Striated Flagellar Roots: Isolation and Partial Characterization of a Calcium-modulated Contractile Organelle

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ABSTRACT We report the isolation of striated flagellar roots from the Prasinophycean green alga Tetraselmis striata using sedimentation in gradients of sucrose and flotation on gradients of colloidal silica. PAGE in the presence of 0.1% SDS demonstrates that striated flagellar roots are composed of a number of polypeptides, the most predominant one being a protein of 20,000 M_r. The 20,000 M_r protein band represents ~63% of the Coomassie Brilliant Blue staining of gels of isolated flagellar roots. Two-dimensional gel electrophoresis (isoelectric focusing and SDS PAGE) resolves the major 20,000 M_r flagellar root protein into two components of nearly identical M_r, but of differing isoelectric points (i.e., pI's of 4.9 and 4.8), which we have designated 20,000-M_r-α and 20,000-M_r-β, respectively. Densitometric scans of two-dimensional gels of cell extracts indicate that the 20,000-M_r-α and -β polypeptides vary, in their stoichiometry, between 2:1 and 1:1. This variability appears to be related to the state of contraction or extension of the striated flagellar roots at the time of cell lysis. Incubation of cells with 32PO_4 followed by analysis of cell extracts by two-dimensional gel electrophoresis and autoradiography reveals that the more acidic 20,000-M_r-β component is phosphorylated and the 20,000-M_r-α component contains no detectable label. These results suggest that the 20,000-M_r-α component is converted to the more acidic 20,000-M_r-β form by phosphorylation. Both the 20,000-M_r-α and -β flagellar root components exhibit a calcium-induced reduction in relative electrophoretic mobilities in two-dimensional alkaline urea gels. Antiserum raised in rabbits against the 20,000-M_r protein binds to both the 20,000-M_r-α and 20,000-M_r-β forms of the flagellar root protein when analyzed by electrophoretic immunoblot techniques. Indirect immunofluorescence on vegetative or interphase cells demonstrate that the antibodies bind to two cylindrical organelles located in the anterior region of the cell. Immunocytochemical investigations at ultrastructural resolution using this antiserum and a colloidal gold-conjugated antirabbit-IgG reveals immunospecific labeling of striated flagellar roots and their extensions. We conclude that striated flagellar roots are simple ion-sensitive contractile organelles composed predominantly of a 20,000 M_r calcium-binding phosphoprotein, and that this protein is largely responsible for the motile behavior of these organelles.

Motility in eucaryotic cells in general appears to operate under calcium control through the regulatory action of a large family of calcium-modulated proteins (18, 19, 26). Calcium-based regulation of motility is best understood for troponin C and calmodulin in actomyosin contraction (sliding) in skeletal and smooth muscle, and in various nonmuscle actin-based cell movements (11, 13, 17, 47, 50). Evidence suggesting that calcium and calmodulin are involved in ciliary microtubule-based movement, i.e., sliding, (15, 22, 30) and in regulation of the mitotic apparatus (48, 49) is accumulating. All of these processes represent highly derived motility mechanisms that involve complex interactions between several distinct proteins and their regulatory components. A group of calcium-sensitive contractile organelles distinct from the actomyosin- and
microtubule-based systems have recently come to attention (cf. references 7–9). Such organelles have been most completely characterized for the ciliates *Vorticella* and *Zoanthidium*, and have been shown to be composed primarily of a low-molecular-weight protein called “spasmin” (2–4, 36). We present new evidence for the wider occurrence of spasmin-like proteins in organelles called striated flagellar roots. Striated flagellar roots occur in association with the basal apparatus of many flagellated and ciliated eucaryotic cells (35, 43). Striated flagellar roots are contractile organelles (40). Functional considerations have been somewhat speculative (28, 29, 31, 39, 40), however, due to the paucity of information concerning striated flagellar root composition and responsiveness to physiological changes in free calcium. We present new observations on striated flagellar roots, which suggest that these calcium-sensitive contractile organelles are simple in composition. Flagellar roots appear to be composed, in large part, of a 20,000-M, phosphoprotein, which undergoes a mobility shift in alkaline urea gels, characteristic of several known calcium-binding proteins.

**MATERIALS AND METHODS**

* Cultures: *Tetraselmis striata* Batch. (No. 443) was obtained from Dr. J. C. Green, Plymouth Culture Collection, United Kingdom. Cultures were grown in filtered seawater enriched with 10% soil water extract, 200 mg/liter NaNO3, and 20 mg/liter NaHPO4.7H2O at 18°C on a 16-h light/8-h dark cycle and constantly bubbled with air. For flagellar root isolation, 18–20 liters of cells were harvested by centrifugation, washed in 0.3 M NaCl and 0.1 M CaCl2, and stored frozen at −20°C.

* Flagellar Root Isolation: Frozen cells were defrosted in the isolation buffer containing 0.1 M NaCl, 0.5 mM MgCl2, 30 mM 2-(morpholino)-ethanesulfonic acid, 3 mM Na2SO4, 2 mM EGTA, 0.5% Triton X-100, and 5 mM phenylmethylsulfonyl fluoride. The free calcium level was adjusted to pCa 4 by the addition of CaCl2 and pH adjusted to 6.8 with KOH. Cells were washed two or three times in the isolation buffer by centrifugation at 121 g for 5 min to remove old mother cell walls (thecae) and other debris. Cells were lysed by a 20% suspension (packed cell vol/isolation buffer) at 0–4°C by sonication (100 W) using a Heat Systems Sonifier model W185D (Plainview, NY) equipped with a macroprobe. Eight periods of sonication (10 s each) were applied between intermittent 30-s cooling periods. Cell debris was sedimented at 121 g for 5 min, and the supernatant fraction was collected and centrifuged at 12,000 g for 10 min. The resulting pellet was resuspended in 30 ml of isolation buffer with 12 strokes of a tight-fitting Dounce homogenizer and centrifuged again at 12,000 g for 10 min. This was repeated three to five times until no further chlorophyll was extracted into the supernatant fraction. The pellet was then resuspended by homogenization in 5 ml of isolation buffer, and layered onto a discontinuous sucrose gradient composed of a 1.0 M sucrose zone was harvested, diluted, and pelleted by centrifugation at 121 g for 5 min. The final pellet, corresponding to ~106 cells was resuspended in 300 ml of isoelectric focusing lysis buffer, sonicated, and centrifuged to remove undissolved residue and theca. After electrophoresis and staining, gels were dried and autoradiographed according to Kodak XAR film (Eastman Kodak Co., Rochester, NY), using a Dupont Cronex Quanta III screen (DuPont Instruments, Wilmington, DE) for the times indicated in the figure legend.

* Immunoprecipitation of 32P-labeled protein was carried out after lysis of whole cell preparations in 100 ml of 2% SDS, 0.5% deoxycholate, and 0.5% Nonidet P-40, and brief sonication. Insoluble material was pelleted by centrifugation at 50,000 g for 3 min, and the pellet was dissolved with 9 vol of buffer containing 2% Triton X-100, 150 mM NaCl, 20 mM Tris-HCl (pH 8.6), 2 mM EDTA, 1 ml of preimmune serum or antisera directed against the 20,000 M, flagellar root protein was added and allowed to react overnight at 4°C. 20 ml packed volume of Protein A-Sepharose (Pharmacia Fine Chemicals) was added and, after 1 h of incubation, washed three times and then dissolved in SDS PAGE sample buffer and analyzed by electrophoresis and autoradiography.

* Indirect Immunofluorescence: Cells were fixed in 3% fresh formalin and washed in ASW for 30 min. Cells were then permeabilized through ethanol, cleared with xylene and infiltrated with paraffin. Sections, ~3 μm thick, were mounted on glass slides, deparaffinized with xylene, hydrated through a descending ethanol series, and brought into PBS, pH 7.2. Sections were incubated for 30 min at 37°C in primary anti-20K immune serum or preimmune serum (1:250 dilution), washed in PBS, incubated in fluorescein-conjugated goat anti-rabbit IgG (Cappel Laboratories, Cochranville, PA), washed and mounted in 10% glycerol in PBS. Observations were made with a Zeiss Axiosmat microscope (Carl Zeiss, Inc., New York) equipped with epi-illumination for fluorescence excitation and were recorded on Kodak Tri-X film developed in Acufine developer.

* Immuno-Gold Labeling: Sections from 3% formalin-fixed tissue were cut and fixed again through ethanol, cleared with xylene and infiltrated with paraffin. Sections, ~3 μm thick, were mounted on glass slides, deparaffinized with xylene, hydrated through a descending ethanol series, and brought into PBS, pH 7.2. Sections were incubated for 30 min at 37°C in primary anti-20K immune serum or preimmune serum (1:250 dilution), washed in PBS, incubated in fluorescein-conjugated goat anti-rabbit IgG (Cappel Laboratories, Cochranville, PA), washed and mounted in 10% glycerol in PBS. Observations were made with a Zeiss Axiosmat microscope (Carl Zeiss, Inc., New York) equipped with epi-illumination for fluorescence excitation and were recorded on Kodak Tri-X film developed in Acufine developer.

* Electron Microscopy: "Calcium Shock": Living cultures were harvested, washed in ASW lacking added calcium (ASW-Ca) and incubated in this medium for 30 min. Samples were "calcium-shocked" by the addition of 2 mM CaCl2 and then fixed within 30 s. Fixation was carried out for 1 h at

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**Abbreviation used in this paper:** ASW, artificial seawater.
room temperature by addition of an equal volume of ASW containing 4% glutaraldehyde. Samples were washed twice, postfixed in aqueous 1% OsO4, dehydrated in an ethanol series with propylene oxide as the transition fluid, infiltrated with Epon 812 resin and cured. Thin-sections were stained with 1% uranyl acetate and lead citrate and then observed and photographed using a JEOL 100 CX electron microscope (JEOL USA, Electron Optics Div., Peabody, MA).

RESULTS

Calcium Shock-induced Flagellar Root Contraction

_Tetraselmis_ is a quadriflagellate unicellular green alga. Each cell has two striated flagellar roots which are associated proximally with the flagellar apparatus and distally with the plasmalemma (24, 27, 37, 41). The striated flagellar roots of _Tetraselmis_ are contractile organelles (40). Fig. 1 illustrates two _Tetraselmis_ cells sectioned through the narrow plane of these broadly oval and flattened cells. The cell in Fig. 1A was fixed in ASW-Ca in the absence of added calcium, whereas the cell in Fig. 1B was fixed within 30 seconds of adding CaCl2 (2 mM) to the cell suspension. Contraction of the flagellar roots (Fig. 1B) results in in-pocketing of the plasmalemma distally (double-headed arrow, S), and proximally in deflagellation and displacement of the flagellar apparatus (FA). In-pocketing of the plasma membrane can be monitored at the light microscope in living cells caused to tumble across the field of view. This in-pocketing was observed to be cyclic in some cells. Cells can recover completely from calcium shock, regrowing flagella and becoming motile within several hours of being returned to culture conditions.

Fractions Enriched in Flagellar Roots

Fig. 2 illustrates a preparation enriched in flagellar roots from the final continuous sucrose gradient (see Materials and Methods). Flagellar roots were isolated in a fully contracted

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_Figure 1_ Striated flagellar roots are contractile organelles. (A) A thin-sectioned _Tetraselmis_ cell fixed in the absence of added calcium. One of the two fully extended striated flagellar roots (SFR) of this cell is visible. (B) A thin-sectioned _Tetraselmis_ cell fixed within 30 s of a "calcium shock." Both flagellar roots have contracted (CFR) resulting in deflagellation, displacement of the flagellar apparatus (FA), and in an in-pocketing of the plasmalemma (S). V, vacuole; N, nucleus; P, pyrenoid; T, theca; F, flagella. Bar, 1 μm. × 15,000.
FIGURE 2 Fraction from a continuous sucrose density gradient enriched for striated flagellar roots. Striated flagellar roots isolated in buffers with pCa 4 are fully contracted. (A) Low-magnification view. (B) Higher magnification. Bar, 1 μm.

state in an isolation buffer of pCa 4. Clearly, flagellar roots are the predominant organelle in this fraction (Fig. 2). The principal identifiable contaminant appears to be short segments of thecae (cell wall). Isolated flagellar roots have a triangular appearance, measure ~0.75 μm when sectioned longitudinally, and consist of 10–12 densely staining amorphous zones. Cross-sectioned roots have various shapes; often they appear as triangular structures that display an amorphous substructure.

Flagellar Root Composition and Anti-20,000-M, Serum Characterization

Analysis of isolated flagellar roots by SDS PAGE indicates that the predominant Coomassie Brilliant Blue staining band has a relative mobility corresponding to an apparent molecular weight of 20,000 (Fig. 3A). The 20,000-Mr protein represents ~63% of the Coomassie Brilliant Blue staining material in gels of the isolated striated flagellar roots. A number of other components are present at low levels in flagellar root preparations, including several between 50,000 and 60,000 Mr, and several in the lower molecular-weight range.

The 20,000 Mr protein was purified by preparative SDS PAGE (Fig. 3B) and used to elicit antibodies in rabbits. Fig. 3C illustrates immune serum staining of only the 20,000 Mr band of a flagellar root SDS polycrylamide gel lane (similar to Fig. 3A) after transfer to a nitrocellulose sheet, whereas preimmune serum (Fig. 3D) shows no detectable reaction. Immunoelectrophoretic analysis demonstrates immune specific (Fig. 3E, trough I) precipitation resulting in a single arc when tested against flagellar root extracts.

Analysis of 6 M urea extracts of flagellar root preparations by two-dimensional PAGE utilizing isoelectric focusing in the first dimension and SDS PAGE in the second dimension, demonstrates that the 20,000 Mr root protein is composed of two acidic components, which we call 20,000-Mr-α and 20,000-Mr-β (Fig. 4A). These components have estimated isoelectric points of 4.9 and 4.8, for α and β, respectively. Both 20,000-Mr-α and -β polypeptides can be identified in two-dimensional O'Farrell gels of whole cell preparations on the basis of their migration (Fig. 4B) and by immunoblot analysis of whole cell two-dimensional gel transfers using antisera specific for the 20,000-Mr flagellar root protein (Fig. 4C). Both 20,000-Mr-α and 20,000-Mr-β flagellar root components react with immune serum, whereas no other proteins show detectable reaction.

Variable Stoichiometry of 20,000-Mr-α and 20,000-Mr-β

Comparison of the relative amounts of 20,000-Mr-α and -β by densitometric scans of Coomassie Brilliant Blue-stained gels (Fig. 5) illustrates a variable stoichiometry of α to β, which ranges from 2:1 to 1:1. Changes in the ratio of α to β relate to the state of contraction or extension of striated flagellar roots immediately before cell lysis. Preparations from calcium-shocked cells, where most of the flagellar roots are contracted, show a higher ratio of α to β (Fig. 5, trace a). Preparations from cells that were not calcium shocked contain a mixture of extended, and partially contracted striated flagellar roots and these show a lower α to β ratio (Fig. 5, trace b).
Protein Phosphorylation

*Tetraselmis* cells rapidly incorporate $^{32}$PO$_4$ label into a number of proteins. Two dimensional O'Farrell gel analysis and radioautography demonstrates that the 20,000-$M_r$-$\alpha$ component of striated flagellar roots is a major acidic phosphoprotein of the cell (Fig. 6, B and C), whereas the 20,000-$M_r$-$\alpha$ component shows no detectable incorporation of label.

Prolonged exposure of the radioautogram (Fig. 6 C) reveals many phosphoproteins in addition to the 20,000-$M_r$-$\alpha$ component. In addition, the anti-20,000-$M_r$ serum specifically immunoprecipitates a $^{32}$P-labeled 20,000-$M_r$ protein from *Tetraselmis* whole cell extracts (Fig. 6 D). Thus, the variable stoichiometry of $\alpha$ to $\beta$ Coomassie staining bands probably corresponds to conversion of 20,000-$M_r$-$\alpha$ into 20,000-$M_r$-$\beta$ by phosphorylation.

Alkaline Urea Gel Electrophoresis

Both the $\alpha$ and $\beta$ components undergo a reduction in mobility when electrophoresed in polyacrylamide gels containing 6 M urea and millimolar free calcium (Fig. 7). This mobility shift was best demonstrated in two-dimensional gels where the protein was electrophoresed in low free calcium levels in the first dimension and either low or high free calcium levels in the second dimension. Under these conditions the 20,000-$M_r$-$\alpha$ and -$\beta$ components undergo a 16 and 24% reduction, respectively, in relative mobility in the presence of excess calcium.

Indirect Immunofluorescence and Immuno-Gold Labeling

Because the theca or cell wall of *Tetraselmis* posed a barrier to antibody penetration, both immunofluorescence and immuno-electron microscopic investigations were carried out on sectioned material. Analysis of fluorescent images (Fig. 8 B) of sections treated with the anti-20,000-$M_r$ immune serum reveals two cylindrical structures, often forming a V shape,
located in the anterior (flagellated) region of vegetative or interphase cells. Not all cells in a given section stain in this manner due to the limited probability of a section including the immunoreactive structures in an appropriate orientation. The lower portion of Fig. 8 illustrates several examples at higher magnification of selected cells stained with the anti-20,000-Mr serum. Fig. 8, G–H illustrate cells that had been subjected to a calcium shock before fixation. Preimmune controls (Fig. 8A) show only a low level fluorescence. A significant portion of the fluorescence in preimmune-treated samples can be attributed to residual pigment remaining in the cells, in that similar levels of fluorescence are observed in cells that have not been exposed to the secondary fluorescein conjugate (not shown).

Immunoelectron microscopy of anti-20,000-Mr-treated thin sections confirm that striated flagellar roots are the reactive structure because they label with the secondary gold conjugate (Fig. 9). Clearly, both extended (Fig. 9A) and contracted (Fig. 9B) flagellar roots show dense labeling, whereas preimmune treated controls (Fig. 9C) show only a sparse gold deposit.

DISCUSSION

Striated flagellar roots are nearly ubiquitous in occurrence among ciliated and flagellated eucaryotic cells. Their structure varies from the "massive" roots seen in green algae such as Tetraselmis, in ciliated epithelia (14) and in retinal rod cells (42), to the less prominent roots associated with the primary cilia of quiescent Balb/c 3T3 fibroblasts (46). Though the fine structure of striated flagellar roots was described early in the development of biological electron microscopy (see references 14 and 23) little was known of their function, and until now, of their composition.

Here we demonstrate that preparations highly enriched for striated flagellar roots are composed predominantly of an acidic protein of 20,000 Mr. This protein has been resolved into two components by isoelectric focusing and by two-dimensional electrophoresis in alkaline urea gels run in either low or high free calcium. These two components have been designated α and β. We were interested in the physical basis for two polypeptides with nearly identical Mr's but different molecular charges. We suspected that they were similar polypeptides differing in some form of posttranslational modification. Our analysis of variable ratios of α to β in cell extracts and our demonstration that the more acidic 20,000-Mr-β component is phosphorylated leads us to suggest that the 20,000-Mr-β arises through a phosphorylation of the 20,000-Mr-α component during flagellar root extension. At this time we do not know details concerning the mechanism of 20,000-Mr-β phosphorylation; these are the subject of current investigation. We have published, in a preliminary report (39),

**Figure 6** Analysis of phosphorylated proteins. Cells were incubated in $^{32}$PO$_4$ for 1 h before lysis. Two-dimensional gel of cell extracts, 12 μg of protein, (A) stained with Coomassie Brilliant Blue show the 20,000-Mr-α and -β flagellar root proteins (arrows). (B) Autoradiograph of the same gel shown in (A) after 1-h exposure illustrates that the 20,000-Mr-β flagellar root protein is a major acidic phosphoprotein, no $^{32}$P-label is associated with the 20,000-Mr-α component. (C) 2-h exposure of the gel illustrates other phosphoproteins labeled under the conditions used. (D) Immunoprecipitation of $^{32}$P-labeled flagellar root proteins. Cell lysates were treated with preimmune (P) or anti-20,000-Mr immune serum and precipitated as described in Materials and Methods. Clearly, the immune serum (I) precipitates a $^{32}$P-labeled protein of 20,000 Mr, and no other $^{32}$P-labeled bands. Molecular weight markers indicated at right are listed in Fig. 3.

**Figure 7** Two-dimensional alkaline urea gels of 6 M urea extracts of striated flagellar roots, 2 μg protein, run either in the presence of low or high free calcium reveal a retardation in mobility of both α and β polypeptides in the presence of excess free calcium. The samples shown here were run in the same first dimension gel in the presence of EGTA, individual lanes excised and equilibrated in either 2 mM EGTA (left) or 2 mM calcium (right) and run simultaneously in the second dimension in the presence of 2 mM EGTA (left) or 2 mM calcium (right). A separate lane from the first dimension gel has been placed above each slab to illustrate first dimension migration.
ATPase cytochemistry demonstrating the localization of activity to the cross striations of the flagellar roots, and Anderson (5) has reported ATPase activity associated with isolated basal bodies and associated structures from oviduct. Perhaps these activities are related to the phosphorylation and dephosphorylation of striated flagellar root proteins. We must, therefore, now consider two energy-requiring steps in striated flagellar root action. One involved in regulation of cytoplasmic free calcium levels (38) and a second more directly involved in 20,000-Mr protein phosphorylation probably during flagellar root extension.

Alkaline urea gels run in the presence of elevated calcium
illustrate that both polypeptides undergo a reduction in relative mobility suggesting direct binding of calcium to the protein. Such binding may alter the net charge and molecular conformation of the protein. In preliminary studies, we have assembled anastomosing networks of 3-nm diameter filaments from urea extracts of flagellar roots. These filaments convert into clusters of 16-nm diameter “globettes” at raised free calcium levels. These in vitro changes in morphology of reassembled filaments appear to correspond to changes in flagellar root filament morphology observed in intact organelles during contraction (cf. reference 39, Fig. 5). Our earlier observations (39) revealed distinct stages of filament shortening in situ that appeared as a twisting and supercoiling of individual filaments. The immunofluorescent and immunogold labeling studies confirm that the 20,000 Mr protein is a major structural component of striated flagellar roots. Based on the observations presented above, we suggest that the 20,000 Mr protein is the principal functional component of striated flagellar roots.

We do not know what relationship the polypeptides between 50,000 and 60,000 Mr, or the present, exist in low amounts in our preparations, have to the flagellar roots. These proteins may be components of basal bodies or other fibrous material associated with the isolated flagellar roots. Alternatively, certain of these proteins may be accessory molecules related to striated flagellar root organization or possibly to phosphorylation.

Flagellar Roots and Spasmonemes

Fibrous flagellar roots are of at least two morphologically distinct types: (a) microtubule-associated striated fibers with pattern repeat of 25-35 nm (also known as System I fibers, cf. reference 27), and (b) striated flagellar roots, such as those studied here, composed of a bundle of 3-7-nm diameter filaments with variable striation patterns of repeat <50 nm (also known as System II fibers, cf. reference 27). System II flagellar roots of the amoeboflagellate Naegleria guberi appear to be composed of a distinct class of high-molecular-weight proteins of 170,000 Mr (21). In contrast, another study (44) on the composition of flagellar roots of the System-II type isolated from gill cells of the bay scallop Aequipecten suggest that these organelles are composed of a high molecular weight doublet of proteins, which Stephens (44) named “ankyrin.” Analysis of the published gels, however, also reveals a major polypeptide component in the lower molecular weight region that may correspond to the 20,000 Mr protein. Further experimental analyses of striated flagellar roots from Aequipecten will be necessary in order to clarify the composition of these organelles.

Amos and coworkers (2-4, 36) have shown that the contractile fiber within the stalk of the ciliates Vorticella, Zoothera, and Carchesium is primarily composed of a calcium-binding contractile protein of 20,000 Mr, that they call “spasmin.” A preliminary investigation (Salisbury, J. L., and A. Baron, unpublished observation) demonstrates immunological cross-reactivity between the anti-20K immune serum and a 20,000-Mr component of Vorticella whole cell extracts by immunoblot analysis. On the basis of our results, we would suggest that striated flagellar roots of the type we have studied and the vorticellid spasmoneme are homologous organelles, and consequently that the 20,000 Mr flagellar root protein is a spasminlike molecule. If this hypothesis is correct, spasmin-like contractile machines may have developed before the ancient evolutionary divergence of these two groups of organisms.

Calcium-modulated Proteins

The major 20,000 Mr, striated flagellar root protein shares a number of features in common with members of the calcium-modulated regulatory protein family that includes calmodulin, parvalbumin, and troponin C (10, 18, 19). Common features of these proteins include low molecular weight (generally below 20,000), acidic isoelectric point, binding of calcium with high affinity (usually around pH 5-6) even in the presence of mild denaturants, and calcium-induced alterations in molecular conformation. In addition, a number of these proteins have phosphorylated forms, including calmodulin (32), myosin light chain (1) and the flagellar root protein (this study). Currently, there are no primary sequence data available for the flagellar root protein or other spasminlike proteins. Consequently, we do not know if these ion-sensitive contractile proteins share sequence homology within their calcium-binding domain(s), or with the calcium-binding domains of the calcium modulated regulatory proteins mentioned above (19). This is of particular interest in view of the fundamental role of calcium modulated regulatory proteins in motility phenomena. We postulate that ion-sensitive contractile systems such as the flagellar root represent a “primitive” motility mechanism possessing the qualities of simplicity of composition, and direct mediation of contraction by calcium binding. Perhaps, as more complex motility mechanisms arose (i.e., actomyosin- and microtubule-based systems) an evolutionary schism or divergence occurred, in which aspects of the primitive system were retained for either regulation (i.e., the calmodulin-troponin C group) or contractile (i.e., the spasminlike proteins) function.

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REFERENCES

1. Adelstein, R. S., and E. Eisenberg. 1980. Regulation and kinetics of the actin-myosin-ATP interaction. Annu. Rev. Biochem. 49:921-956.
2. Amos, W. B. 1971. Reversible mechanochemical cycle in the contraction of Vorticella. Nature (Lond.). 239:127-128.
3. Amos, W. B. 1975. Contraction and calcium binding in vorticellid ciliates. In Molecules and Cell Movement. R. E. Stephens and S. Inouye, editors. Raven Press, New York. 411-436.
4. Amos, W. B., L. M. Routledge, and F. F. Yew. 1975. Calcium-binding proteins in a vorticellid contractile organelle. J. Cell Sci. 14:313-326.
5. Anderson, R. G. W. 1977. Biochemical and cytochemical evidence for ATPase activity in basal bodies isolated from eukaryotic cells. J. Cell Biol. 74:547-560.
6. Andrews, A. T. 1981. Electrophoresis: Theory, Techniques, and Biochemical and Clinical Applications (Monographs on Physical Biochemistry Series) Oxford University Press, London. 336 pp.
7. Beckerle, M. C., and K. R. Porter. 1983. Analysis of the role of microtubules and actin in extracellular actin-based motility. J. Cell Biol. 96:354-362.
8. Cachon, J. M., and M. Cachon. 1981. Movement by non-actin filament mechanisms. Biosystems 14:313-326.
9. Cachon, M. J. M. Cachon, L. G. Tilney, and M. S. Tilney. 1977. Movement generated
by interactions between the dense material at the ends of microtubules and non-actin-containing microfilaments in *Sticholonche zanclea*. J. Cell Biol. 72:314-338.

10. Chen, W. Y. 1980. Calmodulin plays a pivotal role in cellular regulation. Science (Wash. DC). 207:19-27.

11. Dabrowska, R., J. M. F. Sherry, D. K. Aromatorio, and D. J. Hartshorne. 1978. Modulator protein as a component of the myosin light chain kinase from chicken gizzard. Biochemistry. 17:253-258.

12. De Mey, J., M. Moeremans, G. Geuens, R. Nuydens, and M. De Brabander. 1981. High resolution light and electron microscope localization of tubulin with IGS (immunogold staining) method. Cell Biol. Int. Rep. 5:889-899.

13. Ebashi, S., and M. Endo. 1968. Calcium and muscle contraction. Prog. Biophys. Mol. Biol. 18:123-183.

14. Fawcett, D. W., and K. R. Porter. 1954. A study of the fine structure of ciliated epithelia. J. Morph. 94:221-282.

15. Gietelman, S. E., and G. B. Witzman. 1980. Purification of calmodulin from *Chlamydomonas* calmodulin occurs in cell bodies and flagella. J. Cell Biol. 87:764-770.

16. Head, J. F., and S. V. Perry. 1974. The interaction of the calcium-binding protein (troponin C) with bivalent cations and the inhibitory protein (troponin I). Biochem. J. 137:145-154.

17. Kaküchi, S., and K. Sobue. 1983. Control of the cytoskeleton by calmodulin and calmodulin-binding proteins. Trends Biochem. Sci. 8:59-62.

18. Klee, C. B., T. H. Crouch, and P. G. Richman. 1980. Calmodulin. Annu. Rev. Biochem. 49:489-515.

19. Kretzinger, R. H. 1980. Structure and evolution of calcium-modulated proteins. Crit. Rev. Biochem. 8:119-174.

20. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.). 277:680-685.

21. Larson, D. E., and A. D. Dingle. 1981. Isolation, ultrastructure, and composition of the flagellar rootlet of *Naegleria gruberi*. J. Cell Biol. 89:424-432.

22. Mahtle, N. J., R. J. Redman, A. R. Means, J. G. Chaofueas, and B. Satir. 1981. Presence and indirect immunofluorescent localization of calmodulin in *Paramecium tetraurelia*. J. Cell Biol. 89:695-699.

23. Mason, L. 1959. Plast cilia and associated organelles. In Cellular Mechanisms in Differentiation and Growth. D. Rudnick, editor. Princeton University Press, Princeton, N.J. 61-72.

24. Mason, L., and M. Parke. 1965. Observations on the fine structure of two species of *Planomonas* with special reference to flagellar structure in plants. Recent Adv. Bot. Res. 2:1-21.

25. Mayer, R. J., and J. H. Walker. 1980. Immunocytochemical Methods in the Biological Sciences: Enzymes and Proteins. Academic Press, Inc., New York, pp 168.

26. Means, A., and J. G. Chaofueas. 1982. Regulation by and of calmodulin in mammalian cells. Cold Spring Harbor Symp. Quant. Biol. 46:903-908.

27. Melkonian, M. 1979. An ultrastructural study of the flagellate *Tetraselmis subcordiformis* Stein (Chlorophyceae) with emphasis on the flagellar apparatus. Protoplasma. 98:139-152.

28. Melkonian, M. 1980. Ultrastructural aspects of basal body associated fibrous structures in green algae: a critical review. Biosystems. 12:83-104.

29. Melkonian, M. 1983. Functional and phylogenetic aspects of the basal apparatus in green algae. *Submicrosc. Cytol.* 15:105-110.

30. Naitoh, Y., and H. Kaneko. 1972. Reactivated triton-extracted models of *Paramecium*: modification of ciliary movement by calcium ions. Science (Wash. DC). 176:521-524.

31. Norris, R. E. 1980. Pratichorphytes. In Phytoflagellates. E. R. Cos, editor. Elsevier North-Holland, Inc., New York. 82-145.

32. Plancke, Y. D., and E. Laxaires. 1983. Evidence for a phosphorylated form of calmodulin in chicken brain and muscle. Mol. Cell. Biol. 3:1412-1420.

33. O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 230:4007-40.

34. Oakley, B. R., D. R. Kirsch, and N. R. Morris. 1980. A simplified ultrarapid silver stain for detecting proteins in polyacrylamide gels. *Anal. Biochem.* 105:361-363.

35. Plutzek, D. R. 1974. Basal bodies and root structures. In *Cilia and Flagella*. M. A. Sleigh, editor. Academic Press, Inc., New York. 427-464.

36. Routledge, L. M., W. B. Amos, E. F. Yew, and T. Weis-Fogh. 1976. New calcium-binding contractile proteins. In Cell Motility. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Conference on Cell Proliferation, Vol. 3, pp 93-114.

37. Robenek, H., and M. Melkonian. 1979. Rhizoplast-membrane associations in the flagellate *Tetraselmis subcordiformis* Stein (Chlorophyceae) revealed by freeze fracture and thin sections. Arch. Protistenkd. 122:340-351.

38. Salisbury, J. L. 1982. Calcium sequestering vesicles and contractile flagellar roots. *J. Cell Sci.* 58:433-443.

39. Salisbury, J. L. 1983. Contractile flagellar roots: the role of calcium. *J. Submicrosc. Cytol.* 15:105-110.

40. Salisbury, J. L., and G. L. Floyd. 1978. Calcium induced contraction of the rhizoplast of a quadriflagellate green alga. *Science (Wash. DC).* 202:975-978.

41. Salisbury, J. L., J. Swanson, G. L. Floyd, R. Hall, and N. J. Mabille. 1981. Ultrastructure of the flagellar apparatus of the green alga *Tetraselmis subcordiformis* with special consideration given to the function of the rhizoplast and rhizoplast. *Protoplasma.* 107:1-11.

42. Sjostrand, F. S. 1953. The ultrastructure of the inner segments of the retinal rods of the guinea pig eye as revealed by electron microscopy. *J. Comp. Physiol. 42*:45-70.

43. Sligh, M. A. 1979. Contractility of the roots of flagella and cilia. *Nature (Lond.).* 277:283-284.

44. Stephens, R. E. 1975. The basal apparatus. *J. Cell Biol.* 64:408-420.

45. Towein, H., T. Stechelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:5300-5304.

46. Tucker, R. W., A. B. Pardee, and K. Fujisawa. 1979. Centriole ciliation is related to quiescence and DNA synthesis in 3T3 cells. Cell. 7:527-535.

47. Wasko, D. M., T. J. Singh, and J. H. Wang. 1978. The modulator-dependent protein kinase: a multifunctional protein kinase activatable by the calcium-dependent modulator protein of the cyclic nucleotide system. *J. Biol. Chem.* 253:3387-3392.

48. Welsh, M. J., J. R. Redman, B. R. Britskley, and A. R. Means. 1978. Calcium-dependent regulator protein: localization in mitotic apparatus of eukaryotic cells. *Proc. Natl. Acad. Sci. USA.* 75:1867-1871.

49. Wolniak, S. N., P. K. Hepler, and W. T. Jackson. 1983. Ionic changes in the mitotic modification of ciliary movement by calcium ions. *Science (Wash. DC).* 176:521-524.