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-Mr) 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 eukaryotic 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/cenriole 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*. 

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**Materials and Methods**

**Cell Culture**

*Chlamydomonas reinhardtii*, wild-type strain 173c derivative NORT 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 Gra-

**Monoclonal and Polyclonal Antibodies**

The polyclonal serum, 08/28, was raised against SDS-PAGE purified cen-

**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% polyethylene- 

**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 0.1% osmium tetroxide and 0.8% K3Fe(CN)6 in 4 mM phosphate buffer for 60 min, followed by dehydration through an ethanol series, cleared with propylene oxide, and embedded in Lowicryl resin (Pella, Inc., Redding, CA). Sections were washed for 15 min, followed by 30 min at 4°C. Samples were washed with deionized water, and post stained as above through an ethanol series, cleared with propylene oxide, and embedded in Lowicryl resin (Pella, Inc., Redding, CA). Sections were hydrated with deionized water for 10 min, followed by 30 min at 4°C. After a buffer 10 mM Hepes (pH 7.2) wash the samples were post-fixed in 0.1% osmium tetroxide and 0.8% K3Fe(CN)6 in 4 mM phosphate buffer for 60 min, followed by dehydration through an ethanol series, cleared with propylene oxide, and embedded in Lowicryl resin (Pella, Inc., Redding, CA). Sections were hydrated with deionized water, and post stained as above.
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 anteriormost mitochondrion 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 μm long, 80–200-nm wide, and 10-nm thick. The descending fibers therefore occur in only one or two consecutive 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 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
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 reextends (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...
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 eukaryotic cells to serve a variety of functions.

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