Developmental Reorganization of the Skeletal Framework and Its Surface Lamina in Fusing Muscle Cells

ALICE B. FULTON, JOAV PRIVES, STEPHEN R. FARMER, and SHELDON PENMAN
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; Department of Anatomical Science, State University of New York, Stonybrook, New York 11740. Dr. Farmer's present address is the Boston University Medical School, Boston, Massachusetts 02118.

ABSTRACT The skeletal framework of cells, composed of internal structural fibers, microtrabeculae, and the surface lamina, is revealed with great clarity after extraction with detergent. When muscle cells fuse to form a multinucleated myotube, their skeletal framework reorganizes extensively.

When myoblasts prepare to fuse, the previously continuous surface lamina develops numerous lacunae unique to this stage. The retention of iodinated surface proteins suggests that the lacunae are not formed by the extraction of lamina proteins. The lacunae appear to correspond to extensive patches that do not bind concanavalin A and are probably regions of lipid bilayer devoid of glycoproteins. The lacunae appear to be related to fusion and disappear rapidly after the multinucleated myotube is formed.

When muscle cells fuse, their internal structural networks must interconnect to form the framework of the myotube. Transmission electron microscopy of skeletal framework whole mounts shows that proliferating myoblasts have well developed and highly interconnected internal networks. Immediately before fusion, these networks are extensively reorganized and destabilized. After fusion, a stable, extensively cross-linked internal structure is reformed, but with a morphology characteristic of the myotube. Muscle cells therefore undergo extensive reorganization both on the surface and internally at the time of fusion.

In the interior of vertebrate cells, there are dense, heterogeneous and highly interconnected networks; these include the well known structural filaments (micro- and intermediate filaments, microtubules) and the extensive system of highly cross-linked microtrabeculae described by Porter and co-workers in intact cells (2, 3, 6, 8, 9, 20, 21). These networks appear largely unaffected by extraction with Triton or glycerol under gentle conditions (4, 5, 13, 17–19). Such extraction permits much clearer visualization of many aspects of internal cell structure because the detergent removes most phospholipid and obscuring soluble cytoplasmic proteins. An important aspect of cell structure seen after extraction with Triton is the surface lamina, an external protein sheet derived from the plasma membrane, that retains the overall morphology and many details of the intact cell. We have suggested that this extracted structure, bounded by the protein sheet or lamina formed by the plasma membrane proteins (1), be designated the "skeletal framework" to distinguish it from a more narrowly defined cytoskeleton.

The formation of muscle fibers by myoblasts fusing is a striking example of the skeletal framework reorganizing during development. Myoblasts fuse to form multinucleated myotubes which then synthesize and organize the banded actomyosin contractile apparatus. Cells develop under optimum in vitro conditions to mature, twitching muscle fibers. To form the multinucleated myotube, plasma membranes of fusion-competent myoblasts must meld: at this time, large openings, or lacunae, in the surface lamina appear. The junction formed at fusion then disappears and the internal structural networks reorganize to form a continuous structure oriented along the muscle fiber axis. The specialization of the skeletal framework and its surface lamina that accompanies these highly specific events is described here.

MATERIALS AND METHODS

Muscle Cultures

Primary chick embryonic muscle cultures were made from 11- to 13-d-old (usually 12-d-old) chick embryo pectoral muscles following the method of Paterson and Prives (14). The cultures were maintained at 37°C on 5% CO_2 in a medium containing 88% Dulbecco's Modified Eagle's Medium (DMEM), 2% chick embryo extract, 10% horse serum, and penicillin and streptomycin. Under

THE JOURNAL OF CELL BIOLOGY · VOLUME 91 OCTOBER 1981 103-112
© The Rockefeller University Press · 0021-9525/81/10/0103/10 $1.00

these conditions, cultures kept from 6 to 10 d generally displayed spontaneous twitching. This medium, which allows one to a few rounds of mitosis before cells prepare to fuse, is called fusion medium, to distinguish it from a richer medium also used. Growth medium, which contained 30% fetal calf serum and 10% chick embryo extract in Dulbecco's MEM, maintained myoblasts proliferating for several days; <10% fusion was seen at 48 h. Thus, at 24 h on growth medium, >95% of the myoblasts were proliferating; at the same time on fusion medium, most of the cells were at some stage of preparing for fusion and will be called "postproliferative myoblasts."

Staining Intact Cells with Fluorescent Concanavalin A

Myoblasts and fibroblasts that were to be stained intact with fluoresceinated concanavalin A (Con A) were rinsed three times with cold phosphate-buffered saline (PBS), covered with a cold solution of 100 μg/ml of fluoresceinated Con A in PBS, and maintained in a humid atmosphere at 4°C for 30 min. After being exposed to Con A, cells were rinsed three times in cold PBS. The cells were fixed, intact, in cold 3% formaldehyde in PBS for 1 h, rinsed with PBS and distilled water, dehydrated through a graded ethanol series, and then air-dried. No differences in staining were seen when cells were fixed and then stained; in both cases, debris being cleared from the cell surface was associated with bright patches and strands.

Scanning Electron Microscopy

Cells for scanning electron microscopy were grown on circular glass cover slips that had been ethanol sterilized and soaked in PBS for 30 min before being used for cell growth. Cells were prepared for scanning electron microscopy by being rinsed several times with PBS, left intact or extracted with lysis buffer as described below, and then fixed for 30 min in 2% glutaraldehyde in lysis buffer. They were rinsed twice with lysis buffer and then fixed with 0.5% osmium tetroxide for 5 min on ice. Cells were rinsed with distilled water, dehydrated through a graded ethanol series, critical point dried, and sputter-coated with gold and paladium.

Radioiodination of External Proteins

Cells to be radioiodinated were rinsed three times with PBS and were then incubated at room temperature for 10 min with a reaction mixture that contained 5 mM glucose, 0.1 U/ml glucose oxidase, 20 μg/ml horseradish peroxidase, and 400 μCi/ml Na ^{125}I in PBS. After iodination, the cells were rinsed three times with phosphate-buffered iodide solution. The soluble proteins were removed by a 1-min room temperature extraction with lysis buffer. The remaining proteins were scraped off the plates in SDS buffer. All samples were acetone precipitated with 2 vol of cold acetone and washed three times with cold acetone to remove free iodine. Samples were suspended in running buffer as described below for polyacrylamide gel electrophoresis. Control dishes treated with collagen and allowed to stand with complete medium in them for comparable lengths of time show that the diffuse background in the skeletal fraction is due to adsorption onto the collagen of polypeptides from serum and embryo extract.

Transmission Electron Microscopy of Whole Mounts

Myoblasts and fibroblasts were grown on carbon- and formvar-coated gold grids. For transmission electron microscopy, they were rinsed with PBS, left intact or extracted with lysis buffer, and then fixed for 30 min with 1% glutaraldehyde in lysis buffer. They were rinsed twice, and then postfixed for 5 min in 0.5% osmium tetroxide. They were rinsed twice in distilled water and then dehydrated with ethanol by dilution. Samples were critical point dried and then examined at 100 kV in a Jeolco electron microscope.

[^{35}S]Methionine Labeling of Myoblast Proteins

Cells to be labeled with [^{35}S]methionine were rinsed three times with methionine-free MEM medium and then incubated for 30 min in methionine-free medium plus 10% dialyzed serum and appropriate levels of [^{35}S]methionine: 5 μCi/ml for plates that would be used for quantitation only, and 25 μCi/ml for plates that would be used for gel electrophoresis of proteins. After the 30-min pulse, the isotope was chased with complete medium containing 2 mM methionine for 3 h. Cells were rinsed three times with cold medium and were then extracted with lysis buffer for 1 min at room temperature. The skeletal frameworks were scraped off the plate, centrifuged down, and the supernatant fluid was removed as the soluble cytoplasmic fraction. The skeletal framework remaining was suspended in the reduced salt buffer described below, vortexed vigorously, and then spun at 3,000 rpm for 5 min to remove nuclei. Aliquots of samples were mixed with sample buffer (1:1) and loaded onto gels for electrophoresis. Sample loadings for every day represent an equal fraction of the plate.

Lysis and Fractionation Buffers

The lysis buffer used here is a slightly hypertonic, moderately saline solution. It contains 3 mM MgCl, 50 mM NaCl, 300 mM sucrose, 0.5% Triton and is buffered with 10 mM HEPES to pH 7.4. Phenylmethylsulfonyl fluoride (PMSF) was added to a final concentration of 150 μg/ml. Skeletal frameworks were stripped from nuclei in a reduced salt buffer that contained 10 mM NaCl, 3 mM MgCl, 1.0% Tween 40, and 0.5% Na desoxycholate, buffered with 10 mM Tris to pH 7.4. (15).

RESULTS

Cell Surface Lamina of Fibroblasts and Myoblasts

Myoblasts for these studies are dissociated from breast muscle of 12-d chicken embryos. During the first 24 h in culture, the myoblasts proliferate and then leave the proliferative cycle and prepare for cell fusion. Under proper conditions, cells fuse, and the resulting myotubes continue to mature. The morphology and surface structures of postproliferative cells close to fusion are unique and probably related to the act of fusion and later orderly development.

The distribution of cell surface proteins in proliferating and post proliferative myoblasts is clearly visible in the skeletal framework prepared by detergent extraction. After extraction, plasma membrane proteins constitute a lamina that retains much of the configuration of the plasma membrane of the intact cell. This surface lamina retains most plasma membrane proteins and surface-specific structures such as binding sites for virus and lectins (1). However, the surface lamina of postproliferative myoblasts is quite unlike the surface structure of either fibroblasts or proliferating myoblasts, in that it contains lacunae, apparently corresponding to lipid-rich regions of the plasma membrane and concentrated in regions that apparently may participate in fusion.

The surface lamina seen in cultured mammalian fibroblasts and epithelial cells (1) is nearly continuous. Myoblasts that are still proliferating also form an almost continuous surface lamina (Fig. 1). Freshly explanted myoblasts can be maintained in

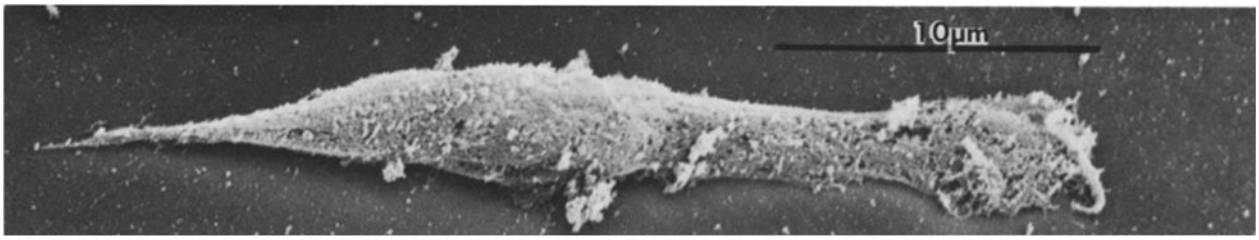


FIGURE 1 Scanning electron micrograph of skeletal framework of proliferating myoblast: maintained for 1 d in culture on growth medium containing 30% fetal calf serum and 10% chick embryo extract. Cell is small and bipolar; surface lamina is largely intact.

the proliferative state and prevented from fusing by using highly enriched culture medium. These proliferating myoblasts are typically short, bipolar cells with ruffled or knobbed tips; their surface lamina is almost as continuous (Fig. 1) as that of the fibroblasts described earlier (1). A few small perforations, <0.2 μm, can be seen near the nucleus; occasionally, the lamina over the tip of the cell may have one or two perforations that are as large as 1 μm. Similar small perforations were seen in fibroblasts (1).

In sharp contrast, both the morphology and surface lamina os postproliferative myoblasts show marked specialization. The myoblasts take on a new morphology as they prepare to fuse and form multinucleated myotubes, becoming elongated and more spindlelike. One or both cell tips generally have a complex ruffled and knobbed configuration; the other end may be pointed. The remainder of the plasma membrane appears relatively smooth. The surface lamina of these postproliferative myoblasts is revealed by detergent extraction (Fig. 2). The extracted structure retains the overall shape of the intact myoblast. However, unlike the almost continuous surface lamina of the proliferative myoblast, many large openings or lacunae are seen in the surface lamina of the postproliferative myoblast.

The extremities of the postproliferative myoblast framework have largely collapsed and the surface lamina in these regions has a very discontinuous, reticulate appearance. At higher magnification, an extracted tip (Fig. 2 *C*) shows the typical

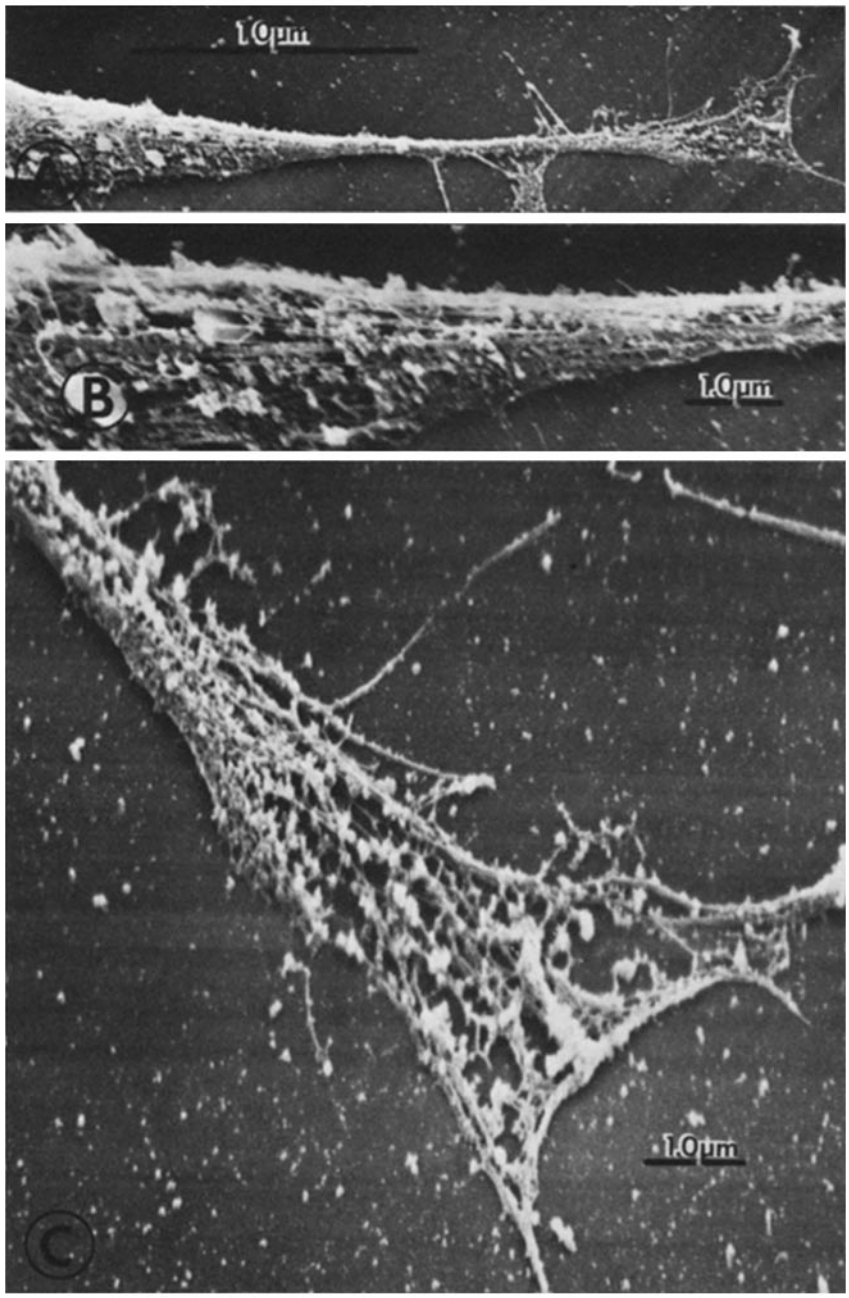


FIGURE 2 (*A*) Scanning electron micrograph of skeletal framework of postproliferative myoblast: maintained for 1 d in culture on fusion medium containing 10% horse serum and 2% chick embryo extract. Cell is more elongated; surface lamina shows many lacunae, particularly at the tip, revealing the underlying filaments. (*B*) The lacunae near the nucleus and the underlying cables. (*C*) Tip of myoblast showing the filamentous network.

fibrillar substructure underlying the lacunae. In many regions where the surface lamina is absent, prominent fibrous structures are seen in the exposed layer (Fig. 2 *B*). The lamina appears more continuous in regions between the periphery and the nucleus, but often some lacunae are clustered in the nuclear region itself. The lacunae in the surface lamina of the postproliferative myoblasts are very likely related to the formation of cell-to-cell junctions during fusion and are found principally in regions where junction formation is expected. The lacunae seen in postproliferative myoblasts disappear quickly after fusion, and the lamina becomes almost entirely continuous. The skeletal framework of a day-2 myotube is shown in Fig. 3; the lamina is a smooth, continuous sheet, with few breaks. No lacunae are visible at this time.

Lacunae in the Surface Lamina Arise from Protein-deficient Regions of the Plasma Membrane

Lacunae in the surface lamina appear in the postproliferative myoblasts and disappear shortly after fusion. Because the surface lamina is formed by plasma membrane proteins, these lacunae indicate that myoblasts organize plasma membrane proteins in an exceptional and characteristic way. Such lacunae could arise either from unstable membrane regions that release proteins extensively during extraction with detergent or from protein-deficient, lipid-rich domains in the plasma membrane.

The partitioning of surface proteins between the skeletal framework and the soluble, detergent-extracted phase is measured by iodinating the surface proteins of the intact cell with ^{125}I and then analyzing the iodinated polypeptides released from and retained by the skeletal framework during extraction with detergent. The electropherogram in Fig. 4 shows that the skeleton fraction of postproliferative and fusing myoblasts almost completely retains surface iodinated proteins (days 1 and 2), even though lacunae in the surface lamina of the postproliferative myoblast often cover nearly a fifth of the cell. Clearly, surface peoteins that can be iodinated are not extensively released. Myotubes after fusion (day 3), that have developed a continuous surface lamina, retain a similar amount of surface proteins (Fig. 4, lane 3 *B*). Indeed, the results in Fig. 4 for all days are similar to those obtained using fibroblasts, which retain ~95% of their surface proteins after detergent extraction (1). The myoblast lacunae do not, therefore, appear to result from extensive surface protein extraction.

If the lacunae in the myoblast surface lamina result from extraction of lipid-rich, protein-poor areas in the plasma membrane, then such areas would also have few surface glycoproteins and should bind lectins poorly. The distribution of Con A bound to the surfaces of intact cells is visualized by fluorescence microscopy using fluorescein isothiocyanate (FITC)-Con A. Surfaces of intact fibroblasts display a relatively uniform distribution of Con A, with occasional bright patches and strands where debris (visible by phase-contrast optics) is being cleared from the cell surface (Fig. 5 *A*). The surface of intact proliferating myoblasts stains diffusely; high magnification reveals, in addition, a finely punctate pattern of bright staining (Fig. 5 *B*). Although a similar pattern of staining is seen over most of the intact postproliferative myoblast, these postproliferative cells have peripheral regions that are free of Con A sites (arrows, Fig. 5 *C*); such Con A-free regions have only been seen in these postproliferative myoblasts. Thus, there are unstained regions in the intact myoblast plasma membrane that correspond in size and location to the lacunae seen in the surface lamina after extraction; these regions very likely represent the postulated protein-poor membrane regions.

The fusion-related lacunae persist for only a brief time after cell fusion and are essentially gone by day 2–3 in culture. Scanning electron microscopy of the extracted myotube skeletal framework at this time shows an essentially continuous surface lamina (Fig. 3). The disappearance of the large lipid-rich, protein-deficient regions should accompany a uniform distribution of surface proteins and, probably, of lectin binding sites. No lectin-free regions are seen in the intact cell at day 2, just shortly after fusion (Fig. 5 *D*). The absence of dark regions on

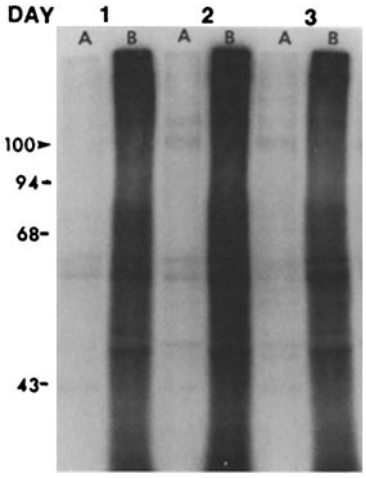


FIGURE 4 Polyacrylamide gel electrophoresis of externally ^{125}I-iodinated proteins of myoblasts, day 1-3 of culture. Lanes *1*, *2*, and *3* represent days *1*, *2*, and *3*, respectively, in culture. Lane *A* for each day contains the soluble fraction, proteins extracted by a gentle Triton extraction. Lane *B* contains the skeletal fraction, those proteins that remain with the skeletal framework after the gentle Triton extraction.

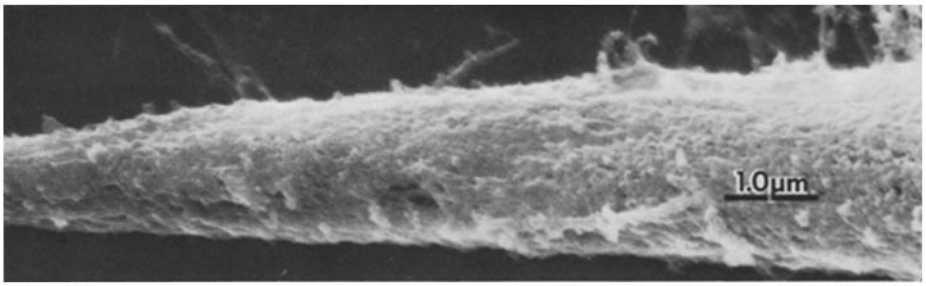


FIGURE 3 Scanning electron micrograph of skeletal framework of day-2 myotube. The cell shown contained at least four nuclei. Surface lamina has become intact, with no lacunae visible.

the surface of intact day-2 myotubes is consistent with the postulated direct relation between lacunae seen by scanning microscopy before and during fusion and the distribution of surface proteins in the plasma membrane.

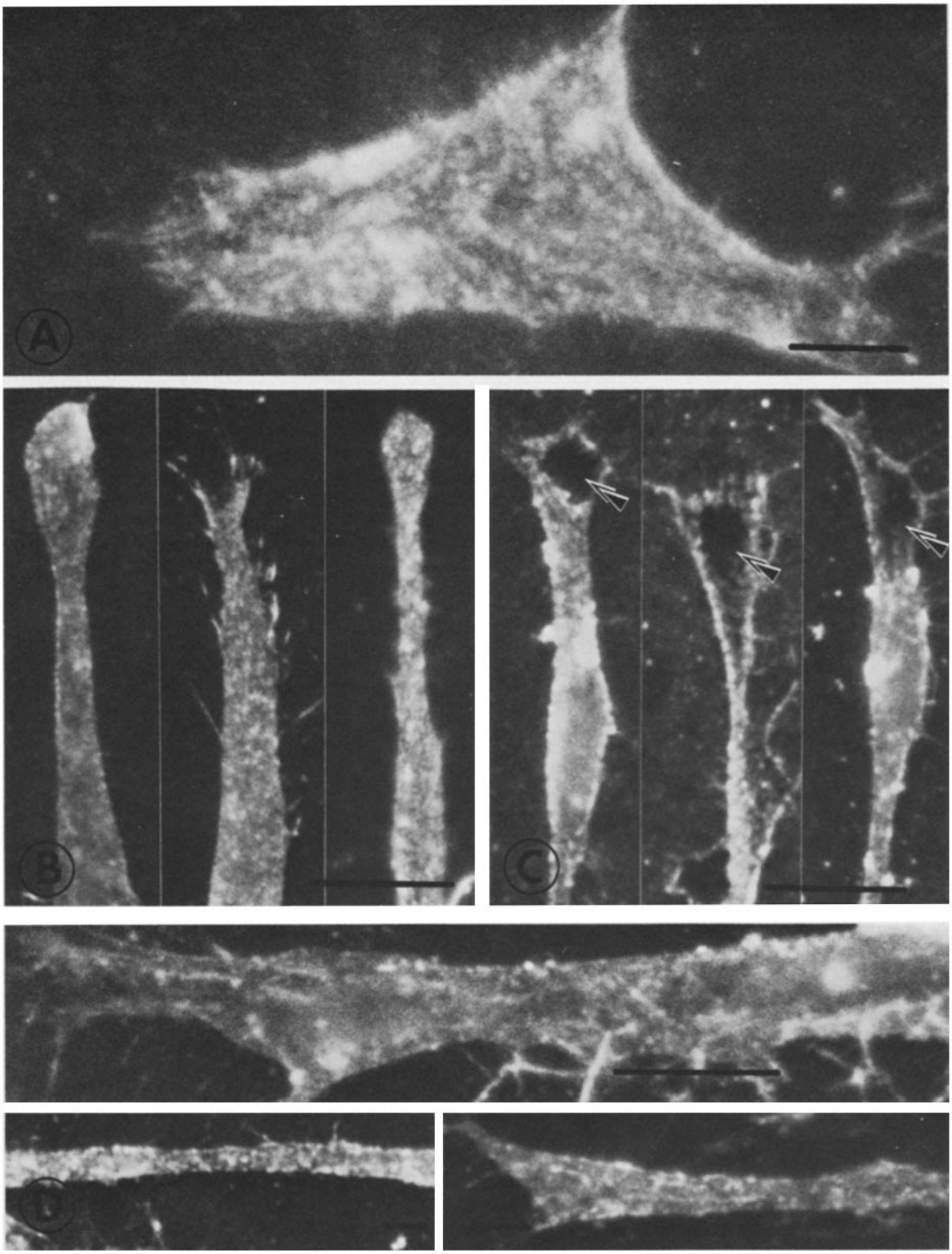


FIGURE 5 Fluorescence micrographs of intact cells stained with fluoresceinated Con A and fixed without extraction. All bars represent 5 μm. (*A*) Intact chicken embryonic fibroblast; bright patches are associated with debris (visible by phase-contrast optics). (*B*) Intact proliferating myoblasts at day 1 in culture on growth medium. High magnification reveals diffuse staining with punctate brightness. (*C*) Intact postproliferative myoblasts at day 1 in culture on fusion medium. Arrows indicate Con A-free regions seen only at this stage at locations where lacunae are seen after extraction. (*D*) Intact myotubes at day 2 in culture. No Con A-free regions are visible.

Transitional State of the Internal Skeletal Networks during Myoblast Fusion

At the time that postproliferative myoblasts show profound surface modifications, the elaborate structural networks that fill the cytoplasmic space also undergo extensive changes. These networks must rearrange after fusion and become continuous in the maturing myotube.

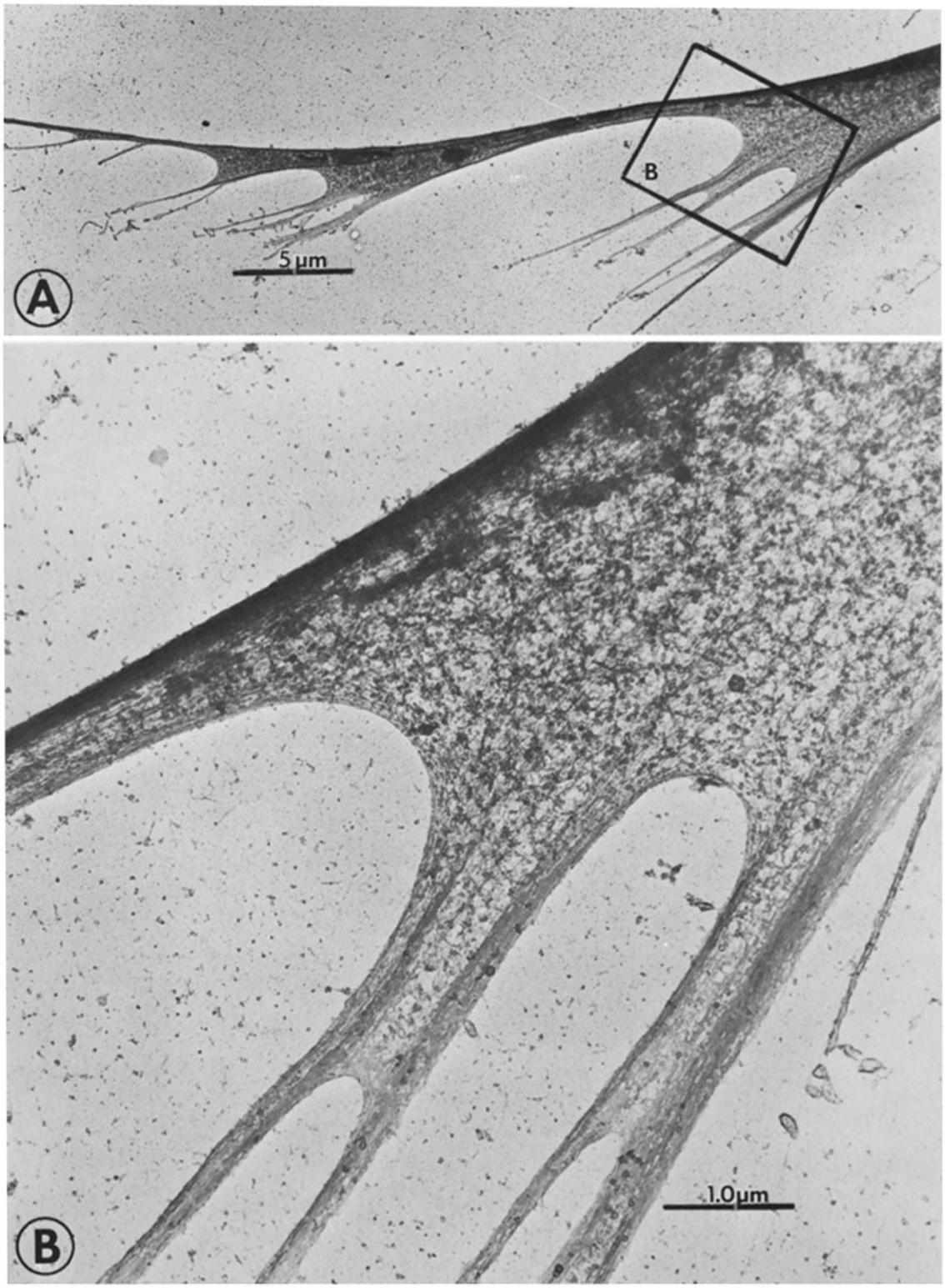
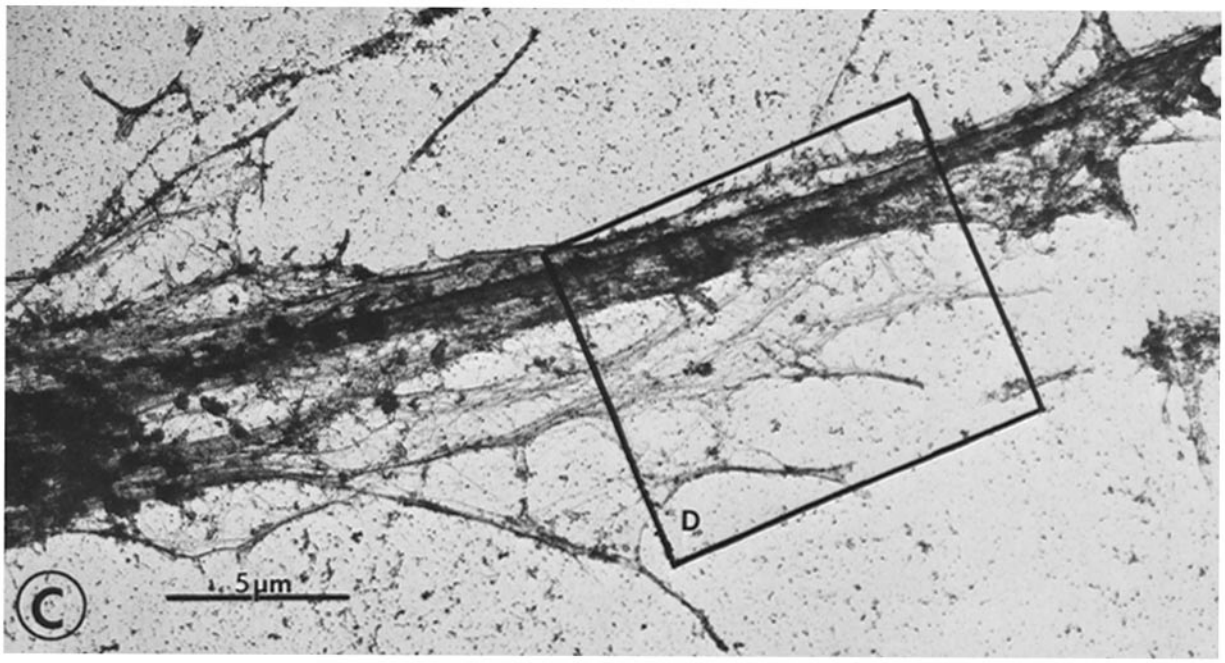
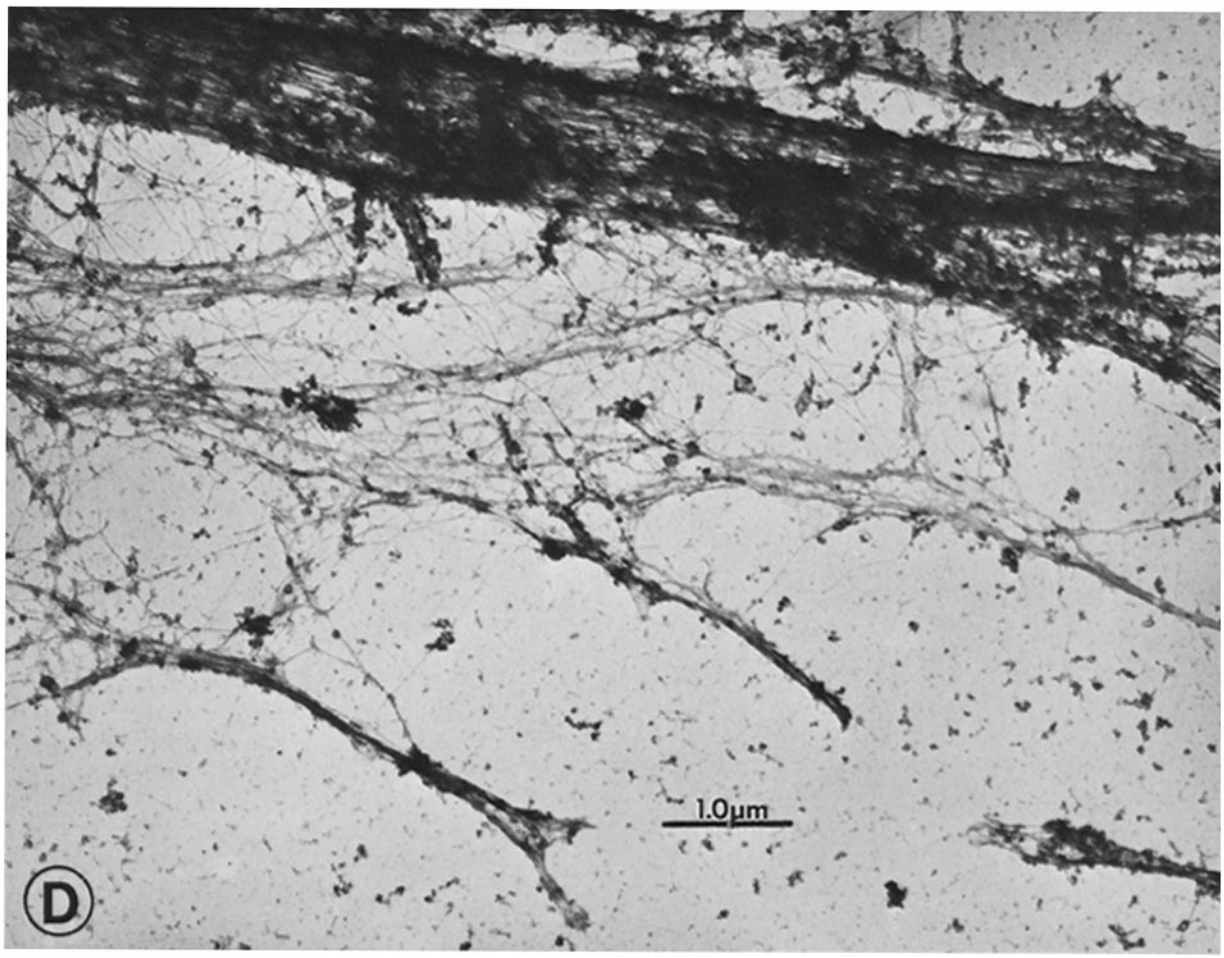


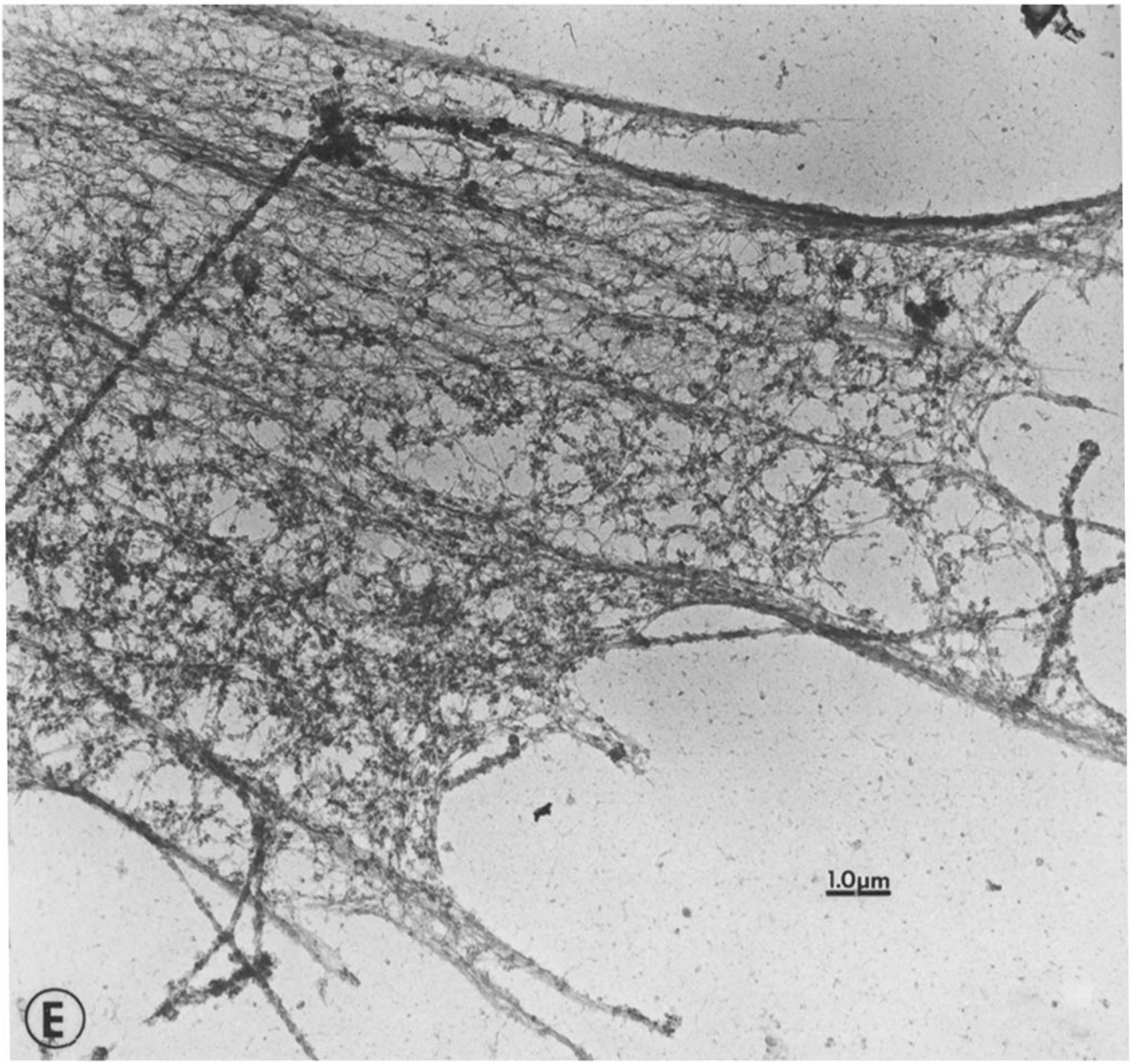
FIGURE 6 Transmission electron micrographs of whole mounted muscle cell skeletal frameworks. (*A*) The skeletal framework of a proliferating myoblast grown on growth medium. Note the well-integrated and extensively cross-linked microtrabecular framework. (*B*) *Inset* at higher magnification. (*C*) Post-proliferative myoblast, grown on fusion medium. Note the sparsely cross-linked skeletal framework and the cables running the length of the cell. (*D*) Inset at higher magnification. (*E*) Peripheral tip of multinucleated myotube taken on day 2 in culture. Note the increased integrity of the skeletal framework and the extensive cross-linking between the cables that run the length of the cell.

The internal networks can be best seen in muscle cells grown on grids and Triton extracted and then examined in peripheral regions of the cytoplasm; here, the thinness of the cell minimizes confusion from overlapping filaments. The electron micrographs of extracted whole mounts (Fig. 6) show the major stages during which internal networks are reorganized in the muscle cell. The proliferating myoblast (Fig. 6 *A and B*) has a dense and highly interconnected trabecular network that resembles (but is not identical to) that seen in fibroblasts.

Preparation for fusion occurs rapidly when the myoblasts are switched from the medium supporting proliferation to the medium inducing fusion. The cytoplasmic network at the periphery of a postproliferative myoblast is shown in Fig. 6 *C* and *D*. The myoblasts in this culture are the same age as that







shown in Fig. 6*A* but were shifted to fusion medium 24 h before the sample was prepared. The organization of the filamentous networks has changed drastically. Large, nearly empty regions are now seen, with only a few trabecular structures, and there are now major cables aligned along the long axis of the cell, which often splay at the periphery. The cytoplasmic networks are either depolymerized in the intact cell at this stage or are more labile during extraction.

Notable structures in the postproliferative myoblast are the major filament bundles or cables extending from deep within the cytoplasmic structure to the outer cell periphery. These cables, traversing large, nearly empty regions, are characteristic of and always found in postproliferative myoblasts. The cables, much less prominent in the proliferative myoblast (Fig. 6*A*), apparently form or increase in size as cells prepare for fusion.

After myoblasts fuse and form multinucleated cells, further changes occur rapidly in the networks of the skeletal framework. These changes can be seen in whole mounts at those tips distal to the junctions; the junction regions themselves are too thick for transmission electron microscopy without sectioning. In such a cell tip (Fig. 6*E*), the fiber networks are either reformed or stabilized against extraction and now form a dense, interconnected complex, distinct from the pattern seen in postproliferative myoblasts (Fig. 6*C*). The major filament cables are still present, but now the interlinked trabecular network appears connected with and organized around these cables.

The cytoplasmic filaments and microtrabeculae clearly undergo a profound change in organization and stability when the myoblast prepares for fusion. Not surprisingly, the protein content of the skeletal framework relative to total cytoplasmic protein is lowest at this time and rises sharply after fusion. The electropherograms in Fig. 7*A* show the partition of proteins between the skeletal framework and the soluble phase, for muscle cells in fusion-inducing medium at days 1 (postproliferative) through 4 (fully fused). The fraction of cytoplasmic protein in the skeletal framework is plotted as a function of days in culture in Fig. 7*B*. The framework preparation here includes both the cytoplasmic networks and surface lamina; nuclei and their protein content, also a component of the framework, have been removed by a second fractionation. The

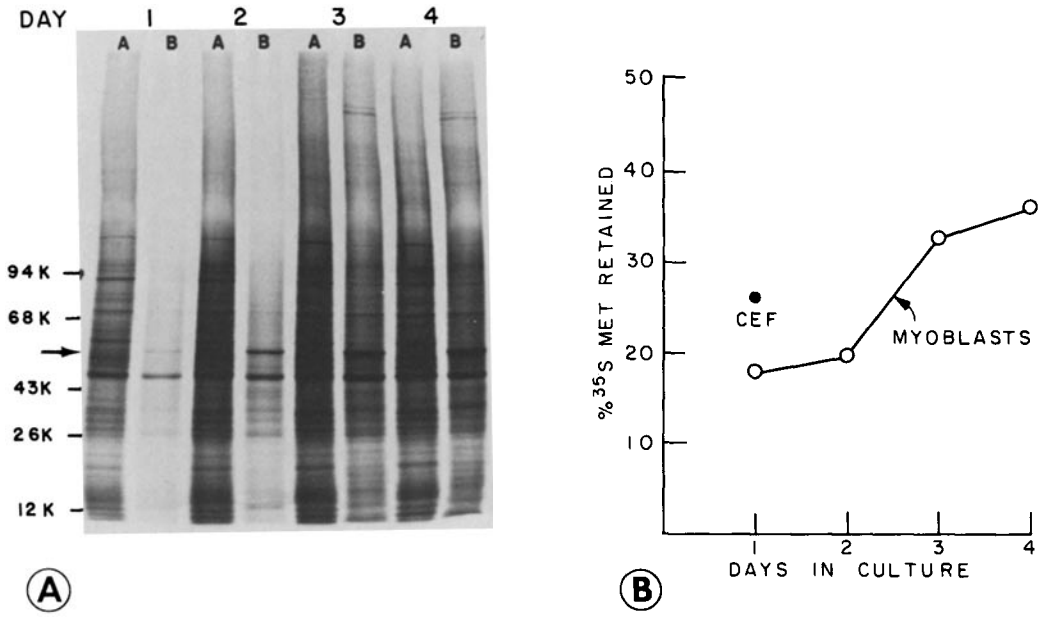


FIGURE 7 Increasing stability of [^{35}S]methionine-labeled proteins after fusion. (*A*) Polyacrylamide gel electrophoresis of myoblast proteins labeled with [^{35}S]methionine. Lanes *1*, *2*, *3*, and *4* represent days *1*, *2*, *3*, and *4* in culture, respectively. Lane *A* of each day represents the soluble proteins, extracted under gentle Triton extraction; lane *B* of each day represents the skeletal framework. Arrow indicates band at 58,000 mol wt of presumptive intermediate-filament protein; a fraction of this cytoskeletal protein can be extracted only from the postproliferative myoblasts (day 1). (*B*) The graph shows the fraction of nonnuclear [^{35}S]methionine-labeled proteins that remains associated with the skeletal framework for each day of culture. *CEF* represents skeletal framework protein of chicken embryonic fibroblasts. Values shown are the means of three experiments. For a given day, values from different experiments differed between 6 and 10%. The large increase between day 2 and day 3 was seen in every case; day 3 was between 65 and 69% >day 2.

fraction of protein in the skeletal framework is initially only 18% on day 1, but this rises abruptly between day 2 and day 3, approaching 36% of total cytoplasmic protein by day 4. For comparison, the fraction of cytoplasmic protein in a fibroblast skeletal framework is 22%. The differences in protein extraction are not a consequence of cell loss from the culture dish during fractionation, as no cells are detected in the extraction buffer by microscopic examination. Also, the data in Fig. 4 show clearly that nearly all surface protein, and hence, cell structures from similar myoblasts remain attached.

The extent of disruption of cytoplasmic structural networks during preparation for fusion is indicated by the partial release of a normally unextractable structural component, the presumptive 58,000-mol wt intermediate-filament protein. Intermediate filaments are completely retained in the skeletal frameworks of all cells examined, with the sole exception of the day-1 postproliferative myoblast. The transient extractability of intermediate-filament protein in Triton buffer is seen in the electropherograms in Fig. 7*A*. The band corresponding to the 58,000-mol wt protein, indicated by the arrow on the electropherogram, is partially extracted and partially retained on day 1; it is completely retained in the skeletal framework at all subsequent times.

DISCUSSION

The skeletal frameworks of postproliferative myoblasts revealed by gentle detergent extraction undergo profound alterations in preparation for and after fusion. Many of these changes in cellular organization are not easily visualized in the intact cell but are seen with great clarity in the Triton-extracted structures.

The surface lamina, formed by plasma membrane proteins remaining after extraction, has been described previously in cultured fibroblasts and epithelial cells (1) and appears to be part of the skeletal framework. Both the internal networks and surface lamina in the myoblast undergo profound, concurrent, yet distinct, changes in preparation for and after fusion.

In contrast to both proliferating myoblasts and mature muscle fibers, the surface lamina of the postproliferative myoblast has many clustered lacunae. The nearly complete retention in the skeletal framework of surface proteins labeled by iodination suggests that these lacunae are not formed by extensive loss of surface protein. Furthermore, only the postproliferative myoblasts show extensive dark patches when stained with FITC-Con A; these two findings suggest that the lacunae in the surface lamina correspond to regions deficient in lectin binding protein that are presumably lipid-rich domains in the plasma membrane.

It seems likely that the lacunae in the surface lamina relate to the requirements for cell fusion. Myotube formation requires juxtaposing fusion-competent cell surfaces. The lacunae seen in the postproliferative myoblast may reflect an early stage of this process. The act of melding together two cell surfaces must require unusual membrane properties, perhaps met by the lipid-rich patches characteristic of this stage of muscle development.

Transient lipid-rich patches, relatively free of protein, may explain the observation that myoblast lipid fluidity changes with fusion (16). Fluorescence depolarization measurements have indicated a large increase in lipid fluidity just before fusion followed by a decline to normal values after fusion. This fluidity change probably does not result from alterations in cell lipids, because the phospholipid composition and cholesterol content of cultured muscle cells remain relatively constant (10). Recent reports of greater fluidity at the cell tips and nuclei (7)

and of similarly located, particle-free regions in the freeze-fractured myoblast plasma membrane (11) suggest that the lacunae seen in the surface lamina result from these regions of protein-free, highly fluid lipid; thus, the change in plasma lamina organization may account for the transient increase in lipid fluidity.

The structural networks underlying the surface lamina undergo corresponding profound changes in organization during fusion, changes best studied in whole mounts at the flattened cell tips. Three distinct stages of internal cytoskeletal structure organization are discerned. The dense, highly interconnected filamentous network of the proliferative myoblast resembles the microtrabecular networks of fibroblasts and other cell types. When cells prepare to fuse, they have a sparse skeletal structure, lightly cross-linked and nearly empty, with a few major filamentous cables that terminate at the cell periphery. These cables, observed by Pudney and Singer (17), are characteristic of this specific stage in muscle development. In the cells observed by Pudney and Singer, these cables were predominantly peripheral. This difference in location and the differences in cell flattening are likely due to the different substrates and media used. Finally, after fusion, a dense interconnected framework re-forms but with a pattern distinctly different from the proliferating myoblast; this stage is characterized by a significant increase in the fraction of cytoplasmic protein associated with the skeletal framework.

The relatively empty framework of the postproliferative myoblast appears to result, in part, from the extraction of skeletal elements, as at least one major skeletal component can be extracted only during this brief transition stage. Intermediate-filament protein is partially removed from the day-1 myoblast skeleton (Fig. 7*A*). Shortly after fusion (day 2), the 58,000-ml wt protein is no longer extractable but appears well anchored in the skeletal framework. Thus, in myoblasts preparing to fuse, both the surface lamina and the internal networks show highly specific spatial rearrangement; in addition, the internal networks become more extractible. After fusion, both the internal networks and the surface lamina rapidly reorganize in a stable arrangement as the muscle cell begins to construct the extensive contractile apparatus. Thus, a critical stage in muscle development is accompanied by rapid, extensive, and transient reorganization of the skeletal framework and its surface lamina.

It is a pleasure to thank Dr. R. Singer for many valuable discussions. We also thank Elisabeth Beaumont for her excellent transmission electron microscopy of challenging samples, and Dr. R. O. Hynes for help with radioiodination.

This work was supported by fellowships to Drs. A. B. Fulton and S. R. Farmer from the Muscular Dystrophy Association and by grants CA08416-14 and CA12174-10 from the National Institutes of Health and NP-263 from the American Cancer Society.

Received for publication 27 January 1981, and in revised form 15 June 1981.

REFERENCES

1. Ben-Ze'ev, A., A. Duerr, F. Solomon, and S. Penman. 1979. The outer boundary of the cytoskeleton: a lamina derived from plasma membrane proteins. *Cell.* 17:859–865.
2. Blose, S. H., M. Shelanski, and S. Chacko. 1977. Localization of bovine brain filament antibody on intermediate (100A) filaments in guinea pig vascular endothelial cells and chick cardiac muscle cells. *Proc. Natl. Acad. Sci. U. S. A.* 74:662–665.
3. Brinkley, B., G. Fuller, and D. Highfield. 1975. Cytoplasmic microtubules in normal and transformed cells in culture: analysis by tubulin antibody immunofluorescence. *Proc. Natl. Acad. Sci. U. S. A.* 72:4981.
4. Brown, S., W. Levinson, and J. Spudich. 1976. Cytoskeletal elements of chick embryo fibroblasts revealed by detergent extraction. *J. Supramol. Struct.* 5:119–130.
5. Buckley, I., and T. R. Raju. 1976. Form and distribution of actin & myosin in non-muscle cells: a study using cultured chick embryo fibroblasts. *J. Microsc. (Oxf.).* 107:129–149.
6. Goldman, R. D., E. Lazarides, R. Pollack, and K. Weber. 1975. The distribution of actin in non-muscle cells. *Exp. Cell Res.* 90:333–344.
7. Herman, B. A., and S. M. Fernandez. 1978. Changes in membrane dynamics associated with myogenic cell fusion. *J. Cell Physiol.* 94:253–264.
8. Hynes, R. O., and A. T. Destree. 1978. 10 nm filaments in normal and transformed cells. *Cell.* 13:151–163.
9. Ishikawa, H., R. Bischoff, and H. Holtzer. 1969. Formation of arrowhead complexes with a heavy meromyosin in a variety of cell types. *J. Cell Biol.* 43(2, Pt.2):312*a* (Abstr.).
10. Kent, C., S. D. Schimmel, and R. P. Vagelos. 1974. Lipid composition of plasma membranes from developing chick muscle cells in culture. *Biochim. Biophys. Acta.* 360: 312–321.
11. Kalderon, N., and N. B. Gilula. 1979. Membrane events involved in myoblast fusion. *J. Cell Biol.* 81:411–425.
12. Lenk, R., L. Ransom, Y. Kaufmann, and S. Penman. 1977. A cytoskeletal structure with associated polyribosomes obtained from HeLa cells. *Cell.* 10:67–78.
13. Osborn, M., and K. Weber. 1977. The detergent-resistant cytoskeleton of tissue culture cells includes the nucleus and the microfilament bundles. *Exp. Cell Res.* 106:339–349.
14. Paterson, B., and J. Prives. 1973. Appearance of AChR in differentiating cultures of embryonic chick breast muscle. *J. Cell Biol.* 59:241–245.
15. Penman, S. 1966. RNA metabolism in the HeLa cell nucleus. *J. Mol. Biol.* 17:117–130.
16. Prives, J., and M. Shinitzky. 1977. Increased membrane fluidity precedes fusion of muscle cells. *Nature. (Lond.).* 268:761–763.
17. Pudney, J., and R. Singer. 1979. Electron microscopic visualization of the filamentous reticulum in whole cultured presumptive chick myoblasts. *Am. J. Anat.* 156:321–325.
18. Small, J. V., and J. E. Celis. 1978. Filament arrangements in negatively stained culture cells: the organization of actin. *Cytobiologie.* 16:308–325.
19. Webster, R. D., D. Henderson, M. Osborn, and K. Weber. 1978. Three-dimensional electron microscopical visualization of the cytoskeleton of animal cells: immunoferritin identification of actin- and tubulin-containing structures. *Proc. Natl. Acad. Sci. U. S. A.* 75:5511–5515.
20. Wolosewick, J., and K. R. Porter. 1976. Stereo high-voltage electron microscopy of whole cells of the human diploid line, W1-38. *Am. J. Anat.* 147:303–332.
21. Wolosewick, J., and K. R. Porter. 1979. Microtrabecular lattice of the cytoplasmic ground substance. Artifact or reality. *J. Cell Biol.* 82:114–139.