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Contractile Eukaryotic Flagella: Centrin Is Involved

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Summary

Isolated transverse flagella of *Peridinium inconspicuum* (*Dinophyceae*) undergo a rapid Ca^{2+}-induced (50 µM Ca^{2+}) contraction in the absence of exogenous ATP. Longitudinal flagella from the same species do not contract under these conditions. Contraction leads to a supercoiling of the axoneme and a shortening of the paraxonemal fiber that accompanies the axoneme over most of its length. Using a polyclonal antibody generated against centrin, a 20 kDa Ca^{2+}-modulated contractile protein of striated flagellar roots of the green flagellate *Tetraselmis striata*, we have found that the paraxonemal fiber in transverse flagella of three taxa of *Dinophyceae* is immunoreactive by indirect immunofluorescence. The localization of the antigen in the paraxonemal fiber of transverse flagella was confirmed by two-colour double immunofluorescence using monoclonal mouse-anti-β-tubulin for identification of the axoneme. No structure was immunoreactive to anticentrin in the longitudinal flagella of all taxa. Electrophoretic and immunoblot analysis of isolated flagella of *P. inconspicuum* show that the antigen is a 21 kDa protein, indicating that it is either centrin or a closely related protein. We conclude that centrin confers contractility to the transverse flagellum of dinoflagellates and possibly to other contractile eukaryotic flagella.

Keywords: Eukaryotic flagella; Contractility; Centrin; Ca^{2+}-modulated contractile protein; Dinoflagellates; Transverse flagellum.

Abbreviations: ASP-H artificial seawater medium with Hepes-buffer; BSA bovine serum albumine; DTT dithiothreitol; EGTA ethylene glycol bis(2-amino-ethylether)tetraacetic acid; FITC fluorescein isothiocyanate; MT-buffer microtubule stabilizing buffer; PBS phosphate buffered saline; SDS sodium dodecyl sulfate; TLCK Nα-p-tosyl-l-lysine chloromethyl ketone; TRITC tetramethylrhodamine isothiocyanate.

1. Introduction

Eukaryotic flagella exhibit a variety of motile behaviours (GRAY 1928, JAHN and BOVEE 1967, SLEIGH 1974). The two most common types of flagellar motion, the undulatory-type motion with bending wave propagation and the cilia-type motion with effective and recovery strokes have been well characterized (recent reviews: NAITOH and SUGINO 1984, SATIR 1985, GIBBONS *et al.* 1985, MACHEMER 1986). The force-generating system during these types of flagellar motions involves interactions between dynein-ATPases and tubulin to produce relative sliding of adjacent microtubule doublets in the axoneme (for reviews, see GIBBONS 1981, JOHNSON 1985). Little attention has so far been paid for other types of flagellar motions except for the flagellar cell surface motility system of *Chlamydomonas* (BLOODGOOD 1987). It has long been known that the longitudinal flagellum of some *Dinophyceae* is capable of rapid contractions (SCHÜTT 1895, METZNER 1929, PETERS 1929, AFZELIUS 1969). Only relatively recently has this process been studied in some detail in *Ceratium tripos* (MARUYAMA 1981, 1982, 1985 a, 1985 b). The 220 µm long flagellum of this species retracts into the sulcus within 28 msec after mechanical stimulation of the cell (MARUYAMA 1981). Reextension of the folded longitudinal flagellum takes usually about 1–5 seconds (MARUYAMA 1981). It was also shown that contraction in reactivated models of longitudinal flagella required at least 10 µM Ca^{2+} but was independent of ATP (MARUYAMA 1985 b). An ultrastructural analysis of the longitudinal flagellum of *C. tripos* revealed the presence of a cross-striated fiber (R-fiber; MARUYAMA 1982) consisting of fine filaments that supercoil upon contraction of the flagellum (MARUYAMA 1982). In the longitudinal flagellum of dinoflagellates such cross-striated fibers seem to be confined to only a few taxa (perhaps only those that are capable of rapid flagellar contractions), whereas similar types of fibers (known

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as striated strands or paraxial rods) (for a review, see MOESTRUP 1982) are presumably universally present in the transverse flagella of dinoflagellates. Interestingly, MARUYAMA (1982) reported that, when the longitudinal flagellum of *C. tripos* contracts, the transverse flagellum stops beating for about one second before it resumes motility. GAINES and TAYLOR (1985) studying the transverse flagellum of other dinoflagellates have suggested that the stop response and an accompanying pull of the axoneme toward the cell surface is caused by a contraction of the striated strand.

The characteristics of the contractile behaviour of dinoflagellate flagella suggested to us that the contractile mechanism could be a centrin-mediated process. Centrin, a Ca^{2+}-modulated contractile protein is universally associated with eukaryotic basal bodies or centrioles (SALISBURY *et al.* 1984, SALISBURY *et al.* 1986, COLING and SALISBURY 1986, SCHULZE *et al.* 1987). It is involved in a variety of extremely rapid motile responses in protists such as basal body reorientations during photophobic responses (MCFADDEN *et al.* 1987) and contraction of cross-striated flagellar roots upon flagellar shedding (SALISBURY and FLOYD 1978, MCFADDEN and MELKONIAN 1986a, SALISBURY *et al.* 1987). In this study we present evidence that the transverse flagellum of dinoflagellates is contractile and that contractility is presumably mediated by centrin, which is localized in the striated strand or paraxonemal fiber.

2. Materials and Methods

2.1. Strains and Culture Conditions

Peridinium inconspicuum Lemmermann (UTEX LB 2255) (STARR and ZEIKUS 1987) was cultured in 5 l Erlenmeyer flasks in a modified WARIS-solution (WARIS-H) (KIES 1967 modified by MCFADDEN and MELKONIAN 1986b). *Gyrodinium resplendens* Hulburt (UTEX LB 1655) and *Scrippsiella trochoidea* (Stein) Loeblich III (kindly provided by Dr. K. Roberts, University of Southwestern Louisiana, Lafayette, LA, U.S.A.) were cultured in Petri-dishes in the synthetic seawater medium ASP-H (MCFADDEN and MELKONIAN 1986 b). All cultures were maintained at 15 °C in a 14/10 hr light/dark cycle (light intensity: 15 W/m^2).

2.2. Light Microscopy of Living Cells

Vegetative cells of *S. trochoidea* were embedded in 0.8% ultra low gelling temperature agarose (FMC BioProducts, Rockland, ME, U.S.A.) in ASP-H (REIZE and MELKONIAN, in prep.). For comparison cells were fixed in 1% OsO_4 and 2% glutaraldehyde in ASP-H at room temperature for 5 minutes and washed in ASP-H (MELKONIAN 1982). Cells were viewed with a Zeiss IM 35 inverted microscope using Nomarski interference contrast optics (100 × oil immersion lens). Photographs were taken on Kodak Technical Pan film (2415) in large-scale (4 ×) format.

2.3. Isolation of Flagella

Cells from 250–500 ml of a logarithmically growing culture of *P. inconspicuum* were concentrated by careful filtration without application of external pressure to a final volume of 2–3 ml using a 5 µm membrane filter (Millipore, no. SMWP 047000). Cells were then deflagellated by rapid dilution of the suspension with 3–5 times the volume ice-cold MT-buffer (15 mM Hepes, 10 mM EGTA, 15 mM KCl, 5 mM $MgSO_4$, pH 7.2) and 5 seconds vortexing at full speed (Heidolph Reax 2000). The deflagellated cells were sedimented at 60 **g** for 8 minutes (Heraeus Labofuge I). The supernatant including the flagella were pelleted at 2,000 **g** for 10 minutes (Heraeus Labofuge I) and resuspended in MT-buffer.

2.4. Calcium Induced Contraction of Isolated, Transverse Flagella

Isolated flagella of *P. inconspicuum* were transferred to MT-buffer containing 5×10^{-5} M Ca^{2+} (Ca^{2+}-buffered solution according to BESSEN *et al.* 1980) and immediately fixed in 3% freshly prepared paraformaldehyde on ice for 15 minutes. Flagella were washed in MT-buffer and viewed with a 63 × oil immersion lens using Nomarski interference contrast optics.

2.5. Electron Microscopy of Isolated Flagella of P. inconspicuum

Whole mounts of isolated flagella for electron microscopy were prepared by placing a drop of paraformaldehyde-fixed flagella on a pioloform-coated EM-grid for 30 minutes. The flagella were negatively stained with 2% aqueous uranylacetate according to standard techniques. Flagella were viewed with a Siemens Elmiskop 102.

2.6. Indirect Immunofluorescence

For indirect immunofluorescence cells and/or isolated flagella were prepared using three different techniques:

P. inconspicuum: flagella were isolated as described above and allowed to settle on pre-cleaned polylysine-coated coverslips. Flagellar membranes were permeabilized with 0.5% Nonidet P-40 (Sigma) in ice-cold MT buffer containing 1.5% freshly prepared paraformaldehyde for 15 minutes. Subsequently, the flagella were fixed in 3% paraformaldehyde (in ice-cold MT-buffer) for further 15 minutes.

G. resplendens: cells were allowed to adhere to polylysine-coated coverslips for 10 seconds. After removal of the culture medium, the coverslips were plunged into cold acetone (–20 °C) for 5 minutes, followed by rinsing with MT-buffer.

S. trochoidea: cells were fixed on ice in culture medium containing 0.5% paraformaldehyde for 15 minutes. The cells and detached flagella were pelleted at 10,000 **g** for 5 minutes (Heraeus Biofuge B). The pellet was resuspended in 1% Nonidet P-40 in MT-buffer and further incubated on ice for 30 minutes. Cells and flagella were washed twice with ice-cold MT-buffer, resuspended into cold acetone (–20 °C), incubated in acetone for 30 minutes, washed in MT-buffer and allowed to adhere to polylysine-coated coverslips.

The subsequent steps were identical for all preparations. The cells and/or flagella were washed with phosphate buffered saline (PBS), quenched with PBS/1% bovine serum albumine (BSA; RIA-grade; Sigma) for 15 minutes at room temperature, then incubated with the polyclonal anti-centrin (1 : 200 dilution in PBS/1% BSA; for source and characterization of the antibody, see SALISBURY *et al.* 1984) for 60 minutes at 37 °C. Coverslips were washed extensively in PBS, followed by quenching with PBS/1% BSA for 15 minutes. TRITC-conjugated goat-anti-rabbit IgG (Sigma) diluted 1 : 40 with PBS/1% BSA was used as the secondary antibody. Specimens were incubated

for 60 minutes with the secondary antibody at 37 °C, thereafter washed extensively in PBS and mounted in 0.1% p-phenylenediamine (Sigma) in glycerine/PBS (1 : 2).

Monoclonal mouse-anti-β-tubulin IgG (Amersham) was used to visualize tubulin in axonemes of isolated flagella of *P. inconspicuum*. The antibody was diluted 1 : 1,000 in PBS/1% BSA, and as secondary antibody FITC-conjugated goat-anti-mouse IgG (1 : 20 dilution in PBS/1% BSA) was used. Details of incubation were as described above for the anti-centrin immunofluorescence. The specimens were observed with a Zeiss IM 35 equipped with epifluorescence (63× or 100× oil immersion lenses). Fluorescence photographs were taken with Ilford HP5 film, phase contrast controls with Kodak Technical Pan (2415) film.

2.7. *Double Immunofluorescence*

For two colour double immunofluorescence flagella of *P. inconspicuum* were in sequence incubated in anti-centrin IgG, TRITC-conjugated goat-anti-rabbit IgG, mouse-anti-β-tubulin, and FITC-conjugated goat-anti-mouse IgG (for details of the procedure see above). Flagella were photographed using Kodak Ektachrome 400 film.

2.8. *Electrophoretic and Immunoblot Analysis*

Flagella of *P. inconspicuum* were isolated in MT-buffer with 0.1 mM TLCK (Sigma) and 1 mM DTT (Boehringer, Mannheim, Federal Republic of Germany) as described above. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was prepared following the method of LAEMMLI (1970) in 13% mini-slab gels and 0.1% sodium dodecyl sulfate. For immunoblot analysis proteins were electrophoretically transferred to nitrocellulose membranes (Millipore, Immobilon, ref. no. IPVH 00010) essentially using the method of TOWBIN *et al.* (1979). Immunostaining was done according to SUREK and LATZKO (1984) using protein A-coated 15 nm gold particles.

3. Results

A distinctive characteristic of most dinoflagellates is the presence of a transverse flagellum that encircles the cell within a surface groove or cingulum (Figs. 1 and 2). In agreement with observations by other authors (BERDACH 1977, REES and LEEDALE 1980, GAINES and TAYLOR 1985) we find that the axoneme of the transverse flagellum in the three taxa investigated is helical irrespective of whether living cells or carefully fixed cells are studied (Figs. 1 and 2). It is also clear that the helix is left-handed but not isotropic, a number of loops (in *S. trochoidea* usually 11) exist: the helix is less steep at its outer edges (the outer edge is nearly vertical; Fig. 2) than at its inner edge. The distance between loops is rather constant in a given species: in *S. trochoidea* it is about 4 μm. Optical sections of living cells embedded in agarose reveal that the outer edges of the loops protrude considerably beyond the cell surface and that the diameter of one loop is about 2 μm (Fig. 1). When flagella of *P. inconspicuum* are isolated using cold shock in Ca^{2+}-free MT-buffer (see Materials and Methods) transverse and longitudinal flagella are shed from the cells. Flagella are stable in MT-buffer for several hours in the cold (Fig. 3). Isolated transverse flagella can be easily distinguished from longitudinal flagella at the light microscope level because they are longer (60–80 μm compared to 30–40 μm) and exhibit a more irregular coiled outline than the longitudinal flagella (Figs. 3–13). Isolated transverse flagella do not retain their *in situ* and *in vivo* shape (compare Figs. 1 and 2 with Figs. 3 and 5): in Ca^{2+}-free MT-buffer the regular appearance of loops is lost and isolated flagella are less and more irregularly coiled than *in situ*. Electron microscopy revealed that isolated flagella retain most of their flagellar membrane, but that the membrane does not seal over the shear region (not illus-

Figs. 1 and 2. Nomarski interference contrast light micrographs of *Scrippsiella trochoidea*

Fig. 1. Optical cross section through the cingulum revealing circular axonemal coils of the transverse flagellum on opposite sides of the cell. Living cell embedded in agarose (see Materials and Methods). Bar: 10 μm

Fig. 2. Tangential view of the cingulum (oblique view from the anterior end of the cell). Helical coils of the transverse flagellum are clearly visible. Fixed cell. Bar: 10 μm

Figs. 3 and 4. Nomarski interference light micrographs of isolated transverse flagella of *Peridinium inconspicuum*

Fig. 3. Four transverse flagella isolated in Ca^{2+}-free MT-buffer (for details see Materials and Methods). Bar: 20 μm

Fig. 4. Three transverse flagella after transfer to 50 μM Ca^{2+} (in MT-buffer). The flagella occur in a contracted state. Bar: 20 μm

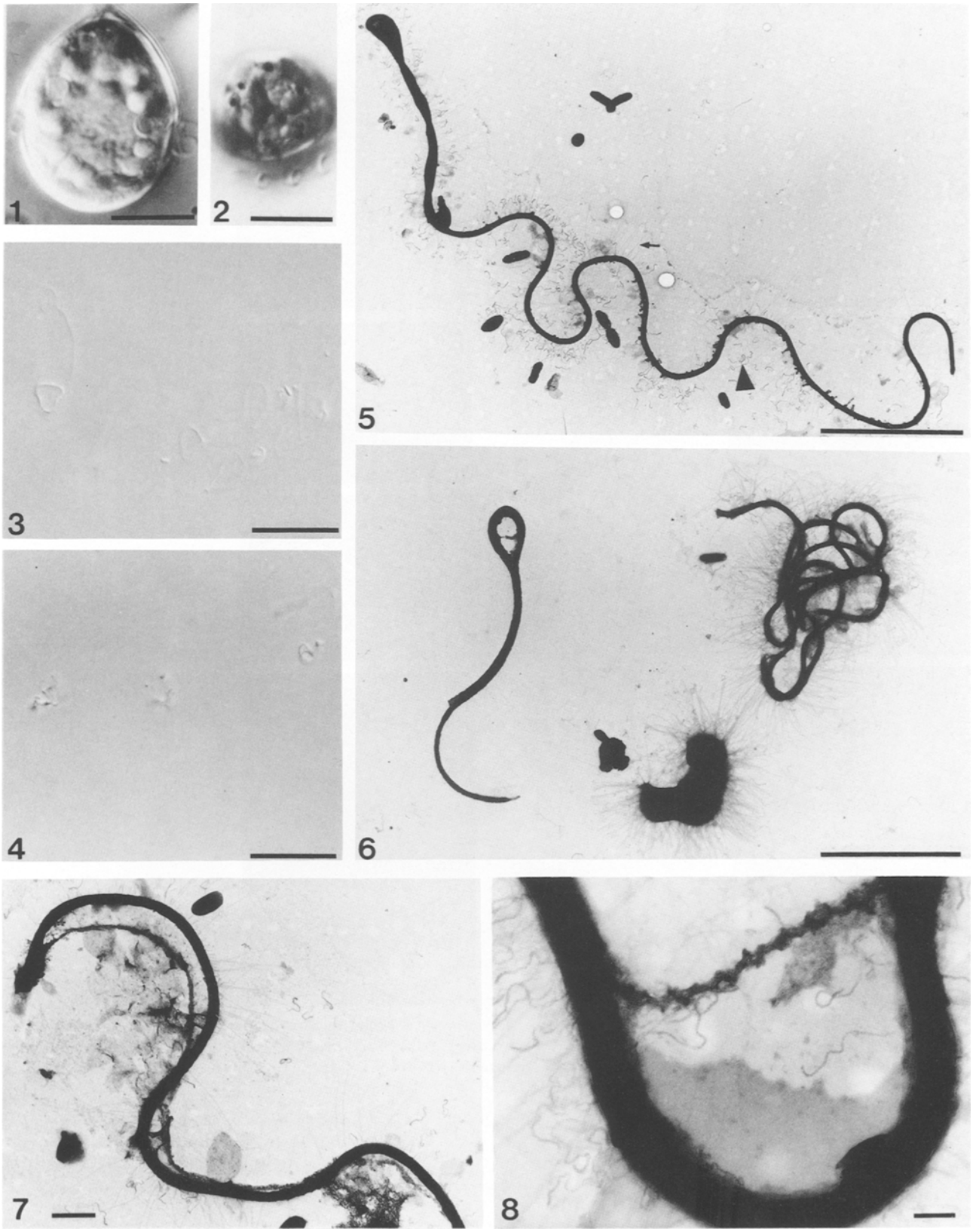
Figs. 5–8. Whole-mount electron microscopy of isolated transverse flagella of *P. inconspicuum*

Fig. 5. Transverse flagellum isolated in Ca^{2+}-free MT-buffer. → Long, thin hairs; ▶ short, thick hairs. Bar: 5 μm

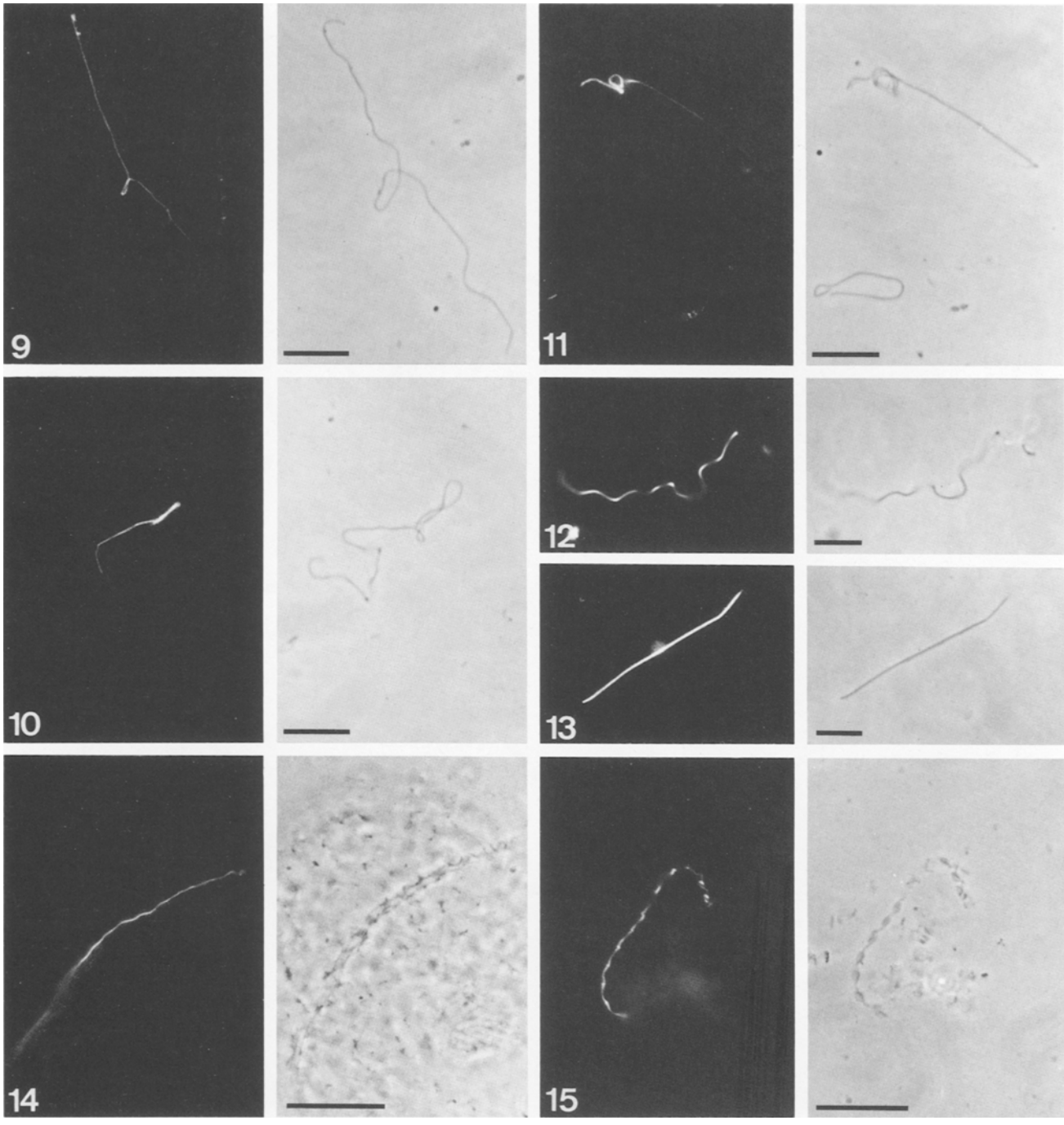
Fig. 6. Two transverse flagella and one longitudinal flagellum (left) after exposure to 50 μM Ca^{2+} in MT-buffer. The transverse flagella have contracted to different degrees (compare with Fig. 5; flagellar hairs still attached), the longitudinal flagellum does not contract in response to Ca^{2+}. Bar: 5 μm

Fig. 7. A partially demembranized transverse flagellum. A paraxonemal fiber accompanies the axoneme. Bar: 1 μm

Fig. 8. A demembranized part of a transverse flagellum. The paraxonemal fiber is capable of significant contraction (apparently by coiling). Bar: 0.2 μm



Figs. 1–8



Figs. 9–11. Indirect immunofluorescence of isolated flagella of *P. inconspicuum* using polyclonal anti-centrin. Left micrographs: fluorescent flagella, right micrographs: corresponding phase contrast images of the same flagella. Notice that the immunofluorescent rods do not correspond with the major structural elements of the flagella as seen in the corresponding phase contrast images (especially Figs. 9 and 10). The longitudinal flagellum (bottom of Fig. 11) does not show immunofluorescence when exposed to anti-centrin

Figs. 12 and 13. Indirect immunofluorescence of isolated flagella of *P. inconspicuum* using mouse monoclonal anti-β-tubulin. Left micrographs: fluorescent flagella, right micrographs: corresponding phase contrast images. Bar: 10 μm

Fig. 12. Transverse flagellum

Fig. 13. Longitudinal flagellum

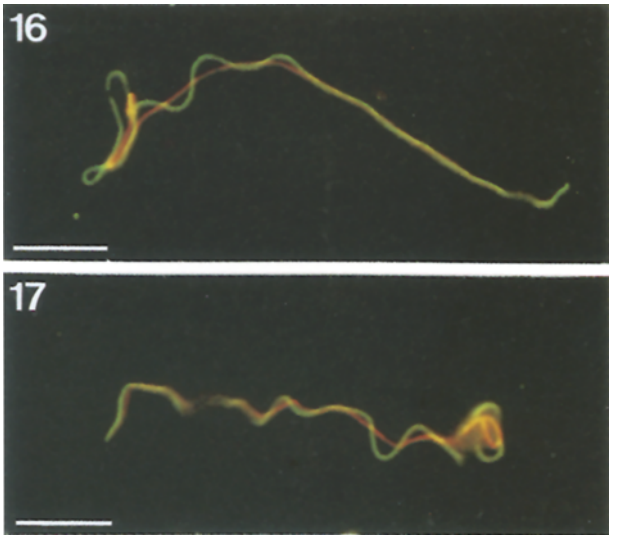
Fig. 14. Indirect immunofluorescence of the transverse flagellum of *Gyrodinium resplendens in situ* using anti-centrin. Left micrograph: fluorescent flagellum, right micrograph: corresponding phase contrast image. Bar: 20 μm

Fig. 15. Indirect immunofluorescence of a detached transverse flagellum of *Scrippsiella trochoidea* using anti-centrin. Left micrograph: fluorescent flagellum, right micrograph: corresponding phase contrast image. Bar: 20 μm

trated). At the EM-level each transverse flagellum contains a thin fiber (here termed paraxonemal fiber) that runs parallel to the axoneme; in whole mount preparations this fiber is only visible when the flagellum is partially demembranized (compare Fig. 7 with Fig. 5). In addition the surface of the transverse flagellum is covered by two types of flagellar hairs that are attached to the flagellar membrane in a single row near the axoneme: long, thin flagellar hairs (diameter: 5 nm; length: 2.8 μm; Fig. 5, arrow) form bundles of 5–10 hairs, and short, thicker, non-tubular hairs (diameter: 15 nm; length: 670 nm; Fig. 5, arrowhead) often detach during isolation and preparation of the flagella. When isolated flagella of *P. inconspicuum* are resuspended in 50 μM Ca^{2+} the transverse flagella undergo dramatic changes in shape: flagella supercoil within a few seconds to a highly contracted state (Figs. 4 and 6). Interestingly, longitudinal flagella are unaffected by 50 μM Ca^{2+} and do not change shape (Fig. 6). Contraction of transverse flagella occurs in MT-buffer in the absence of exogenous ATP. Electron microscopy of partially demembranized transverse flagella shows that the paraxonemal fiber is capable of significant contraction (Fig. 8). We have localized an antigenic homolog to centrin by indirect immunofluorescence in isolated flagella of *P. inconspicuum* (Figs. 9–11). Immunolocalization occurs only in transverse flagella, not in longitudinal flagella (Fig. 11). The antigenic structure is a thin fluorescent filament that extends over most of the flagellar length (Figs. 9–11). Careful examination of the phase contrast images reveals that the fluorescent strand corresponds not to the major longitudinal structural component of transverse flagella–the axoneme–but is separate from it (Figs. 9 and 10).

Transverse flagella of two other taxa of *Dinophyceae, Gyrodinium resplendens* (Fig. 12) and *Scrippsiella trochoidea* (Fig. 13) are also immunoreactive to anti-centrin. Pre-immune controls do not show any recognizeable labelling in flagella of the three taxa investigated (not illustrated).

To verify that anti-centrin labelling is distinct from the axoneme we sought to immunolabel the axoneme with antitubulin. After several trials with different antibodies we found that a mouse monoclonal anti-β-tubulin reacts strongly with the axonemes of both types of flagella (Figs. 12 and 13). Using a two-colour double-immunofluorescence approach we can clearly show that anti-centrin immunolabelling is structurally distinct from the axoneme and that the two fluorescent rods run parallel to each other over most of the length of the transverse flagellum (Figs. 16 and 17).



Figs. 16 and 17. Two-colour double-immunofluorescence of isolated transverse flagella of *P. inconspicuum* using anti-centrin (secondary antibody: TRITC-conjugated goat-anti-rabbit IgG; red fluorescence) and mouse monoclonal anti-β-tubulin (secondary antibody: FITC-conjugated goat-anti-mouse IgG; green fluorescence). Bar: 10 μm

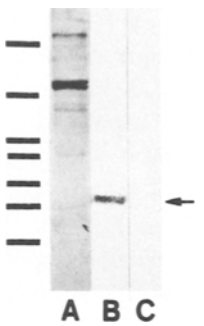


Fig. 18. SDS-PAGE analysis of isolated flagella of *Peridinium inconspicuum*. *A* Amido-black stained nitrocellulose blot of isolated flagella. *B* Nitrocellulose blot of flagella labelled with anti-centrin and protein A/colloidal gold showing one antigenic protein band of Mr 21,000 (arrow). *C* Nitrocellulose blot of isolated flagella incubated in preimmune IgG and protein A/colloidal gold. Molecular weight markers indicated at left are from top to bottom bovine serum albumin (67,000), ovalbumin (45,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), trypsinogen PMSF-treated (24,000), trypsin inhibitor (20,100), α-lactalbumin (14,200)

Immunoblots of isolated flagella of *P. inconspicuum* as separated by SDS-PAGE (13%) and labelled with anti-centrin detect a single antigenic polypeptide of Mr 21,000 (Fig. 18). The major protein in the amido-black stained nitrocellulose blot (Fig. 18 *a*) has an Mr of 50,100 and is presumably tubulin.

4. Discussion

In recent years considerable evidence has accumulated that in eukaryotic cells a cell motility system exists which is based on the contraction (*i.e.*, supercoiling) of non-actin filaments (HOFFMANN-BERLING 1958, AMOS 1971, AMOS *et al.* 1975, SALISBURY and FLOYD 1978, CACHON and CACHON 1981, SALISBURY 1983, SALISBURY *et al.* 1984, SALISBURY *et al.* 1986, MCFADDEN *et al.* 1987). The phenomenological characteristics of this cell motility system are a) a rapid contraction of a fibrillar structure within less than 20 msec to usually less than 50% of its original length and b) a much slower reextension of the fibrillar structure to its original length (in the range of a few seconds up to about one hour in one example). We may therefore call this motile system a "shock-motility system". Other common aspects of the shock-motility system of eukaryotes are a) it is based on a supercoiling of filaments not on a sliding mechanism (SALISBURY 1983), b) it is initiated by the binding of Ca^{2+} to the major filament protein which then presumably undergoes large conformational changes that lead to the contraction of the filaments (AMOS 1971), c) the Ca^{2+}-modulated contractile proteins belong to a family of low molecular weight (20–22 kDa) acidic phosphoproteins that have been termed centrins or spasmins (AMOS *et al.* 1975, SALISBURY *et al.* 1984, COLING and SALISBURY 1987) and which show considerable sequence homology to calmodulin (COLING and SALISBURY 1987, HUANG *et al.* 1987), d) reextension of the filaments requires removal of Ca^{2+} from the protein (SALISBURY *et al.* 1984, SALISBURY *et al.* 1987), e) phosphorylation of the protein is necessary for repeated cycles of contraction/reextension (SALISBURY *et al.* 1987).

The use of polyclonal and monoclonal antibodies against centrin (isolated from the striated flagellar roots of the green flagellate *Tetraselmis striata*; SALISBURY *et al.* 1984) has made possible the localization of this antigen in a great variety of eukaryotic organisms (WRIGHT *et al.* 1985, SALISBURY *et al.* 1986, MCFADDEN *et al.* 1987, SCHULZE *et al.* 1987, MELKONIAN *et al.* 1988, and own unpublished observations). From these studies it can be concluded that centrin is universally associated with eukaryotic basal bodies or centrioles. The present study extends this conclusion in several ways: we demonstrate the presence of centrin for the first time in dinoflagellates, a phylogenetically isolated group of protists (TAYLOR 1988). In addition we show that centrin may be located inside a eukaryotic flagellum and is therefore not exclusively associated with the basal apparatus. Centrin apparently confers contractility to eukaryotic flagella, at least to the transverse flagellum of dinoflagellates. That centrin is involved in the contractile behaviour of the transverse flagellum of dinoflagellates is evident from the following observations: contraction of isolated transverse flagella occurs upon exposure to 50 μM Ca^{2+} in the absence of exogenous ATP; contraction is apparently a rapid response; a polyclonal antibody against centrin localizes an antigen in the paraxonemal fiber of the transverse flagellum but not in the non-contractile longitudinal flagellum of the species studied; immunoblot analysis shows that the antigen is a 21 kDa polypeptide; the paraxonemal fiber consists of filaments that upon exposure to Ca^{2+} undergo contraction by supercoiling. In addition to localization of centrin in the paraxonemal fiber of transverse flagella in dinoflagellates we have also found this antigen in structures associated with the basal apparatus of several dinoflagellates (HÖHFELD *et al.* unpublished observations).

Compared to the wealth of information that is now available about the occurrence of centrin in various eukaryotic organisms, still very little is known about the physiological significance of this cell motility system. Present evidence, exclusively obtained from studies of two centrin-containing structures in green algae, namely the nucleus-basal body connector (SALISBURY *et al.* 1984, WRIGHT *et al.* 1985, MCFADDEN and MELKONIAN 1986, SCHULZE *et al.* 1987, SALISBURY *et al.* 1987) and the connecting fiber (MCFADDEN *et al.* 1987), suggests that centrin-mediated cell motility represents a phobic shock-motility system. As a result of a light shock or a mechanical shock (deflagellation) an influx of Ca^{2+} into the cell is initiated that triggers a rapid contraction of centrin-containing filaments. The cell responds in different ways depending on the types of structures that the centrin-containing filaments are linked to: basal bodies reorient (MCFADDEN *et al.* 1987), the nucleus moves towards the basal bodies (WRIGHT *et al.* 1985, SALISBURY *et al.* 1987) or a cell wall opening is closed (MCFADDEN and MELKONIAN 1986 a). In a similar way the spasmoneme of vorticelloid ciliates and the myonemes of other contractile ciliates undergo rapid contractions upon mechanical shock (AMOS 1971, HUANG and PITELKA 1973). What is then the physiological significance of centrin-mediated contraction in the dinoflagellate transverse flagellum? The fact that isolated transverse flagella of *P. inconspicuum* upon exposure to 50 μM Ca^{2+} exhibit a strong contractile response that leads to a supercoiling of the whole flagellum indicates that the intraflagellar

Ca^{2+}-concentration must be strictly regulated since such a strong response does not occur *in vivo*. Since isolation of transverse flagella in Ca^{2+}-free (about 10^{-9} M Ca^{2+}) buffers yields a relatively extended, not regularly coiled configuration of the axoneme that differs from the *in vivo* configuration (see Results) we conclude that the paraxonemal fiber *in vivo* is partially contracted and that the differences in length between the paraxonemal fiber and the axoneme are responsible for the appearance of tight axonemal coils during flagellar movement and for retaining the transverse flagellum inside the cingulum by providing a constant curvature to the flagellum. Such a function for the paraxonemal fiber has previously also been suggested by Rees and Leedale (1980) and we agree with these authors that the occurrence of special "anchoring threads" as postulated by other authors (Taylor 1975, LeBlond and Taylor 1976) is not necessary to explain the unique location of this flagellum inside the cingulum.

The possibility should also be discussed that a contraction of the paraxonemal fiber caused by a rise in the intraflagellar Ca^{2+}-concentration could exert an effect on axonemal beat. Several possibilities can here be envisaged: number, steepness or diameter of the axonemal coils may be modified thus possibly affecting the swimming behaviour of the cell. Additionally axonemal wave propagation may be inhibited by a contraction of the paraxonemal fiber, *i.e.* a stop response of the cell would result. Stop responses are a common feature of swimming dinoflagellates (Jahn *et al.* 1963, Forward 1974, Hand and Schmidt 1975). Now that intact transverse flagella can be isolated from dinoflagellates it should be possible to reactivate flagellar motility *in vitro* and to study the functional relationships between the paraxonemal fiber and the axoneme in greater detail. Although we have not yet tried to localize centrin in the longitudinal flagellum of *Ceratium* spp., the characteristics of the contractile reponse of the longitudinal flagellum (see Introduction) strongly suggest to us that the R-fiber (Maruyama 1982) should also contain centrin. Finally we would like to point out that in the *Dinophyceae* there are a number of non-actin filament systems known, some of which are apparently involved in rapid motile responses (Cachon and Cachon 1981, Cachon *et al.* 1983, Cachon and Cachon 1984, Cachon and Cachon 1985).

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