

The Centrin-based Cytoskeleton of *Chlamydomonas reinhardtii*: Distribution in Interphase and Mitotic Cells

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Abstract. Monoclonal and polyclonal antibodies raised against algal centrin, a protein of algal striated flagellar roots, were used to characterize the occurrence and distribution of this protein in interphase and mitotic *Chlamydomonas* cells. *Chlamydomonas* centrin, as identified by Western immunoblot procedures, is a low molecular (20,000-*M*_r) acidic protein. Immunofluorescence and immunogold labeling demonstrates that centrin is a component of the distal fiber. In addition, centrin-based flagellar roots link the flagellar apparatus to the nucleus. Two major descending fibers extend from the basal bodies toward the nucleus; each descending fiber branches several times giving rise to 8–16 fimbria which surround and embrace the nucleus. Immunogold labeling indicates that

these fimbria are juxtaposed to the outer nuclear envelope. Earlier studies have demonstrated that the centrin-based linkage between the flagellar apparatus and the nucleus is contractile, both in vitro and in living *Chlamydomonas* cells (Wright, R. L., J. Salisbury, and J. Jarvik. 1985. *J. Cell Biol.* 101:1903–1912; Salisbury, J. L., M. A. Sanders, and L. Harpst. 1987. *J. Cell Biol.* 105:1799–1805). Immunofluorescence studies show dramatic changes in distribution of the centrin-based system during mitosis that include a transient contraction at preprophase; division, separation, and re-extension during prophase; and a second transient contraction at the metaphase/anaphase boundary. These observations suggest a fundamental role for centrin in motile events during mitosis.

THE flagellar apparatus of *Chlamydomonas reinhardtii* is a useful model system for the characterization of the basal body/centriole (Johnson and Porter, 1968; Coss, 1974; Cavalier-Smith, 1974) and centrosome (Mazia, 1984) cycles in eucaryotic cells. Recent studies with polyclonal and monoclonal antibodies have demonstrated that the “centrins” are ubiquitous centrosome-associated proteins of eukaryotic cells of diverse origins, including: algae, protozoa, mammals (Salisbury et al., 1984, 1986, 1987; Schulze et al., 1987; Baron, A., and J. L. Salisbury, manuscript in preparation), and higher plants (Cho, S., and S. Wick, University of Minnesota, St. Paul, MN, personal communication). The study of centrin, therefore, has direct bearing on our understanding of the centrosome and cell cycle.

Centrin is a component of several morphologically well-defined centrosome-associated structures, which are composed of 6-nm filaments and show calcium-sensitive contractile or elastic behavior (Salisbury et al., 1983, 1984; and this study). We have shown that centrin-based flagellar roots are contractile under conditions of elevated calcium in a variety of algae, including *Chlamydomonas* and *Tetraselmis* (Salisbury and Floyd, 1978; Salisbury et al., 1984; 1986a; 1987; McFadden et al., 1987). Recent molecular cloning studies have demonstrated that centrin is a member of the calcium-binding protein superfamily, which includes parvalbumin,

calmodulin, and troponin C. Centrin has also been shown to bind calcium and undergo mobility shifts in alkaline urea polyacrylamide gels (Salisbury et al., 1984); this behavior is characteristic of proteins in this superfamily. In addition, centrin has a phosphorylated isoform (Salisbury et al., 1984). Studies on *Tetraselmis* and detergent extracted models of *Chlamydomonas* indicate that ATP and presumably phosphorylation are required for cycles of calcium-induced contractile behavior (Salisbury et al., 1984; 1987).

In mammalian cells, centrin homologs components of centriolar are basal feet, pericentriolar satellites, pericentriolar matrix, and the spindle poles and spindle matrix of dividing cells (Salisbury et al., 1986; Baron, A., and J. L. Salisbury, manuscript in preparation). In “lower” eukaryotes, centrin is a component of contractile striated flagellar roots and distal fibers (McFadden et al., 1987; Salisbury et al., 1984; 1987; Schulze et al., 1987; and this study). Centrin is the structural basis of a novel cytoskeletal system, which appears to be involved in basal body/centriole positioning and reorientation (Wright et al., 1985; McFadden et al., 1987; Baron, A., and J. L. Salisbury, manuscript in preparation), and, in certain cells, in nuclear movement and nuclear shape changes (Salisbury et al., 1987). Here we examine, in detail, the dynamics of centrin distribution in interphase and mitotic cells of the unicellular alga, *Chlamydomonas reinhardtii*.

Materials and Methods

Cell Culture

Chlamydomonas reinhardtii, wild-type strain 137c derivative N0mt⁺ and the cell wall-less mutant cw92 (provided by Dr. J. Jarvik, Carnegie-Mellon University, Pittsburgh, PA), were maintained in Medium I of Sager and Grannick (1954) in 10-ml tubes under a 14:10 h light/dark cycle at 24°C.

Monoclonal and Polyclonal Antibodies

The polyclonal serum, 08/28, was raised against SDS-PAGE purified centrin from the alga *Tetraselmis striata* and has been characterized elsewhere (Salisbury et al., 1984). The monoclonal antibody, 17E10, was raised against centrin of *T. striata* prepared according to the procedure of Salisbury et al. (1986b). Female Balb/c mice (Jackson Laboratories, Bar Harbor, ME) were primed by subcutaneous injection of 25 µg centrin, which had been emulsified in complete Freund's adjuvant. Three subsequent boosts of 25 µg centrin in PBS (10 mM KH₂PO₄ and K₂PO₄, 150 mM NaCl, pH 7.2) were given at 21-d intervals by i.p. injection. Spleens were removed from immunized mice 3 d after the final injection for hybridoma production. Spleen cells (6 × 10⁸) were fused with 6 × 10⁷ SP-2/0 cells (Shulman et al., 1978) as described by Goldsby and coworkers (1977). After fusion the cells were diluted in HAT (hypoxanthine/aminopterin/TdR; Sigma Chemical Co., St. Louis, MO) supplemented RPMI-1640 medium containing 20% fetal bovine serum, 20 mM Hepes (pH 7.3), 2 mM L-glutamine, 1 mM Na-pyruvate and antibiotic/antimycotic solution (Biologos, Naperville, IL). All cell culture reagents were purchased from Gibco (Grand Island, NY) except where otherwise indicated. The cells were distributed in 0.1-ml aliquots to 96-well microtiter plates. Hybridomas were screened by an ELISA on 96-well plates coated with 25 µg centrin per plate, using alkaline phosphatase-conjugated secondary anti-mouse Ig (Organon Teknica-Cappel, West Chester, PA) according to the methods of Engvall and Perlmann (1971). Selected hybridomas were cloned by limiting dilution according to Goldsby and Zipser (1969). Monoclonal antibody, 17E10, was used where indicated as a concentrated culture supernate.

Immunofluorescence

Cells were fixed in 4% fresh paraformaldehyde buffered with 10 mM Hepes (pH 7.0) for 15 min and allowed to adhere to 8-well glass slides (Carlson Scientific, Peotone, IL), which had been pretreated with 0.1% polyethylenimine (Sigma Chemical Co.). For studies on mitotic cells, cultures were harvested and fixed at 30-min intervals for up to 3 h into the dark cycle. After three washes in PBS the cells were permeabilized in -20°C methanol for 10 min, followed by -20°C acetone for 5 min, and air dried. The cells were incubated in primary monoclonal antibody (17E10, diluted 1:100 in PBS, 5% fetal bovine serum, and 5% glycerol) for 1 h, washed three times in PBS, and reacted with secondary fluorescein-conjugated goat anti-mouse Ig (1:400 dilution in PBS, Organon Teknica-Cappel) for 1 h. The preparations were washed for 15 min in three changes of PBS, mounted in a 50% glycerol/50% PBS, pH 8.5, containing 2% n-propylgallate (Kodak, Rochester, NY), and observed using a Nikon Optiphot microscope equipped for epifluorescence (excitation 450–490 nm, 520 nm barrier filter). Immunofluorescence images were recorded on Hypertech film (Microfluor Ltd., Stony Brook, NY) with 10–20 s exposures, and developed in D-19 developer at 68°F for 4 min.

Electron Microscopy

Fixation of *Chlamydomonas* cells for EM was carried out according to the procedure of Dr. K. McDonald (University of Colorado, Boulder, CO; personal communication) as modified below. Cultures were harvested and fixed in 3% glutaraldehyde buffered with 10 mM Hepes (pH 7.2) for 2 h at 4°C. After a buffer 10 mM Hepes (pH 7.2) wash the samples were post-fixed in 1% osmium tetroxide and 0.8% K₃Fe(CN)₆ in 4 mM phosphate buffer for 30 min at 4°C. Samples were washed with deionized water, mordanted with 0.15% aqueous tannic acid for 1 min at room temperature, washed with deionized water, and stained with 2% aqueous uranyl acetate for 2 h in the dark. After washing with deionized water, the samples were dehydrated through an ethanol series, cleared with propylene oxide, and embedded in Poly/Bed 812. Blocks were polymerized at 60°C for 48 h. Silver-gold sections were collected on copper grids, post-stained at room temperature with aqueous 2% uranyl acetate for 15 min followed by Reynold's lead citrate for 15 min. Sections were observed and photographed on a JEOL 1200 electron microscope.

Immunogold Labeling

Cells were fixed in 3% glutaraldehyde buffered with 10 mM Hepes (pH 7.2) for 15 min at 4°C and washed three times in 10 mM Hepes buffer. Free aldehyde groups were reduced by treatment with aqueous sodium borohydride (7 mg/ml) for 15 min at 4°C and washed three times with 10 mM Hepes. The material was dehydrated through an ethanol series at -20°C. Samples were infiltrated with Lowicryl resin (Pella, Inc., Redding, PA), cured at -20°C using UV light, sectioned, and collected on formvar-coated nickel grids. Sections were hydrated with deionized water for 10 min, blocked in 0.1% BSA and 5% normal goat serum in Tris-buffered saline (TBS¹; 10 mM Tris-HCl, 150 mM NaCl, pH 7.4) for 1 h, incubated in primary monoclonal antibody (17E10 diluted 1:100 in TBS) for 2 h, washed for 15 min with several changes of TBS, and reacted with secondary goat anti-mouse Ig conjugated to 10-nm colloidal gold (1:40 dilution in TBS, pH 8.2, containing 0.1% BSA and 5% normal goat serum, Janssen Pharmaceutica, Piscataway, NJ) for 1 h. Sections were washed as above, postfixed in aqueous 1% glutaraldehyde, washed with deionized water, and post stained as above before observation.

Western Immunoblot Analysis

Tetraselmis cells were harvested by centrifugation (121 g for 5 min). The cell pellet was resuspended to 30 times its volume with SDS-PAGE sample buffer (Laemmli, 1970) sonicated for 10 s, and boiled for 2 min before gel loading. *Chlamydomonas*, strain cw92, nuclear flagellar apparatus were prepared according to a modified procedure of Wright and coworkers (1985). Cells were harvested by centrifugation as above, resuspended in a microtubule-stabilizing buffer (MSB; 30 mM Tris-acetate, pH 7.3, 5 mM MgSO₄, 5 mM EGTA, 25 mM KCl), allowed to equilibrate for 5 min, centrifuged as above, and lysed in lysis buffer (LB; 2% NP-40 in MSB). Lysis was achieved by adding 10 vol LB to 1 vol of pelleted cells, gently inverting the tube five times to completely resuspend the cells; the lysate was then brought to 100 times the volume of the original cell pellet with MSB. After a 121 g centrifugation for 5 min, the supernate was collected and centrifuged at 3,000 g for 10 min to pellet the nuclear flagellar apparatus. Pellets were resuspended to four times their volume with SDS-PAGE sample buffer and processed as above. Proteins were separated by SDS-PAGE through a 5–15% polyacrylamide gradient (Laemmli, 1970). Protein transfer to nitrocellulose and immunostaining were carried out according to Towbin and coworkers (1979) with the addition of 6 mM CaCl₂ to the transfer buffer to optimize the binding of centrin. Monoclonal antibody, 17E10, and polyclonal serum, 08/28, were used at 1:100 and 1:500 dilutions in TBS, respectively. The appropriate HRP-conjugated secondary antibodies (1:500 dilution in TBS, Organon Teknica-Cappel, West Chester, PA) were used to visualize the reactive proteins.

Results

Chlamydomonas Centrin by Western Immunoblot Techniques

SDS-PAGE and Western immunoblot analysis of *Chlamydomonas* and *Tetraselmis* show that centrin is a 20,000-mol wt protein (Fig. 1). Both polyclonal antisera, 08/28 (lane A), and monoclonal antibody, 17E10 (lane C), recognize this protein in *Chlamydomonas*. Lane B demonstrates that the monoclonal antibody, 17E10, recognizes the same protein in *Tetraselmis*; no other bands are labeled.

Centrin Organization in Interphase Cells

Fig. 2 a illustrates a phase contrast image of an interphase *Chlamydomonas* cell in longitudinal profile. The immunolocalization of centrin stained with the monoclonal antibody, 17E10, in a cell with a similar orientation is illustrated in Fig. 2 b. Another cell viewed from the anterior end is also shown (Fig. 2 c). The monoclonal antibody, 17E10,

1. Abbreviation used in this paper: TBS, Tris-buffered saline.

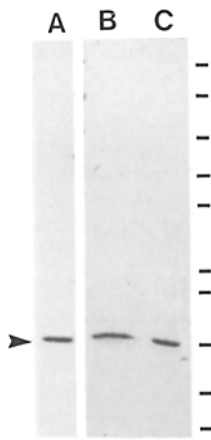


Figure 1. SDS-PAGE and Western blot analysis of *Chlamydomonas* and *Tetraselmis* centrin. (Lane a) Western blot of *Chlamydomonas* nuclear flagellar apparatus probed with polyclonal anti-centrin serum, 08/28. (Lane b) Western blot of *Tetraselmis* whole cell extracts probed with monoclonal anti-centrin antibody, 17E10. (Lane c) Western blot of *Chlamydomonas* nuclear flagellar apparatus probed with monoclonal anti-centrin antibody, 17E10. Note that only a single protein of $\sim 20,000$ mol wt is identified in each preparation. Molecular weight markers indicated at the right are, top to bottom: 180,000, 116,000, 84,000, 58,000, 48,000, 36,500, 26,600, 20,100, 18,400, and 14,200 M_r .

stains these cells with remarkable detail and resolution when compared with images obtained with polyclonal serum, 08/28 (see Salisbury et al., 1984; 1986a; Wright et al., 1985). The distal fiber which links the two basal bodies is distinctly labeled. Also labeled are flagellar roots which link the basal bodies of the flagellar apparatus to the nucleus. The flagellar roots are comprised of two major descending fibers, which extend from the flagellar apparatus toward the nucleus; each descending fiber branches several times giving rise to 8–16 fimbria which surround and embrace the nucleus (see also Fig. 5). The basal body-nuclear linkage is robust since isolated nuclei remain associated with the flagellar apparatus through several centrifugation steps (Wright et al., 1985).

Transmission electron microscopy of *Chlamydomonas* prepared by conventional fixation procedures (Ringo, 1967; Johnson and Porter, 1968; Goodenough and Weiss, 1978)

has not revealed the centrin-based flagellar root system observable by immunofluorescence microscopy. To visualize these structures we have found it necessary to prepare *Chlamydomonas* cells using procedures that use iron containing mordants and tannic acid (Fig. 3). A median longitudinal section of a cell prepared in this way reveals a pair of darkly staining thin descending fibers, which extend from the lateral wall of each basal body toward the nucleus. These fibers are closely adherent to the membrane of the anterior-most mitochondria of the cell (see also Fig. 3, d–f). Fig. 3, a–c illustrate that the descending fibers originate above the proximal fibers of the basal bodies; they attach to the outer wall of basal body microtubules of triplet number 7 and 8 as defined by the nomenclature of Hoops and Witman (1983). Each descending fiber is composed of a bundle of fine filaments ~ 6 -nm in diameter (Fig. 3, d–f). Careful analysis of serial sections indicates that each descending fiber is a flat ribbon ~ 1 to $3 \mu\text{m}$ long, 80 – 200 -nm wide, and 10 -nm thick. The descending fibers therefore occur in only one or two consecutive serial thin sections of cells in longitudinal orientation.

Immunogold staining using monoclonal antibody, 17E10, at electron microscopic resolution illustrates that the distal fiber and the descending fibers contain centrin (Fig. 4 a). Note that the proximal fibers which attach the basal bodies to one another near their base do not label with anti-centrin antibodies. Based on immunofluorescence each descending fiber branches at the level of the nucleus giving rise to a system of fimbria. Transverse cross-sections through the cell at the level of the nucleus reacted with monoclonal antibody, 17E10, and gold-conjugated secondary antibody, reveal that the centrin-based fimbria are intimately associated with the nuclear envelope (Fig. 4 b).

Our observations demonstrate that *Chlamydomonas* cells elaborate a novel cytoskeletal system that is based, at least in part, on centrin (Wright et al., 1985; Salisbury et al.,

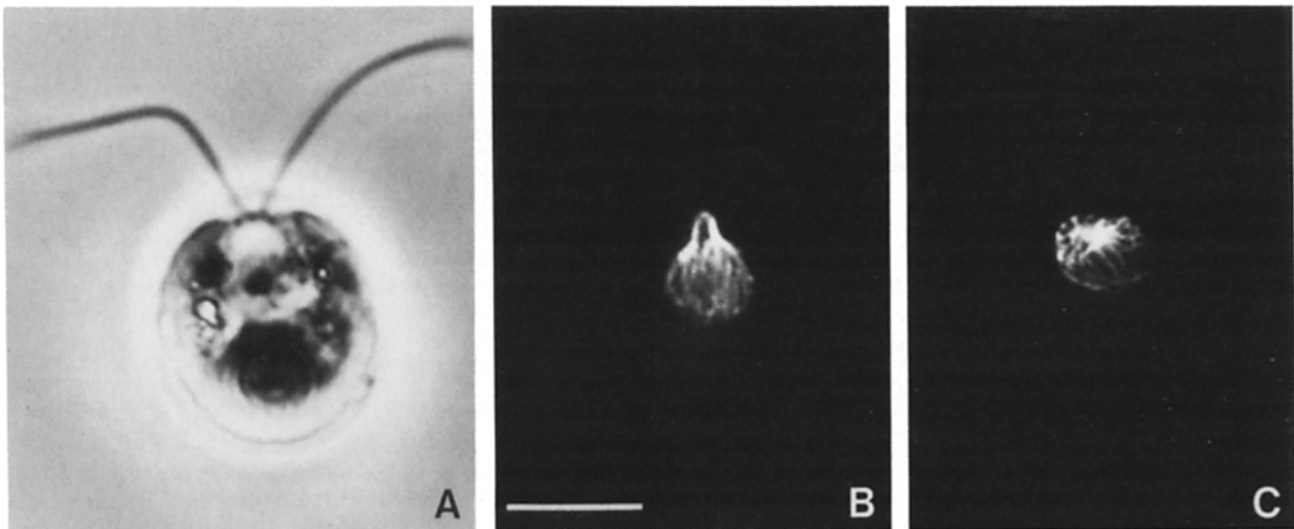


Figure 2. Localization of *Chlamydomonas* centrin. (a) Phase contrast image of a *Chlamydomonas* cell viewed in longitudinal profile. (b) Indirect immunofluorescence of centrin localization using monoclonal antibody, 17E10, in a cell in the same orientation as in (a). (c) Indirect immunofluorescence of centrin localization using monoclonal antibody, 17E10, in a cell viewed from the anterior end. Anti-centrin labeling of the distal fiber, two descending fibers, and their fimbria (~ 16) are evident. Bar, $5 \mu\text{m}$.

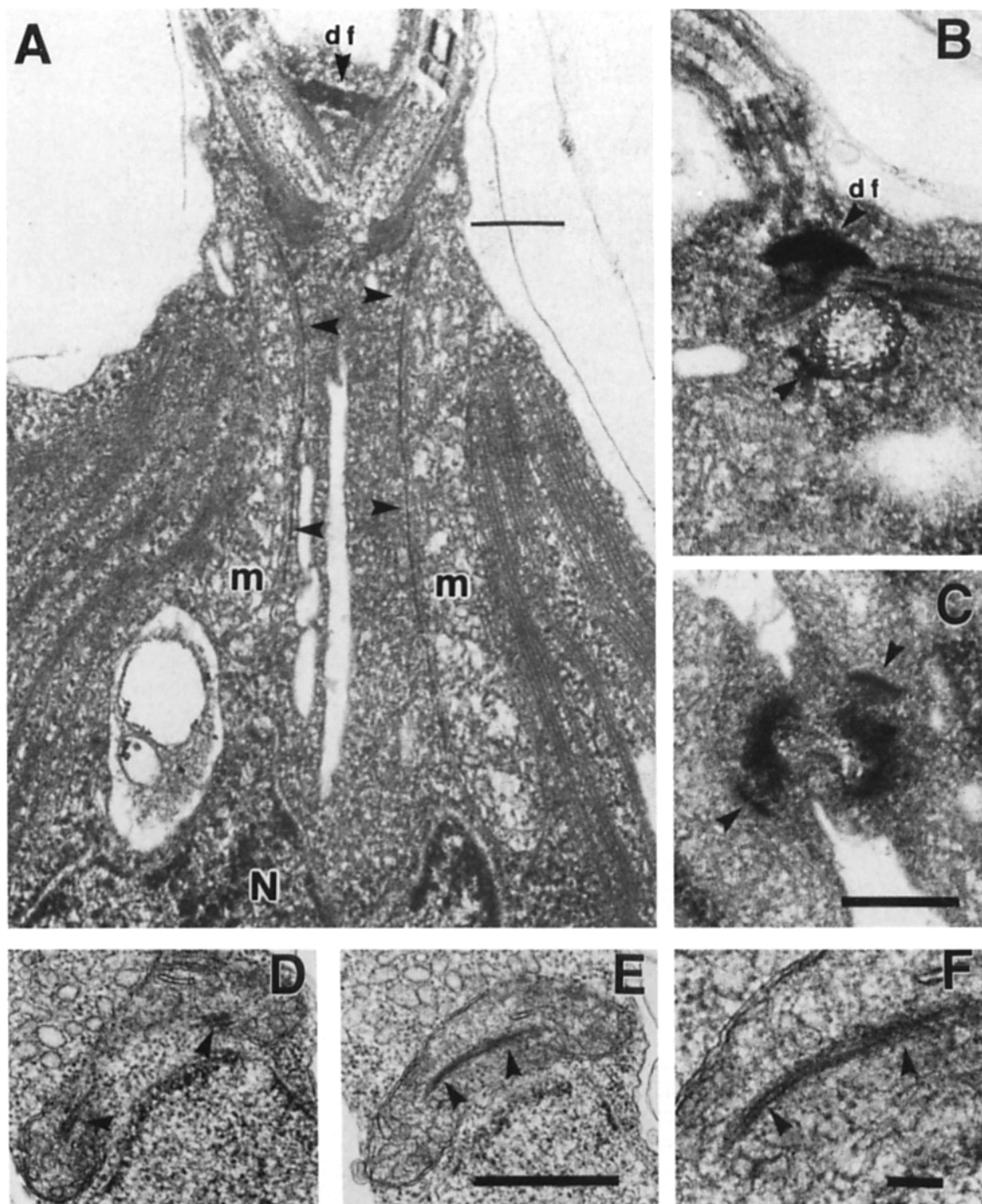


Figure 3. Ultrastructure of the centrin-based fiber system of *Chlamydomonas*. (a) Longitudinal section through the flagellar apparatus illustrating the distal fiber (df) and the darkly staining descending fibers (arrowheads). (b) Cross section through the flagellar apparatus showing the origin of the descending fiber (arrowhead) at triplets 7 and 8 of the basal body. (c) Cross section (at the level of the basal plaque; see indication in a) just proximal to the basal bodies illustrating position, width, and thickness of the two descending fibers (arrowheads). (d-e) Consecutive serial sections of the surface of a mitochondrion showing the descending fibers. (f) Higher magnification illustrating the filaments (arrowheads) of a descending fiber. Mitochondria (m); nucleus (N). Bars: (a-e) 0.25 μm ; (f) 0.1 μm .

1987; and this study). To integrate the organization of the centrin-based cytoskeletal system with earlier studies on the distribution of microtubules in interphase cells (Ringo, 1967; Goodenough and Weiss, 1978), we have illustrated the three-dimensional organization of the *Chlamydomonas* cytoskeleton in Fig. 5.

Centrin Organization during Mitosis

The centrin-based linkage between the flagellar apparatus and the nucleus undergoes a dramatic reorganization during mitosis. Centrin immunolocalization throughout the cell cycle is illustrated in Fig. 6. The centrin-based fiber system of

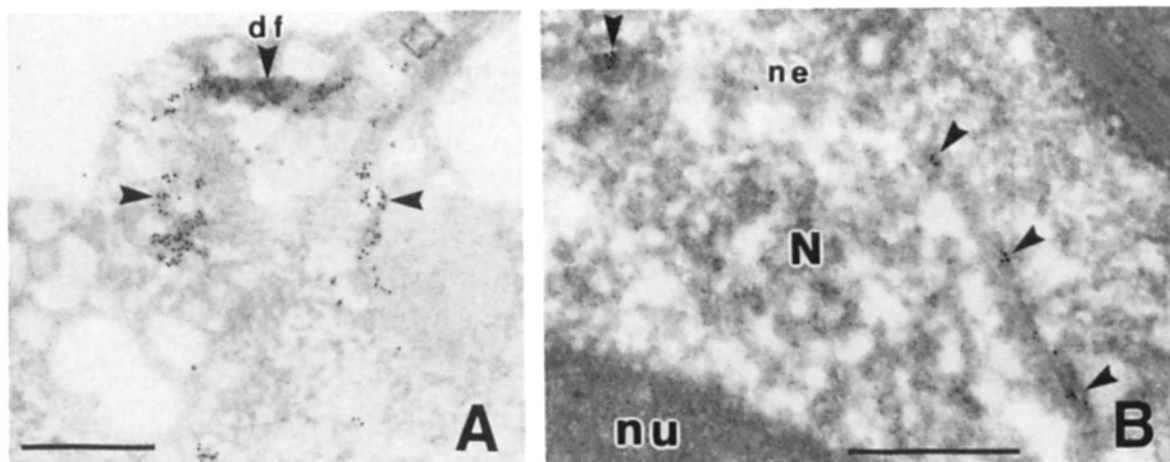


Figure 4. Immunogold localization of centrin in *Chlamydomonas*. (a) Longitudinal section through the flagellar apparatus labeled with monoclonal antibody, 17E10, and secondary 10-nm colloidal gold Ig-conjugate illustrating labeling of the distal fiber (df) and descending fibers (arrowheads). (b) Cross section of the nucleus (N) illustrating gold-labeled centrin containing fimbria (arrowheads) in close association with the nuclear envelope (ne). Nucleolus (nu). Bars, 0.25 μ m.

interphase cells (Fig. 6 a) undergoes a pronounced contraction at the interphase/preprophase boundary to form a tight aggregate of material in the anterior-most region of the cell (Fig. 6 b). This coincides temporally with the movement of the nucleus toward the flagellar apparatus (cf. Coss, 1974; Triemer and Brown, 1974), loss of flagella, and a subtle cell shape change that is particularly evident in cell wall-less mutants (e.g., cw92, not shown; also see Doonan and Grief, 1987). During prophase the centrin cytoskeleton divides (Fig. 6, c–d). The separated centrin foci move to opposite poles of the nucleus, re-extend and outline a symmetrical (crescent-shaped) spindle at metaphase (Fig. 6 d). At the onset of anaphase the crescent-shaped centrin array undergoes a second transient contraction and separation, thus delineating two half-spindles (Fig. 6 e). As anaphase proceeds each half-spindle continues to separate (Fig. 6 f). During telophase the centrin array of each daughter nucleus again re-extends (Fig. 6, f–g). Thus, by the time of cytokinesis the centrin-based cytoskeleton has returned to an interphase organization (Fig. 6 h). It is also evident from the micrographs that post-mitotic cells show a reduction to approximately half the number of fimbria. In *Chlamydomonas* this entire process may either repeat itself to give rise to four daughter cells, or the newly divided pair of cells can grow flagella and swim away.

Discussion

The Centrin-based Cytoskeleton of *Chlamydomonas*

Centrin of *Chlamydomonas* represents a novel cytoskeletal fiber system distinct from the actin (Detmers et al., 1985) and tubulin (Ringo, 1967; Goodenough and Weiss, 1978; Doonan and Grief, 1987) cytoskeletons of this cell. Centrin is a component of the distal fiber which links adjacent basal bodies to one another (McFadden et al., 1987; and this study). In addition, centrin containing flagellar roots link the flagellar apparatus to the nucleus through a pair of descending fibers. These fibers extend into the cytoplasm and branch into 8–16 fimbria, which associate with the nuclear envelope.

Although the early light microscopic investigation of Kater (1929) described this system in remarkable detail, more recent ultrastructural analyses (Ringo, 1967; Johnson and Porter, 1968; Goodenough and Weiss, 1978) did not reveal the centrin-based flagellar roots. Our studies (Wright et al., 1985; Salisbury et al., 1987; Salisbury, 1988; and this study) of the *Chlamydomonas* flagellar apparatus using iron containing mordants and tannic acid, and antibodies directed against centrin demonstrate that this cytoskeletal system does indeed exist.

The Function of Centrin in Interphase *Chlamydomonas*

There is a growing body of knowledge regarding the function of centrin in interphase cells. Distal fiber contraction mediates *in vivo* changes in flagellar position during the photophobic response in the alga *Spermatozopsis* (McFadden et al., 1987). High free calcium levels have also been shown

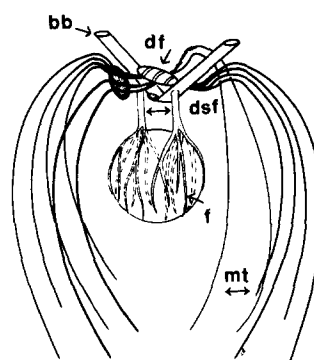


Figure 5. Diagram illustrating the organization of microtubule- and centrin-based cytoskeletal systems of *Chlamydomonas*. Two basal bodies (bb) are connected by a distal (df) and two proximal fibers. Four groups of microtubules (mt) with a 4/2/4/2 organization originate below the distal fiber and extend into a cortical array into the cytoplasm; one of the two membered microtubule roots has an associated mating structure (oval, top left cf. Ringo, 1967; Goodenough and Weiss, 1978). Two descending fibers (dsf) originate at the lateral wall of each basal body, extend into the cytoplasm, and branch into 8–16 fimbria (f) which embrace the nuclear envelope. Centrin-containing structures include the distal fiber, the two descending fibers and their fimbria.

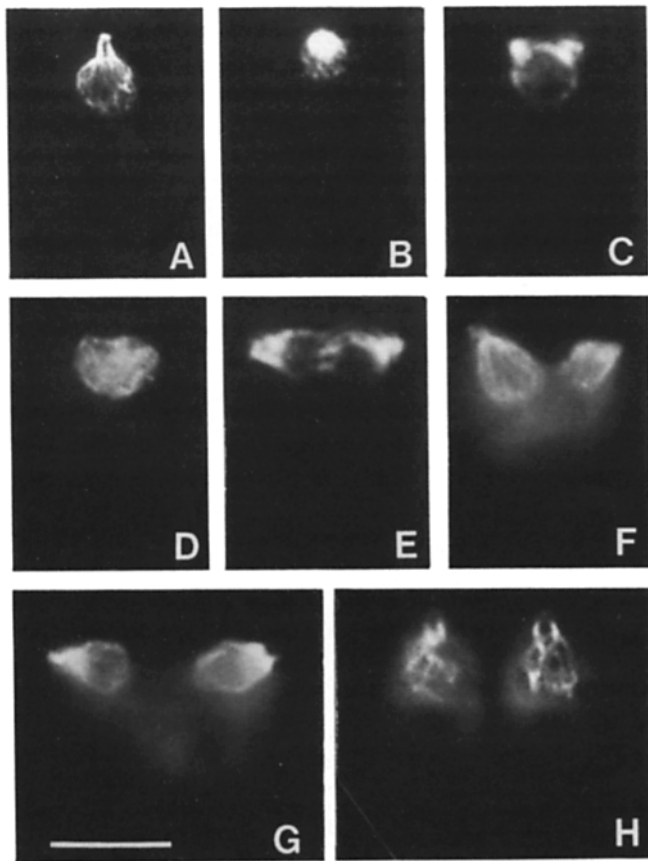


Figure 6. Centrin distribution during mitosis in *Chlamydomonas* as revealed by indirect immunofluorescence using monoclonal antibody, 17E10. (a) Interphase centrin distribution. (b) Preprophase centrin contraction. (c) Prophase separation of centrin foci and beginning of re-extension of centrin. (d) Metaphase spindle outlined by an extended centrin system showing foci at the spindle poles. (e) Metaphase/anaphase boundary illustrating a second transient centrin contraction. (f-g) Telophase centrin distribution within mother cell undergoing cytokinesis. (h) Cytokinesis is complete. This final image illustrates that the fimbria have been reduced to approximately eight in each daughter cell. Each image is printed with the anterior region of the cell at the top and shows the space occupied by the entire cell. Bar, 5 μ m.

to result in flagellar reorientation of the isolated flagellar apparatus of *Chlamydomonas* (Hyams and Borisy, 1975; 1978). However, there are no observations of flagellar reorientation in living *Chlamydomonas* cells. This may be due to the presence of a (presumably) rigid cell wall (and flagellar tunnels), which precludes gross physical displacement of the basal bodies.

In *Chlamydomonas*, the centrin-based flagellar root system shows a dramatic contraction at the time of flagellar excision, which is followed by re-extension during flagellar regeneration (Salisbury et al., 1987). This contraction physically displaces the nucleus toward the flagellar apparatus and results in nuclear shape changes. The functional implications of these movements and shape changes are unclear. However, their coincidence with the period of flagellar precursor induction has led us to question whether this phenomenon may represent a physical (cytoskeletal) signaling mechanism related to flagellar precursor gene induction (Salisbury, et

al., 1987). Contraction of flagellar roots in *Chlamydomonas* at preprophase (see below) may, likewise, signal a burst of tubulin synthesis, which is known to occur prior to the onset of mitosis in this organism (Ares and Howell, 1982; Piperno and Luck, 1977).

Centrin Dynamics in Mitotic *Chlamydomonas* Cells

Microtubule dynamics and the mitotic cycle of *Chlamydomonas* have been well described at both the ultrastructural (Johnson and Porter, 1968; Cavalier-Smith, 1974; Coss, 1974) and immunofluorescence level (Doonan and Grief, 1987). Interphase microtubules occur in the flagellar axonemes, basal bodies, and in a cortical cytoplasmic array that converges on the flagellar apparatus (Fig. 5). Flagellar and cytoplasmic microtubules become reorganized during mitosis through disassembly, and are replaced by the spindle and metaphase band microtubules (Johnson and Porter, 1968; Doonan and Grief, 1987). Spindle microtubules originate near the basal bodies, which persist throughout mitosis and are located near each spindle pole (Coss, 1974). The nuclear envelope also persists throughout mitosis and is fenestrated at the poles, where the spindle microtubules pass into the mitotic nucleus (Johnson and Porter, 1968).

Our study shows pronounced changes in the interphase organization of centrin during mitosis. Although dramatic changes occur, the centrin-based cytoskeletal system always remains convergent on the region of the basal bodies and maintains its association with the nuclear envelope. At the interphase/preprophase boundary the centrin-based descending fibers and their branches show a conspicuous contraction, which draws the nucleus toward the flagellar apparatus (cf. Coss, 1974; Triemer and Brown, 1974). This movement coincides with the loss of flagella, subtle cell shape changes, and a burst of tubulin synthesis (Ares and Howell, 1982; Piperno and Luck, 1977). The preprophase contraction is transient and is followed in prophase by division and separation of the centrin-based cytoskeleton; the timing of basal body separation is coincident with this event. Before metaphase, the two newly formed centrin foci migrate toward opposite poles of the spindle. By metaphase, centrin has reformed an array of fibers that extend from the poles and delineate the mitotic spindle. At the metaphase/anaphase boundary a second transient contraction of centrin occurs; the timing of chromosome separation is coincident with this event. At telophase the contracted centrin fibers re-extend; thus two distinct cytoplasts are delineated. By the time of cytokinesis, the two daughter nuclei have re-established an interphase-like organization of centrin.

The essential features of centrin dynamics described here for *Chlamydomonas* mitosis are also common to other mitotic cells that we have studied, including mammalian cells (Baron, A., and J. L. Salisbury, manuscript in preparation). It is conceivable that the centrin-containing cytoskeleton of mitotic cells is responsible for basal body/centriole segregation (Kuchka and Jarvik, 1982; Wright et al., 1985; Jarvik, J., Carnegie-Mellon University, Pittsburgh, PA, personal communication), and for the poleward movement of chromosomes during anaphase (cf. Pickett-Heaps et al., 1982).

Concluding Remarks

Although centrin containing structures are morphologically

diverse, they are always associated with the flagellar apparatus or centrosome of eukaryotic cells and show calcium-sensitive contractile or elastic behavior. Centrin is therefore yet another cytoskeletal protein, which, like tubulin, actin, and intermediate filament proteins, are elaborated in eucaryotic cells to serve a variety of functions.

We thank the members of our laboratory for their comments on the manuscript, and D. Coling for preparation of centrin used in the monoclonal antibody production.

This work was supported by a grant from the National Institutes of Health (GM-35258) to J. L. Salisbury.

Received for publication 18 February 1988, and in revised form 13 April 1988.

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