Cell Motility and the Cytoskeleton 22:117–126 (1992)

Actin Dynamics During the Cell Cycle in *Chlamydomonas reinhardtii*

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We have used two monoclonal antibodies to demonstrate the presence and localization of actin in interphase and mitotic vegetative cells of the green alga *Chlamydomonas reinhardtii*. Commercially available monoclonal antibodies raised against smooth muscle actin (Lessard: *Cell Motil. Cytoskeleton* 10:349–362, 1988; Lin: *Proc. Natl. Acad. Sci. USA* 78:2335–2339, 1981) identify *Chlamydomonas* actin as a ~43,000-M_r protein by Western immunoblot procedures. In an earlier study, Detmers and coworkers (*Cell Motil.* 5:415–430, 1985) first identified *Chlamydomonas* actin using NBD-phallacidin and an antibody raised against *Dictyostelium* actin; they demonstrated that F-actin is localized in the fertilization tubule of mating gametes. Here, we show by immunofluorescence that vegetative *Chlamydomonas* cells have an array of actin that surrounds the nucleus in interphase cells and undergoes dramatic reorganization during mitosis and cytokinesis. This includes the following: reorganization of actin to the anterior of the cell during preprophase; the formation of a cruciate actin band in prophase; reorganization to a single anterior actin band in metaphase; rearrangement forming a focus of actin anterior to the metaphase plate; reextension of the actin band in anaphase; presence of actin in the forming cleavage furrow during telophase and cytokinesis; and finally reestablishment of the interphase actin array. The studies presented here do not allow us to discriminate between G and F-actin. None the less, our observations, demonstrating dynamic reorganization of actin during the cell cycle, suggest a role for actin that may include the movement of basal bodies toward the spindle poles in mitosis and the formation of the cleavage furrow during cytokinesis.

Key words: algae, cell division, cytokinesis, mitosis

INTRODUCTION

Actin and tubulin are abundant cytoskeletal proteins of eukaryotic cells that play major roles in cytoplasmic organization and motility in both interphase and mitotic cells. Plant cells were first shown to contain actin not long after the development of sensitive analytical techniques of gel electrophoresis and HMM decoration of F-actin [Condeelis, 1974; Forer and Jackson, 1976; Palevitz et al., 1974]. Actin has long since been known to play an active role in organelle movements in giant algal cells [reviewed by Williamson, 1980; Kuroda, 1990], but its role in higher plants is less clear [reviewed by Lloyd, 1988, 1989].

With higher plants, fluorescence microscopy studies have employed antiactin antibodies and F-actin-binding probes such as rhodamine phalloidin and NBD-phallacidin. They demonstrate actin distribution in fine

Received October 9, 1991; accepted January 14, 1992.

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cortical networks close to the plasma membrane, in large oriented cables or bundles located in the subcortical region of the cytoplasm, and in a "basket" that appears to surround the nucleus in interphase cells [Parthasarathy et al., 1985; Seagull et al., 1987]. During mitosis in higher plant cells, these interphase actin arrays often are replaced by actin in the preprophase band and spindle, and by actin localization along the phragmoplast of dividing cells [Clayton and Lloyd, 1985; Gunning and Wick, 1985; Kakimoto and Shibaoka, 1987; McCurdy and Gunning, 1990; McCurdy et al., 1988; Palevitz, 1987; Schmit et al., 1986; Schmit and Lambert, 1987; Seagull et al., 1987; Traas et al., 1987].

In the motile unicellular green alga *Chlamydomonas reinhardtii* actin filaments have been demonstrated to play a role in the formation of the fertilization tubule in mating cells [Detmers et al., 1983, 1985; Friedmann et al., 1968; Goodenough and Weiss, 1975]. In addition, an actin-like protein has been shown to be a component of the inner dynein arms of the flagellar axoneme of this organism [Piperno and Luck, 1979]. However, prior to the present study there have been no reports of involvement of actin in the vegetative cell cycle of *Chlamydomonas*.

Chlamydomonas has a well characterized microtubule-based cytoskeleton [Doonan and Grief, 1987; Johnson and Porter, 1968; LeDizet and Piperno, 1986; Ringo, 1967]. In interphase cells, microtubules form the structural basis for the pair of flagellar axonemes and their associated basal bodies. Also, a cortical cytoskeletal array, consisting of four distinctive acetylated microtubule rootlets, originate between the two basal bodies. From these rootlets a large number of secondary microtubules radiate in the cell periphery [LeDizet and Piperno, 1986]. Before mitosis, the flagellar microtubules are resorbed [Randall et al., 1967] and interphase microtubules become largely disassembled and replaced by several distinct sets of cell division microtubules: 1) those that form the mitotic spindle proper; 2) a "metaphase band" of microtubules that arcs over the nucleus, indicating the future cleavage plane and are derived from a reorganization of the persistent four-membered microtubule rootlets of interphase cells; 3) internuclear microtubules, which form between daughter nuclei at right angles to the spindle axis; and finally 4) cleavage microtubules (the phycoplast), which lie along the path of the cleavage furrow [Doonan and Grief, 1987; Gaffal and el-Gammel, 1990; Harper and John, 1986; Johnson and Porter, 1968]. Basal body replication begins at preprophase, and at the time of spindle assembly basal bodies have separated to the region of the mitotic spindle poles [Coss, 1974; Gaffal 1988; Holmes and Dutcher, 1989; Triemer and Brown, 1974].

Another cytoskeletal system of *Chlamydomonas* involves calcium-sensitive centrin-based contractile fibers linking the flagellar basal bodies to one another and to the nucleus [Salisbury et al., 1988; Wright et al., 1985]. In *Chlamydomonas,* the centrin-based cytoskeleton appears to play a role in basal body segregation, positioning, and orientation and in the process of microtubule severing during flagellar excision [Salisbury et al., 1988; Sanders and Salisbury, 1989; Wright et al., 1989]. During the cell cycle, the centrin-based fibers that link the flagellar apparatus to the nucleus show dramatic changes in distribution that include 1) a transient contraction at preprophase coincident with the movement of the nucleus toward the flagellar apparatus; 2) division and separation, marking the nascent spindle poles, and reextension during prophase; 3) a second transient contraction at the metaphase/anaphase boundary, and 4) reestablishment of the typical interphase array by the time of cytokinesis [Salisbury et al., 1988].

Here, we describe for the first time the dynamic behavior of the actin-based elements of the *Chlamydomonas* cytoskeleton during the cell cycle. Our observations on the reorganization of actin during mitosis have important implications for the movement of basal bodies toward the mitotic spindle poles and for the establishment of the cleavage furrow at cytokinesis. Due to the insensitivity of mitosis and cytokinesis to cytochalasins, we also recognize the interesting possibility that the actin observed in vegatative cells may be G-actin associated with different cytoskeletal structures during the cell cyle.

MATERIALS AND METHODS

Cultures

Wild-type *Chlamydomonas reinhardtii* strain 137c mt^+ (cc-125 mt^+) was obtained from the *Chlamydomonas* culture collection at Duke University (E.H. Harris, Durham, NC). Cultures (100 ml) were synchronized in Sager and Granick [1953] medium I in 250 ml flasks under a 14:10 hr light/dark cycle at 20°C. Samples were fixed just after the lights went out, at which time ~6% of the cells showed mitotic figures [Harper et al., 1990]. Gametes were prepared by nitrogen starvation as described by Harris [1989].

Antibodies and Western Immunoblotting

Two commercially available monoclonal antibodies raised against smooth muscle actin were used in this study; N350, an IgM monoclonal antiactin developed by Lin [1981] was obtained from Amersham (Amersham International, Sydney, Australia), and C4, an IgG antiactin developed by Lessard [1988], was obtained from ICN (ICN Immunologicals, Lisle, IL). Each of these monoclonal antiactin antibody preparations is widely re-

active against actin from a variety of species and has been shown to recognize actin in higher plants [McCurdy et al., 1988].

Cells were ground in liquid nitrogen with a mortar and pestle and extracted as described by John and coworkers [1989]. SDS-PAGE was carried out according to the procedures of Laemmli [1970], and Western transfer and immunoblotting were carried out according to Towbin and coworkers [1979]. Ponceau S total protein staining on blots was achieved by immersing the blot in a solution of 0.2% Ponceau S and 3% trichloroacetic acid (Sigma, St. Louis, MO) for 5 min.

For immunoblotting, the N350 antiactin was used at 1:1,000 dilution, and C4 antiactin was used at 30 μg/ml. Secondary alkaline phosphatase-conjugated affinity purified F(ab') antimouse Ig (Sigma) was used at 1:1000 dilution.

Indirect Immunofluorescence

Several fixations were tried to best preserve actin (see below). The best preservation was seen using the following technique: Cells were fixed for 30 min on a rotating shaker in 4% formaldehyde from a 16% EM grade stock solution; (Electron Microscopy Sciences, Fort Washington, PA) in PEMD buffer containing 50 mM PIPES [piperazine, N,N'-bis (2-ethane sulfonic acid)], 5 mM EGTA (ethyleneglycol-bis[β-aminoethyl ether]N,N'-tetraacetic acid), 1 mM $MgSO_4 \cdot 7H_2O$, and 1% dimethylsulfoxide (DMSO). The pH of the PEMD buffer and buffered fixative was 6.8. After fixation, cells were washed in three changes of PEMD buffer (5 min each) and resuspended for 1 hr in *Chlamydomonas* culture medium containing autolysin. Autolysin was prepared as described by Harris [1989] using the high-efficiency mating strains cc-620 mt^+ and cc-621 mt^- supplied by the *Chlamydomonas* culture collection at Duke University (E.H. Harris, Durham, NC). Following autolysin treatment, cells were washed in phosphate-buffered saline (PBS; 8 mM Na_2HPO_4, 2 mM NaH_2PO_4, 140 mM NaCl, pH 7.4) and allowed to settle onto poly-L-lysine (390 kD, 1 mg/ml in H_2O; Sigma)-coated eight-well microslides (Carlson Scientific, Peotone, IL) for 20 min. Excess liquid was carefully removed from the slides with filter paper, and the slides were immersed in −20°C methanol for 10 min. For the N350 antibody, an additional 5 min −20°C acetone step was found to be essential to reveal the perinuclear actin staining. Slides were then rehydrated in PBS before incubation in primary antibody.

Primary and secondary antibodies were diluted in PBS containing 1% bovine serum ablumin (BSA, fraction V; Sigma) and 0.02% sodium azide. Antibody incubations were for 1 hr or overnight at 20°C. For visualization of actin staining, a fluorescein isothiocyanate (FITC)-conjugated sheep antimouse antibody (Amersham) was used at 1:40 dilution. Antibody controls included the use of nonreactive IgG or the omission of the primary antibody step. Slides were then washed in PBS (3 × 5 min). To allow the identification of mitotic stages by DNA staining, slides were washed for 10 min in PBS containing 0.2 μg/ml 4,6-diamidine-2-phenylindole dihydrochloride (DAPI). Slides were then rinsed briefly in PBS and mounted in Citifluor antifade mountant (AF1; City University, London, England). Coverslips were sealed with nail varnish to prevent evaporation.

Slides were viewed using a Nikon Microphot-FXA photomicroscope equipped with 100 W mercury lamp and epifluorescence illumination, standard FITC and UV filter sets, and a Fluor 100×, 1.30 N.A. oil immersion objective. Automatic exposures were between 30 and 45 sec duration. Photographs were recorded on either Kodak T-max 400 film, rated at 1600 ASA, and developed in Kodak T-max developer for 10 min at 20°C, or Hypertech film (Microfluor Ltd., Stony Brook, NY), rated at 800 ASA and developed in Kodak D-19 developer for 6 min at 20°C.

The following methods sections document other protocols we investigated for preserving and staining actin with antibodies or phalloidins and attempts to arrest cell division with cytochalasins in *Chlamydomonas*. Since our results with these trials were largely negative, they are included with the methods for completeness and clarity.

Other Protocols Investigated for Preserving Actin

The best actin preservation and staining technique that we have obtained is described above. We have also, however, tested several fixation, actin stabilization, and cell extraction protocols in our studies. For fixation, the following concentrations of formaldehyde were tried: 8%, 4%, 2%, 1%, 0.5% with or without 0.1% or 0.2% glutaraldehyde in either 50 mM phosphate buffer or 50 mM PIPES buffer also with or without $MgSO_4$ or EGTA. Different concentrations of DMSO (i.e., 0, 1%, 2%, and 5%) in the fixative were tried. After aldehyde fixation, permeabilization using either acetone or methanol was found to be essential to obtain any antibody labeling. Further extractions with different concentrations of nonionic detergents such as Nonidet P-40 [Doonan and Grief, 1987] or Triton X-100 gave progressively poorer staining with higher concentrations and incubation times. The actin stabilization compound m-maleidobenzoyl N-hydroxysuccinimide ester (MBS; from Pierce Chemicals, Rockford, IL) was tried at 100 μM according to Sonobe and Shibaoka [1989], both by preincubating cells in the compound and by adding it to the fixatives. None of these treatments improved the preservation of actin. To avoid aldehyde fixation altogether, cells were fixed in

−20°C methanol for various times. No staining was observed with this treatment.

Phalloidin Staining

Autolysin-treated cells were fixed for 30 min in 4% formaldehyde in 50 mM phosphate or PIPES buffer, final pH 6.8, with or without 5% DMSO and 25 mM EGTA, followed by detergent extraction (1% Triton X-100). Small volumes (70 μl) of phallotoxin stocks (Molecular Probes, Eugene, OR), described below, were taken, the methanol was allowed to evaporate off, and the phallotoxin was reconstituted in 50 mM phosphate or PIPES buffer containing 5% DMSO, but no EGTA, at a 1:20 dilution. Staining was for 2 hr in a dark, moist chamber. All protocols and phalloidins gave good staining of the microfilament-containing fertilization tubule in mating *Chlamydomonas* cells, as did the C4 and N350 actin antibodies (not shown). However, the FITC-phalloidin, rhodamine phalloidin, or NBD phallacidin gave only a bright overall fluorescence of vegetative or mitotic cells, which made it impossible to distinguish any localized staining. Therefore, antibody labeling was employed to describe actin localization in vegetative cells.

Cytochalasin Treatments

Cytochalasins B and D (Sigma) were prepared in DMSO and added to cells in culture to give a final concentration of 1% DMSO. Concentrations of up to 400 μg/ml of cytochalasin were added to synchronous cells at various times throughout the cell cycle. Autolysin was also added to some wild-type cultures or cell wall-less mutants were employed to determine if the cell wall was proving to be a barrier to cytochalasin uptake. None of these treatments had an appreciable effect on mitosis or cytokinesis, suggesting that cytochalasins do not get into the cells, that they do not bind well to *Chlamydomonas* actin, or that the actin labeling with antibodies that we describe is not cytochalasin-sensitive F-actin (see Discussion).

RESULTS

Identification of *Chlamydomonas* Actin

Western transfer and immunoblot procedures using the commercially available antiactin monoclonal antibodies N350 and C4 identify *Chlamydomonas* actin (M_r ~43,000) from whole cell lysates in late G1 cells (9 hr; Fig. 1) and in dividing cells (14 hr; Fig. 1). Monoclonal antibody C4 identifies a single protein of 43,000 M_r (Fig. 1, lane C4), and monoclonal antibody N350 identifies the 43,000 M_r protein and a second minor reactive band at ~85,000 M_r (Fig. 1, lane N350). Both monoclonal antibodies gave essentially identical immunofluorescence localization (see below), with the exception that

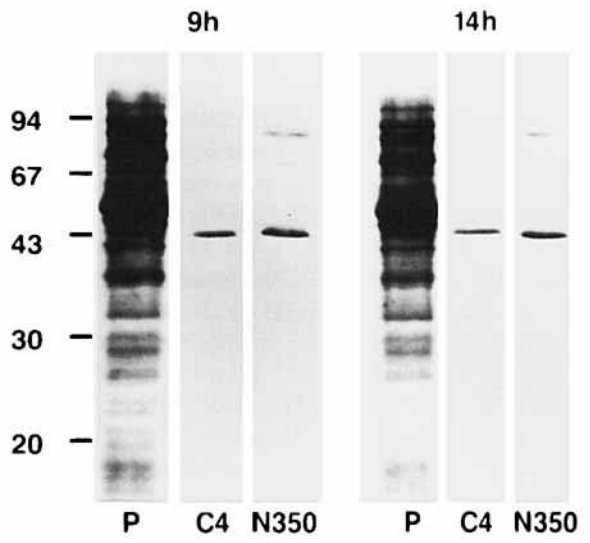

Fig. 1. Immunoblot analysis of *Chlamydomonas* actin. *Chlamydomonas* whole cell extracts were probed with Ponceau S (P) stain for total protein or actin using the monoclonal antibodies C4 and N350. Samples were taken from late G_1 (9 hr) and actively dividing (14 hr) cells in synchronous culture. Note that the 43,000 M_r actin band is the major protein identified.

N350 occasionally also stained the region of the eyespot (not shown), while C4 never stained eyespots. We do not know the nature of eyespot reactivity with monoclonal N350. However, we recognize that it may be related to the 85,000 M_r band identified in the Western immunoblot analysis. Controls in which the primary monoclonal antibody was omitted resulted in detection of no bands by these Western transfer and immunoblot procedures.

Localization of *Chlamydomonas* Actin: Interphase Cells

Indirect immunofluorescence localization using the monoclonal antiactin antibodies and staining of nuclear and chloroplast DNA using DAPI is illustrated for interphase and mitotic vegetative cells of *Chlamydomonas* in Figures 2–4. Interphase cells can be identified by their ellipsoid shape, the position and uniform diffuse DAPI staining of the nucleus in a "central" position ~3 μm below the flagellar basal apparatus, and the presence of a pair of flagella. Actin in interphase cells occurs in a diffuse perinuclear localization (Fig. 2A). All interphase cells we observed showed this pattern of actin labeling, but with the N350 antibody a 5 min acetone extraction was necessary to reveal it. Vegetative cells do not have a fertilization tubule, nor do they show actin localization in the region occupied by the fertilization tubule of gametic cells [Detmers et al., 1985].

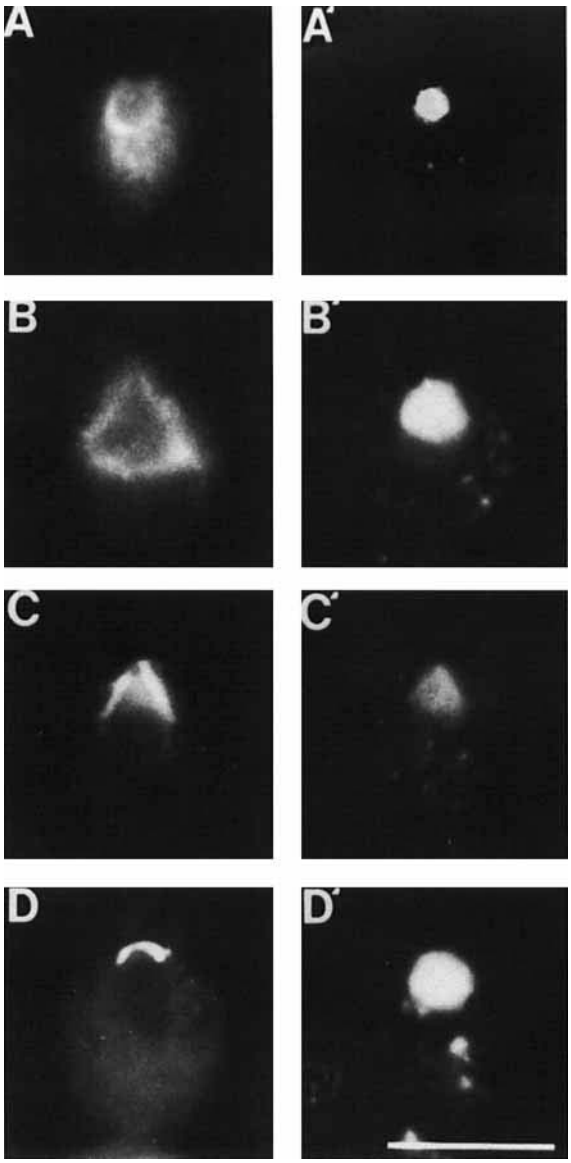

Fig. 2. Immunofluorescence localization of actin (**A–D**) and DNA (**A′–D′**) during interphase and preprophase in *Chlamydomonas* cells. Cells are positioned with the anterior end (flagellar basal apparatus) toward the top of the photograph. A: Interphase diffuse perinuclear actin localization. B–D illustrate the changes in actin distribution during preprophase. The perinuclear actin distribution becomes more angular (B), then redistributes to a cone-shaped apical zone (C), and finally redistributes to a band just beneath the flagellar basal apparatus (D). Bar = 10 μm.

Preprophase Cells

Mitotic cells can be identified by several criteria, including their larger size and more rotund shape compared with interphase cells; their loss of flagella [Johnson and Porter, 1968; Cavalier-Smith, 1974]; the position of the nucleus just below the flagellar basal apparatus [Doonan and Grief, 1987; Salisbury et al., 1988]; and a more intense, irregular, and granular DAPI staining of condensing nuclear DNA. Figure 2B–D illustrates the changes in actin distribution of preprophase cells. In these cells, actin localization first becomes more angular (Fig. 2B), then redistributes toward the flagellar basal apparatus as a cone-shaped apical zone (Fig. 2C), and finally appears as an arc or band just below the flagellar basal apparatus (Fig. 2D).

Prophase and Metaphase Cells

At the time of prophase, DAPI staining of nuclear DNA appears highly granular and peripheral in localization (Fig. 3A′). At this time, through focusing of the microscope reveals that many cells show actin localization in two short, crescent-shaped arcs or bands forming a cruciate structure in the extreme anterior region of the cell (Fig. 3A). Each of these actin bands curves over the surface of the nuclear envelope. One of the bands is more transient; it suggests the future plane of the cleavage furrow and is no longer observed by metaphase. At the time of metaphase, the DAPI staining of nuclear DNA clearly shows alignment of chromosomes at the metaphase plate (Fig. 3B′). During metaphase, the original persistent actin band elongates in the region between the two mitotic spindle poles and becomes more distinctive (Fig. 3B). In ~5–10% of metaphase cells, actin localization defines a focus near the plasma membrane anterior to the metaphase plate (Fig. 3C,C′). This focal pattern of actin appears to be consistent for a particular period in metaphase cells; it predicts the site of initiation of the future cleavage furrow; and it may represent a transient reorganization of actin, perhaps late in metaphase.

Anaphase Cells

Anaphase cells can be identified by the DAPI staining pattern of nuclear DNA which appears as two distinct dense ellipsoid masses separated by ~2–3 μm in the anterior region of the cell (Fig. 3D′). Actin localization in anaphase cells defines a diffuse band extending anterior to the elongating nucleus (Fig. 3D). It appears that the original metaphase arc of actin has become less distinctive by anaphase; it is diffuse, broader, and somewhat less regular in shape.

Telophase and Cytokinesis

The transition from telophase to cytokinesis is marked by a distinctive anterior depression in cell shape and by the movement of nuclei toward one another (Fig. 4A′). Throughout cytokinesis, actin is localized along the progressing cleavage furrow (Fig. 4A–D). The cleavage furrow localization of actin resembles the reorganization of microtubules in the early stages of cytokinesis, and at later stages both actin and tubulin become distinctive cytoskeletal features of the cleavage furrow [Doonan

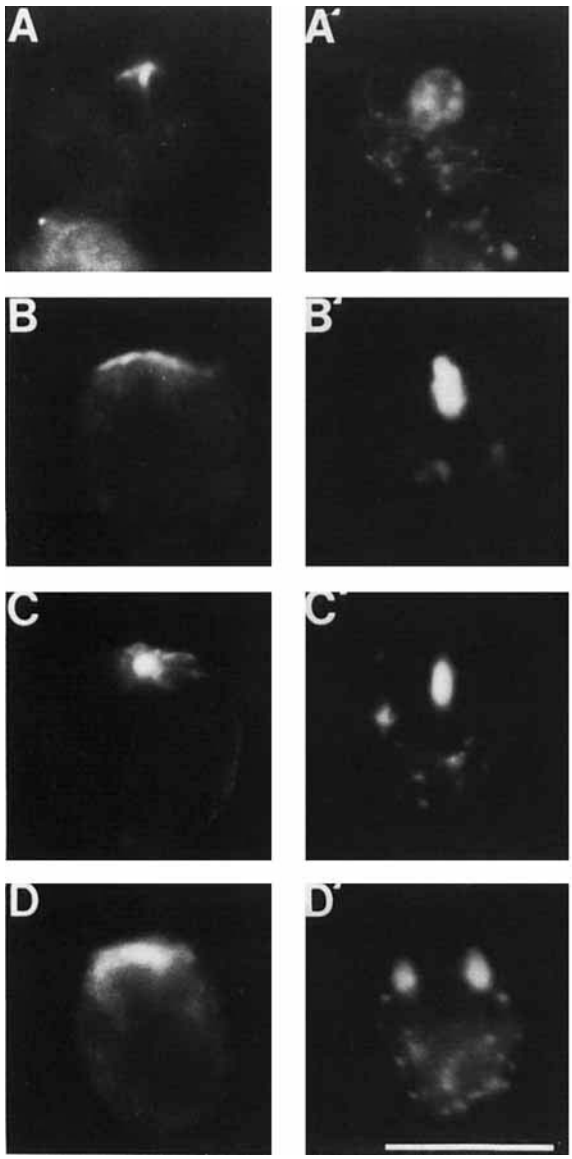

Fig. 3. Immunofluorescence localization of actin (**A–D**) and DNA (**A′–D′**) from prophase to anaphase. Cells are positioned with the anterior end (flagellar basal apparatus) toward the top of the photograph. A: Prophase distribution demonstrating the cruciate bands of actin at the cell anterior. The persistent actin band has elongated to the region of the two mitotic spindle poles by metaphase (B). C: A transient aggregation of actin, anterior to the metaphase plate. In anaphase (D), the actin distribution is seen as a diffuse band that extends anterior to the elongating nucleus. Bar = 10 μm.

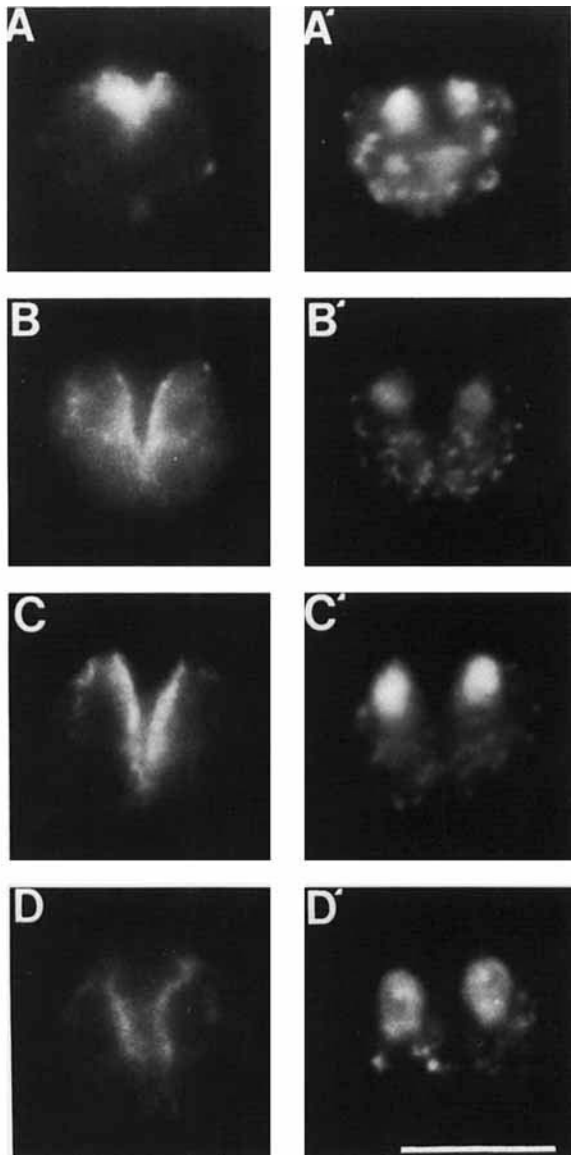

Fig. 4. Immunofluorescence localization of actin (**A–D**) and DNA (**A′–D′**) from telophase through cytokinesis in *Chlamydomonas*. Cells are positioned with the anterior end (flagellar basal apparatus) toward the top of the photograph. A: Telophase distribution of actin in the incipient cleavage furrow at the cell anterior. B–D demonstrate the reorganization of actin distribution along the progressing cleavage furrow during cytokinesis. Bar = 10 μm.

and Grief, 1987; this study]. During the final stages of cell division, the nuclei begin to move toward a more central position within each daughter cell, the nuclei expand in volume, and the DAPI staining of nuclear DNA becomes more diffuse and uniform (Fig. 4D′). As cytokinesis proceeds, the actin also begins to encircle each daughter nucleus (Fig. 4B–D) in a manner similar to that in interphase cells (cf. Fig. 2A–B). This is most clearly seen in Figure 4B.

DISCUSSION

We present studies on the localization of actin during the vegetative cell cycle of *Chlamydomonas*. We demonstrate that two monoclonal antibodies, raised against smooth muscle actin, recognize a 43,000 M_r pro-

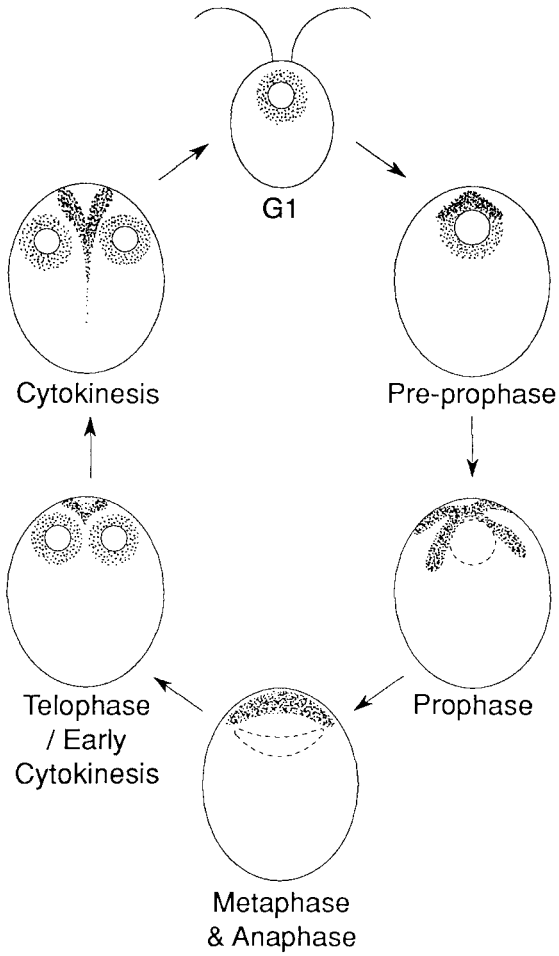

Fig. 5. Diagram summarizing actin dynamics through the cell cycle of *Chlamydomonas*. In interphase (G1) cells, there is a perinuclear actin localization, which becomes more angular in preprophase and redistributes to the cell anterior. In prophase, a cruciate structure is observed arcing over the nucleus. One of the actin bands in this cruciate structure is transient and predicts the future cleavage plane. In metaphase and anaphase, a band of actin is seen in the anterior of the cell above the nucleus and extending between the mitotic spindle poles. There is also a transient aggregation of actin to a focus in metaphase (see Fig. 3C). In telophase and cytokinesis, actin is observed in the cleavage furrow and around the newly divided nuclei.

tein in both actively growing and dividing cells. Furthermore, immunofluorescence studies with these monoclonal antibodies show distinct changes in the distribution of actin throughout the cell cycle. A diagrammatic representation summarizing our observations is presented in Figure 5. Although antibody staining of actin in these cells does not distinguish between G- and F-actin, the established precedent strongly favors F-actin to be the predominant functional cytoskeletal form. Our observations suggest that actin plays a role in the *Chlamydomonas* cell cycle that may include the movement of basal bodies toward the spindle poles during prophase and in the formation of the cleavage furrow during cytokinesis.

In interphase *Chlamydomonas* cells, actin is localized in a diffuse region around the nucleus. In preprophase, there are dramatic changes in actin distribution. Early in preprophase, the actin becomes more angular in shape, then subsequently redistributes to the region of the nucleus lying proximal to the flagellar basal apparatus. At this time, the centrin-based nucleus basal body connector contracts [Salisbury et al., 1988], and the nucleus moves toward the anteriormost region of the cell. Also at this time, the cytoplasmic microtubules begin to disassemble [Doonan and Grief, 1987]. During early prophase, there are two bands of actin that together form a cruciate structure at the anterior of the cell. The cruciate nature of the actin bands, observed by through focusing of the microscope, suggests that they may be in close association with the anterior most region of microtubule rootlets [see Weiss, 1984]. One of these bands lies anterior to the plane of the developing spindle; the other is a more transient band and may lie in the plane of the "metaphase band" of microtubules. This transient band, like the metaphase band, may predict the site of the future cleavage plane. However, unlike the metaphase band microtubules, which persist from preprophase to telophase [Doonan and Grief, 1987; Johnson and Porter, 1968], the band of actin disappears by the end of prophase. During prophase, the duplicated centrosomes separate and move toward the poles [Gaffal, 1988]. It has been suggested that the centrin-based nucleus basal body connector may be involved in equal segregation of the basal bodies and associated structures to the spindle poles and ultimately to the daughter cells [Gaffal and el-Gammal, 1990; Salisbury et al., 1988; Wright et al., 1985, 1989]. It is unclear how the centrin system alone could provide the appropriate force considering its location between the poles and its contractile properties. However, actin is a likely candidate for mediating the movement of basal bodies toward the mitotic poles. Evidence exists for the involvement of actin in the positioning and motility of the centrosome in animal cells [Euteneuer and Schliwa, 1985]. Electron microscopy studies indicate that at least one basal body at each spindle pole in *Chlamydomonas* appears to be attached to the plasma membrane [Coss, 1974; Triemer and Brown, 1974]. During the time of basal body duplication and separation, the actin localization at the anterior plasma membrane suggests that an actin-based system may interact with the centrin-based system to ensure basal body segregation. This could be accomplished by actin polymerization or filament sliding to provide the force necessary to move the basal bodies to either pole. Alternatively, actin dynamics may effect growth or fluidity of the plasma membrane in the anterior of the cell resulting in

basal body movement [cf. Adams and Pringle, 1984; Heath, 1987; Novick and Botstein, 1985; Cho and Wick, 1990, 1991]. In either case, centrin may maintain the nucleus-basal body association. In the marine phytoflagellate *Apedinella radians* it has been demonstrated that an elaborate array of actin and centrin filament bundles act in concert to reorientate the external spine-scales of the organism [Koutoulis et al., 1988]. Likewise, centrin and actin may be involved in the events leading to basal body repositioning in *Chlamydomonas*.

By anaphase, the actin band is again seen to arc across the cell anterior. This localization is consistent with an involvement of actin in the correct positioning of the spindle axis relative to the cleavage site. Evidence for a similar role for actin in higher plants is suggested by drug studies where cytochalasins have disrupted the plane of cytokinesis [see, e.g., Cho and Wick, 1990; 1991; Lloyd and Traas, 1988].

The presence of actin in the cleavage furrow of *Chlamydomonas* raises the possibility that actin may provide the generative force for cytokinesis in a manner similar to the contractile ring of microfilaments in dividing animal cells and in the green alga *Spirogyra* [Schroeder, 1981; Goto and Ueda, 1988]. In this regard, Pickett-Heaps [1975] has argued that the microtubules in the *Chlamydomonas* phycoplast do not provide the generative force for cleavage but instead may act in the spatial positioning of the cleavage furrow to ensure equal segregation of daughter cell components. The localization of actin in the cleavage furrow suggests that actin may be involved in the process of cytokinesis. However, we have not been able to inhibit this process using concentrations of cytochalasins B and D as high as 400 μg/ml. While this concentration is eight to 40 times that required to disrupt actin filaments in higher plant cells [McCurdy et al., 1991], the studies of Detmers and coworkers [1985] demonstrate that concentrations of cytochalasin D as high as 200 μg/ml are required to affect the microfilaments in the fertilization tubule of mating *Chlamydomonas* cells. This observation suggests that cytochalasin may not penetrate *Chlamydomonas* cells freely or may not bind well to *Chlamydomonas* actin. We recognize the possibility, however, that the antibody labeling of actin that we describe is of G-actin. If indeed the staining represents changes in G-actin, this is a novel and intriguing observation and raises questions regarding the function of G-actin during the *Chlamydomonas* cell cycle. Presumably the changes in distribution correspond to G-actin associated with other structures or cytoskeletal elements. The interphase localization suggests an association with the nuclear envelope and/or Golgi apparatus. The mitotic actin localization pattern suggests an association with microtubules and/or centrin and the cleavage furrow localization indicates an association with the ingrowing plasma membrane and/or microtubules. G-actin or short actin oligomers could be attached to microtubules and other cytoskeletal elements by MAP-2 proteins or other actin binding proteins [Bershadsky and Vasiliev, 1988; Schliwa, 1986].

In higher plant cells, an actin "basket" has been reported to surround the nucleus in interphase [Seagull et al., 1987; Traas et al., 1987] and may play a role in nuclear positioning [see Cho and Wick, 1990, 1991]. The perinuclear *Chlamydomonas* actin may, as part of the centrin-based system, play a similar role. Parallels with *Chlamydomonas* actin redistribution in preprophase have been observed in higher plants [Lloyd and Traas, 1988]. In carrot suspension cells, nucleus-associated actin is drawn into a transvacuolar disc. The presence of actin in the preprophase band of higher plants has been demonstrated by a number of workers [Kakimoto and Shibaoka, 1987; Palevitz, 1987; Traas et al., 1987]. Johnson and Porter [1968] noted that the preprophase band in higher plants is in some ways analogous to the metaphase microtubule band of *Chlamydomonas* in that both appear around preprophase and mark the site of future cytoplasmic division. We suggest that actin may also be associated with the metaphase microtubule band in *Chlamydomonas*.

Observations of actin localization near the anterior plasma membrane region of *Chlamydomonas,* in both mating and mitotic cells [Detmers et al., 1983, 1985; this study], suggest that there may be an actin organizing center involved in local polarization or differentiation of the plasma membrane [cf. Adams and Pringle, 1984; Cho and Wick, 1990, 1991; Kropf et al., 1989; Quader and Schnepf, 1989; Quatrano and Kropf, 1989]. In dividing *Chlamydomonas* cells, localized growth of the plasma membrane might contribute to the movement of basal bodies and progression of the cleavage furrow.

The observations reported here illustrate, for the first time, the dynamic behavior of actin in the vegetative *Chlamydomonas* cell cycle. Two observations of unique and particular interest are the band of actin arcing between the basal bodies and the presence of actin in the cleavage furrow. It is proposed that actin, in association with other cytoskeletal elements, is involved in the processes of basal body separation and cleavage furrow progression in *Chlamydomonas*.

ACKNOWLEDGMENTS

We thank Drs. Ursula Goodenough and André Baron and Ms. Tammy Greenwood for helpful comments. We also thank Dr. Ann Cleary for technical advice about phalloidin staining. This work was supported in part by National Institute of Health grant GM35258 to

J.L.S. and by a visiting fellowship from The Australian National University to J.D.I.H..

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