A Novel Mitotic Spindle Pole Component that Originates from the Cytoplasm during Prophase

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Abstract. Several unique aspects of mitotic spindle formation have been revealed by investigation of an autoantibody present in the serum of a patient with the CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, schlerodacytyly, and telangiectasias) syndrome. This antibody was previously shown to label at the spindle poles of metaphase and anaphase cells and to be absent from interphase cells. We show here that the serum stained discrete cytoplasmic foci in early prophase cells and only later localized to the spindle poles. The cytoplasmic distribution of the antigen was also seen in nocodazole-arrested cells and prophase cells in populations treated with taxol. In normal and taxol-treated cells, the microtu-

bules appeared to emanate from the cytoplasmic foci and polar stain, and in cells released from nocodazole block, microtubules regrew from antigen-containing centers. This characteristic distribution suggests that the antigen is part of a microtubule organizing center. Thus, we propose that a prophase originating polar antigen functions in spindle pole organization as a coalescing microtubule organizing center that is present only during mitosis. Characterization of the serum showed reactions with multiple proteins at 115, 110, 50, 36, 30, and 28 kD. However, affinity-eluted antibody from the 115/110-kD bands was shown to specifically label the spindle pole and cytosolic foci in prophase cells.

THE transition from interphase to mitosis is marked by the reorganization of the interphase microtubule network into the mitotic spindle. In interphase cells, microtubules arise generally from one perinuclear microtubule organizing center (MTOC)[1] consisting in most animal cells of the centrioles and associated pericentriolar material. At the onset of mitosis, or slightly before, the centrioles separate and migrate to take up their positions at the opposite spindle poles (Kuriyama and Borisy, 1981; Robbins and Gonatas, 1964; Robbins et al., 1968; Roos, 1973; Vorobjev and Chentsov, 1982). The role of the pericentriolar material in this migration and in the redistribution of microtubules into the spindle poles is not well understood. Indeed, the mechanisms by which the rearrangement of microtubules is effected are unknown.

A number of proteins or antibodies whose antigens are associated with the mitotic spindle (McCarthy et al., 1981; Zieve and Solomon, 1982) have been described. Several were found in the interphase nucleus and localized with the spindle after nuclear envelope breakdown (Izant et al., 1982; Lydersen and Pettijohn, 1980; Pettijohn et al., 1984; Price et al., 1984). Other proteins and phosphoproteins specific to

mitotic cells are reported to appear in the cytoplasm and to bind to mitotic chromosomes (Davis et al., 1983).

We describe here the distribution of a unique antigen recognized by a human autoantibody present in the serum of a patient with CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, schlerodacytyly, and telangiectasias). This antigen was originally identified as a constituent of the mitotic spindle (Oliver et al., 1983; Senecal et al., 1985). We show here that it arises in cytoplasmic foci during prophase and is found subsequently until telaphase in association with the spindle poles. The molecular mass of the antigen was established by specific elution from Western blots and evidence adduced that the antigen is a constituent of an MTOC.

Materials and Methods

Human Serum

Cells

HeLa S3 cells (ATCC CCL 2.2) were grown as described (Sager et al.,

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1. *Abbreviations used in this paper*: MTOC, microtubule organizing center; POPA, prophase-originating polar antigen.
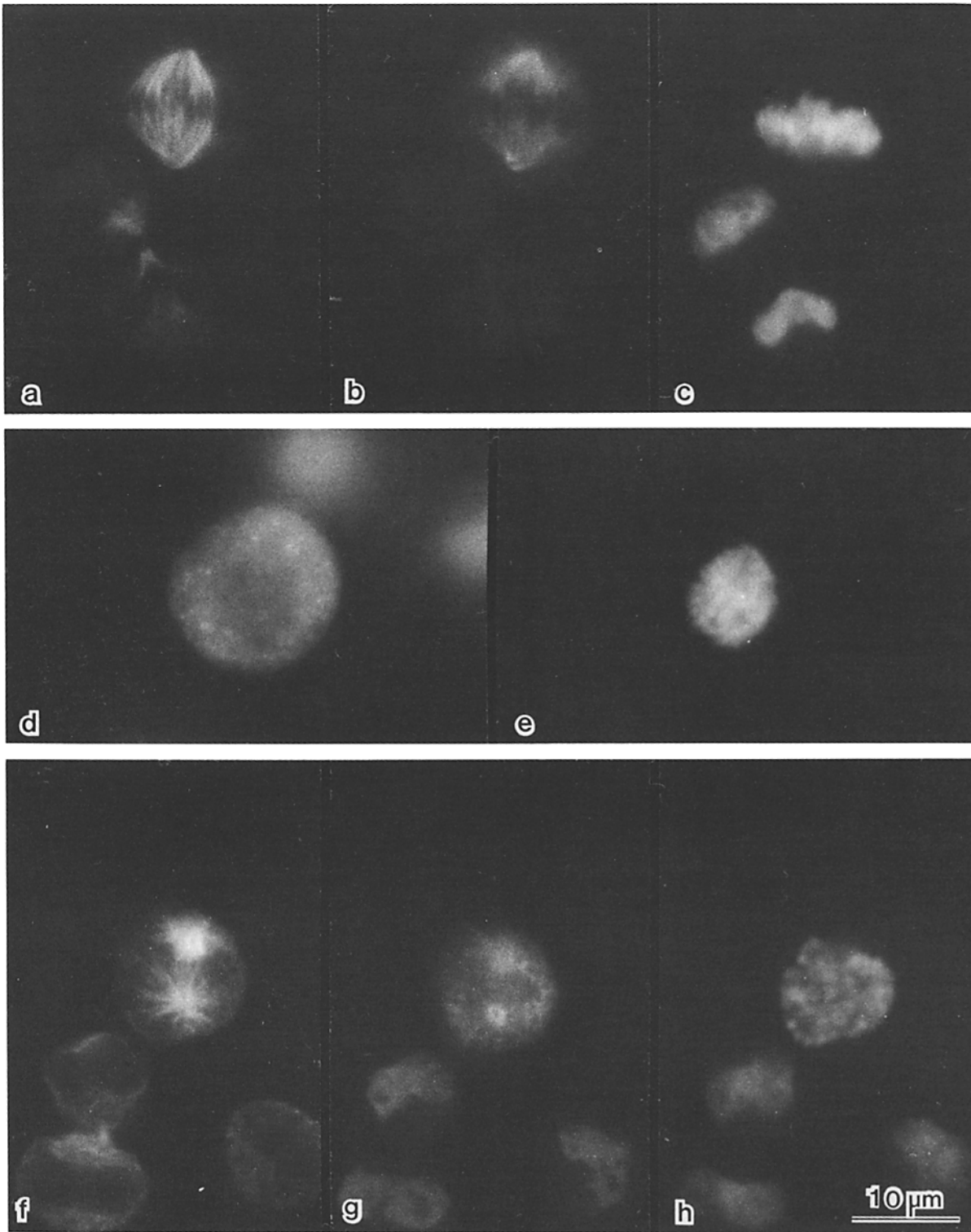
Figure 1. The distribution of human autoantibody, POPA, and antitubulin stains in mitotic HeLa cells. (*a–c*) Metaphase (*top*) and late telaphase (*bottom*). A midbody is nearly formed in the latter. (*a*) Antitubulin: mitotic spindle (metaphase) and midbody region (telaphase) are labeled. (*b*) Autoantibody: the label is concentrated at the poles. Stain is absent in late telaphase. (*c*) Hoechst stain identifying the metaphase (*top*) and telaphase (*bottom*) cells. (*d–e*) Early prophase. (*d*) Autoantibody: many stained punctuate areas are scattered through the cytoplasm. (*e*) Hoechst stain of chromosomes just beginning to condense. (*f–h*) Late prophase. (*f*) Antitubulin: spindle asters are clearly formed. (*g*) Autoantibody is concentrated at poles but some diffuse stain also present. (*h*) Hoechst stain: nuclear envelope is probably still intact.
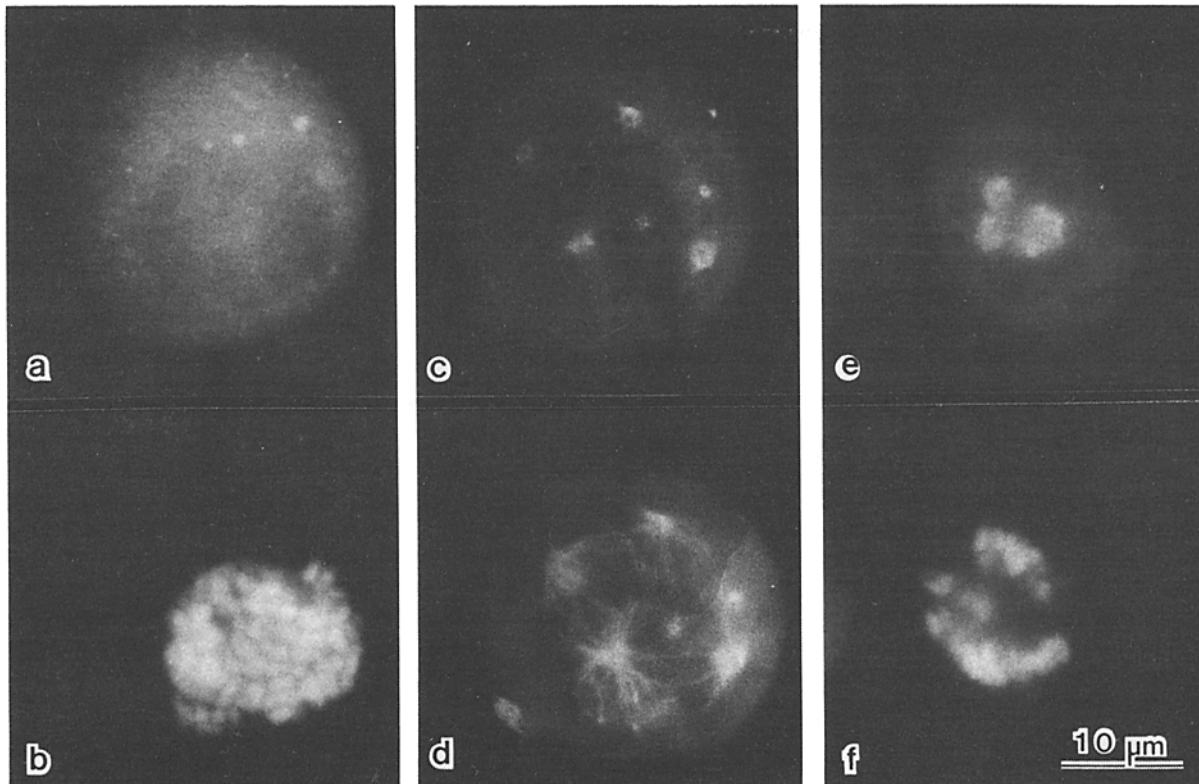
Figure 2. The distribution of POPA in taxol-treated mitotic HeLa cells. Cells were treated with 10 μM taxol for 4 h. (*a-b*) Prophase cell. (*a*) Autoantibody: punctate stain is diffuse in cytoplasm. (*b*) Hoechst stain. (*c-d*) Late prophase. (*c*) Autoantibody: rings at the centers of microasters are stained. (*d*) Antitubulin: small multiple microtubule asters surround the chromosomes. (*e-f*) Metaphase taxol-treated cell. (*e*) Autoantibody: large aggregates of stain are surrounded by chromosomes. (*f*) Hoechst stain.

1984) in Dulbecco's modified Eagle's medium (DME; Gibco, Grand Island, NY) supplemented with L-glutamine, nonessential amino acids, and 7% FBS (HyClone Laboratories, Sterile Systems, Inc., Logan, UT). Cells were plated either directly into 35-mm tissue culture plastic dishes (Falcon Labware, Becton, Dickinson & Co., Oxnard, CA) or into dishes containing three 13-mm diameter glass coverslips. Taxol, provided by the National Cancer Institute, was diluted from a 10-mM stock in DMSO to a final concentration of 10 μM in the culture medium. Nocodazole (Sigma Chemical Co., St. Louis, MO) was also diluted from a 10-mM stock in DMSO to a final concentration of 1 or 10 μM. In some cases, nocodazole-treated cells were incubated further in drug-free medium to allow microtubule recovery.

Labeling of Cells

Cells were fixed for 20–30 min in 2% paraformaldehyde in PBS, postfixed in −20°C acetone for 5 min, and stained with either a mAb against β-tubulin (Amersham Corp., Arlington Heights, IL) and FITC-conjugated anti-mouse IgG (Cappel Laboratories, Cochranville, PA) or human serum containing a mitotic-specific autoantibody (Oliver et al., 1983) and rhodamine isothiocyanate-conjugated anti-human IgG (Cappel Laboratories). All cells were counterstained with a DNA dye (Hoechst 33242; Sigma Chemical Co.) to identify stages of mitosis as described by Berlin et al. (1978).

Preparation of Antigens

After treatment with 10 μM taxol for 15–17 h, cells that were >80% mitotic were aspirated from tissue culture dishes and lysed for 10 min on ice in lysis buffer (Kuriyama et al., 1984), containing 2 mM Pipes, pH 6.8, 0.25% Triton X-100, 20 μg/ml taxol, 1 mM EGTA, and 100 kallikrein inhibitor units (KIU)/ml aprotinin. The cells were pelleted in a microfuge and resuspended in rinse buffer containing 0.25% Triton X-100, 20 μg/ml taxol, 1 mM EGTA, and aprotinin in PBS. DNAse was added to a final concentration of 0.45 mg/ml and the suspension was incubated for 5 min at 37°C. The resulting microfuge pellets and supernatants were frozen in rinse buffer. Interphase cells, incubated with taxol for only 3 h (<10% mitotic), were prepared in

a similar manner. The thawed samples were sonicated and dialysed in Laemmli sample buffer before electrophoresis on 7.5% SDS slab polyacrylamide gels (Laemmli, 1970). Sections of gels were stained with Coomassie Brilliant Blue in acidic isopropanol.

Immunoblotting Procedure

Electrophoretic transfer of proteins from gels to nitrocellulose sheets was performed as described (Towbin et al., 1979). Blots were incubated for 2 h at 30°C in heat-sealed freezer bags with antisera diluted 1:20 or 1:50 in TS buffer (10 mM Tris, 0.9% NaCl, pH 7.4) with 1% BSA. After rinsing in TS-Tween buffer (TS buffer + 0.05% Tween-20), the blots were incubated in peroxidase-conjugated anti-human IgG diluted 1:500 (Cappel Laboratories). After extensive rinsing, the blots were developed in 100 ml TS buffer, containing 40 μl 30% H_2O_2 and 60 mg 4-chloro-1-naphthol (Bio-Rad Laboratories, Richmond, CA) dissolved in 20 ml ice-cold methanol.

Elution of Antibody from Blots

Conditions for affinity-elution of antibody fractions from nitrocellulose were determined according to the scheme presented by Olmsted (1986). Briefly, 0.5-cm strips of the blotted nitrocellulose were incubated with antiserum and, after rinsing, treated for 7 min with various elution solutions, including: 0.2 M glycine, pH 2.8; 0.3 M citric acid, pH 3.0; 0.5 M acetic acid, pH 2.5; 5 M potassium iodide, pH 8.0; 3 M magnesium chloride, pH 8.0; and 3 M potassium isothiocyanate, pH 8.0. After rinsing, the strips and untreated control strips were incubated with peroxidase-conjugated secondary antibody and developed. The treated strips were compared with control strips for efficiency of removal of the antibody stain.

To prepare affinity-eluted antibody, strips were cut from either end of the blot and stained as above. Horizontal sections ∼0.5-cm wide were cut from the blot in areas aligned with the stained strips. These horizontal strips were incubated with antiserum and rinsed in TS-Tween buffer. Each strip was minced, placed in a microfuge tube, and extracted twice with 1 ml 0.5 M acetic acid, 0.5 M NaCl, pH 2.5 for 7 min. Eluted fractions were neutralized with NaOH, desalted on a column (PD-10, G-25 Sephadex, bed volume 3.5
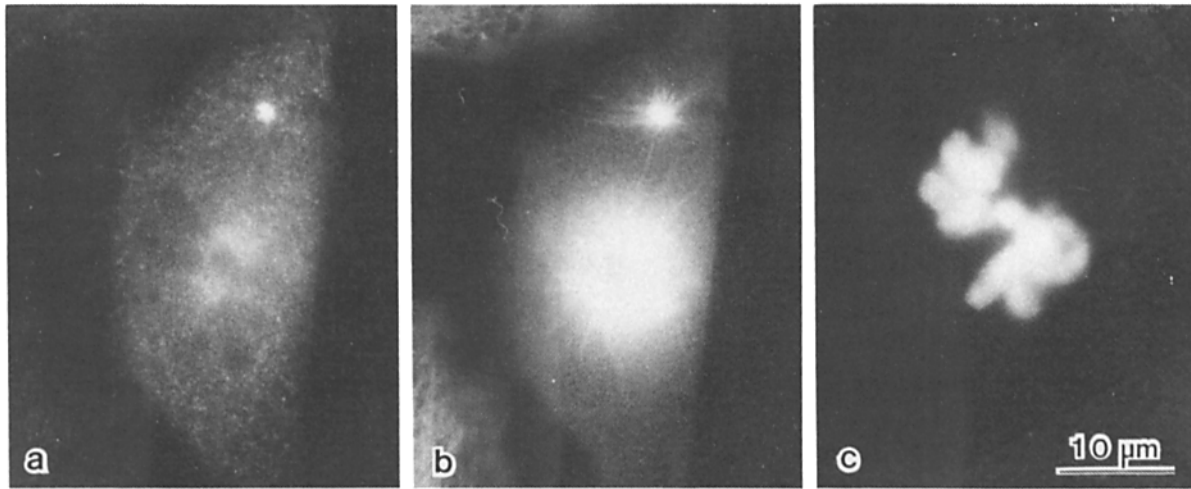
Figure 3. Microtubule growth from coalesced POPA stained center separated from chromosomes in nonhuman cell. PtK2 cell, treated with 10 µM taxol for 4 h, showing two widely separated asters. (*a*) Autoantibody: diffuse stain is seen at core of the larger aster; discrete focus of "satellite" POPA at core of smaller aster. (*b*) Antitubulin: microtubules radiate from central aster as well as from smaller peripheral aster. (*c*) Hoechst stain.

ml; Pharmacia Fine Chemicals, Piscataway, NJ) and concentrated fourfold in microconcentrators (Centricon 10; Amicon Corp., Danvers, MA). Eluted antibody fractions were used to stain cells and to reprobe nitrocellulose strips.

Results

Autoantibody Prophase-originating Polar Antigen (POPA) Stained Mitotic Spindle Poles

In HeLa cells, as well as Chinese hamster ovary (CHO) and PtK2 cells, the only others tested, human serum described here stained spindle poles from late prophase through late anaphase; midbodies in telophase and early G_1 cells were not labeled. As shown in Fig. 1 *a–c*, in cells double-labeled for tubulin (Fig. 1 *a*) and autoantibody (Fig. 1 *b*), the label localized around the ends of microtubules at the spindle poles. When viewed down the long axis of the spindle, the antigen was seen as a hollow circular structure reminiscent of the staining patterns for pericentriolar material (Calarco-Gillam et al., 1983) and NuMA protein (Pettijohn et al., 1984). Importantly, no specific stain of centrioles or pericentriolar material was observed in interphase cells (Senecal et al., 1985).

Autoantibody POPA Stained Multiple Coalescing Cytoplasmic Foci in Prophase

A unique pattern of stain was seen in cells at the onset of mitosis (Fig. 1 *d* and *e*). In early prophase cells, the antigen (Fig. 1 *d*) appeared as small discrete areas of stain in the cytoplasm. As prophase progressed (Fig. 1 *f–h*), the label was associated with the developing asters forming doughnut-shaped areas of stain (Fig. 1 *g*). A similar distribution was seen in taxol-treated cells (Fig. 2 *a* and *b*) where stain first appeared as discrete cytoplasmic foci. Taxol-treated later-prophase cells contain many microasters arranged in a cage around the nucleus; the bases of these microasters (Fig. 2 *d*) were stained with autoantibody in a ring-like pattern (Fig. 2 *c*). After nuclear envelope breakdown, POPA (Fig. 2 *e*) was aggregated within clusters of chromosomes (Fig. 2 *f*) local-

ized at the bases of multiple asters and pseudospindles characteristic of taxol-mitotic cells. Thus, we will refer to the antigen as prophase-originating polar antigen (POPA), reflecting its appearance first during prophase and its movement from the cytoplasm to the spindle poles.

Rarely, Not All POPA Aggregated to the Spindle Poles but Remained Cytoplasmic

Occasionally, prophase cells could be found that simultaneously contained both cytoplasmic and aster-associated foci of POPA. In these cells, microtubules were associated not only with the asters but also with the smaller cytoplasmic areas of POPA. These latter microtubules appeared to be free, but stained for POPA at one end. Rarely, in other cell types, peripheral antigen was observed in the presence of a well-formed spindle. This can be seen readily in the taxol-treated PtK2 cell in Fig. 3. Here POPA was localized in a peripheral focus, from which shorter microtubules radiated, as well as at the spindle; this pattern is similar to the harder-to-visualize peripheral clusters of microtubules emanating from peripheral POPA in untreated prophase cells (not shown). This figure also emphasizes the presence of antigen in nonhuman cell lines.

POPA Marked Areas of Microtubule Initiation in Nocodazole-treated Cells

The apparent association of POPA with microtubules in the spindle pole suggested that the coalescence of antigen might be related to the presence of microtubules. Thus, we examined the distribution of antigen in cells treated with nocodazole, which disrupts microtubules (Fig. 4). Cells treated with 1 or 10 µM nocodazole for 1 h contained no microtubules visible by immunofluorescence staining (Fig. 4 *b*). In cells arrested at prophase–prometaphase, POPA was visible as many small foci throughout the cytoplasm (Fig. 4 *a* and *d*, right); these cells presumably entered mitosis while in nocodazole. Other, less frequent cells that may have been already in mitosis when nocodazole was added were metaphase-like in appearance (Fig. 4 *e*, left). In these cells the
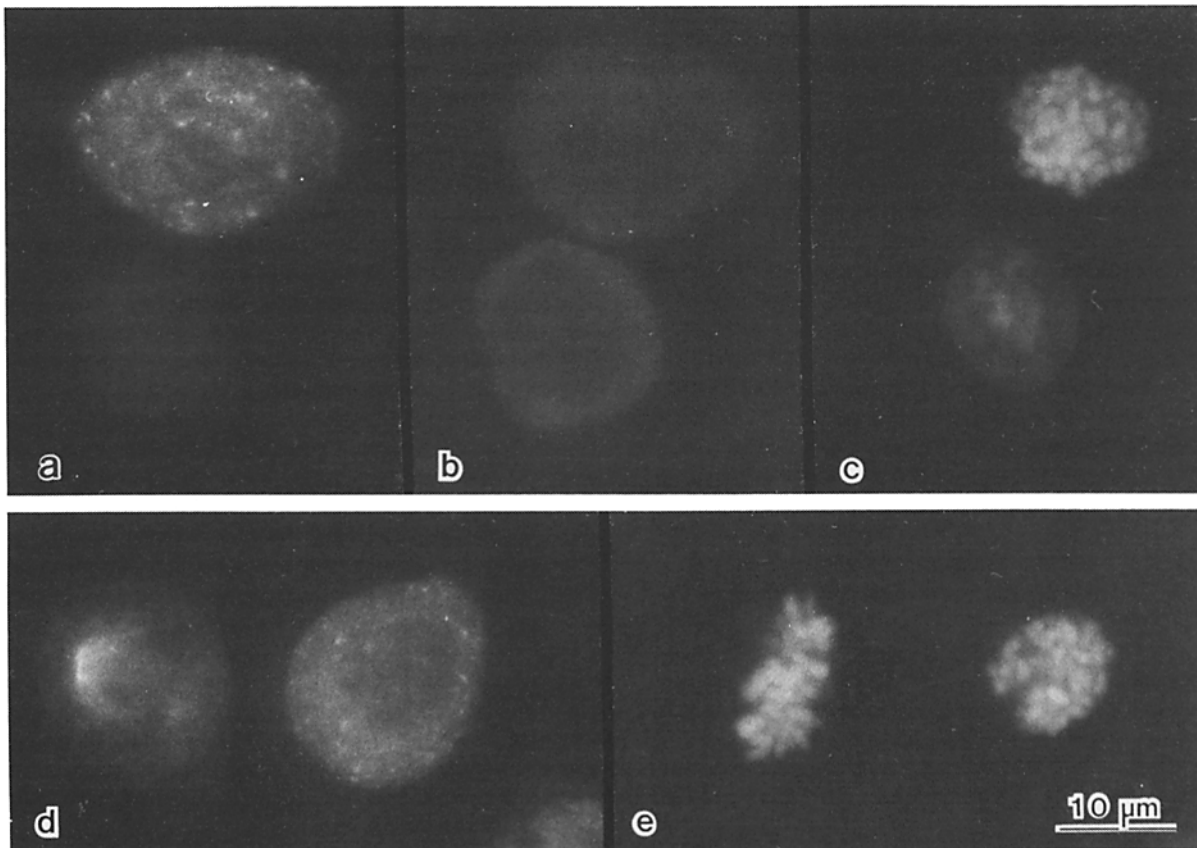
Figure 4. The distribution of POPA autoantibody in nocodazole-treated cells. Cells were treated with 10 μM nocodazole for 1 h. (*a–c*) Late prophase (*top*) and interphase cell. (*a*) Autoantibody: cytoplasmic foci stain in the mitotic cell; no stain is seen in the interphase cell. (*b*) Anti-tubulin: no microtubules remain. (*c*) Hoechst stain. (*d–e*) Arrested metaphase (*left*) and prophase (*right*). (*d*) Autoantibody: POPA is localized in the spindle pole region of disassembled spindle in metaphase cell but remained as cytoplasmic foci in the prophase cell which had not assembled a spindle. (*e*) Hoechst stain.

antigen remained localized at the spindle poles although the spindle had disassembled (Fig. 4 *d*, left).

As cells were allowed to recover from nocodazole treatment, microtubules reformed in both arrested mitotic and interphase cells. In mitotic cells, microtubule reassembly was coincident with the coalescence of POPA. At the earliest stages of recovery (e.g., 5 min, Fig. 5 *a–b*), short microtubules appeared to emanate from several aggregates of POPA. Smaller, more peripheral foci remained but were without obvious microtubule structures. The number of POPA foci was reduced and the size of aggregates increased during initial recovery (compare Figs. 4 *a* and *d* and 5 *a*). During further incubation in drug-free medium for 15–25 min, many cells formed bipolar or multipolar spindles (Fig. 5, *c–e*). A tripolar spindle formed 25 min after release from nocodazole block is shown in Fig. 5, *e–h*. Microtubules appeared anchored in POPA-autoantibody-stained material at the three poles. In this cell, as in many other multipolar and bipolar cells (Fig. 5 *c, f,* and *g*), no additional free microtubules or peripheral POPA foci were seen.

Thus, with or without microtubule drugs, the antigen (POPA) recognized by the autoantibody was first seen as cytoplasmic foci. During subsequent stages of mitosis (in the absence of antimicrotubule drugs), the antigen appeared at the spindle poles or at the base of taxol-pseudospindles.

Identification of Antigens Recognized by Autoantibody

The visualization by immunofluorescence of abundant POPA in taxol-treated mitotic cells, and the accumulation of HeLa cells in mitosis in taxol (>80% at 17 h) suggested a convenient source of antigen. One-dimensional gels (Fig. 6) of Triton X-100–insoluble and –soluble material, derived from mitotic and interphase cell populations, were blotted onto nitrocellulose and probed with patient serum; only proteins in the Triton-insoluble pellet from mitotic cells reacted with anti-POPA serum (compare Fig. 6, lane *b* with lane *d*). As shown, the antiserum consistently recognized six proteins with approximate molecular masses of 115, 110, 50, 36, 30, and 28 kD. Interphase proteins (Fig. 6, lanes *e–h*) did not show specific stain, although occasional faint label of high molecular mass material was seen in the Triton pellet of interphase cells (Fig. 6, lane *f*). Control human serum gave rise to no specific staining (Fig. 6, lane *l*). The serum failed to recognize antigens in nitrocellulose blots of whole nuclei or chromosomal proteins (Earnshaw, W. C., and N. L. Rothfield, personal communications).

Characterization of Affinity-eluted Antibody Fraction

Affinity-eluted antibody fractions were prepared from nitrocellulose blots of mitotic HeLa cells. Four regions, corre-
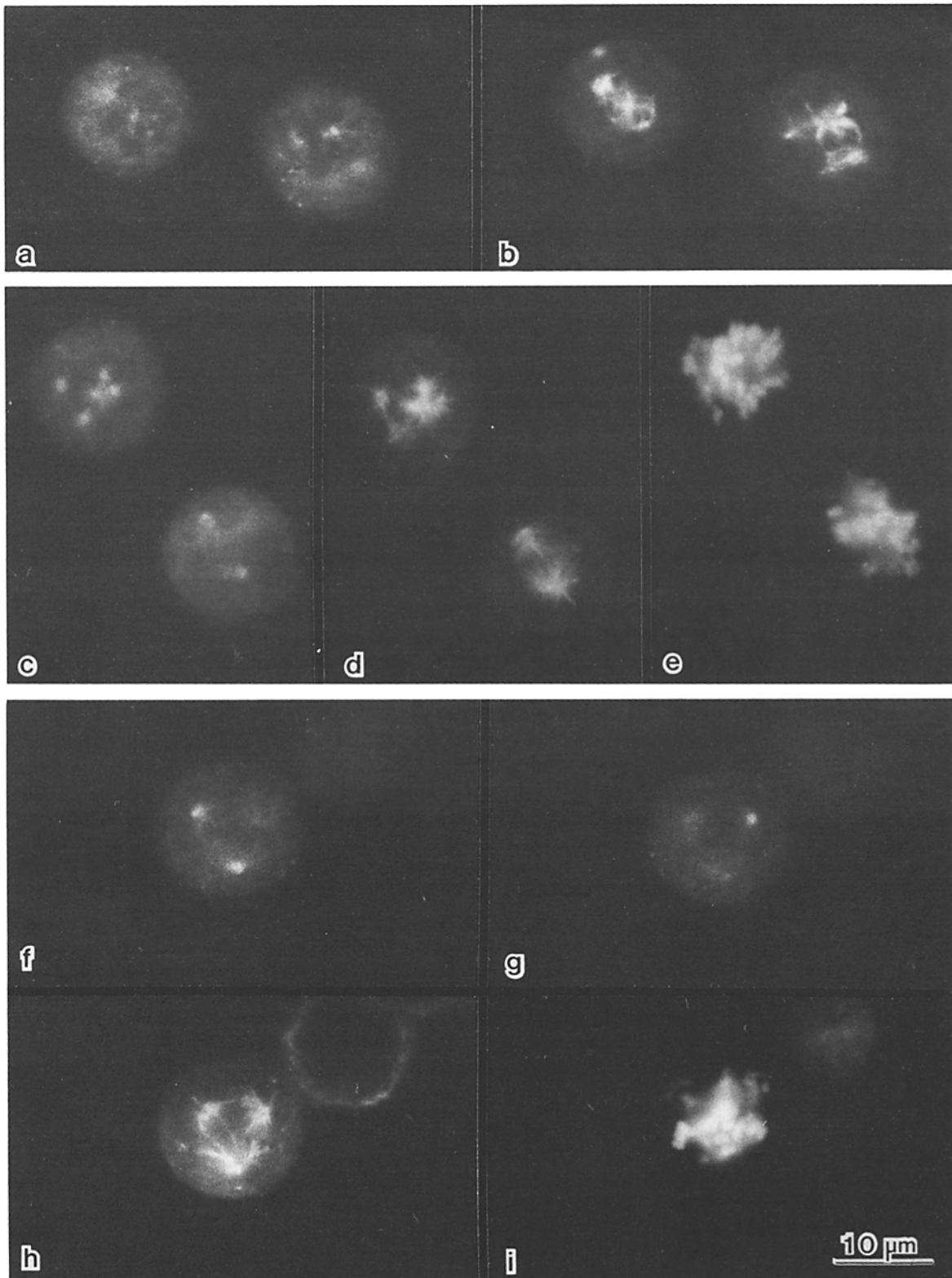
Figure 5. Nucleation of microtubules from multiple foci of POPA in mitotic cells recovering from nocodazole treatment. Cells were exposed to 1 μM nocodazole for 1 h and then allowed to recover in drug-free medium for 5–25 min. (*a–b*) Prometaphase cells, 5-min recovery from nocodazole treatment. (*a*) Autoantibody: POPA appears localized at the base of the aster structures as well as in smaller, more peripheral foci. (*b*) Antitubulin: reassembly of microtubules began, forming several aster-like structures centered on larger aggregates of POPA. (*c–e*) Prometaphase cells, 15-min recovery from nocodazole treatment. (*c*) Autoantibody: POPA coalesced to multiple centers in these cells. The bottom cell nearing metaphase appears to contain only two aggregates of POPA. Small peripheral foci of POPA have disappeared. (*d*) Antitubulin: microtubules emanate from the areas of POPA stain; a bipolar spindle appears to be forming in the cell at the bottom. (*e*) Hoechst stain: the chromosomes in the cell at the bottom have nearly formed a metaphase plate. (*f–i*) Tripolar metaphase cell: 25-min recovery from nocodazole treatment. (*f–g*) Autoantibody: POPA is aggregated at the two poles seen in *f* and the third pole at a different focal plane (*g*); no peripheral POPA stain remains. (*h*) Antitubulin: reassembling microtubules form a tripolar spindle. (*i*) Hoechst stain: chromosomes are aligned between the three spindle poles.
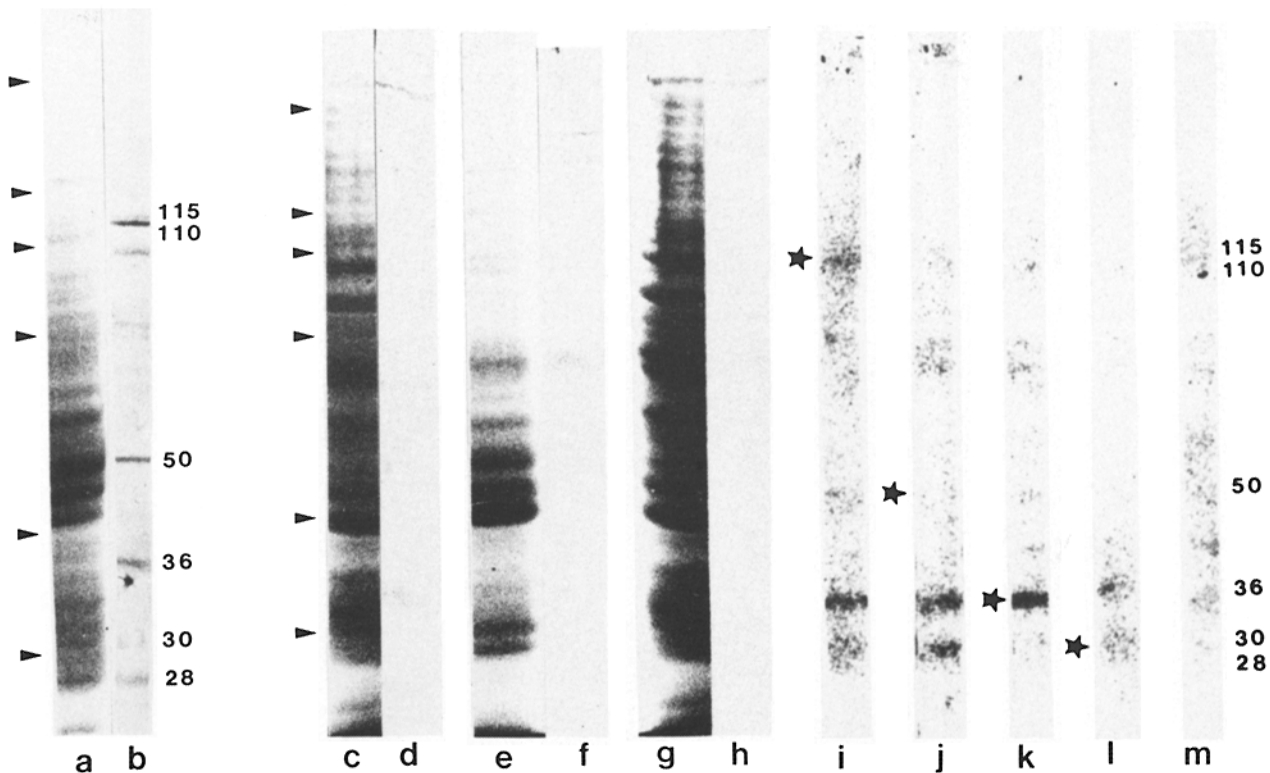
Figure 6. Immunoblotting analysis of mitotic antigens recognized by anti POPA serum. Lanes *a–h*, Triton X-100 pellets (*a, b, e, f*) and soluble supernatants (*c, d, g, h*) were prepared from mitotic cells (lanes *a–d*) in taxol 15–17 h and interphase cells (lanes *e–h*) treated with 10 μM taxol for 2 h. Lane *a*, Coomassie Blue–stained gel of mitotic Triton pellet; lane *b*, nitrocellulose blot probed with whole serum and peroxidase-conjugated anti–human IgG; antigens identified at 115, 110, 50, 36, 30, and 28 kD; lane *c*, Coomassie-stained gel of mitotic-soluble extract; lane *d*, immunoblot; lane *e*, Coomassie-stained gel of interphase Triton pellet; lane *f*, immunoblot; lane *g*, Coomassie-stained gel of interphase-soluble extract; lane *h*, immunoblot. *Arrows* on left indicate molecular mass markers at 205, 116, 97.4, 66, 45, and 29 kD. Lanes *i–l*, binding of affinity-eluted antibody to nitrocellulose blots of Triton-insoluble mitotic proteins (lanes *a* and *b*). Antibodies were eluted with acetic acid and used to reprobe blots. Eluates were collected from four regions of the blot: lane *i*, 115/110 kD (anti–115/110-kD fraction); lane *j*, 50 kD (anti–50-kD); lane *k*, 36 kD (anti–36-kD); lane *l*, 30/28 kD (anti–30/28 kD). Stars mark the region from which antibody was eluted. All fractions show binding to the 36-kD region. Lane *m*, Control serum from nonpatient; positions of antigens are marked on the right.

sponding to the 115/110-kD doublet, the 50-kD region, the 36-kD region, and the 30/28-kD doublet areas of antibody stain, were excised and treated with acetic acid as described in Materials and Methods. This treatment was found to be effective in reducing antiserum labeling of all bands.

Antibody fractions were used to reprobe immunoblots. Reincubation of eluted fractions with nitrocellulose blots (Fig. 6, lanes *i–l*) showed that antibody eluted from the 115/110-kD region (anti–115/110 fraction) relabeled the 110–115-kD region. Stain in the 36-kD region was seen with the anti–115/110-kD, anti–50-kD, anti–36-kD, and anti–30/28-kD fractions. This label probably represents nonspecific sticking of antibody, since faint, diffuse stain in this area was also seen in blots probed with nonpatient sera.

The eluted antibody fractions were used to stain cells to determine which antigens were responsible for the cytosolic prophase and spindle pole stain. As shown in Fig. 7, *a* and *c*, the anti–115/110-kD fraction localized at the spindle poles and also in prophase asters and peripheral foci. Other fractions displayed no specific stain (the anti–36-kD antibody is shown as an example, Fig. 7 *e*). Thus the 115/110-kD bands appear to be distinct from the other proteins labeled by the

autoantibody and appear to represent the antigen present in the cytoplasmic foci and spindle poles.

Discussion

While the mechanisms underlying the transition of the interphase microtubule complex to the mitotic apparatus remain an enigma, the human autoantibody described here offers some hints as to the pathway of microtubule reorganization. The antigen POPA, recognized by the antiserum, has been characterized by a scheme followed for several other autoantibodies associated with spindle microtubules, e.g., autoantibodies recognizing centromeres (Cox et al., 1983; Earnshaw and Rothfield, 1985). The binding of antisera to several proteins is not unusual and complicates interpretation of immunofluorescence staining. Affinity–elution fractionation of serum has proved useful therefore in identifying the antigen of interest (Earnshaw and Rothfield, 1985; Olmsted, 1986). Under conditions described here only spindle pole stain was observed, although in initial studies labeling PtK2, 3T3, and HEp-2 cells with serum from this patient, midbody stain as well as spindle pole label was observed (Oliver et al., 1983;
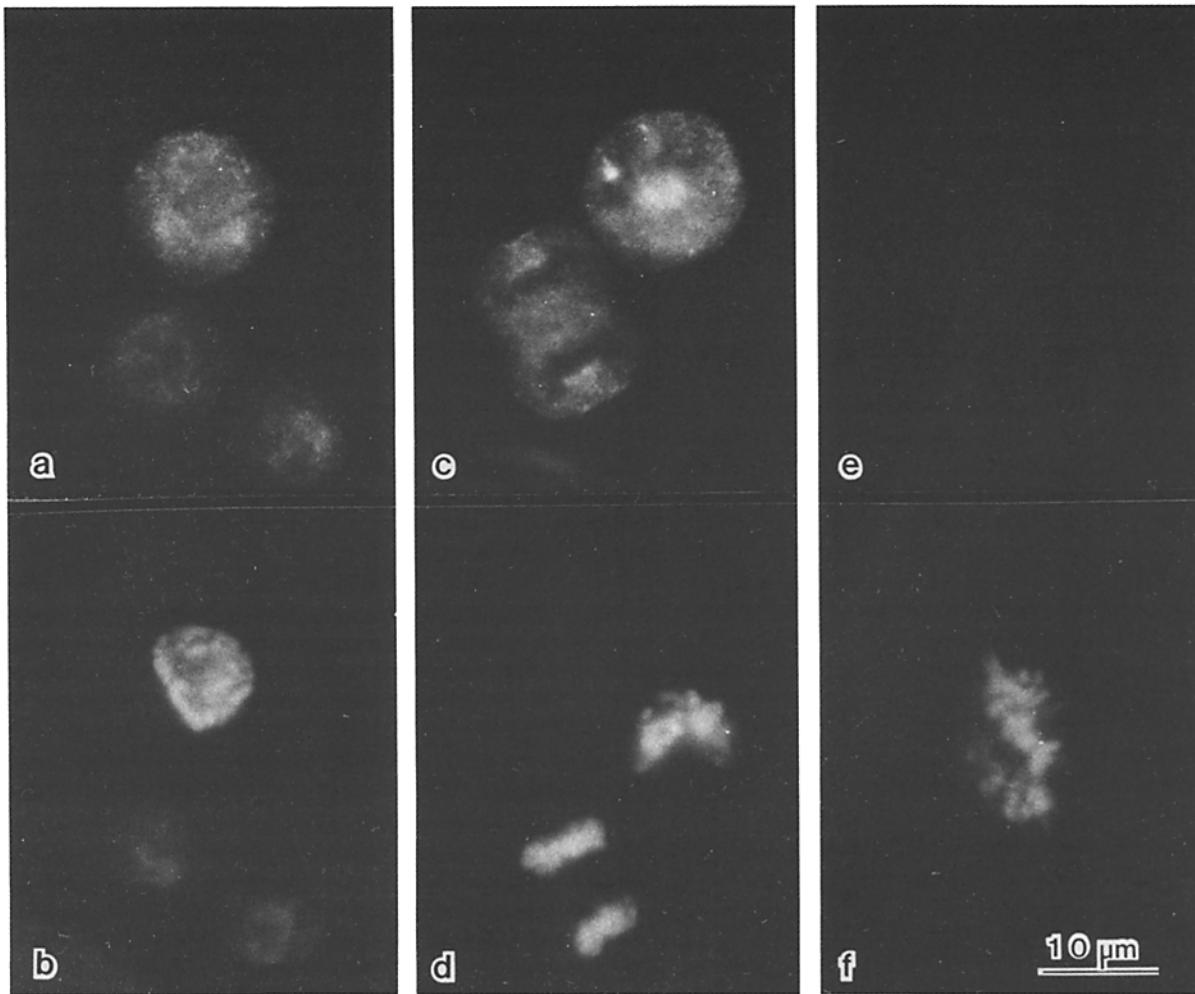
Figure 7. Restaining of cells with affinity-eluted antibody fractions. (*a–d*) Distribution of anti–115/110-kD fraction (eluted from 115/110-kD bands). (*a*) Anti–115/110-kD antibody fraction: label in prophase cell (*center*) at developing asters and in the cytoplasm; (*b*) Hoechst stain; (*c*) anti–115/110-kD antibody fraction: spindle poles stained in anaphase (*bottom*) and prometaphase (*top*) cells. Separated foci are also seen in the prometaphase cell; (*d*) Hoechst stain. (*e–f*) Distribution of anti–36-kD fraction. (*e*) Anti–36-kD antibody fraction. No specific label of spindle poles was seen in the metaphase cell; (*f*) Hoechst stain.

Senecal et al., 1985). Whether this difference is due to fixation methods, cell type, or factors within the serum is unclear.

The specificity of stain of the eluted antibody fractions, by repeated immunoblotting and most importantly by immunofluorescence, indicated that the 115/110-kD antigens were those responsible for mitosis-specific stain in cells. This 115/110-kD species appears distinct from other previously identified mitosis-specific antigens such as the 250-kD NuMA protein that has been found only in human cells (Lydersen and Pettijohn, 1980; Pettijohn et al., 1984; Price et al., 1984) and the mitotic antigens recognized by sera from other patients with connective tissue diseases (reviewed by Price et al., 1984). While several of these sera recognized proteins of 105–115 kD, chromosome or kinetochore stain was demonstrable in addition to polar stain attributable to the 250-kD protein. Neither does POPA correspond by molecular mass to other mitosis-specific proteins (Davis et al., 1983; Zieve and Solomon, 1982).

The absence of detectable antigenicity in interphase cells, detectable by either immunoblotting or specific immuno-

fluorescence stain, distinguishes POPA from microtubule-associated proteins (Izants et al., 1982), and from centrioles and pericentriolar material (Anderson and Floyd, 1980; Lin et al., 1981; Shyamala et al., 1982; Turksen et al., 1982), which form part of the spindle pole. It is, of course, possible that the antigen recognized by the patient serum is present during other parts of the cell cycle but is modified at mitosis. Indeed, other antibodies directed towards mitosis-specific proteins appear to recognize the phosphorylated form (Davis et al., 1983). We are unable to detect POPA antigens in nonmitotic cells, suggesting that POPA precursors, if indeed such exist, are not reactive with the antibodies used to identify the mitotic proteins.

It is the appearance of the antigen during prophase in the cytoplasm, its movement to the spindle poles, and its disappearance during telaphase that makes POPA unique among mitosis-specific proteins. Characteristics of antibody localization revealed in our study may be summarized as follows. (*a*) POPA was present at discrete loci in the cytoplasm at early prophase. In untreated cells, this stage appears to be very short. In taxol-treated cells, the loss of cytoplasmic

POPA appeared to have been slowed. POPA was also cytoplasmic in nocodazole- and colcemid-arrested mitotic cells. (b) At later stages of mitosis, prometaphase through anaphase, POPA was localized near or at the spindle poles. In mitotic cells recovering from nocodazole, POPA became increasingly associated with the spindle poles, including the many cells that developed multipolar spindles. (c) POPA reactivity was not restricted to cells of human origin but was recognized in CHO and PtK cells.

POPA appears to be a constituent of a novel MTOC, as defined by Pickett-Heaps (1969). The antigen forms a core from which microtubules radiate. Moreover, in rare cases, cytoplasmic arrays of microtubules were seen in the presence of developed asters (Fig. 3); these cytoplasmic microtubules were centered on POPA and no other free microtubules were observed. Vandre et al. (1984) reported that free cytoplasmic microtubules could be found in the cell periphery of late prophase PtK cells. It is our conjecture that the ends of these microtubules might have been buried in aggregates of POPA.

The distribution of POPA in mitotic cells recovering from nocodazole treatment lends further support to the role of POPA as a constituent of an MTOC. In these cells, aggregation of POPA and microtubule assembly appear to coincide (Fig. 5). Initially, microtubules were seen in association with several of the larger POPA foci. Subsequently, spindles were formed from the poles to which the majority of POPA had aggregated. POPA stain was localized at the poles of all the tripolar and tetrapolar spindles we observed. Occasionally, more peripheral smaller foci remained but few microtubules could be distinguished in association with these. The patterns of POPA and microtubule localization suggest that initiation of microtubule assembly may be quantitatively correlated with the mass of POPA in an aggregate. Alternatively, coalescence of POPA with some other polar factor may be requisite for efficient initiation of microtubule assembly.

The appearance of POPA in the cytosol occurred at a unique stage of the cell cycle. It was not detectable in centromeres, nor in interphase centrosomes, although we can not rule out the presence of a modified form of the protein. Non-centriolar MTOCs have been reported previously but differ considerably from those reported here. Several aggregates of pericentriolar material surround the nuclei of myogenic syncytia during interphase (Tassin et al., 1985); this is apparently unique to this tissue. Another human autoantibody that was originally described as labeling pericentriolar material has been used to stain cells that do not contain centrioles. The antigen has been localized in the cytoplasm of metaphase II–arrested mouse oocytes (Calarco-Gillam et al., 1983; Maro et al., 1985) and at the spindle poles of higher plants (Clayton et al., 1985). The pericentriolar-like material in oocytes was simultaneously present at the spindle poles and in the cytoplasm. After fertilization, the foci of pericentriolar material remained in the cytoplasm throughout interphase. By contrast, cytoplasmic POPA occurred only in early prophase and appeared to migrate quantitatively (with rare exception) into the spindle. On the other hand, if assimilation into the spindle did not occur (Fig. 3), the cytoplasmic POPA still appeared to coalesce.

It is intriguing to speculate that POPA could play a role in the formation of multipolar spindles. Roughly half the tripolar spindles in CHO cells studied by Keryer et al. (1984) had one pole lacking a centriole. Thus it appears that some

pericentriolar material, but not centrioles, are required for spindle pole formation. POPA also was coalesced at the poles of tripolar and tetrapolar mitotic HeLa cells (Fig. 5) obtained under conditions similar to release from antimicrotubule drug block in Keryer et al. (1984). It is our hypothesis that the additional poles are formed by the aggregation of POPA and perhaps other pericentriolar material that has not been captured by the normal centriolar poles.

Indeed, once coalesced into the spindle, the distribution of POPA, by fluorescence at least, is similar to that of pericentriolar material. Pericentriolar material (or osmophilic fuzz) has been shown to increase at the spindle poles around the centrioles beginning at prophase and continuing through metaphase, and declining at anaphase and telaphase (Robbins et al., 1968; Reider and Borisy, 1982). This resembles, in both localization and abundance, the appearance of POPA. We must emphasize, however, that because of its locus of origin and its appearance exclusively during mitosis, POPA is distinct from other components of the centrosome identified to date.

The special significance of POPA may be that it serves to mark the pathway by which the mitotic spindle is assembled. Whereas all spindle constituents are in some sense of cytoplasmic origin, POPA appeared to coalesce into definite foci separate from the centrioles before its assimilation into the spindle. These foci, which are likely to contain other constituents, may function as MTOCs. When cells were treated with nocodazole, the foci failed to coalesce near the spindle poles and remained dispersed throughout the cytoplasm. However, once coalesced near the poles, POPA was not redispersed after nocodazole treatment; when released from nocodazole block, POPA coalesced to the poles of bipolar or multipolar spindles concurrently with microtubule assembly. This suggests that the assimilation of POPA into the spindle is coordinate with and perhaps dependent upon microtubule assembly. POPA may thus be instrumental in spindle formation.

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Sager et al. Novel Spindle Pole Antigen Arises during Prophase

1871

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