CYCLIC PRODUCTION OF TENSION FORCE IN THE PLASMODIAL STRAND OF PHYSARUM POLYCEPHALUM AND ITS RELATION TO MICROFILAMENT MORPHOLOGY

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SUMMARY

Cyclic contraction and relaxation of plasmodial strands of Physarum polycephalum were measured under both isotonic and isometric conditions, and their relation to changes in microfilament (MF) morphology was investigated. The contraction-relaxation rhythm of a strand segment was insignificant and irregular immediately after isolation from the mother plasmodium. It became regular half an hour later when local minute rhythms were synchronized spontaneously. If a strand kept under isotonic conditions was loaded with a heavier weight or a strand kept under isometric conditions was stretched a few times, the amplitude of each contraction wave was enhanced. After a strand had been thus conditioned, it was fixed at a selected phase of the contraction-relaxation cycle under both isotonic and isometric conditions.

The state of MFs changed strikingly according to the phase of the contraction cycle. In the shortening phase of the strand under isotonic contractions, MFs with a diameter of 6-7 nm were arranged parallel to each other to form large compact bundles in which adjacent filaments were bridged with cross linkages. Among these MFs, thicker filaments were sporadically scattered. At about the phase of minimal strand length, most of the MFs became kinky and formed networks. In the elongating phase, new loose bundles of MFs developed from the network. These loose bundles became compact again when the strand reached its maximal elongation phase. In the isometric contraction, MFs in the increasing tension phase were nearly the same as those in the shortening phase in isotonic contraction. Around the maximal tension phase, dense areas of MFs appeared along the bundles in place of the network formed in the isotonic contraction phase. These areas were closely packed, with MFs arranged parallel to each other. In the decreasing and minimal tension phases in isometric contraction, MFs were arranged similarly to those in the elongating and maximally elongated phases, respectively, in isotonic contraction. Alternation between the straight bundle and fine network configuration of the MFs observed in isotonic contraction was inconspicuous in isometric contraction. This was probably due to spatial restriction of shortening under isometric contraction. The results are interpreted in terms of cyclic changes of the aggregation pattern of the MFs in the form of F-actin, as opposed to the possibility that the contraction-relaxation cycles depend on cyclic G-F transformation of actin.

INTRODUCTION

It is generally accepted that contraction of the ectoplasmic gel of the myxomycete plasmodium gives rise to a certain level of internal pressure locally and that a difference in the local internal pressure is in turn the cause of the endoplasmic streaming so
impressively manifested in this organism (Kamiya, 1959). The difference in internal pressure, i.e. the motive force responsible for endoplasmic streaming, is a quantity measurable by the double-chamber method, but the absolute contractile force of the ectoplasmic gel was not known until it was measured directly in an excised segment of plasmodial strand with a sensitive electromagnetic tension transducer (Kamiya, 1970, 1973; Kamiya, Allen & Zeh, 1972; Kamiya & Yoshimoto, 1972). Characteristic features of the contractile behaviour of the plasmodial strand were described in some detail.

We have briefly pointed out remarkable changes in the aggregation pattern of microfilaments (MFs) corresponding to the phase of the contraction-relaxation cycle of the strand under isotonic conditions (Nagai, Yoshimoto & Kamiya, 1975). The filament bundles, which are known to be composed mainly of F-actin (Alléa, Beck, & Wohlfarth-Bottermann, 1971; Nagai & Kato, 1975), may be mixed with myosin (Nagai & Kamiya, 1966, 1968; Alléa & Wohlfarth-Bottermann, 1972; Kessler, 1972) and regulatory protein molecules (Kato & Tonomura, 1975a, b). Fleischer & Wohlfarth-Bottermann (1975) and Wohlfarth-Bottermann & Fleischer (1976) also constructed a tensiometer similar to ours and attempted to correlate the morphological changes with tension production under isometric conditions. Their observations partly coincide but partly disagree with ours. We do not know whether the discrepancies are due to differences in experimental conditions, i.e. the difference between isotonic and isometric contractions.

The purpose of the present paper is, first, to describe the important contractile characteristics of the slime mould strand and second, to clarify the relation between the cyclic production of tension and cyclic changes in MF morphology under both isotonic and isometric conditions.

MATERIALS AND METHODS

The plasmodium of Physarum polycephalum was used. To keep the physiological conditions of the plasmodia for experimental use as constant as possible, we used only those reactivated from sclerotia 15-20 h beforehand.

The stock culture of the plasmodium was fed with compressed oats and grown on sheets of wet filter paper lining the bottom and side wall of a covered 12-l. plastic bucket. Outgrowths of the plasmodium were detached, together with the filter paper from the side wall of the bucket and dried slowly for 1 day. Sclerotia thus obtained were stored in a desiccator.

To reactivate the sclerotia, a sheet of 1.5% agar was prepared with non-nutrient tap water on the flat bottom of a shallow covered rectangular plastic container (20 x 30 cm²). A piece of sclerotium ca. 10 cm³ was placed on one edge of the agar plate and left there overnight. During 15-20 h, the sclerotium developed a large active plasmodium over the agar plate. Except for the advancing front zone, the plasmodium took the form of ramifying strands. A segment of smooth plasmodial strand, 10-40 mm long and 0.5-0.9 mm in diameter, was detached carefully from the network of the plasmodium creeping on the agar gel surface.

A specially constructed apparatus consisting of a vertical-type tension transducer (sensitivity, 0.1 mg; capacity, 150 mg) and a suitable recording set-up (Kamiya, 1970; Kamiya & Yoshimoto, 1972; Kamiya et al. 1972; Kamiya, 1973) enabled us to record isometric changes in tension as well as isotonic changes in strand length. Fig. 1 is a diagram of the whole layout.

A segment of plasmodial strand was set in the moist chamber between 2 hooks, one of which was affixed to the base of the moist chamber and the other connected to the tension transducer. As will be described later, the strand was subjected to repeated stretching or repeated...
loading with weights. These procedures effectively augmented and stabilized the amplitude of cyclic changes in tension or length. After the strand had been thus conditioned, it was fixed for electron-microscopic examination at a selected phase of a contraction-relaxation wave, which was registered on a chart of the pen recorder.

Fig. 1. Diagram of the tensiometer. Either isometric or isotonic contraction of the plasmodial strand is measured by setting the switch (S) at the upper or lower position, respectively. The plasmodial strand (PS) is held between the upper hook connected to the electrobalance (EB) and the lower hook affixed to the bottom of the moist chamber (MC). C, clutch; L, light source; P1, P2, potentiometers; PR, photoresistor; RP, rack and pinion; V, vent for introducing OsO4 vapour; WG, worm gear.

For fixation, the air of the moist chamber (7.5 cm³) was replaced quickly with air saturated with vapour from a 2% OsO4 solution. A possible lag of actual fixation after introduction of the vapour will be considered later. The strand was kept in the OsO4 vapour for 1–2 min before being removed from the tensiometer and submerged in 2% glutaraldehyde solution (50 mM cacodylate buffer, pH 6.4) for 1 h. The material was postfixed for 1 h in 1% OsO4 solution in the same buffer. The specimen was then dehydrated with a graded series of ethanols and cut into 2 or 3 segments, each 5–8 mm long, prior to embedding in Epon 812. The specimen was sectioned in both longitudinal and transverse directions on an LKB ultratome with a diamond knife, then stained with uranyl acetate and lead citrate. JEM-100B and JEM-100C electron microscopes were used.
RESULTS

Before describing changes in MF aggregation patterns in relation to tension force production of the plasmodial strand, certain important dynamic properties of this material must first be made clear.

Conditioning of the plasmodial strand

A strand segment isolated from the mother plasmodium and set between the 2 hooks of the tensiometer under isometric conditions, did not immediately exhibit

Fig. 2A. A record of isometric contraction under stepwise stretching. For explanation, see text.

Fig. 2B. A record of isotonic contraction under stepwise loading. For explanation, see text.
periodicity as shown in the initial part of Fig. 2A, where tensiometry was started (time 0) within 1 min after the specimen had been isolated. After 10 min, the tension began to oscillate. Thereafter the strand was stretched by ca. 10%. The level of the tension waves increased suddenly with concomitant increase in amplitude. The increased amplitude, however, diminished rapidly as the level of the tension waves decreased (Fig. 2A). The second stretch by 3 mm (19% of the total length) caused another marked increase in amplitude of the tension waves. By repeating the stretching a few times, tension waves stable both in amplitude and period were recorded without being followed by a rapid decrease of the tension level.

To condition the strand under isotonic conditions, the load was increased stepwise in place of stepwise stretching. The amplitude of the contraction-elongation waves then increased each time and the wave form became regular (Fig. 2B). In isotonic contraction, the enhanced amplitude persisted as there was no tension relaxation. Only when the tension decreased, did the amplitude again diminish.

When the strand is subjected to repeated stretching or repeated loading with higher tension, the Young's modulus of the strand increases conspicuously (Kamiya & Yoshimoto, 1972). Morphologically, MFs in the ectoplasmic layer of the strand not only reorient themselves but increase in number under higher tension (Kamiya, 1973; Nagai et al. unpublished). The characteristic cyclic changes in the filamentous structures become more obvious under these conditions.

Synchronization of the contraction-relaxation cycle over the strand

To correlate the ultrastructural changes with the phase of the contraction-relaxation cycle, the phase of cyclic activities must be synchronized sufficiently over the entire length of the strand. For this purpose, we resorted to the following 2 methods (cf. Yoshimoto & Kamiya, 1978a).

For isotonic conditions, we attached resin particles to the strand as index markers. This method showed that the contraction and elongation cycles of local segments between adjacent index markers became almost completely in phase within 0.5 to 1 h after a strand segment had been isolated and tension imposed upon it.

When only a short-range synchrony, but not overall synchrony, is attained under isometric conditions, tension forces produced in different loci of the strand in different phases would interfere with one another, and hence the amplitude of the isometric tension waves tends to be larger, the shorter the strand. Once long-range synchrony has been established over the entire strand length, however, the magnitude of the isometric tension force must be the same, independent of the strand length. We can check synchrony under isometric conditions using this characteristic of the strand, i.e., by modifying its net length during tensiometry (Yoshimoto & Kamiya, 1978a). To perform such an experiment, we constructed a special moist chamber equipped with a pair of horizontal sliding bars which can clamp the strand laterally between the 2 terminal ends while the tension force is being measured under isometric condition. We noticed that shortly after the strand had been set up in the tensiometer, the amplitude of the wave increased abruptly at the moment when the bars clamped part of the strand (data not shown) to shorten its net length. However, this was no
Fig. 3. Isometric tension changes of the plasmodial strand before and after its net length was shortened. \(a\), original length; \(b\), length of the upper free part; \(c\), length of the lower clamped part. After the strand was clamped at the time indicated by the arrow on the wave, the recorded tension represented only that produced in part \(b\). Note that there were no changes in period, phase, and amplitude of the isometric contraction waves.

Fig. 4. Alternation between isometric and isotonic contraction of the plasmodial strand. The ordinates for isotonic contractions are shown inverted. No phase shift occurred after the conversion. Upper curve: isometric tension changes; lower curve: isotonic length changes.
longer the case 0.5 to 1 h after the strand had been isolated from the mother plasmodium and conditioned. In the conditioned strand, the amplitude and phase of the tension waves were independent of the strand length (Fig. 3). This testifies that sufficient synchrony of isometric contraction rhythms existed over the whole strand.

Phase relation between isotonic and isometric contraction cycles

The apparatus constructed by Kamiya (1970) permitted instantaneous conversion of isometric contraction into isotonic contraction, or vice versa. Fig. 4 shows an example of this kind of experiment. The upper curve represents isometric contraction and the lower one isotonic contraction of a single strand segment (cf. Yoshimoto & Kamiya, 1978b).

Comparison of the phases of the waves of isotonic and isometric contraction at the moment of interconversion clearly shows that the following distinct phase relation exists between the two:

<table>
<thead>
<tr>
<th>Isotonic contraction</th>
<th>Isometric contraction</th>
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<tr>
<td>phase of maximal length ↔ phase of minimal tension</td>
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<tr>
<td>shortening phase ↔ phase of increasing tension</td>
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<tr>
<td>phase of minimal length ↔ phase of maximal tension</td>
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<td>elongating phase ↔ phase of decreasing tension.</td>
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Note that the phase of maximal tension in isometric contraction corresponds to the shortest phase and not the phase of maximal shortening velocity where maximal mechanical work per unit time is done. These phase relations find their counterparts in cyclic changes in the morphology of MFs under isotonic and isometric conditions as will be described later.

Behaviour of the strand in response to exposure to fixatives

To correlate changes in MF morphology with cyclic tension development, we must know how long it takes for the MFs to be fixed after the fixative has been applied. This is a difficult problem but the following phenomenon accompanying introduction of OsO4 vapour is instructive. The onset of introduction of OsO4 vapour to a strand under isotonic conditions is followed by sudden elongation, usually within 10–15 s (cf. insets in Figs. 5, 8 and 10). In a strand kept under isometric conditions, decrease in tension followed just as rapidly and abruptly as elongation under isotonic conditions (cf. insets in Figs. 13 and 15). It also took 10–15 s for a plasmodium spreading on the agar plate to stop streaming after it had been exposed to OsO4 vapour. Thus it seems reasonable to assume that the moment of abrupt length change or tension change represents the moment when the MFs are fixed. Each inset of the following electron micrographs shows the normal time course of tension or length changes of the strand before the fixative was applied, the time at which introduction of OsO4 vapour was begun (arrow) and the time course of length or tension changes after application of the fixative.
This rapid elongation or slackening of tension of the strand on application of OsO₄ vapour raises a further problem of whether the MF morphology in the specimen thus fixed is a real representation of that phase or a distorted one after elongation or tension slackening. Therefore, an experiment was conducted in which glutaraldehyde solution was used as the first fixative in place of OsO₄ vapour. A slight contraction, instead of elongation, occurred in this case soon after the strand was immersed in the fixative under isotonic conditions. Under isometric conditions, the tension tended to increase rather than decrease. The filamentous structures in the strand fixed with glutaraldehyde nevertheless preserved essentially the same MF morphology as when the specimen was prefixed with OsO₄ vapour. Thus rapid decrease or increase in tension or strand length after application of fixatives is not likely to distort the MF morphology. Therefore, our tentative conclusion is that the characteristic filamentous structures must have been fixed before the length or tension of the strand was changed.

**Patterns of MF assembly in relation to phases of isotonic contraction cycle**

Segments of the plasmodial strand showing well synchronized dynamic activities over their entire length were chosen for observation. The structure of the MFs and the state of their assembly are characteristic of the phase of the contraction-relaxation cycle, though not all filaments in a section exhibit exactly the same pattern. Next, we describe the structure of the MFs and their assembly which we encountered most frequently, as representative of the phase when the strand was fixed.

*Shortening phase.* Fig. 5 shows a longitudinal section of the contracting strand near the surface. The strand, ca. 0.6 mm in diameter, was kept under a tension of 10 mg. The specimen was fixed in its shortening phase as shown by the arrow in the inset. On application of OsO₄ vapour, the strand soon started to elongate. The dotted line in the inset shows what the length change would have been if the OsO₄ vapour had not been applied. Note that the bundle is composed of nearly straight MFs. They run parallel to the longitudinal axis of the strand. Adjacent parallel filaments are linked with many cross-bridges (thin arrows). These MFs can be decorated with heavy meromyosin of rabbit skeletal muscle, showing that they represent F-actin (Fig. 6). Spindle-shaped thicker filaments, which remain undecorated, are sporadically found (arrows). These filaments may represent aggregates of myosin molecules.
Fig. 7. Cross-section of a bundle of MFs in the strand shown in Fig. 5. The region surrounding the invaginated membrane is replete with electron-dense dots, each of which in most cases represents the cross-section of a MF. × 60000.

(cf. Kessler, 1972). Thick filaments seen in the MF bundle in Fig. 5 (thick arrows) are probably of the same nature. A characteristic feature of the filament bundles in the shortening phase is the abundance of MFs, their high density and the regularity of their orientation.

The filament bundles were found only in the ectoplasmic region. They were located near the surface membrane as well as near the invaginated membrane. Fig. 7 shows an electron micrograph of a cross-section of the same strand shown in Fig. 5 in longitudinal section. Fig. 7 clearly shows that the region surrounding the invaginated membrane is replete with electron-dense dots, each of which represents mostly the cross-section of a MF. Note that the area rich in dots is discrete from other areas of the cytoplasm, although there is no membranous structure at the boundary of the bundle. These MFs do not form a regular geometrical array, but their density is fairly uniform in the dense area, averaging 2·0 × 10³ MF/(μm)².

Fig. 8. Longitudinal section of the strand shown in Fig. 5. The invaginated membrane is partially surrounded by straight MFs arranged in parallel array and partially by randomly oriented kinky MFs. × 58000.

Fig. 9. Structure similar to that shown in Fig. 8 under higher magnification, showing the coexistence of parallel MFs and randomly oriented MFs. × 70000.
In electron micrographs of a specimen fixed in the shortening phase we occasionally found the invaginated membrane surrounded partially by straight MFs arranged in parallel order and partially by randomly oriented kinky MFs; Fig. 8 shows an example. Coexistence of parallel MFs and the randomly oriented MFs was more clearly visible under higher magnification (Fig. 9).

**Phase of minimal length.** When the strand was fixed in the phase of minimal length, i.e. the phase of maximal contraction, most of the MFs were kinky (Fig. 10). They seemed not to be a mere entanglement of flexible MFs but a 3-dimensional network, the MFs being connected with one another. The randomly oriented MFs forming the network, which is what we previously called the 'skein body' or 'feltwork' (Kamiya, 1968; Nagai et al. 1975), began to appear in the shortening phase and became most conspicuous around the phase of maximal contraction. The density of the MFs was higher at the core of the network surrounding the invaginated membrane (arrows) and less dense distally. The region occupied by the MF aggregations, regardless of whether the MFs were straight and in bundles or kinky and in a network, was usually connected with another such region, to maintain structural continuity over the entire strand length. A similar network is shown in Fig. 11 under higher magnification.

**Elongating and maximally elongated phase.** In a strand which has started elongation, new loose bundles of MFs develop from the network. They are not straight and not markedly parallel with one another, do not form compact bundles such as shown in Fig. 5, and their assembly resembles those found in Figs. 8 and 9.

By the phase of maximal strand elongation, alignment of the filaments and bundle formation are nearly completed. As far as the morphology and aggregation pattern are concerned, MFs in this phase are similar to those in the shortening phase shown in Fig. 5.

**Microfilament cycle.** The coexistence of the 2 distinctly different patterns of MF aggregation shown in Figs. 8 and 9 strongly suggests that the MF network is not a mere artifact but a real entity. These figures are interpreted as showing that the MF bundles transform themselves into the network when the strand contracts. On the basis of the electron micrographs taken at different phases of cyclic isotonic contractions, we can reasonably conclude that the MFs periodically change their aggregation pattern as the contraction-relaxation cycle advances. These observations can be summarized diagrammatically (Fig. 12). Each pattern of MF aggregation is a representative structure corresponding to phases a, b, c, d, and e of isotonic contraction. In the contracting phase, the MFs are arranged parallel to each other to form compact bundles. They begin to lose their parallel order and become flexible, leading to formation of the network when the strand approaches its state of maximal

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**Fig. 10.** Longitudinal section of the plasmodial strand in a contracted phase (minimal length, see inset). Bundles of straight filaments have been converted into the network surrounding the invaginated membrane (arrows). The randomly oriented MFs forming the network are more or less kinky and usually connected with one another. × 29,000.

**Fig. 11.** Network structure similar to that shown in Fig. 10. Randomly oriented MFs are more clearly seen. × 80,000.
Fig. 12. Diagrammatic representation of changes in MF morphology in the isotonic contraction-relaxation cycle. Each pattern of MF aggregation is a representative structure corresponding to phases a, b, c, d, and e of isotonic contraction (see text).

contraction. As the strand starts relaxing, the MFs forming the network gradually regain their parallel order and bundle structure. The bundle structure is nearly completed in the phase of maximal elongation. The filament cycle seems to represent reversible transformation between 2 states of polymerization of F-actin. There is no evidence to support the view that G-F transformation of actin is essential in the contraction-relaxation cycle of the strand under the above experimental conditions.

Patterns of microfilament assembly in relation to the phase of the isometric contraction cycle

A characteristic pattern of MF aggregation for each phase of tension oscillation was observed similarly under isometric conditions.

Increasing tension phase. Fig. 13 shows a longitudinal section of the strand, ca. 0.4 mm in diameter, near the surface. As shown in the inset, the peak-to-peak amplitude and the period of the tension waves were about 18 mg and 2 min, respectively. On application of OsO₄ vapour to the strand (the arrow in the inset), the tension started diminishing quickly. The broken line in the inset shows what the tension

Fig. 13. Longitudinal section of a plasmodial strand in an increasing tension phase under isometric conditions. The well developed compact bundles of MFs are conspicuous alongside the invaginated membrane as well as throughout the bulk of the ectoplasm. × 11000. Inset: Record of isometric contraction of the strand. The dotted line shows what the tension change would have been if OsO₄ vapour had not been applied.

Fig. 14. Higher magnification of the region outlined in Fig. 13. The bundle is composed of many MFs nearly straight and parallel to one another. Somewhat thicker filaments are also present (thick arrows). Bridges (thin arrows) between adjacent MF are also seen. × 53000.
Tension production and microfilaments
production would have been had the fixative not been applied. Well developed compact bundles of MFs run in the direction of the longitudinal axis of the strand alongside the invaginated membrane, as well as through the bulk of the ectoplasm. Fig. 14 is a higher magnification of part of a well developed bundle shown in Fig. 13. The bundle is composed of many MFs nearly straight and parallel to one another. Among these MFs, somewhat thicker, more electron-dense filaments are sporadically scattered (thick arrows). Bridges (thin arrows) between adjacent MFs are also seen. This structure is strikingly similar to that observed in the isotonic contracting phase (cf. Fig. 5).

Maximal tension phase. In the specimen fixed near its maximal tension, large filament bundles usually remain. The characteristic MF network that appears in the phase of minimal length under isotonic conditions, rarely appears in the phase of maximal tension under isometric conditions. An example of an electron micrograph in this stage is shown in Fig. 15. In place of the network, note that there are dense areas. The electron micrograph in Fig. 16 shows the dense area indicated by the arrow in Fig. 15 at higher magnification. This micrograph was obtained from one of a number of serial sections of the same area. This area is packed more closely than elsewhere with MFs arranged in parallel order. Thicker filaments (arrows) are also found more frequently here.

Decreasing and minimal tension phases. When the strand begins to relax, the bundles become less compact and the dense areas disappear sooner or later, but the bundle structures per se remain throughout isometric contraction and relaxation phases, even though their morphology changes according to the phase. Namely, the MFs are straight and form a compact bundle in the contracting phase, but are less straight and form a loose bundle in the relaxing phase, some MFs parting from it occasionally. These are more kinky than the others. MFs assemble in the phase of minimal tension in such a way that their aggregation pattern is no longer distinguishable from that in the phase of increasing tension shown in Fig. 14. The dense areas which are abundant in the maximal tension phase do not appear in this phase.

DISCUSSION

As described in the foregoing, a close relationship exists between cyclic tension force production and cyclic changes in the aggregation pattern of MFs (F-actin). MFs in the contracting phase under isotonic conditions and increasing tension phase under isometric conditions exhibit similar patterns, namely, they assemble in

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Fig. 15. Longitudinal section of a plasmodial strand at about its maximal tension (see inset). Bundles of MFs similar to those in Fig. 13 are seen. Note the dense areas on the MF bundles. × 4000.

Fig. 16. Higher magnification of the dense area shown at the arrow in Fig. 15. The micrograph was obtained from another section belonging to the same set of serial sections. The dense area is packed more closely than elsewhere with the MFs arranged parallel to each other. A number of thicker filaments (arrows) are also present. × 80000.
parallel order to form straight compact bundles. Aggregation patterns of the MFs in the relaxing and maximally relaxed phases under isometric conditions are virtually the same as those in the elongating and maximally elongated phases in isotonic contraction. However, under isotonic conditions, where filaments are allowed to shorten, overcoming a tension applied externally, a network is formed in the phase of minimal length. Under isometric conditions, such a structure rarely appears. This seems to be due to there being little room for the MF bundles to shorten under isometric conditions. Instead, filament-dense zones appear localized in the straight filament bundles. MFs in the dense areas orient themselves more or less in parallel, rarely forming a network of entangled filaments.

Fleischer & Wohlfarth-Bottermann (1975) reported on the correlation between tension force generation, fibrillogenesis and ultrastructure of the MFs during isometric contraction of the strand. Their observations agree well with ours with regard to the formation of the longitudinally arranged fibrils, or bundles composed of MFs (F-actin), at the beginning of the increasing tension phase in isometric contraction. According to them, many longitudinally oriented fibrils are generated de novo in the endoplasm from G-actin at the beginning of the isometric contraction phase. At the end of the contraction phase, both the number and diameter of MFs decrease. This description, however, does not agree wholly with our observations. The discrepancy between their observations and ours may partly be due to the fact that our plasmodial strands were sufficiently conditioned beforehand, while theirs were not. This may also be why Fleischer & Wohlfarth-Bottermann (1975) overlooked the persistence of MF bundles throughout the entire cycle of isometric contraction and the appearance of filament-dense areas in the phase of maximal tension development. They stressed that the fibrils are transformed into the non-fibrillar form of cytoplasmic actomyosin when isometrically contracting strands are allowed to contract freely under zero tension, a special case of isotonic contraction, where the strand does no mechanical work. This fact is interesting in itself, but cyclic changes in MF morphology share a common characteristic in that the filamentous structures are maintained throughout the contraction-relaxation cycle, whether the strand is under isometric conditions or constant tension. Their view (Fleischer & Wohlfarth-Bottermann, 1975; Wohlfarth-Bottermann & Fleischer, 1976) that cyclic G-F transformation of actin is essential for each contraction-relaxation cycle seems unlikely for the plasmodial strand. Instead, we would like to stress the alternating changes in the aggregation patterns of actin filaments between straight bundles and a fine network, a transformation which is easily demonstrated in isotonic contraction with a proper load. The transformation as such seems to be an event on the ultrastructural level underlying each contraction-relaxation cycle in the strand of the myxomycete plasmodium. Poor development of the network in the isometric contraction seems to be due to spatial restriction.

In the spread-out plasmodium, Kamiya (1973) has demonstrated by means of a polarizing microscope that the appearance and disappearance of birefringent fibrils are repeatedly observed in the zone close to the front with the same period as the back-and-forth streaming. Namely, when streaming takes place away from the front
(backward streaming), more fibrils appear than when the streaming takes place toward the front (forward streaming). In the phase of forward streaming, birefringent fibrils in the front zone tend to disappear, except for some thicker fibrils forming knots or compact regions. These changes in birefringence in relation to the 'pulsation' of the plasmodium coincide very well with our observations, since parallel assembly and bundle formation of the MFs are always enhanced in the isotonic shortening phase or isometric tension-developing phase.

The network is what we called previously 'skein body' or 'feltwork' (Kamiya, 1968; Nagai et al. 1975). This structure was found in the ectoplasmic layer of a plasmodium from which a considerable amount of endoplasm had been artificially sucked out. In this case, the ectoplasmic layer of the plasmodium was brought forcibly into a state of contraction by a local suction force applied artificially. The skein state of the filaments did not last long. It was transformed into the bundle structure again after the endoplasmic flow started in the plasmodium (cf. Takata, Nagai & Kamiya, 1967).

We noted above that MFs in the compact bundles are connected with one another by bridges. The bridges are comparable in dimension to those found at the brush border by Mooseker & Tilney (1975). The actin filaments at the brush border are not only attached to the membrane by bridges all along their length but are also connected with one another within the bundles. They stressed that the bridges are comprised of α-actinin, which is known to bind laterally to actin filaments in vitro (Podlubnaya, Tskhovrebova, Zaalishvili & Stefaneko, 1975). Although the existence of α-actinin in the Physarum plasmodium has not been demonstrated yet, an α-actinin-like protein may also participate in the lateral association of actin filaments in vivo. Physarum actomyosin is known to be very actin-rich compared with rabbit skeletal muscle actomyosin, the actin/myosin ratio being as high as (19–33)/1 by weight (Kessler, Nachmias & Loewy, 1976). Therefore, connexion of the actin filaments with one another by means of another protein may be necessary for the entire filament bundle to interact with myosin filaments.

MFs in the loose bundles which appear in the elongating (isotonic contraction) and the decreasing tension phase (isometric contraction) are more or less curly. Morphologically they resemble what was called 'Mg-polymer' by Hatano (1972). Mg-polymer is formed in vitro when plasmodium G-actin is polymerized in the presence of Mg²⁺ and β-actinin. β-actinin-like protein is known to be present in Physarum plasmodium and called plasmodium actinin (Maruyama, Kamiya, Kimura & Hatano, 1976; Hatano & Owaribe, 1976). Whether curly MF is an in vivo counterpart of the Mg-polymer in vitro is not known. However, the finding of Maruyama et al. (1977) is interesting as β-actinin decreases the dynamic rigidity modulus to less than one-tenth of that of the α-actinin-containing F-actin solution. The flexibility of the Mg-polymer is known to decrease as the ATP concentration of the solution increases from 0.05 to 0.5 mM (Fujime & Hatano, 1972). Thus, MFs probably change their flexibility in the contraction-relaxation cycle through participation of α-actinin and plasmodium actinin, depending upon the ATP concentration in the ectoplasm. In the case of the F-actin-tropomyosin-troponin complex, myosin increases the
flexibility in the presence of Ca²⁺ (Oosawa & Asakura, 1977). It is, however, not known whether the cyclic changes in MF morphology reported above are related to the binding and unbinding of myosin with/from F-actin.

This study has shown that MFs change their morphology and aggregation pattern cyclically corresponding exactly to the tension force generation of the plasmodial strand. However, no plausible evidence exists to show that the contraction-relaxation cycle of the slime mould strand operates on the basis of a sliding mechanism. Nor is there any convincing evidence that the changes in aggregation patterns on the part of the MFs produce the tension force. There is a possibility that entanglement and network formation are the visual manifestation of the events accompanying the sliding of MFs against one another in the presence of Mg-ATP, myosin and regulatory proteins.

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