer [0.6 M KCl, 10 mM imidazole (pH 7.0), 1 mM dithiothreitol, 0.5 mM ATP, and 0.1 mM $MgCl_2$] was used. Crude actomyosin obtained after three precipitations was dissolved in a small volume of KI–ATP buffer (0.6 M KI, 5 mM dithiothreitol, 1 mM $MgCl_2$, and 20 M imidazole (pH 7.0) and clarified by centrifugation. Aliquots of the supernatant (0.2–0.3 ml) containing about 3 mg of protein were applied to the column, which had been prerun with a 15% bed volume of KI–ATP buffer. After the sample was washed on with an appropriate volume of KI–ATP buffer, the protein was eluted with column buffer at 5 ml/hr and 0.5 ml fractions were collected. ATPase was assayed as described in Table 1. Absorbance at 290 nm and the radioactivity of each fraction were measured. A similar profile was obtained from all cell types studied. Cultures were labeled with 10 μCi/100 mm dish [^{3}H]leucine (30 Ci/mmol) for 24–48 hr before the living cells were collected and actomyosins extracted (23). Radioactive samples represent the newly synthesized proteins. Fractions 6–8 (I), 10–12 (II), and 13–16 (III) were pooled, dialyzed against 5 mM phosphate buffer (pH 6.5), and concentrated by freeze-drying. Protein was dissolved in 1% sodium dodecyl sulfate–1% mercaptoethanol–10 mM phosphate buffer–35% glycerol and analyzed by 0.1% sodium dodecyl sulfate–12.5% polyacrylamide gel electrophoresis. The myosin light chain patterns were visualized either by Coomassie brilliant blue stained electrophoretograms, or densitometry tracing at 600 nm, or radioactivity profiles.

blocked from fusing, but the transition from presumptive myoblasts to myoblasts is essentially unimpeded (15). BrdUrd-suppressed myogenic cells were grown as described in Bischoff and Holtzer (13).

Myosin Light Chains. Crude actomyosin (16), or myosin partially purified on a KI–Bio-Gel A-15m column (17) was analyzed in 12.5% polyacrylamide gels in 0.1% sodium dodecyl sulfate and 10 mM sodium phosphate, pH 7.0, for myosin light chains (18, 19). Using these procedures, we confirmed earlier findings that standard muscle cultures synthesize the three light chains, MLC_1, MLC_2, and MLC_3, whereas cultured fibroblasts and chondroblasts synthesize two light chains, FLC_1 and FLC_2 (12). The molecular weights of the light chains were estimated, using a calibrated curve obtained by plotting the electrophoretic mobilities of purified proteins against the logarithm of known molecular weights. Partially purified myosins from presumptive myoblasts, myoblasts, pure myotubes, fibroblasts, chondroblasts, or BrdUrd-suppressed myogenic cells were coelectrophoresed with the standard as described (20).

Light Meromyosin. Rabbit antibody against chicken skeletal light meromyosin (A-LMM) was prepared and conjugated with fluorescein isothiocyanate (10). Antisera from hyperimmunized rabbits were discarded, for, though their titers were high, such antisera contained components against minor antigens. In double diffusion tests in 1% agar containing 0.6 M KI in phosphate buffer (pH 7.0), the A-LMM formed a single precipitin band against KI extracts of (*1*) breast muscle from adult chicken, (2) muscle from 12-day chick embryos, and (3) standard 6-day muscle culture from 10-day chick embryos. The A-LMM did not form precipitin bands in Ouchterlony diffusion tests with KI extracts from (*1*) 2-day chick somites, (2) 5- or 15-day embryonic spinal cord, (3) cultured fibroblasts, chondroblasts, or BrdUrd-suppressed myogenic cells, (*4*) 15-day embryonic gizzard or liver, (*5*) mature chicken sperm. Glycerinated models of the various cultured cells, freeze-dried thick and thin sections, as well as formaldehyde-fixed material (21, 22), were treated with labeled A-LMM. The freeze-dried and sectioned cells and the formaldehyde-fixed material reacted to the A-LMM as did glycerol-extracted cells. This finding suggests that glycerol extraction did not result in loss of soluble antigen that might have reacted with A-LMM. Further details regarding the immunological findings will be published elsewhere. In one series of experiments living cells were incubated in A-LMM to determine whether antigenic sites were available on the exterior of cells.

RESULTS

Myosin light chains

Actomyosins were extracted from the following kinds of cultured cells: (*1*) presumptive myoblasts; (2) standard muscle cultures; (3) pure myotubes; (*4*) EGTA/Ara-C myoblasts; (*5*) fibroblasts; (*6*) chondroblasts; (7) BrdUrd-suppressed myogenic cells. The myosins were partially purified on a KI–Bio-Gel A-15m column. Essentially similar elution profiles were obtained with the actomyosin or myosin extracts from all cells. Fig. 1 shows a representative elution pattern of the actomyosin from 24 hr presumptive myoblast cultures. The ATPase activity coincided with the first eluted peak (Peak I) based on either the radioactivity profile or on absorbance at 290 nm. Samples from Peak I proved to be partially purified myosin as judged by sodium dodecyl sulfate-polyacrylamide gels. The ATPase activity from all mononucleated cell cultures was lower than that from standard 6-day muscle cultures or from pure 6-day myotube cultures (**Table** 1). The lower value from standard muscle cultures, in contrast to that of pure myotube cultures, is likely to be due to the fact that the former contains fibroblast and presumptive myoblast myosin with specific activities lower than that of myotube myosin. The ATPase activity in the EGTA/Ara-C enriched myoblast cultures is consistent with the modest amount of myofibrillar myosin (Fig 5b) diluted by the myo-

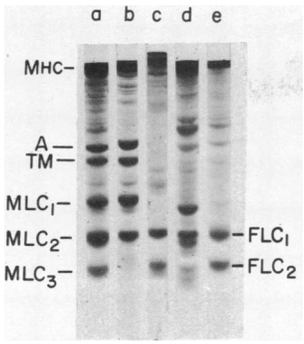

FIG. 2. Electrophoresis of myosin in 12.5% polyacrylamide gels in the presence of 0.1% sodium dodecyl sulfate: (a) actomyosin from standard muscle cultures; (b) actomyosin from pure myotube cultures; (c) myosin from presumptive myoblast cultures; (d) myosin from EGTA/Ara-C treated myoblast cultures; (e) myosin from fibroblast cultures; 20–40 μg of protein in a volume of 10–50 μl was applied to each gel. It should be noted that the intermediate-sized light chain from EGTA/Ara-C treated myoblast myosin invariably yields a second band just behind MLC_2. The nature of this trailing band is unknown; however, it appears both in these cultures and in cultures treated with EGTA alone. This band could be (*1*) the FLC_1 that the myoblast either synthesizes or inherited from its mother presumptive myoblast, or (*2*) a component of troponin or some other unidentified molecule. MHC = myosin heavy chain; A = actin; MLC_1 = muscle light chain 1; MLC_2 = muscle light chain 2; MLC_3 = muscle light chain 3; TM = tropomyosin; FLC_1 = fibroblast light chain 1; FLC_2 = fibroblast light chain 2.

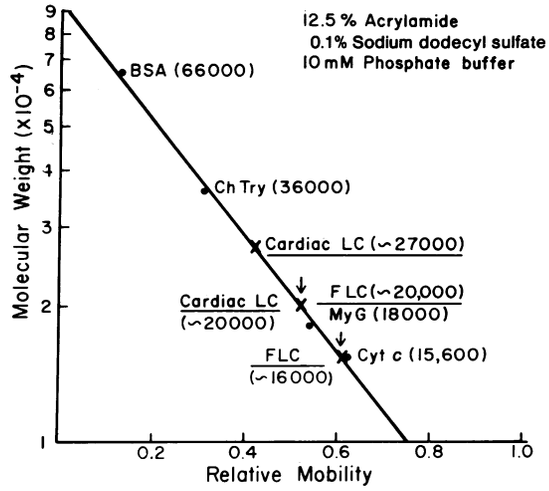

FIG. 3. Molecular weight calibration curve for 0.1% sodium dodecyl sulfate–12.5% polyacrylamide gels. The standard proteins are bovine serum albumin (BSA, 66,000), chymotrypsin (Ch Try, 36,000), myoglobin (My G, 18,000), and cytochrome *c* (Cyt *c*, 15,600). Cardiac or fibroblast myosin (FLC_1 and FLC_2) was run simultaneously or was mixed and coelectrophoresed with standards. The relative mobility of each light chain was determined and the molecular weight was estimated from the standard curve. Cardiac myosin was extracted from 11-day chick embryonic heart as described for pure myotube cultures.

sin from presumptive myoblasts and fibroblasts present in these cultures.

Fig. 2 illustrates the electrophoretograms of the light chains extracted from the different kinds of cultures. The mobility of the two myosin light chains from presumptive myoblasts is indistinguishable from that of the two light chains extracted from fibroblasts, chondroblasts, or BrdUrd-suppressed myogenic cells. Cultures enriched for myoblasts, standard muscle cultures, and cultures of pure myotubes all display a common light chain, MLC_1, which is absent from all other cell types tested. The MLC_3 in standard muscle cultures is likely to be the product of presumptive myoblasts and fibroblasts and corresponds to FLC_2. This statement is based on the observation that when the presumptive myoblasts and fibroblasts in standard cultures are killed with Ara-C, the surviving myotubes only yield MLC_1 and MLC_2 (12). The presence of MLC_1 in the EGTA/Ara-C treated myoblast cultures demonstrated that myoblasts synthesize a myosin light chain not synthesized by their mothers, the replicating presumptive myoblasts. Furthermore, it is clear that the initiation of the synthesis of these definitive myosin light chains is not dependent upon the fusion of myoblasts into myotubes.

Table 1. ATPase activity of myosins from a variety of myogenic and non-myogenic cells

Cell types	Specific activity (nmol/mg protein per min)*	
Standard muscle culture	686	551
Pure myotubes	747	626
EGTA/Ara-C myoblasts	348	242
Presumptive myoblasts	258	167
BrdUrd-suppressed myogenic cells	275	160
Fibroblasts	290	208
Chondroblasts	278	173

ATPase was assayed at 25° for 10 min. The reaction mixture contained 10 mM imidazole·HCl (pH 7.0), 2 mM ATP, 0.6 M KCl, and 2 mM EDTA, and protein in a final volume 1.0 ml. P_i was measured by the modified method of Fiske and Subbarow (24), and protein by the method of Lowry *et al.* (25).

* Results from two separate experiments.

The molecular weights of the myosin light chains from presumptive myoblasts, myoblasts, fibroblasts, and cardiac myoblasts were estimated in sodium dodecyl sulfate-polyacrylamide gels (Fig. 3). As others have reported (18, 19), MLC_1 and MLC_2 from fast skeletal muscle differ in molecular weight from cardiac light chains, CLC_1 and CLC_2. We also found that MCL_2 did not comigrate (not shown here) with the larger light chain, FLC_1, from presumptive myoblasts or the non-myogenic cells. This demonstrates that in addition to initiating the synthesis of a distinctive MLC_1, the myoblasts accumulate a distinctive MLC_2 and that this latter light chain is different from the slower migrating light chain (FLC_1) synthesized by non-myogenic cells and by presumptive myoblasts.

Myosin heavy chains

Actin or actin-like molecules have been localized beneath the plasma membrane in a variety of cell types, including presumptive myoblasts and myoblasts (1, 26). Accordingly, we re-examined the issue of whether a myosin similar to that in mature myofibrils is present either in the cell surface or in the cytoplasm of presumptive myoblasts, myoblasts, myotubes, or non-myogenic cells.

Cultures used for the analysis of myosin heavy chains were either freeze-dried and sectioned, fixed in formaldehyde, or glycerinated and treated with fluorescein-labeled A-LMM. Figs. 4, 5, and 6 are dark-field and fluorescence micrographs of (*1*) a standard 6-day muscle culture, (*2*) an EGTA/Ara-C myoblast enriched culture, and (*3*) a 30-hr-

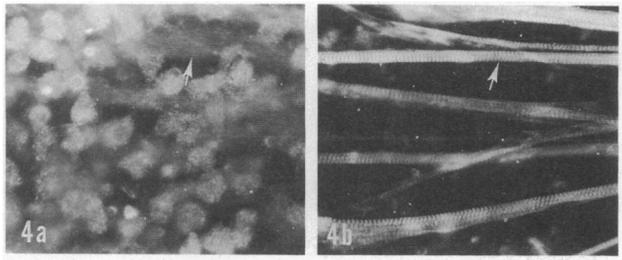

FIG. 4. Darkfield (a) and fluorescence (b) photomicrographs of the same field of cells from a standard 6-day culture that had been glycerinated and treated with fluorescein-labeled A-LMM. The arrows in both panels mark the same myotube. The striated myotubes present in the dark field micrograph (a) are obscured by the large number of mononucleated cells that have settled between and over the myotubes. The binding of the labeled A-LMM shown in (b) demonstrated *in situ* localization *exclusively* along the lateral borders of the A-bands. Observe that the labeled A-LMM is not bound by antigen associated with the plasma membrane, nor by antigen distributed throughout the cytoplasm. This preparation is over 50 nm in thickness, making it impossible to focus on all the myotubes in the field. The nuclei, cell outlines, and debris, barely perceptible in (b), are so, not because they have bound minute amounts of labeled A-LMM, but because of their blue-white autofluorescence (×293).

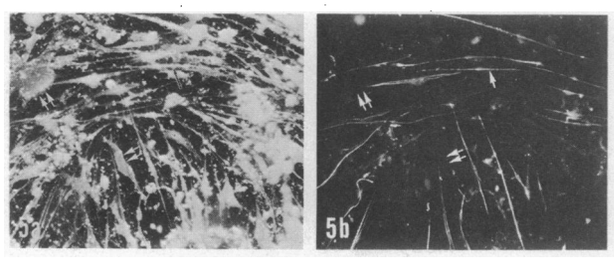

FIG. 5. Darkfield (a) and fluorescence (b) photomicrographs of the same field of cells from 4-day EGTA/Ara-C treated myoblast cultures. The single arrows in both figures mark the same myoblasts. Double arrows mark negatively stained cells, either presumptive myoblasts or fibroblasts. Observe that many of the elongated mononucleated, postmitotic myoblasts bind the labeled A-LMM. At higher magnification, incipient striations may be observed in some of these myoblasts. Only the emerging myofibrils bind the labeled A-LMM; the other cellular material in (b) is barely visible by virtue of its autofluorescence. Neither the nucleus nor the plasma membranes of the myoblasts bind the A-LMM and at no stage in their development do myoblasts display a generalized, cytoplasmic distribution of antigen (×88).

old culture of presumptive myoblasts. Cultures of fibroblasts, chondroblasts, or BrdUrd-suppressed myogenic cells treated with fluorescein-labeled A-LMM responded in the same way as the presumptive myoblasts shown in Fig. 6. These fluorescence micrographs confirm the results of gel diffusion tests—neither presumptive myoblasts, nor the non-myogenic cells tested, nor the BrdUrd-suppressed myogenic cells synthesize an antigen precipitating A-LMM. This suggests that the myosin heavy chains isolated from presumptive myoblasts, fibroblasts, chondroblasts, and BrdUrd-suppressed myogenic cells are immunologically distinct from the heavy chains isolated from myoblasts and myotubes (2, 12).

Of additional interest is the fact that A-LMM is not bound by antigen in the plasma membrane, cytoplasm, or nucleus of presumptive myoblasts, myoblasts, myotubes, or of any non-myogenic cell tested. Willingham *et al.* (22) reported that the plasmalemma of a variety of living cells binds labeled antibody to L-cell myosin. Accordingly, we cultured living myogenic and non-myogenic cells in A-LMM for 1 hr at 37°. The A-LMM was not bound to the surface of any of the cells we tested. Clearly, if a myosin-like molecule interacts with the actin-like molecules associated with the cell surfaces of myogenic and non-myogenic cells, then such a species of myosin is at least immunologically distinct from the myosin in thick filaments. Failure of A-LMM to bind throughout the cytoplasm of myoblasts and myotubes confirms earlier reports which stressed that skeletal myosin was localized only in proximity to both definitive and emerging A-bands (1, 10). These findings are consistent with the proposition that the myosin for thick filaments is synthesized in proximity to the site of its assembly into myofibrils (1, 2, 15).

DISCUSSION

The presumptive myoblast is a differentiated phenotype; it is the only cell with the option of replicating and yielding daughter, post-mitotic myoblasts. As a unique phenotype, the presumptive myoblast must synthesize, or have inherited from its mother cell, some molecule(s) not present in other cell types (15). Efforts to characterize molecules unique to presumptive myoblasts currently are inconclusive. The claims (3, 4) that presumptive myoblasts transcribe "informosome-like," inactive myosin mRNAs that will be translated only in myotubes is rendered doubtful by several recent reports (2, 9), as well as by the data in this paper. Equally untenable are the claims (5–7) that replicating presumptive myoblasts do not synthesize any kind of myosin mRNA. Replicating presumptive myoblasts and their postmitotic daughters, the myoblasts, both transcribe myosin mRNAs. However, the *kinds of myosin mRNAs* translated differ in the mother presumptive myoblasts from those translated in their postmitotic daughter myoblasts.

Our experiments support the following summary of the striking differences in metabolic properties between replicating presumptive myoblasts and their daughter postmitotic myoblasts: (*1*) Presumptive myoblasts synthesize myosin light chains that, electrophoretically, are indistinguishable from the "constitutive" light chains found in many non-myogenic cells (e.g., fibroblasts, chondroblasts, nerve cells, etc). (2) Myoblasts synthesize two different light chains, electrophoretically similar to those found in myotubes and mature muscle (12). (3) Presumptive myoblasts do not, but myoblasts do, synthesize myosin heavy chains similar to

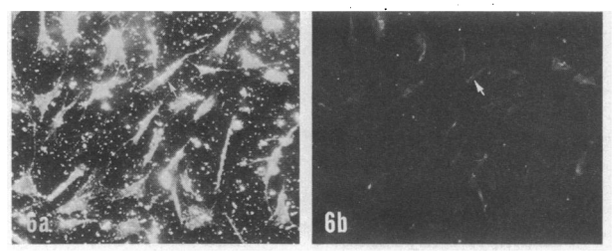

FIG. 6. Darkfield (a) and fluorescence (b) photomicrographs of the same field of a presumptive myoblast culture. The arrows in both panels mark the same cells. The majority of these cells are in the cell cycle and currently it is impossible to distinguish cells in the fibrogenic from cells in the myogenic lineages. No cell in (b) bound the labeled A-LMM. The faint images observed in (b) are due to their blue-white autofluorescence and not their binding of minute amounts of fluorescein-labeled A-LMM. Cultures of fibroblasts, chondroblasts, and BrdUrd-suppressed myogenic cells behave similarly with respect to binding labeled A-LMM as do these cultures of presumptive myoblasts (×279).

those found in thick filaments of mature myofibrils. (*4*) The *in situ* binding of A-LMM shown in Fig. 4 correlates with the first emergence of interdigitating thick and thin filaments as observed in electron microscopy (1, 9, 12). The finding that A-LMM is bound only in myoblasts in which stacked thick and thin filaments are being assembled suggests another difference in metabolic options between presumptive myoblasts and myoblasts, namely, the intracellular regulation of actin synthesis and polymerization. In the course of normal myogenesis large numbers of preformed cytoplasmic 60 Å actin filaments are not found in presumptive myoblasts, but are first found in postmitotic myoblasts, and then always in association with thick filaments (26). Even in myoblasts treated with cytochalasin-B (27) or colcimide (1) where the assembly of myofibrils is distorted, the hexagonal stacking of thick and thin filaments is quite normal. These electron microscopic findings, coupled with the data in this paper, suggest that (*1*) in presumptive myoblasts the synthesis of actin molecules destined for the plasmalemma is coordinated with the synthesis of "constitutive" myosins, whereas (*2*) in myoblasts the synthesis of actin for myofibrils is coordinated with the synthesis of myofibrillar myosins (11, 23). Evidence that the rates of synthesis and degradation of myosin and actin in non-myogenic cells differ from those in myotubes has been presented elsewhere (2, 28).

It is instructive to compare the localization of fluorescein-labeled A-LMM with the localization of antibodies against myosin from (*a*) chicken gizzard, (*b*) mouse L-cells, and (*c*) mouse uterus. Weber and coworkers (21) found that anti-gizzard myosin localizes along the long bundles of fibers that correlate with the microfilament system subtending the plasmalemma. Anti-gizzard myosin also localizes in the perinuclear region. Willingham *et al.* (22) report that antibody against myosin from mouse L-cells binds uniformly over the surface of those cells. Clearly much is still to be learned about the localization of the different myosins in living and fixed cells, as well as in cells in different physiological states.

The finding that A-LMM did not react with the heavy chains from non-myogenic cells and presumptive myoblasts (2, 10) complements the finding that antibodies against platelet myosin (17), gizzard myosin (21), or mouse L-cell myosin (22) did not crossreact against skeletal myosin. It will be interesting if these myosins from different types of non-myogenic cells, as well as from presumptive myoblasts, comprise a single class of "constitutive" myosins. If so, such non-fibrillar myosins may be present in the sarcoplasm or in the plasmalemma of myoblasts, myotubes, and even of mature muscle fibers. In this context it is worth stressing that antibody against skeletal tropomyosin that localizes in the I-band of myofibrils does not bind to the microfilaments subtending the plasmalemma of myoblasts, myotubes or non-myogenic cells (30).

The difference in metabolic options between mother replicating presumptive myoblast and daughter myoblast is reminiscent of that between erythrogenic hematocytoblast and daughter first generation erythroblast. It has been proposed that the quantal cell cycle functions so as to allow the globin gene that was not available for transcription in the hematocytoblast to become available for transcription in the daughter erythroblasts (29). We propose that the quantal cell cycle separating presumptive myoblast from myoblast allows the definitive myosin heavy and light genes to become available for transcription (1, 2, 15, 27, 30).

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