In Situ Reconstitution of Myosin Filaments within the Myosin-extracted Myofibril in Cultured Skeletal Muscle Cells

MIEKO TANIGUCHI and HARUNORI ISHIKAWA
Department of Physics, Faculty of Science, Nagoya University, Nagoya 464, and Department of Anatomy, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan

ABSTRACT We studied the *in situ* reconstitution of myosin filaments within the myosin-extracted myofibrils in cultured chick embryo skeletal muscle cells using the electron microscope and polarization microscope. Myosin was first extracted from the myofibrils in glycerinated muscle cells with a high-salt solution containing 0.6 M KCl. When rabbit skeletal muscle myosin was added to the myosin-extracted cells in the high-salt solution, thin filaments in the ghost myofibrils were bound with myosin to form arrowhead complexes. Subsequent dilution of KCl in the myosin solution to 0.1 M resulted in the formation of thick myosin filaments within the myofibrils, increasing the birefringence of the myofibrils. When Mg-ATP was added, such myosin-reassembled myofibrils were induced either to form supercontraction bands or to restore the sarcomeric arrangement of thick and thin filaments. Under the polarization microscope, vibrational movement of the myofibrils was seen transiently upon addition of Mg-ATP, often resulting in a regular arrangement of myofibrils in register. These myofibrils, with reconstituted myosin filaments, structually and functionally resembled the native myofibrils. The findings are discussed with special reference to the myofibril formation in developing muscle cells.

The formation of myofibrils is one of the main events that occur during myogenesis. Myofibrils are a complex of myosin, actin, and various related proteins. The orderly assembly of those proteins into cross-striated myofibrils may be affected by the lack of any one of the involving proteins, and it appears to take place under delicate control. It is interesting to ask whether or not the myofibril formation can be regarded as a self-assembly system.

There are many reports describing in vivo myofibril formation at the fine structural level (see review, reference 11). The earliest myofibril appears as a bundle or skein of thin filaments into which few thick myosin filaments are incorporated (2, 4, 9, 20, 21, 23). Such a myofibril does not show any cross-striations, although one can see dense patches within it, presumably Z-disk precursors, to which the thin filaments appear to be anchored. As the developing myofibrils contain an increasing number of thick myosin filaments, they become more regularly arranged (9, 10, 11, 17, 23). Thus, an important question which naturally arises is whether or not the assembly of myosin filaments gives rise to the regular sarcomere pattern in developing myofibrils.

This paper describes our attempt to experimentally reconstruct myofibrils structurally and functionally similar to native myofibrils by *in situ* reassembling myosin filaments within myosin-extracted myofibrils in cultured skeletal muscle cells. Such experiments may also provide helpful information on some aspects of muscle contraction.

MATERIALS AND METHODS

Preparation of Muscle Proteins

Myosin was prepared according to the method of Szent-Györgyi (34) and stored in 50% glycerol, 0.5 M KCl, and 10 mM Tris-HCl buffer (pH 8.3) at −20°C (36). Before use, the myosin was washed by repeating association-dissociation cycles and was finally dissolved in 0.6 M KCl, 1 mM $MgCl_2$, and 20 mM phosphate buffer (pH 7.2). The supernate obtained after a high-speed centrifugation was used for the experiments. The purity of the myosin was confirmed by gel electrophoresis (see below) (see Fig. 3*d*). The storage of myosin in glycerol did not alter its ATPase activity or binding ability to F-actin. The protein concentration was determined by the Biuret reaction (13).

Treatments of Muscle Cells

Muscle cells were grown in monolayer culture from 11-d chick embryo breast muscles (6). 4-d muscle cultures grown on collagen-coated plastic or glass cover slips were glycerinated in 50% glycerol in standard salt solution (60 mM KCl, 1 mM $MgCl_2$, and 20 mM phosphate buffer, pH 7.2) at 4°C for 1 d. Before the experiments, the glycerinated muscle cells were washed twice for 30 min each with a standard salt solution containing 1 mM EDTA to remove glycerol. All the

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treatments were performed with the cells on plastic or glass cover slips. The muscle cells were treated with a high-salt solution containing 0.6 M KCl, 1 mM $MgCl_2$, and 20 mM phosphate buffer (pH 7.2) for 20 min to extract myosin and then with the same solution containing 2 mM ATP for 20 min, and again with the same solution lacking ATP for another 20 min. To the myosin-extracted cells was added the myosin from rabbit skeletal muscle (3–4 mg/ml) in a high-salt solution. The myosin solution was then polymerized by dilution in two steps: first to 0.3 M of KCl concentration by adding an equal volume of 1 mM $MgCl_2$ and 20 mM phosphate buffer (pH 7.2), and further to 0.1 M by replacement with the standard salt solution. The ATP treatment on muscle cells containing such myosin-irrigated myofibrils was performed at room temperature with the following solutions: 1 mM ATP, 60 mM KCl, 1 mM $MgCl_2$, and 20 mM phosphate buffer (pH 7.2) with 1 mM EGTA or 1 mM $CaCl_2$.

Polarization Microscopy

Polarization microscope examination was performed with a Nikon Apophoto microscope (Nippon Kogaku, Tokyo) equipped with rectified lenses (19) and a Brace-Köhler compensator (Nippon Kogaku, Tokyo). A 250 W mercury arc lamp (Ushio Electric, Inc., Tokyo) was used for illumination combined with a heat-absorbing filter and an interference filter (Toshiba Kasei, Shizuoka). A glass cover slip with glycerinated muscle was mounted on a strain-free slide so that the cells were situated in the space between. To exchange the solution, several drops of the appropriate solution were introduced from one edge of the cover slip with a Pasteur pipette, and excess solution was removed at the opposite edge with a piece of blotting paper. For observation the microscope stage was turned to orient the myofibrils at 45° or 135° to the polarizer axis. Photomicrographs were taken with exposure times of 30–60 s using Kodak Plus-X film. The temperature of the sample was controlled by circulating water from a Komatsu coolnics (CTR-1B).

The measurement of birefringence was carried out as follows. A He-Ne laser (Toshiba Electric Co., Tokyo) with 25 mW output power was used as a light source. The light beam through a polarizer was condensed with a rectified condenser lens (Nippon Kogaku, Tokyo) on the specimens. After passing through the myofibril which was oriented at 45° to the polarizer axis, the beam was collected with a rectified objective lens and then led to a photomultiplier tube (Hamamatsu TV Co., Hamamatsu) for amplification. The output signals were viewed on an oscilloscope and recorded on a recorder.

Electron Microscopy

Muscle cells grown on plastic cover slips were used for electron microscopy. The samples at successive steps of treatment were fixed for 2 h at room temperature with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3). The samples in high-salt solutions were fixed with the above fixative containing 0.6 M KCl. After being rinsed with cold 10% sucrose in the same buffer, the samples were postfixed with cold 1% OsO_4 in the same buffer, and then stained en bloc with 0.5% uranyl acetate in distilled water for 2 h at room temperature. Thin sections were cut in a LKB Ultrotome III-8800 (LKB Instruments, Inc., Rockville, Md.), sequentially stained with uranyl acetate and lead citrate, and then examined in a Hitachi HU-12 type electron microscope.

Gel Electrophoresis

SDS PAGE was performed according to the procedure of Laemmli (24). The muscle cells at each step of the treatment were collected and washed by centrifugation. The pellets were resuspended at a concentration of ~2–3 mg protein/ml in a denaturing buffer containing 2.3% SDS, 5 mM β-mercaptoethanol, 10% glycerol, and 62.5 mM Tris-HCl buffer (pH 6.8), homogenized, and heated in a boiling water bath for 5 min.

RESULTS

Removal of Myosin from Myofibril

The muscle cells with striated myofibrils were used for the present observation. Under the electron microscope, the myofibrils in glycerinated muscle cells showed the typical sarcomere pattern of interdigitating thick and thin myofilaments: A-band, I-band, and Z-disk (Fig. 1). Polarization microscopy of the myofibrils revealed the same sarcomere pattern with an intense birefringence at the A-bands (Fig. 2 *a* and *b*). SDS gel electrophoresis of such glycerinated muscle cultures showed numerous bands including prominent bands of myosin and actin (Fig. 3 *a*).

When the glycerinated muscle cells were treated with high-salt solution, the birefringence at the A-bands rapidly diminished. The time-course of the extraction of myosin from the cells was estimated from the changes in birefringence. The birefringence was shown to decrease in a single exponential curve with a lifetime (τ) of ~10 min, and it completely disappeared after 60 min, along with the cross-striations (Fig. 2 *c* and *d*, and Fig. 4 *A*). The polarization microscope finding was substantiated by thin-section electron microscopy. In the myosin-extracted cells, no thick myosin filament was detected

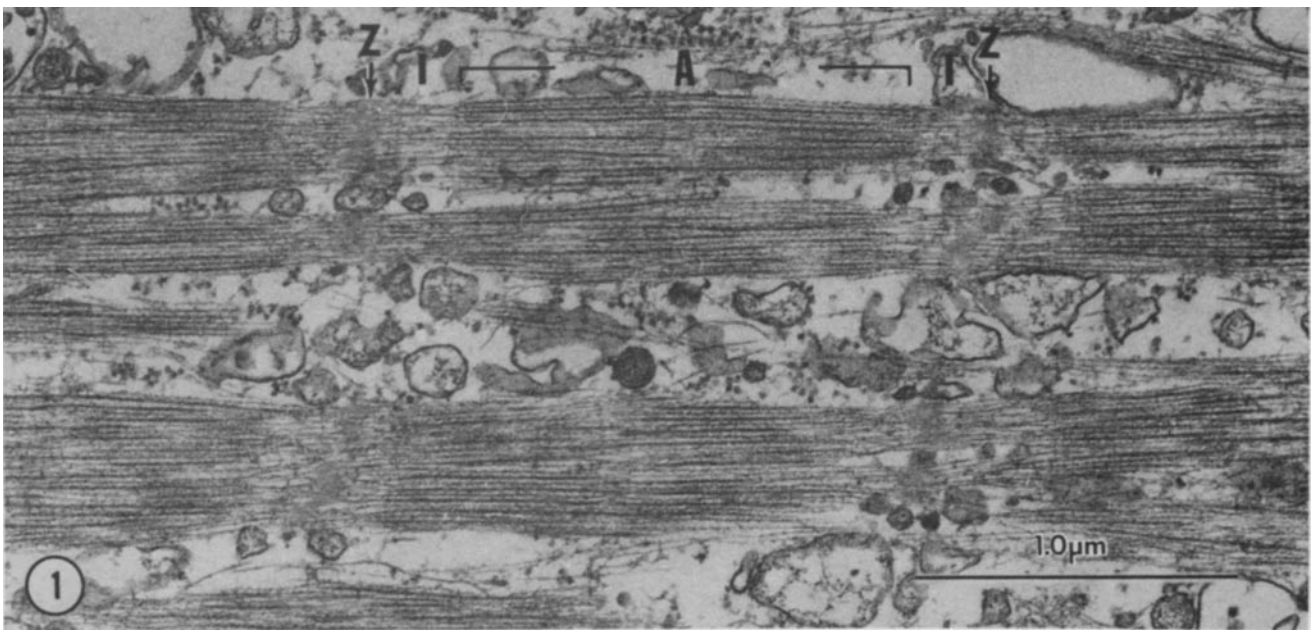

FIGURE 1 Electron micrograph of the longitudinal section of a glycerinated muscle cell. The cell contains relatively well-formed myofibrils in which thick and thin myofilaments interdigitate to form a characteristic sarcomere pattern: A-band (*A*), I-band (*I*), and Z-disk (*Z*). 4-d muscle culture. × 42,000.

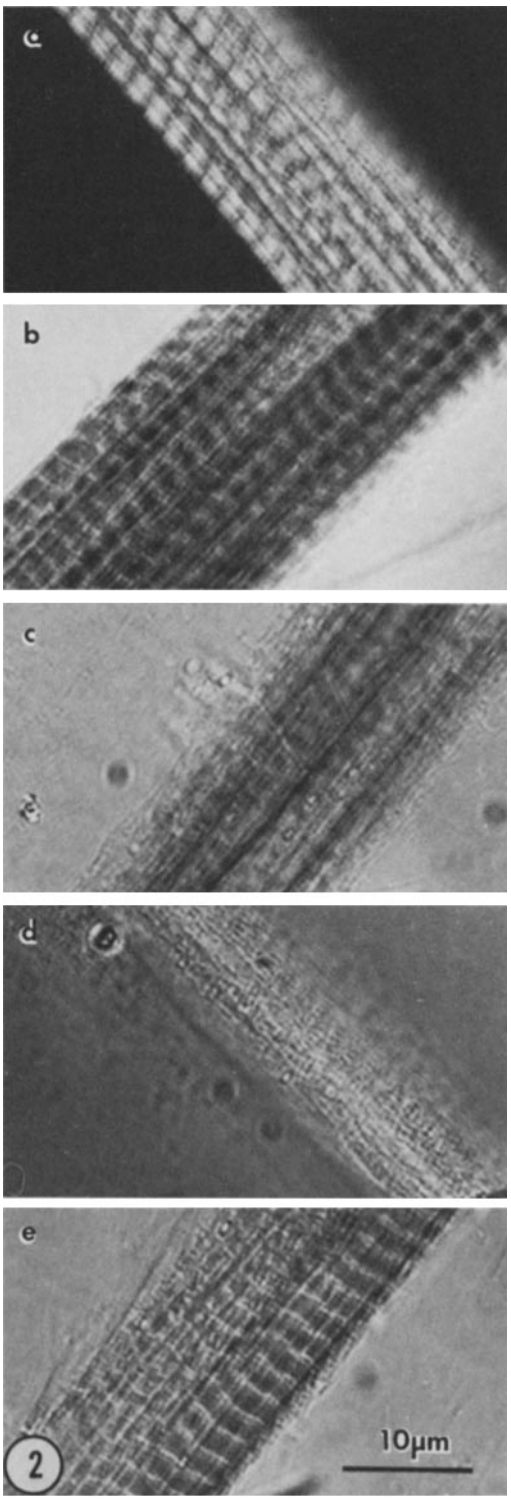

FIGURE 2 Polarization micrographs of glycerinated muscle cells at successive steps of treatment. The cell axis was placed at 45° to the polarizer axis. The dark or light background depends on different compensator settings. 20°C, × 1,750. (*a–b*) Before myosin extraction, 60 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 50 mM Tris-HCl buffer, pH 7.4. (*c–d*) During myosin extraction; 0.6 M KCl, 1 mM $MgCl_2$, 50 mM Tris-HCl buffer, pH 7.4. (*c*) 10 min. (*d*) 60 min. Note the progressive decrease in birefringence in the A-bands, resulting in disappearance of the cross-striation (*d*). (*e*) After myosin reassembly: 60 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 50 mM Tris-HCl buffer, pH 7.4, for 15 min. Note the reappearance of the prominent cross-striation with an increased birefringence.

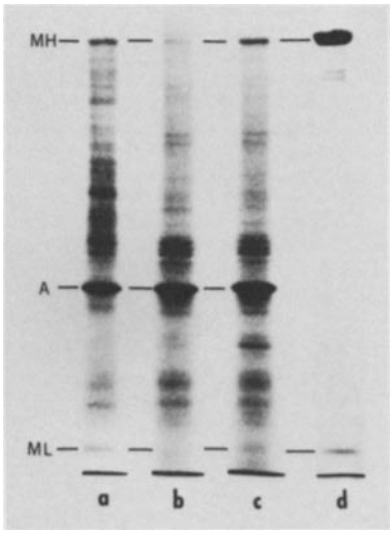

FIGURE 3 SDS gel electrophoresis of glycerinated muscle cells at different steps of treatment. *Lane a:* electrophoretic banding pattern of the original glycerinated muscle cells. Actin (*A*) and myosin (heavy chains: *MH*; light chains: *ML*) show prominent bands. *Lane b:* muscle cells after high-salt extraction. About 90% of myosin was extracted from the cells. *Lane c:* myosin-reassembled muscle cells after Mg-ATP treatment. The myosin reassociated is almost equivalent in relative quantity to the original glycerinated muscle cells. *Lane d:* rabbit myosin preparation used for the present reassembly experiments.

in the myofibrils, whereas the Z-disks and thin filaments remained in position along the course of the myofibrils (Fig. 5). The thin filaments were never decorated with myosin to form arrowhead complexes. Thus, the overall architecture of the myofibrils was fairly well maintained despite the complete loss of the thick myosin filaments. SDS gel electrophoresis showed that >90% of myosin (heavy chains) was extracted by such treatment (Fig. 3*b*), confirming the polarization and electron microscope findings. Interestingly, one could see some fuzzy materials and fine filaments at the H-zones between the ends of opposing thin filaments (Fig. 5, *inset*). The myofibrils from which myosin was extracted did not exhibit any detectable contraction upon addition of Mg-ATP (contraction medium) under the electron microscope as well as the polarization microscope (Fig. 4*A*, see arrow *a*). Intermediate, 10-nm filaments were seen unextracted and freely distributed between the myofibrils.

Binding of Myosin with Ghost Myofibril

When the myosin-extracted muscle cells were incubated with rabbit skeletal myosin in high-salt solution at room temperature, the cross-striations reappeared under the polarization microscope with a moderate increase in birefringence at the regions of the A-bands (Fig. 4*B*, see arrow *b*). Electron micrographs of such incubated cells showed that myosin was bound to thin filaments of the ghost myofibrils, often showing characteristic arrowhead complexes (Fig. 6). No myosin aggregates or thick myosin filaments were found inside or outside the cells, confirming that virtually all the thick myosin filaments had been removed by the preceding treatment. The myofibrils tended to swell at the middle of the sarcomere. The arrowheads, though not so clearly as those with heavy meromyosin, were often observed along the thin filaments with spacing of ~38

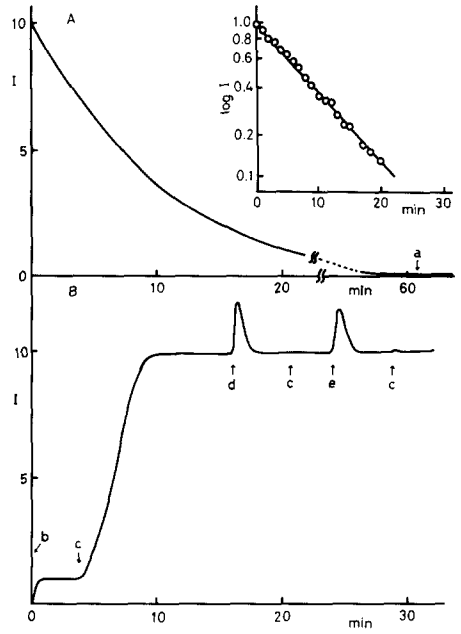

FIGURE 4 The time-course of the change in birefringence of the myofibril in a glycerinated muscle cell. The myofibril was placed between crossed polarizers at 45° to the incident polarized light. 20°C. (*A*) Birefringence of the myofibril during myosin extraction in high-salt solution: 0.6 M KCl, 1 mM $MgCl_2$, 20 mM phosphate buffer, pH 7.2. The decrease in birefringence (*I*) of the myofibril shows a single exponential fit (see *inset*). (*a*) No change in birefringence occurred upon addition of Mg-ATP in high-salt solution; 2 mM ATP 1 mM $MgCl_2$, 0.6 M KCl, 20 mM phosphate buffer, pH 7.2. (*B*) After addition of rabbit myosin (3 mg/ml) to the myosin-extracted ghost myofibril (see *A*). (*b*) Addition of myosin in high-salt solution; 0.6 M KCl, 1 mM $MgCl_2$, 20 mM phosphate buffer, pH 7.2. Note the slight increase in birefringence as caused by myosin binding. (*c*) Dilution of KCl; 60 mM KCl, 1 mM $MgCl_2$, 20 mM phosphate buffer, pH 7.2. Myosin assembly caused a marked increase of birefringence. (*d*) Addition of Mg-ATP; 1 mM ATP, 60 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 20 mM phosphate buffer, pH 7.2. (*e*) Addition of Mg-ATP; 1 mM $CaCl_2$, 1 mM ATP, 60 mM KCl, 1 mM $MgCl_2$, 20 mM phosphate buffer, pH 7.2. Upon addition of Mg-ATP, the birefringence increased transiently and then reversibly recovered to the initial level in this case. No difference in the birefringence response to Mg-ATP could be detected between the presence and absence of added Ca (*d* and *e*).

nm. The arrowheads pointed away from the Z-disk, as did those shown in negatively stained preparations of isolated I-segments (18) and in thin sections (21) (Fig. 6, *inset*). The microfilaments in fibroblasts in the same cultures were also seen decorated with myosin. However, there was no detectable binding of myosin with intermediate, 10-nm filaments or any membranous organelles (Fig. 6). The 10-nm filaments in the muscle cells and fibroblasts were preserved at every step of the treatment.

Assembly of Myosin into Thick Filament

When the ionic strength of the myosin solution was lowered in two steps to a final concentration of 0.1 M KCl, the myosin was assembled into thick filaments inside and outside the cells. Inside the cells most of the thick filaments were closely associated with the myofibrils: thick filaments were arranged parallel to thin filaments at the level of the A-band but somewhat diverged toward the middle of the sarcomere (Fig. 7). The thick filaments appeared as tapered structures and varied in length. Many thick filaments were seen running across the middle of the sarcomere. Thin filaments of the myofibrils were still decorated with myosin, forming arrowhead complexes. Under the polarization microscope the myofibrils showed a further increase in birefringence at the level of the A-band, in good accordance with the electron microscope observation (Fig. 2*e*, and Fig. 4*B*, see arrow *c*).

After the myosin was polymerized, the muscle cells were treated with Mg-ATP to determine whether or not such myosin-assembled myofibrils were contractile. The muscle cells indeed were seen to contract, while viewed under the polarization microscope, upon addition of Mg-ATP with added Ca or with added EGTA. The contraction was closely accompanied by a transient increase in birefringence (Fig. 4*B*, arrows *d* and *e*). In a muscle cell, adjacent myofibrils contracted independently, out of phase with one another. In some cells, the myofibrils contracted vigorously to form contraction bands. In electron microscopy, the myofibrils showed varying degrees of contraction, forming the massive contraction bands (Fig. 8*a*) or filamentous aggregates on both sides of the Z-disks (data not shown). The contraction band was composed of thick and thin filaments packed into a dense mass, where the thick filaments were randomly oriented (Fig. 8*b*). Often, the sarcomeres between the contraction bands were excessively stretched. In the Mg-ATP-treated cells, thin filaments of the myofibril no longer showed arrowhead configurations. Gel electrophoresis for the muscle culture after Mg-ATP treatment showed that the myosin was reassociated with the muscle cultures almost equivalent in relative amount to the original glycerinated muscle cultures (Fig. 3*c*).

The present experiments demonstrated that the myosin-reassembled myofibrils were indeed contractile. Accordingly, we next asked if the contractile activity might correlate with further organization of thick and thin filaments into a regular sarcomere pattern. Under the polarization microscope, some muscle cells were seen to transiently twinkle in the dark background upon addition of Mg-ATP, indicating a vibrational movement (data not shown). This type of response lasted 3–5 min, the presumed time for ATP exhaustion. Such responses often resulted in the formation of a regular sarcomere pattern and the arrangement of adjacent myofibrils in register. Electron microscopy revealed that some cells contained well-defined myofibrils in which myosin filaments are aligned interdigitating with thin filaments parallel to the long axis of the myofibril without any swelling at the middle of the sarcomeres (Fig. 9*a* and *b*). In transverse sections, such reassembled myosin filaments were found to be closely associated with thin filaments, though the degree of hexagonal packing is not complete (Fig. 9*c*).

DISCUSSION

This paper places major emphasis on the electron microscope observation that thick myosin filaments were reconstituted *in situ* within the myosin-extracted ghost myofibrils in cultured skeletal muscle cells. Such reconstituted myofibrils were able to contract upon addition of Mg-ATP. Furthermore, the ATP-induced vibrational movement of the myofibrils occasionally led to the regular arrangement of myosin filaments into a cross-striated sarcomere pattern. These results favor the view that myosin filaments are incorporated into developing myofibrils in vivo basically in conformity with the self-assembly process (17). Recently, the assembly of myosin within the myosin-extracted myofibrils from adult skeletal muscle has been ana-

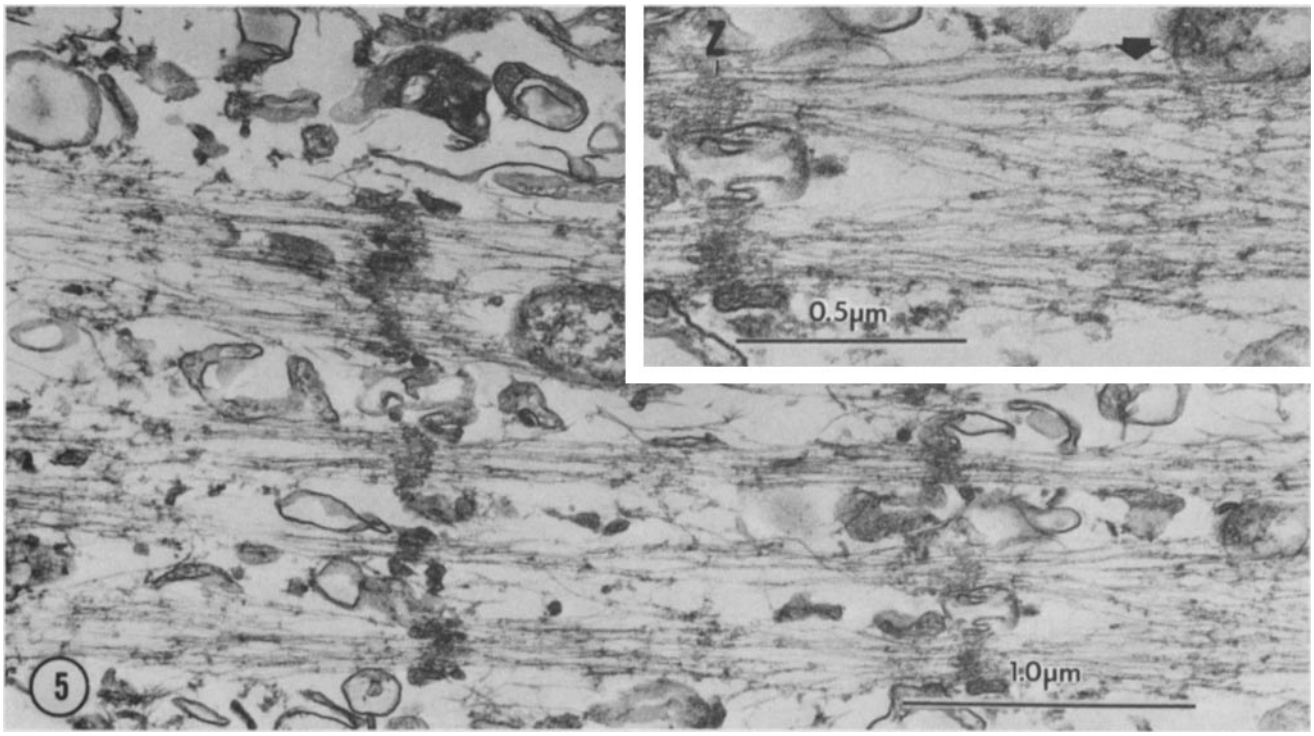

FIGURE 5 Electron micrograph of the longitudinal section of a myosin-extracted muscle cell. The cell was treated with high-salt solution for 80 min at 4°C. No thick myofilament can be seen in the ghost myofibrils. × 38,000. *Inset:* higher magnification. The Z-disks (*Z*) and thin myofilaments remain in position with fuzzy materials and fine filaments at the level of the H-zone (arrow). × 60,000.

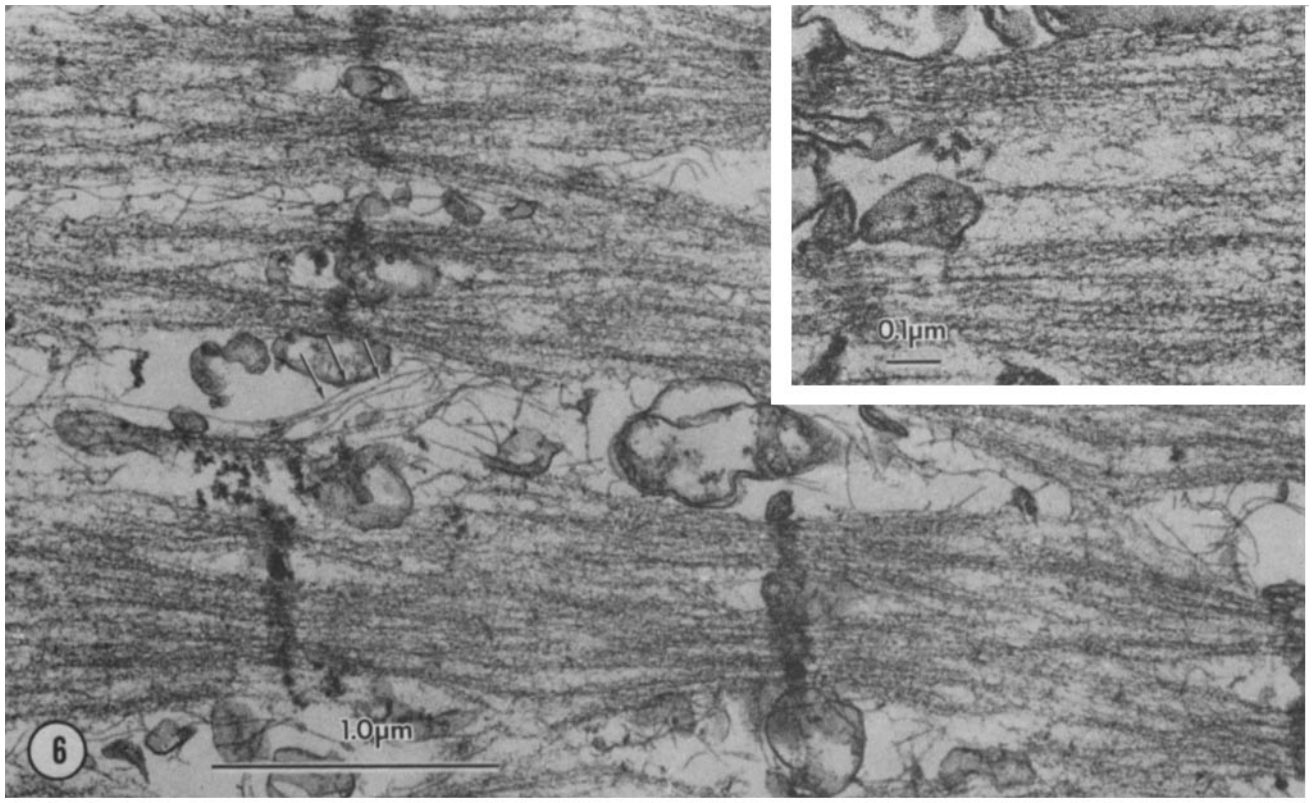

FIGURE 6 Longitudinal section of a myosin-added muscle cell in high-salt solution. The cell were previously treated to extract myosin from the myofibrils (see Fig. 5). Between the myofibrils are seen numerous 10-nm filaments, which do not bind myosin (arrows). × 38,000. *Inset:* higher magnification. All the thin filaments are decorated with myosin to form arrowhead complexes. × 70,000.

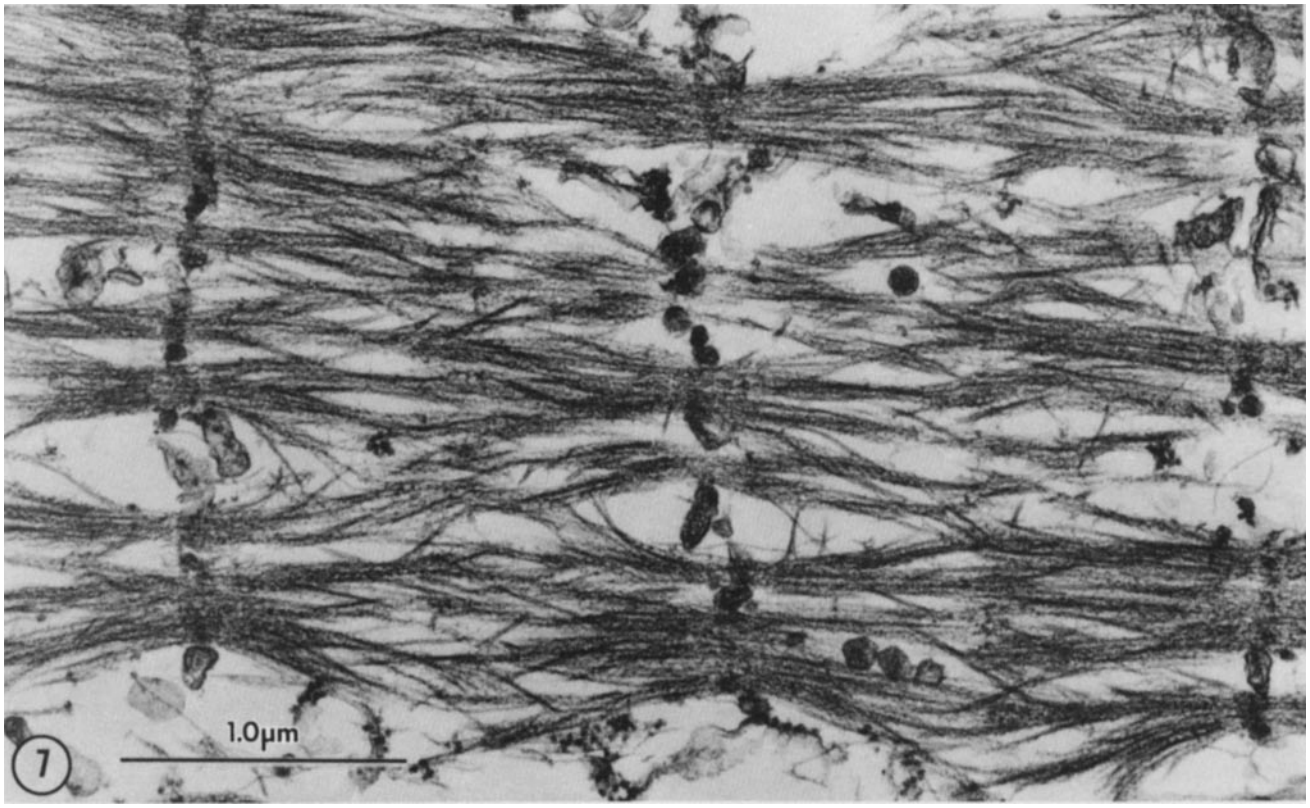

FIGURE 7 Longitudinal section of a myosin-reassembled muscle cell. After the cell was treated with myosin, the high-salt solution was diluted to 0.1 M KCl. Note that most of thick filaments are closely associated with the thin filaments of the original myofibrils, being arranged roughly parallel to the myofibril axis. The thin filaments still exist as arrowhead complexes. × 38,000.

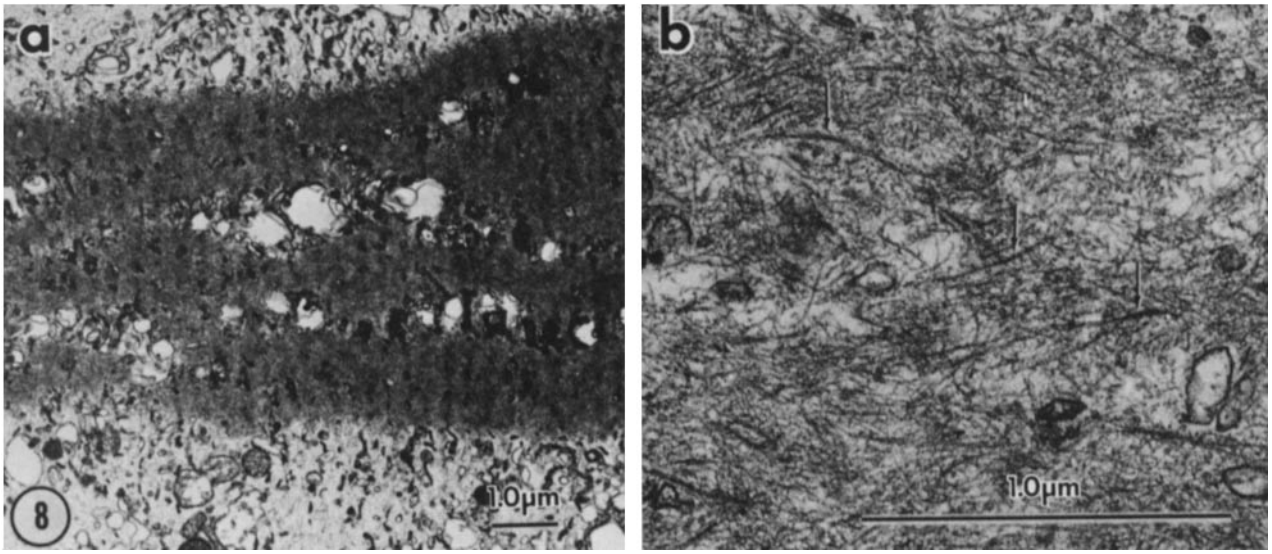

FIGURE 8 Mg-ATP-induced contraction bands of myosin-reassembled myofibrils. (*a*) Low magnification. Myofibrils form characteristic contraction bands. × 8,500. (*b*) High magnification. Thin and thick filaments (arrows) are seen in the contraction band. × 49,000.

lyzed to throw much additional light upon the contractile mechanism (7, 29, 35, 37). Confirming those analyses, our study further provides information on the fine structural aspect of such reconstituted myofibrils.

The results described here are also relevant to a central problem of myofibrillar assembly. Interestingly, myosin was assembled into thick filaments closely related to the pre-existing ghost myofibrils. Only a small number of thick filaments were found to be freely situated between myofibrils. About 10% of myosin still remained unextracted in the myosin-extracted muscle cells. Thus, the possibility can not be excluded that the myosin reassembly might occur on thick filament remnants which remained in the ghost myofibril. However, no partially depolymerized thick filament was found in the myosin-extracted or myosin-irrigated myofibrils. Such unextracted myosin may represent the denatured form of myosin. The

findings obtained seem to be consistent with the view that the interaction between thick and thin filaments occurs in the initial phase of myofibrillogenesis (2, 9, 33). In our *in situ* reconstitution experiments, thick filaments were seen to be closely associated with thin filaments in a manner similar to the original hexagonal pattern. As emphasized by Fischman (10, 11), the cross-bridges between thick and thin filaments would be involved in the underlying mechanism of parallel alignment of filaments and lattice construction. As thick filaments appear in developing muscle cells, the double hexagonal array with thin filaments is demonstrated (2, 9). A similar conclusion has been drawn from an in vitro assembly study in which the association of myosin and actin filaments produced the hexagonal arrangement to some extent (15, 16). However, the regular arrangement of filaments into sarcomeres may not be expected in a one-step process in the early myofibrillogenesis.

In our experiments, the ATP-induced contraction seemed to further cause the filaments to be disposed into a regular sarcomere pattern. During this process the added ATP may act in two ways: first, it dissociates arrowhead complexes of actomyosin into actin filaments and myosin, the latter of which may be further used to form thick filaments; and second, thick and thin filaments slide past each other through repetitive

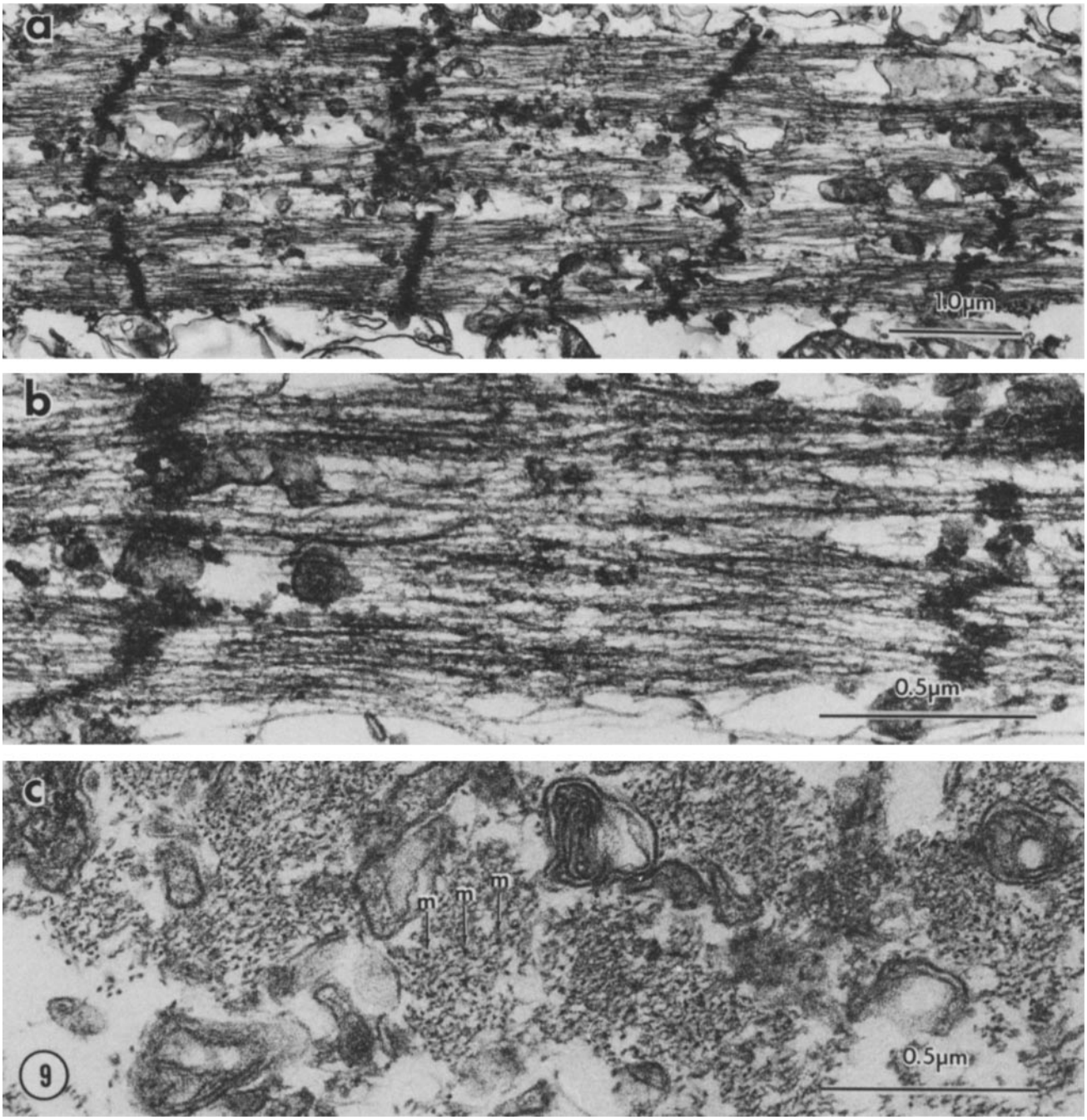

FIGURE 9 Electron micrographs of myosin-reassembled myofibrils. (*a*) Low magnification. After Mg-ATP treatment, some muscle cells are seen to contain well-defined myofibrils. × 22,000. (*b*) Higher magnification. Thick filaments are incorporated into myofibrils in a manner similar to the native developing myofibrils. No arrowhead complex is seen along the thin filaments. × 70,000. (*c*) Transverse section. Note the distribution of thick filaments (*m*) within the myofibrils. × 70,000.

dissociation-association cycles coupled with ATP splitting. Similar processes may work for the orderly incorporation of myosin into developing myofibrils in vivo. In other words, the spontaneous contraction which starts early in development may play a role in the rearrangement of myofibrils. Indeed, the Z-disks appear to develop by coalescence of the Z-bodies or precursors within the earliest, non-striated myofibrils (23).

The question arises whether developing myofibrils may become contractile before the formation of cross-striated sarcomeres. It has been shown that the synthesis of main myofibrillar proteins is initiated almost simultaneously (3, 28). This suggests that the earliest myofibrils, if not cross-striated yet, are equipped to contract. In developing muscle cells, both spontaneous contraction and the formation of cross-striated sarcomeres can be observed almost at the same time. It is likely, however, that before the formation of cross-striated sarcomeres, myofibrils initiate the contraction, *in loco* and out of phase, which may escape detection by ordinary phase-contrast microscopy.

It would be interesting to know how the overall architecture of individual myofibrils is maintained even after complete extraction of myosin. Hanson and Huxley (14) first reported that when the myosin-extracted myofibrils were further treated with 0.6 M KI, much of the material of the thin filaments was removed, but very tenuous backbone structures were still left behind. Walcott and Ridgway (38) and later Remedios and Gilmour (32) demonstrated that a filamentous material, a third type of filament, remained after extraction of myosin and actin from glycerinated myofibrils with high-salt solutions. Our materials also showed the filamentous networks in the H-zones of myosin-extracted myofibrils apparently connecting the ends of opposing thin filaments. These findings taken together suggest that the fine filamentous structures may be important in maintaining the overall integrity of the myofibrils. Connectin, an elastic component of muscle (26, 27), may constitute part of these structures.

In our experiments, ATP-induced vibrational movement also appeared to arrange adjacent myofibrils in register. Little is known about how the adjacent myofibrils are arranged laterally in register in embryonic muscle cells. The interfibrillar membranous system, the sarcoplasmic reticulum and T-system, has been often considered as a candidate for such an arrangement (1, 8, 22, 39, 40). Glycerination caused pronounced breakdown of the membrane system. Thus, there should be additional elements which help to maintain the spatial arrangement of the myofibrils. Recently, Lazarides et al. (25) have proposed that desmin, an intermediate filament protein, may link individual myofibrils laterally by bonding together the individual Z-disks in a Z-plane. Desmin is specifically synthesized in fusing or multinucleate muscle cells (5, 12). In immunofluorescence, the protein becomes redistributed to take a transverse course at the level of the Z-disk as muscle cells mature (5). Thus, desmin (together with vimentin?) may be directly involved in the lateral alignment and organization of myofibrils (12), although the corresponding intermediate filaments have been observed only rarely between the myofibrils (5, 30).

The M-line links thick filaments in the middle of the sarcomere and thus is believed to maintain the thick filaments in hexagonal packing and in register. In our experiments the M-line protein seemed to remain unextracted in the myosin-extracted ghost myofibrils. The remaining M-lines, however, do not seem to serve as centers for myosin reassembly. Likewise, it is too early to assess the role of microtrabecular lattice system in the myofibril formation (31).

This study proposes the importance of the contractile activity in myofibrillogenesis in addition to the self-assembly of the constituent proteins and mutual association. Although the final goal of experimentally constructing cross-striated myofibrils from the earliest non-striated ones is still far from attainment, the experiment described here may serve as the beginning of a direct line of approach to a better understanding of myofibril assembly.

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