Identification of a 102 kDa protein (cytocentrin) immunologically related to keratin 19, which is a cytoplasmically derived component of the mitotic spindle pole

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SUMMARY

The mAb RK7, previously shown to recognize keratin 19, was also found to cross-react with a biologically unrelated 102 kDa protein, which becomes associated with the poles of the mitotic apparatus. This newly identified protein, called cytocentrin, is a stable cellular component, may be at least in part phosphorylated, and displays a cell cycle-dependent cellular localization. In interphase cells, it is diffusely distributed in the cytosol and shows no affinity for cytoplasmic microtubules. It becomes localized to the centrosome in early prophase, prior to nuclear envelope breakdown, separation of replicated centrosomes, and nucleation of mitotic apparatus microtubules. During metaphase, cytocentrin is located predominately at the mitotic poles, often appearing as an aggregate of small globular sub-components; it also associates with some polar microtubules. In late anaphase/early telophase cytocentrin dissociates entirely from the mitotic apparatus and becomes temporarily localized with microtubules in the midbody, from which it disappears by late telophase. In taxol-treated cells cytocentrin was associated with the center of the mini-asters but also showed affinity for some cytoplasmic microtubules. Studies employing G₂-synchronized cells and nocodazole demonstrated that cytocentrin can become associated with mitotic centrosomes independently of tubulin polymerization and that microtubules regrow from antigen-containing foci. We interpret these results to suggest that cytocentrin is a cytoplasmic protein that becomes specifically activated or modified at the onset of mitosis so that it can affiliate with the mitotic poles where it may provide a link between the pericentriolar material and other components of the mitotic apparatus.

Key words: cytocentrin, keratin 19, centrosome, mitotic spindle

INTRODUCTION

Centrosomes play a key role in determining the organization of cytoplasmic and mitotic microtubules (MTs) in metazoans (reviewed by Brinkley, 1985). Ultrastructurally, these microtubule organizing centers (MTOCs) are seen to be composed of two distinct morphological entities: a pair of centrioles, and a surrounding amorphous, fibro-granular cloud of pericentriolar material (Pickett-Heaps et al., 1982). It has been unequivocally demonstrated that it is the pericentriolar portion of the centrosome that is specifically involved in the functions of microtubule nucleation (Gould and Borisy, 1977), regulation of microtubule number (Kuriyama and Borisy, 1981), establishment of microtubule polarity (Heidemann and McIntosh, 1980) and maintenance of microtubule structure (Evans et al., 1985). During the cell cycle, as cells progress from the G₂ phase into mitosis, the cytoplasmic microtubules that are nucleated by the centrosome disassemble, and the centrosome divides into two identical components, each participating in the nucleation of the opposing halves of the MT array that composes the mitotic apparatus (MA) (McIntosh, 1983). The changes in centrosomal activity during mitosis occur concomitantly with an increase in the abundance of electron-dense pericentriolar material (Reider and Borisy, 1982), a corresponding increase in the MT nucleation capacity (Telzer and Rosenbaum, 1979; McIntosh, 1983) and a prominent increase in centrosomal protein kinase activity (Verde et al., 1990; Bailly et al., 1989).

At present little is known in molecular terms about how centrosomes nucleate and control the number of MTs at different stages of the cell cycle, or how they duplicate themselves prior to mitosis. A logical approach for the investigation of these phenomena is the identification of the molecular constituents of the centrosome, particularly those that change in structure or composition during cell division. Several of
them have been recently identified, primarily relying on immunological techniques. A universal component of the centrosomes is gamma tubulin, which is present in low levels during interphase and in higher amounts during mitosis (Oakley and Oakley, 1989; Stearns et al., 1991; Zheng et al., 1991), and has been shown to play a role in the nucleation of MTs (Joshi et al., 1992). Human autoimmune serum # 5051 was found to cross-react with a 230 kDa protein that is a constituent of the centrosome in interphase and mitotic cells (Calarco-Gillam et al., 1983). Likewise, proteins with molecular masses of 43 kDa (Rao et al., 1989), and a series of proteins with molecular masses of 60-65 kDa, 130 kDa and 180-250 kDa (Gosti-Testu et al., 1986; Gosti et al., 1987) have been identified as constituents of the centrosome, but they have not been characterized further. Other centrosomal proteins do display a cell cycle-dependent localization. They include NuMA (Compton et al., 1992) and/or NuMA-related or identical proteins called SPN (Kallajoki et al., 1991, 1993), SP-H (Maekawa et al., 1991) or centrophilin (Tousson et al., 1991), which are located in the nucleus of interphase cells but redistribute to the spindle poles at mitosis, and a protein of 110-115 kDa (POPA) identified with affinity-purified autoantibodies present in the serum of a CREST patient (Sager et al., 1986), which was shown to stain discrete cytoplasmic foci in early prophase cells and the spindle poles at later stages of mitosis. The exact function of the above mentioned proteins has yet to be elucidated. Other proteins with well known affinity for microtubules or other cellular functions unrelated to cell division have been localized to the centrosomes in mitosis; they include dynein (Pfarr et al., 1990), MAP-1A (Sato et al., 1983), kinesin and kinesin related components (Neighbors et al., 1988), the Ca$^{2+}$-binding protein calmodulin (Welsh et al., 1978), and a 165 kDa protein that aggregates in the presence of calcium (Salisbury et al., 1986). The monoclonal antibody MPM-2 identified a family of mitotic phosphoproteins that are associated with the centromeres, centrosomes and midbody (Vandre et al., 1984), however only one of the MPM-2 reactive antigens has been definitively identified (Vandre et al., 1991). Hence the morphological and molecular data, outlined above, portray the centrosome as a very dynamic entity whose composition changes during the cell cycle.

In this report, we describe a newly identified 102 kDa protein, which we have called cytocentrin, and which behaves as a cytosolic cellular component without affinity for microtubular structures during interphase, but becomes affiliated with the mitotic poles at the onset of cell division. This protein is stable throughout the cell cycle and cross-reacts with the MPM-2 antibody indicating that it is a phosphoprotein. Cytocentrin becomes associated with the centrosome in early prophase, prior to nuclear envelope breakdown, suggesting that it may play an important role in the functional changes that characterize the transition between interphase and mitotic centrosomes.

MATERIALS AND METHODS

Cells

The following rat cell lines were obtained from the American Type Culture Collection (Rockville, MD): MH1C1 (Morris hepatoma, ATCC #CCL 144); L2 (adult lung ATCC #CCL 149); L6 (skeletal muscle myoblast, ATCC #CRL 1458); NRK-52E (kidney epithelial, normal ATCC #CRL 1571); and FR (skin, fetal rat ATCC #CRL 1213). The rat intestinal epithelial cell line IEC-17 (Quaroni and Isselbacher, 1981) was established in our laboratory. Cells were cultured in Dulbecco’s modified Eagle’s Medium (DME) containing 4.5 g/l glucose supplemented with 10 mM Hepes, pH 6.5, 2 mM glutamine, 50 units/ml penicillin, 50 µg/ml streptomycin + 10% fetal bovine serum (FBS) at 37°C, 6% CO$_2$.

Microtubule inhibitors

Taxol, purchased from Calbiochem (San Diego, CA), was dissolved in DMSO to yield a 5 mM stock solution and then diluted in serum-free culture medium to a final concentration of 10 µM. Nocodazole (Sigma Chemical Co. St. Louis, MO) was diluted from a 5 mg/ml stock in DMSO to a final concentration of 10 µg/ml. Reversal from the nocodazole blockade was achieved by washing the cells three times in medium without nocodazole.

Synchronization procedures

Cells were accumulated in the G2 phase as described (Tobey et al., 1990) with minor modifications: exponentially growing monolayers were first cultured in the presence of 5 µg/ml aphidicolin (Sigma Chemical Co.; stock solution was 1 mg/ml DMSO, diluted directly into complete culture medium) for 24 hours to arrest the cells in early S phase. The cultures were then placed in the presence of 10 µg/ml Hoechst 33342 (bisbenzimide H 33342, Calbiochem), an inhibitor of topoisomerase II. For 12 hours. Flow cytometric analysis of the DNA content of the cells indicated that the majority were blocked in G2. Reversal of the G2 blockade was achieved by washing the cells twice with drug-free medium.

Antibodies

FITC-conjugated or rhodamine-conjugated goat anti-mouse Ig, F(ab')$_2$ fragment, and FITC-conjugated or rhodamine-conjugated sheep anti-rabbit IgG were from Boehringer Mannheim (Indianapolis, IN). The following mouse monoclonal antibodies were used in this study: RK7, to rat keratin 19 (Quaroni et al., 1991); KMX-1, specific for beta tubulin (Birkett et al., 1985); E-6, specific for lamins (Lehner et al., 1986); MPM-2, recognizing mitotic phosphoproteins (Davis et al., 1983); and MPM-13, recognizing a 43 kDa antigen associated with the mitotic poles (Rao et al., 1989). They were used as diluted ascites fluids, hybridoma-conditioned media, or as purified immunoglobulins (Beaulieu et al., 1989). For double labelling experiments KMX-1 was purified and directly conjugated with Lissamine-rhodamine B sulfonyl chloride (Molecular Probes, Eugene, OR) as described (Scullion et al., 1987). For use in immunoprecipitations affinity-purified antibodies were coupled to Affigel-10 beads following the instructions of the manufacturer (Bio-Rad, Melville, NY).

A rabbit antiserum to mouse lamin B (Traub et al., 1988) was obtained from Dr P. Traub (Max-Planck Institut fuer Zellbiologie, Rosenhof, Ladenburg bei Heidelberg, FRG), and rabbit antisera to synthetic peptides based on the cDNA-derived protein sequences of human lamins A/C (NH$_2$-GSVTKKKKLESTESC-COOH), human and mouse lamin B (NH$_2$-TTRGKRRVDVEES-COOH), were obtained from Drs G. Blobel and N. Chaudhary (Laboratory for Cell Biology and Howard Hughes Medical Institute, The Rockefeller University, New York, NY).

Immunofluorescence staining

Since it is notorious that the method of fixation can affect the degree of spindle preservation, we tested numerous fixation methods to find the optimal conditions for both spindle preservation and localization of cytocentrin, microtubules, and lamins. We have eventually selected the two methods described below. Cells were
typically 50-80% confluent and were fixed and processed for immunofluorescence staining at room temperature.

(a) Cells were sequentially rinsed twice with PHEM (60 mM Pipes, 25 mM HEPES, 10 mM EGTA, 2 mM MgCl₂, pH 7.0), lysed with 0.5% Triton X-100, 10 µg/ml taxol, 2.5% formaldehyde in PHEM for 2 minutes, incubated with 5% paraformaldehyde, 10 µg/ml taxol in PBS, pH 8.3, for 30 minutes with gentle shaking, rinsed twice with PBS, incubated with 100 mM glycine in PBS for 20 minutes, and rinsed twice with PBS.

(b) Cells were rinsed twice with PBS, incubated with 5% paraformaldehyde in PBS, pH 8.3, for 30 minutes, rinsed twice with PBS, incubated with 100 mM glycine in PBS for 30 minutes at 4°C, rinsed twice with PBS, incubated with acetic acid:methanol (1:1, v/v) for 5 minutes at −20°C; rinsed twice (5 minutes each time) in PBS.

In single-label immunofluorescence experiments to detect tubulin, cells were fixed using procedure (a), for detecting cytokeratin or lamin cells were fixed using procedure (b). For double-label immunofluorescence experiments to detect both cytokeratin and tubulin in the same cells, fixation procedure (a) was employed; to detect both cytokeratin and lamin, fixation procedure (b) was employed.

Unlabelled antibodies were used at the following dilutions: KMX-1 ascites, 1:250; RK7 ascites, 1:100; E-6 ascites, 1:100; rabbit anti-lamin B from Dr Blobel, 1:100; rabbit anti-mouse lamin B from Dr Traub, 1:50. For double labelling experiments, Lissamine-Rhodamine B-conjugated KMX-1 antibody was used at a final concentration of 50 µg/ml. Incubations with primary antibodies were for 60 minutes in a humid chamber; after five rinses in PBS, cells were incubated for 60 minutes with the secondary antibodies diluted 1:25 in PBS. Negative control experiments, which were performed with the omission of the primary antibodies, showed negligible diffuse background staining.

DNA was detected, after immunostaining, by incubating the cells in 50 µg/ml propidium iodide for 5 minutes, followed by three washes with PBS (10 minutes each time). Alternatively, in single-label immunofluorescence experiments cells were routinely counterstained with 0.1% Evans blue for 30 seconds, then washed with PBS.

The cells were mounted in glycerol:PBS (9:1, v/v) containing 2.5 % DABCO and then coverslipped. Cells were observed using a laser scanning confocal microscope (model MRC600; Bio-Rad laboratories, Richmond, CA) mounted on a Zeiss Axiovert 10 microscope. Fluorescent images were saved onto an optical disk and subsequently transferred to a Kodak Plus-X pan 125 black/white film.

Protein immunoprecipitation and western blot analysis

Cells were washed three times with PBS and scraped into homogenization buffer consisting of 300 mM NaCl, 50 mM Tris-HCl, 1 mM PMSF, 2.5 mg/ml leupeptin, 5 mg/ml aprotinin and 2.5 µg/ml taxol, pH 7.4. The buffer was made 0.4% in Triton X-100 and the cells were sonicated three times for 5 second periods at setting 7 using a Branson sonifier (Cell Disruptor 200). The homogenate was incubated on ice for 30 minutes and vortexed every 5 minutes. Deoxycholate was added to give a final concentration of 0.08%, and the homogenate centrifuged at 12,000 g for 15 minutes, 4°C. Antigens were immunoprecipitated from the supernatants by incubating with antibodies bound to Affigel-10 beads at 4°C for 4-12 hours as previously described (Cross and Quaroni, 1991). Immunoprecipitated antigens were eluted from the antibody-bead conjugates using sample buffer and subjected to 10% SDS-PAGE under reducing conditions (50 mM DTT) (Beaulieu et al., 1989). Transfer of proteins to nitrocellulose and immunoblotting were performed as previously outlined (Calnek and Quaroni, 1992).

Preparation of cytoskeletons and calcium depolymerization

Exponentially growing monolayers of L6 cells were incubated with either 10 µg/ml nocodazole, 10 µg/ml taxol, or no drug for 5 hours or else treated with 5 µg/ml aphidicolin for 24 hours. The cells from the different treatments were then washed twice with PBS, followed by PEE (100 mM Pipes, 5 mM EDTA, 5 mM MgCl₂, 10 mM EGTA, pH 6.9). The cells were gently resuspended in PEE buffer containing a protease cocktail consisting of 1 mM PMSF, 2.5 mg/ml leupeptin, 5 mg/ml aprotinin, and 2.5 mg/ml antipain. The resuspended cells were made 0.5% with Triton X-100 and gently passed through a wide bore 10 ml pipette ten times. The cytoskeletons were pelleted by centrifuging at 1,000 g, 4°C for 7 minutes. The supernatants were adjusted to 0.08% with deoxycholate and incubated sequentially with RK7 Affigel 10 beads and KMX-1 Affigel 10 beads; bound antigens were eluted as described above. The pellets (cytoskeletons) were resuspended in PEC (100 mM Pipes, 5 mM EDTA, 5 mM CaCl₂) supplemented with protease cocktail and 0.5% Triton X-100 and incubated on ice for 15 minutes; the remaining insoluble material was pelleted by centrifugation at 12,000 g for 15 minutes at 4°C, then the supernatants were prepared for immunoprecipitation as previously outlined.

Purification of RNA and analysis by reverse transcriptase-polymerase chain reaction (RT-PCR) amplification for the presence of keratin 19 transcripts

Amplification of the 3′ coding region of keratin 19 was performed using the RACE (rapid amplification of cDNA ends) protocol (Frohman et al., 1988). Total RNA was extracted from fetal rat intestine, L6, MH1C1, NRK52E, IEC-17, F2 and FR cells as described (Chirgwin et al., 1979). The integrity of the RNA was verified by agarose gel electrophoresis and ethidium bromide staining and the quantity was determined spectrophotometrically. The RNA was converted to cDNA using reagents from a 3′ RACE kit according to manufacturers instructions (Bethesda Research Laboratories, Bethesda, MD). Keratin 19 sequences were then amplified by annealing primers specific for keratin 19, designated 19X (5′GGGCCTCGAAGGGGCCTTGGCATG 3′) and 19E (5′GGGCGGAATTCGGCAGATCCGAGATA 3′). The polymerase chain reaction (PCR) amplification conditions used were: cycle 1, 3 minutes at 94°C, 1 minute at 55°C and 1 minute at 72°C; cycles 2-9, 1 minute at 94°C, 1 minute at 55°C, 1 minute at 72°C; cycle 10, 1 minute at 94°C, 1 minute at 55°C, 10 minutes at 72°C. The PCR products obtained were analyzed by 1.5% agarose gel electrophoresis and stained with ethidium bromide. The predicted size for an amplified keratin 19 sequence is 345 bp.

RESULTS

Keratin 19 and cytocentrin are biologically unrelated proteins which share a common epitope

The mAb RK7 was produced by fusion of myeloma cells with lymphocytes obtained from a mouse immunized with cytoskeletal components purified from isolated rat intestinal villus cells (Quaroni et al., 1991). It was shown to stain keratin-type intermediate filaments in intestinal epithelial cells in vivo (Quaroni et al., 1991). Immunoblotting of cytoskeletal proteins purified from intestinal cells at different stages of development demonstrated a marked specificity of RK7 for rat keratin 19-related polypeptides (Calnek and Quaroni, 1992). Neither keratins 8, 18 or 21, nor other
cytoskeletal proteins known to be present in the cellular fractions examined, were stained with this mAb. The specificity of RK7 is further illustrated by its lack of ability to recognize human keratin 19 on immunoblots (data not shown), in spite of the 84% amino acid sequence identity between the human and rat homologs in the carboxyterminal region of keratin 19 where the RK7 epitope is located (Chandler et al., 1991).

However, immunostaining with RK7 of several different cell lines derived from rat tissues including intestine (IEC-17), liver (MH1C1), kidney (NRK-52E), lung (L2), muscle (L6) and skin (FR), showed, in all cases, intense staining of the mitotic poles (see, for example, Fig. 1B) and a weak, diffuse cytoplasmic fluorescence. Cells derived from animals other than rat (human, hamster, guinea pig) were not stained at all with RK7. Intermediate filaments containing keratin 19 were only detected in two of the cell lines examined (MH1C1, NRK-52E) and were usually expressed by isolated cells or small groups of cells accounting at most for 10% of the overall cell population (two of them are shown in Fig. 1A).

To identify the component(s) of the mitotic poles recognized by RK7, and to determine whether it was keratin 19 or a distinct protein sharing a common epitope, we have

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**Fig. 1.** RK7 antibody (A,B) and DNA staining (a,b) of MH1C1 cells. (A) The RK7 antibody recognizes a network of keratin filaments present in some interphase cells (denoted by arrows) and the mitotic poles of two cells in anaphase. (B) Several cells in different stages of mitoses can be recognized, all stained at the poles with RK7; note in the center of the figure a cell in early prophase (the corresponding partly condensed DNA is evident in panel (b)), with a bright perinuclear dot representing the earliest stage of cytocentrin association with the centrosome observed. The dull, diffuse cytoplasmic fluorescence can barely be appreciated, but was found in all cells under direct microscopic observation. Bar, 25 µm.
examined both cytosolic and insoluble fractions derived from the cell lines mentioned above by immunoblotting using RK7 as a probe. To increase the sensitivity of the technique cytosolic fractions obtained from relatively large numbers of cells were first incubated with RK7 bound to Affigel beads; the entire amount of antigen bound to each set of beads was then eluted by boiling in the presence of SDS-sample buffer and applied as a single sample to the gels. A high salt cytoskeletal extract prepared from freshly dissociated rat intestinal cells (Quaroni et al., 1991) was used as a positive control and to identify keratin 19 on the immunoblots (see, for example, Fig. 2, lane 1).

Using cell lines known to express keratin 19 (MH1C1, NRK-52E), two distinct polypeptides of molecular mass 42 kDa and 102 kDa, respectively, were detected with RK7 in the cytosolic fractions; the 42 kDa antigen had the same gel mobility as the keratin 19 standard, and was the only band observed in the cell pellets (Fig. 2, lanes 4 and 6). With the muscle-derived cell line L6, which should not express any keratin-type intermediate filament protein (Lazarides, 1982), only the 102 kDa antigen was detected in the cytosol, and no band was visible in the pellet fraction (Fig. 2, lanes 7 and 8). The 102 kDa antigen was also detected in the cytosol of all other rat cell lines examined (L2, IEC-17, FR) (data not shown). These experiments also indicated that the 102 kDa polypeptide is not an abundant cellular protein: the entire amount of antigen immunoprecipitated from the cytosol obtained from at least 5×10^7 cells had to be used in order to clearly detect the 102 kDa band in the immunoblots.

To further confirm that keratin 19 could not be expressed by the L6 cells at levels below the limit of detection of immunoblotting, and thus that the 102 kDa antigen could not possibly represent an aggregate of keratin 19, we carried out the more sensitive technique of RT-PCR using primers specific for keratin 19. In Fig. 3, the predicted 345 bp amplified keratin 19 PCR product is present in lane 2, where keratin 19 transcripts were amplified from RNA purified from isolated fetal intestinal epithelial cells. Keratin 19 transcripts were also detected in MH1C1 (lane 3) and NRK-52E (lane 5) cells, but were absent from L6 (lane 4), IEC-17 (lane 6), L2 (lane 7) and FR (lane 8) cells. These observations correlate quite well with the results obtained by immunostaining and immunoblotting described above. It should also be noted that the 102 kDa protein we have identified was not recognized on immunoblots by other, commercially available, monoclonal antibodies specific for keratin 19, and that an anti-human keratin-19 mAb we have produced did not stain the mitotic poles in either human or rat cell lines (data not shown).

![Fig. 2. Identification of the RK7 antigen(s) by immunoblot analysis. Cells were homogenized and centrifuged to yield a cytosolic fraction (C) and a pellet (P). Proteins were immunoprecipitated using the RK7 antibody bound to Affigel 10 beads from the cytosol of MH1C1 cells (lane 3), NRK-52E cells (lane 5) and L6 cells (lane 7); the corresponding pellets from MH1C1 cells (lane 4), NRK-52E (lane 6) and L6 cells (lane 8) were directly solubilized in sample buffer. The proteins immunoprecipitated from the cytosol and those solubilized from the pellets were subjected to 10% SDS-PAGE under reducing conditions (50 mM DTT), transferred to nitrocellulose and probed with the RK7 antibody. A high salt extracted cytoskeletal preparation from rat intestinal epithelial cells served as a positive control for keratin 19 (lane 1); the negative control consists of antibody beads not incubated with cell extracts (lane 2).](image1)

![Fig. 3. Detection of keratin 19 transcripts by RT-PCR. RNA was isolated from cells and converted to cDNA using reverse transcriptase; keratin 19-containing DNA sequences were amplified using primers specific for keratin 19 in a PCR amplification reaction. The products from the reaction were analyzed on a 1.5% agarose gel and stained with ethidium bromide. The predicted keratin 19 product is a 345 bp fragment (marked by an arrow). Lane 1 is a 123 bp ladder of DNA markers. The following tissues or cells were tested for the presence of keratin 19 transcripts: fetal rat intestine (lane 2), MH1C1 (lane 3), L6 (lane 4), NRK-52E (lane 5), IEC-17 (lane 6), L2 (lane 7) and FR (lane 8).](image2)
These results unequivocally demonstrate that the protein seen at the poles of the mitotic cells stained with mAb RK7 is not keratin 19 or an aggregate of keratin 19. Instead, this newly identified 102 kDa antigen is a biologically unrelated protein that shares a common epitope with keratin 19. We have called this protein cytocentrin (cytoplasm-derived centrosome-associated protein).

Cross-reaction of cytocentrin with other anti-centrosomal antibodies

MPM-2, a monoclonal antibody raised against mitotic HeLa cells (Davis et al., 1983), has been used widely to stain the mitotic centrosomes in cultured cells from different species (Davis et al., 1983; Vandre et al., 1984, 1986; Millar et al., 1987). This mAb recognizes a phosphorylated epitope common to several structural and soluble proteins associated with the mitotic apparatus (Davis et al., 1983; Vandre et al., 1991; Vandre and Burry, 1992). The MPM-2 epitope appears on the mitotic centrosome before nuclear envelope breakdown and remains phosphorylated through metaphase (Vandre et al., 1986, 1991), thus closely resembling the behavior and temporal association of cytocentrin with the centrosome (see below). It was therefore of interest to determine whether cytocentrin could cross-react with MPM-2. In these studies we have used L6 cells, either untreated or enriched for mitotic cells by treatment with nocodazole for 5 hours. Following homogenization, cytocentrin was immunoprecipitated from the cytosol as described above and then immunoblotted with either RK7 or MPM-2. Total L6 cell proteins served as a positive control for the latter antibody, which, as expected, stained intensely a large number of phosphorylated proteins (Fig. 4, lane 1). Cytocentrin did appear to cross-react with MPM-2 (Fig. 4, lane 2), but the intensity of the signal was rather weak (compare with the intensity of stain obtained using an equal aliquot of antigen blotted with the mAb RK7; Fig. 4, lane 3). This result suggests that cytocentrin may be, at least in part, phosphorylated, but does not represent one of the major antigens identified by MPM-2 in the mitotic apparatus.

Cytocentrin failed entirely to react with MPM-13, another mAb staining the centrosome in both interphase and mitotic cells (Rao et al., 1989) (not shown).

Distribution of cytocentrin throughout the cell cycle: correlation with lamins and tubulin

The results presented in the following figures were obtained using MH1C1 cells, which, because of their flat mitoses, facilitated the observation of mitotic structures. However, several other cell lines (L6, NRK-52E, SV40G, L2) were examined in detail, and demonstrated the same patterns of cytocentrin localization at different stages of mitosis described for MH1C1 cells.

In Fig. 5, the localization of cytocentrin (A-F) is correlated with that of tubulin (a-f) throughout mitosis. In Fig. 5A,a (prophase), shortly after centrosome duplication, cytocentrin and tubulin are both concentrated in the mitotic centrosomes, although at closer examination staining for cytocentrin often appeared granular and relatively more intense in the center of the developing asters than that of tubulin. At this stage of mitosis some of the cytoplasmic microtubules were often found still assembled, as seen in Fig. 5a, but cytocentrin was clearly not associated with these structures: instead, a diffuse cytoplasmic staining with RK7 is seen throughout the cytoplasm in Fig. 5A; typically, it appeared more intense in cells in prophase than in interphase.

At metaphase (Fig. 5B,b) prominent crescents of cytocentrin, often appearing as clusters of small globular material, are clearly visible at the poles of the mitotic apparatus. This intense staining correlates well with the pronounced increase in dense pericentriolar material seen by electron microscopy (Reider and Borisy, 1982). Some of the polar microtubules also appears weakly stained for cytocentrin. Fig. 5C,c illustrates a tripolar mitosis where cytocentrin is located at the center of all poles.

In Fig. 5D,d (anaphase) a prominent staining for cytocentrin is still observed at the mitotic poles, but a weaker, diffuse staining is also associated with many of the spindle microtubules. At later stages of anaphase, staining of the mitotic poles with RK7 was found to be much less intense or even absent.

In Fig. 5E,e (late telophase) cytocentrin is localized to the central region of the MT bundles located in the midbody of each daughter cell: superimposition of the images shown in this figure clearly confirmed that the center of the midbody and the more peripheral portions of the microtubules were not stained with RK7. By late cytokinesis (Fig. 5F,f) cytocentrin antigen is no longer associated with the remnants of the midbody microtubules.
In Fig. 6 the pattern of cytocentrin localization during the cell cycle is compared to that of lamin B. In Fig. 6A,a (early prophase), the nuclear envelope is still clearly intact, while a prominent accumulation of cytocentrin is observed in a single perinuclear spot. In Fig. 6B,b, the nuclear envelope appears partially disrupted and the daughter centrosomes, both intensely stained with RK7, are starting to separate; this process has progressed further in Fig. 6C,c with the complete dissolution of the envelope and further separation of the asters. In metaphase (Fig. 6D,d) lamin B is dispersed throughout the two cells in mitosis, while the poles are stained intensely for cytocentrin. In Fig. 6E,e (early telophase), as the nuclear envelope is partially reassembled, cytocentrin is still located at the poles and starts to make its appearance at the forming midbody. In Fig. 6F,f (late telophase-early cytokinesis) the nuclear envelope has reformed in the daughter cells and cytocentrin is located exclusively at the midbody.

**Localization of cytocentrin and tubulin in taxol-treated cells**

Treatment of cells with taxol results in the dispersal of pericentriolar material from the centrosomes: it redistributes as expected for a capping or nucleating protein resulting in the formation of multiple miniasters in each mitotic cell (De Brabander et al., 1986). As expected, in taxol-treated cells all of the mitotic asters stained positive for tubulin (Fig. 7b). Although the center of most of the asters was also stained with RK7, the intensity of the fluorescence varied dramatically (Fig. 7A,B) and, in most mitotic cells, only one or two prominent dots were observed. In extreme cases (Fig. 7B), many of the miniasters did not appear to contain cytocentrin at all, and only the two prominent foci/cell were stained intensely with RK7. We speculate that they may have been the ones that formed before nuclear envelope breakdown, and thus the only ones to contain centrioles, whilst the other miniasters may have been nucleated by the dispersed pericentriolar material. In many cells, both with and without mitotic figures, the cytoplasmic bundles of many, but clearly not all (compare Fig. 7B,C with b,c) microtubules were labelled. Superimposition of the two images shown in Fig. 7C,c (and several other similar pictures we have taken) clearly showed that the RK7 and anti-tubulin antibodies stained the same filaments. This suggests that cytocentrin can interact, directly or indirectly, with microtubules but only becomes activated to do so at particular stages of the cell cycle. This association of cytocentrin with cytoplasmic MTs was only observed in taxol-treated cells.

**Localization of cytocentrin in nocodazole-treated cells**

To determine whether the association of cytocentrin with the mitotic centrosome (starting in early prophase) is depen-

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**Fig. 5.** Double-label immunofluorescence staining of MH1C1 cells for cytocentrin (left panels) and tubulin (right panels). (A-F) Cells stained with the RK7 antibody, and (a-f) the same cells stained with the anti-tubulin antibody KMX-1. Cells shown were in prophase (A,a), metaphase (B,b), tripolar mitosis (C,c), anaphase (D,d), late telophase (E,e) and cytokinesis (F,f). Bar, 10 µm.
dent on the presence of microtubules, we have examined its cellular distribution in cells treated with nocodazole, a powerful inhibitor of tubulin polymerization. As expected, neither cytoplasmic nor mitotic spindle microtubules were detected in cells treated with nocodazole for 5 hours (Fig. 8B). In each cell arrested in pro-metaphase only two dots were stained with the anti-tubulin antibody (Fig. 8B): they likely correspond to centrioles, which are known not to be affected by nocodazole (De Brabander et al., 1980). Double labelling experiments demonstrated that they were also stained intensely with the RK7 antibody (Fig. 8A).

To further investigate whether cytocentrin could become associated with the mitotic centrosomes prior to MT nucleation and assembly, cells were synchronized in late G2 and, one hour prior to removal of the Hoechst drug, were also treated with nocodazole. One hour after release from the cell cycle block, but still in the presence of nocodazole, cytocentrin was visible in mitotic cells as two bright foci adjacent to the condensed DNA (Fig. 8C,D), suggesting that cytocentrin becomes sequestered in the mitotic centrosome independently of tubulin polymerization.

Depolymerization of microtubules, induced by 5 mM CaCl2 at the time of fixation and permeabilization of cells not treated with any drugs, produced similar results: double labelling for tubulin and cytocentrin showed that, in mitotic cells devoid of microtubules, the localization of cytocentrin at the mitotic poles was not significantly affected (data not shown).

We have also documented, by double-label immunofluorescence, the location of tubulin and cytocentrin during the process of reassembly of the mitotic apparatus in cells previously treated with nocodazole. As described above, at the time nocodazole was removed each mitotic cell possessed two foci stained for both tubulin and cytocentrin (Fig. 9A,a, '0 minutes'). These two prominent foci of RK7 staining/cell were also clearly visible at later times (see arrows in Fig. 9B-C), but did not usually appear to function as nucleation centers for microtubule assembly. Instead, additional, multiple small foci were already formed in mitotic cells by 15 minutes after nocodazole removal (Fig. 9B,b): they were stained more intensely for tubulin than for cytocentrin and appeared to represent the main nucleation centers for mitotic microtubules. By 30 minutes after release from nocodazole they started to coalesce into 2-3 centers/cell, each nucleating a few short microtubules (Fig. 9C,c) until, by 45-60 minutes, a relatively normal mitotic apparatus (stained for cytocentrin primarily at the poles), was reformed in each mitotic cell (Fig. 9D,d).

**Stability of cytocentrin during the cell cycle**

To investigate whether cytocentrin is subject to cyclic degradation and to confirm its presence in cultures devoid of mitotic cells, L6 cells growing as exponential monolay-

![Fig. 6. Double-label immunofluorescence staining of MH1C1 cells for lamin B (left panels) and cytocentrin (right panels). (A-F) Cells stained with an anti-lamin B antiserum from G. Blobel, (a-f) the same cells stained with the RK7 antibody. Cells shown were in early prophase (A,a), prophase (B,b, and, C,c), metaphase (D,d), early telophase (E,e), and late telophase-early cytokinesis (F,f). Bar, 10 μm.](image-url)
ers were either untreated, or treated with nocodazole or taxol for 5 hours, or aphidicolin for 24 hours (to collect cells at the G1/S interphase) (Pedrali-Noy et al., 1980). Cytocentrin and tubulin were sequentially immunoprecipitated from the cytosolic fractions and analyzed by immunoblotting (Fig. 10, lanes 1-8). The assembled tubulin present in the corresponding cell pellets was subsequently depolymerized, and cytocentrin and tubulin were again sequentially immunoprecipitated and analyzed by immunoblotting. All immunoprecipitates were standardized to the same amount of total cellular protein. All cytosolic samples produced a cytocentrin band (Fig. 10, lanes 1-4), although it appeared significantly more intense in cells treated with nocodazole (lane 2), taxol (lane 3) or aphidicolin (lane 4). Interestingly, in the cytosol of cells treated with nocodazole three additional bands of lower apparent molecular mass were stained with RK7 (Fig. 10, lane 2): we speculate that they may represent degradation products of cytocentrin, suggesting that a prolonged block in metaphase and/or in the absence of microtubules may have

Fig. 7. Localization of cytocentrin (A-C) and tubulin (b,c) in taxol-treated cells. Cells were cultured in the presence of 10 µM taxol for 5 hours and then either processed for single-label immunofluorescence with RK7 followed by DNA staining with propidium iodide (A,a), or double-label immunofluorescence with the RK7 antibody and the anti-tubulin antibody KMX-1 (B,b and C,c). Note the presence of multiple miniasters in each mitotic (A,B,b), many of them showing a markedly different staining intensity with the two antibodies; in (b) miniasters containing both cytocentrin and tubulin are indicated with arrows: all others showed very weak or absent staining with RK7 (compare with B). In (C,c) only interphase cells are present, and intense staining of some cytoplasmic microtubules with RK7 is evident. Bar, 25 µm.
led to cytocentrin’s instability. As predicted a soluble pool of tubulin was also present in all cytosolic samples, and
was reduced in intensity in cells treated with taxol (Fig. 10, lanes 5-8). Under no condition could cytocentrin be
detected associated with the cell pellet (lanes 9-12). The
relative amounts of tubulin immunoprecipitated after solu-
bilization of the microtubules, shown in lanes 13-16, were
as predicted based on the known effects of the drugs used:
absent in nocodazole-treated cells (lane 14), and markedly
increased in cells treated with taxol (lane 15).

DISCUSSION

In this report we have described a novel protein that sequen-
tially relocates from the cytoplasm to the mitotic centro-
somes and eventually to the midbody. This protein, which
we have called cytocentrin, represents a newly identified
component of the mitotic apparatus and first becomes local-
ized to the centrosome in early prophase, before separation
of the diplosomes and breakdown of the nuclear envelope.
Immunoprecipitation, using the mAb RK7, from a variety
of cell lines combined with western blot analysis, identifies
a 102 kDa protein, suggesting this is cytocentrin. However,
it cannot be unequivocally concluded that the spindle-pole-
associated protein detected by immunofluorescence has a
molecular mass of 102 kDa, since it may not be efficiently
immunoprecipitated or recognized on a western blot by
RK7 mAb.

We have identified cytocentrin using a monoclonal anti-
body that was originally characterized as being specific for
keratin 19 (Quaroni et al., 1991). This may appear surpris-
ing, but is not without precedents. Other centrosomal pro-
teins have been found to share common epitopes with
cytoskeletal components. A mAb produced against
Drosophila intermediate filaments was shown to cross-react
with centrosomes in sea urchin eggs, where it specifically
recognized a 68 kDa protein (Schatten et al., 1987). Like-
wise, a panel of monoclonal antibodies produced against
insoluble cytoskeletal residues purified from HeLa cells
identified a 210 kDa protein, called SPN antigen, which
localized to the nucleus in interphase cells and to the poles
during mitosis (Kallajoki et al., 1991, 1993). The opposite
has also been observed: a mAb raised against centrosomes
purified from human lymphocytes was found to stain both
pericentriolar material and intermediate filaments in the
same interphase cells, to recognize an antigen associated
with vimentin-type intermediate filaments, and to stain
intensely cytoplasmic filaments in MDCK cells (Buendia et
al., 1990). The recent description of the molecular structure
of NuMA, a mitotic pole-associated protein (Yang et al.,
1992; Compton et al., 1992) offers a potential explanation

Fig. 8. Localization of cytocentrin
(A,C) and tubulin (B) in
nocodazole-treated MH1C1 cells.
(A,B) Cells were treated with
nocodazole for 5 hours and then
processed for double-label
immunofluorescence with the RK7
antibody and the anti-tubulin
antibody KMX-1: note the
presence of 1-2 foci in each mitotic
cell, stained for both cytocentrin
and tubulin (the latter may
correspond to staining of
centrioles); no cytoplasmic or
spindle microtubules are observed
in (B). (C,D) The cells were
synchronized in late G2 and, 1 hour
prior to removal of the cell cycle
block, were treated with
nocodazole. One hour after
removal of the G2 blockade, but
still in the presence of nocodazole
the cells were stained to detect
cytocentrin (C) and DNA (D). Bar,
12 µm.
for the above findings. DNA sequence analysis has revealed that NuMA contains two nonhelical terminal regions flanking a long central alpha-helical domain, similar to the highly conserved coiled-coil region found in all classes of intermediate filament proteins, including cytokeratins and nuclear laminas.

Ultrastructural examination of the centrosome has shown that, in addition to centrioles and a convergent set of microtubules, it contains a variety of electron-dense structures collectively known as pericentriolar material (PCM). The PCM includes different morphological components: a fibrillar cloud surrounding the centrioles (pericentriolar matrix), compact electron-opaque bodies (pericentriolar satellites), and a variety of centriolar ‘appendages’ (Rieder and Borisy, 1982; reviewed by Brinkley, 1985). It is, at least in part, composed of 3-8 nm diameter filaments forming a three-dimensional lattice (Baron and Salisbury, 1988). A component similar to intermediate filament proteins has been identified in tubulin-depleted sea urchin spindles, where it formed a spindle-shaped matrix (Leslie et al., 1987). Other nonmicrotubule filaments composed of tektin (Steffen and Link, 1992) and spoke (Paddy and Chelsky, 1991) proteins were observed throughout the spindle apparatus. The role of PCM, and not centrioles, in nucleation and anchoring of cytoplasmic microtubules is well established (Gould and Borisy, 1977; Telzer and Rosenbaum, 1979; Kuriyama and Borisy, 1981; Rieder and Borisy, 1982). It seems likely, therefore, that different filamentous proteins containing coiled-coil alpha helical regions may be either constitutive or facultative components of the interphase and mitotic centrosomes. The fact that cytocentrin shares an epitope located in the carboxyterminal (alpha-
helical) domain of keratin 19 suggests that it may belong to such a class of proteins.

**Comparison of cytocentrin with other previously described mitotic pole proteins**

In recent years, immunological techniques have proven highly successful in identifying new components of the centrosome and of the mitotic apparatus. A careful review of the rather extensive literature on this subject has however revealed that cytocentrin can be distinguished from all other previously identified centrosomal and pole proteins based on its localization during the cell cycle, apparent molecular mass, association with microtubules, and other key properties.

Its size and cytoplasmic location in interphase cells clearly distinguish cytocentrin from a group of pole components, many with masses in excess of 200 kDa, which are restricted to the nucleus of non-mitotic cells. Included in this group are the above mentioned NuMA (Lydersen and Pettijohn, 1980) and identical or related NUMA-like proteins called SPN antigen (Kallajoki et al., 1991, Yang et al., 1992), Centrophilin (Tousson et al., 1991), SP-H antigen (Maekawa et al., 1991), H1B2 antigen (Nickerson et al., 1992), and a 225 kDa protein identified in sea urchin eggs (Kuriyama, 1989). Other nuclear proteins that associate with the centrosomes only during cell division are a 280 kDa protein immunologically related to MAP1 (Bonifacino et al., 1985) and a kinesin-like protein of 110 kDa (Sellitto and Kuriyama, 1988b; Nislow et al., 1992).

Other pole proteins and antigens differ from cytocentrin primarily because they are associated with both interphase and mitotic centrosomes; most of them can also be distinguished from cytocentrin based on their molecular size and localization during the various phases of mitosis. This group includes: gamma tubulin (Zheng et al., 1991; Stearns et al., 1991; Joshi et al., 1992); the S051 antigen (Calarco-Gillam et al., 1983); a 43 kDa protein defined by the mAb MPM-13, which stains a membranous perinuclear area in interphase cells (Rao et al. 1989); the antigens of molecular masses 180-250 kDa, 130 kDa, and 60/65 kDa recognized by a non-immune rabbit serum (Gosti-Testu et al., 1986); the 74 kDa and 170 kDa polypeptides defined by a mAb raised against centrosomes from human lymphocytes, which in interphase cells stained both pericentriolar material and intermediate filaments and, after nocodazole treatment, labelled a large ribbon-like structure in addition to interphase centrosomes (Buendia et al., 1990); the 20 kDa centrin and an immunologically related 165 kDa protein, recognized by an antibody that stains a broad juxtanuclear region in the cytoplasm of interphase cells, at higher magnification appearing as a constellation of spots arranged around a point of origin (Baron and Salisbury, 1988); and the 120 kDa kinesin, associated in small amounts with a cytoplasmic, membranous, reticulum in interphase cells and concentrated on centrosomal structures during all stages of the cell cycle (Neighbors et al., 1988).

Of all the previously identified mitotic pole-associated proteins, cytocentrin most closely resembles POPA. This 110/115 kDa doublet antigen was identified by carrying out affinity-elution fractionation of a human autoantiserum (Sager et al., 1986). The apparent molecular masses of POPA and cytocentrin are slightly different, and in contrast to cytocentrin, POPA did appear on gels as a doublet of polypeptides, but they are both localized in the cytosol in early prophase, associate with the centrosomes in prophase, and dissociate from the poles in telophase. At cytokinesis cytocentrin is present in the midbody, whilst POPA is absent, but this might have resulted from differences in staining procedures. There are, however, two key properties, which clearly separate cytocentrin from POPA: (i) while cytocentrin is a cytosolic component present during all phases of the cell cycle, POPA could not be detected by

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**Fig. 10.** Immunoprecipitation of cytocentrin (CC) and tubulin (T) from the cytosol (1-8) and from the pellets following tubulin depolymerization (9-16). L6 cells, which were growing as exponential monolayers were either untreated (C), treated with nocodazole (N) or taxol (T) for 5 hours, or aphidicolin (A) for 24 hours. Following cells' homogenization, cytosolic and pellet fractions were separately separated by centrifugation. Cytocentrin (lanes 1-4) and tubulin (lanes 5-8) were immunoprecipitated sequentially from the cytosolic fractions. The assembled tubulin present in the pellets was depolymerized, and cytocentrin (lanes 9-12) and tubulin (lanes 13-16) were again sequentially immunoprecipitated from the supernatants obtained. Immunoprecipitates were standardized to the same amount of total cellular protein. Cytocentrin and tubulin were detected on the immunoblots with the RK7 and KMX-1 antibodies, respectively.
either immunofluorescence staining or immunoblotting in interphase cells; and (ii) when cells were fractionated into insoluble and soluble components, only proteins in the Triton-insoluble pellet from mitotic cells reacted with the anti-POPA serum; in contrast, cytocentrin could be detected exclusively in the soluble fraction of various cell lines tested (Figs 2 and 10).

Thus, to the best of our knowledge, cytocentrin represents a newly identified component of the mitotic centrosome, displaying a unique cellular localization during the cell cycle.

**Biological properties and possible function of cytocentrin**

Perhaps the most important characteristic of cytocentrin is its diffuse cytoplasmic localization in interphase cells, where its shows no tendency to bind to microtubules, coupled with its ability to bind specifically to centrosome-associated structures (and temporally to the midbody) during mitosis. While we cannot entirely exclude the fact that the lack of staining of interphase centrosomes with the RK7 antibody was due to masking of the corresponding epitope, several lines of evidence strongly suggest that this was not the case: (i) as mentioned above, RK7 did produce a weak fluorescence throughout the cytosol of interphase cells, higher than background and significantly more intense in some cells that may have been approaching mitosis (this was clear under direct microscopic observation, although in the pictures presented here it was obscured by the much more intense staining of the mitotic structures); (ii) cytocentrin could be readily detected by immunoprecipitation and immunoblotting in the cytosol of G1/S-blocked cells (Fig. 10), as well as of untreated cells or cells enriched for mitoses by treatment with taxol or nocodazole; (iii) cytocentrin could be visualized in association with a portion of the midbodies and with some cytoplasmic microtubules in taxol-treated cells. This suggests that the affinity of cytocentrin for microtubules (and the PCM) is affected by some reversible mitosis-specific alteration. It should be noted however that, even when activated, cytocentrin did not appear to bind very strongly to tubulin, since it could not be detected in the pellet fraction obtained from cells treated with taxol (Fig. 10).

It appears, therefore, that starting in early prophase, cytocentrin is activated to bind primarily to centrosomal components that are part of the PCM, both when compact poles are present and when the PCM is fragmented by treatment with taxol or nocodazole (Figs 7 and 9). The association of cytocentrin with the mitotic centrosome appears to be independent of microtubules polymerization (Fig. 8), contrary to what has been observed for several other pole-associated proteins (Price and Pettijohn, 1986; Buendia et al., 1990). Interestingly, at the stage of the cell cycle during which cytocentrin first localizes to the centrosome this organelle is known to increase in volume and to undergo marked morphological changes (Rieder and Borisy, 1982).

The increase in microtubule nucleating activity of the centrosome early in mitosis is known to be accompanied by the phosphorylation of many centrosomal proteins, as evidenced by the intense staining observed with the phosphoprotein-specific monoclonal antibody MPM-2 (Davis et al., 1983; Vandre et al., 1986; Engle et al., 1988; Centonze and Borisy, 1990; Vandre et al., 1991). At this time other proteins involved in accommodating the mitotic event, including histones, lamins and vimentin (Gurley et al., 1978; Gerace and Blobel, 1980; Evans and Fink, 1982) also become phosphorylated. Phosphorylation and the nucleating activity of the mitotic centrosome both decline in parallel in late anaphase (Kuriyama and Borisy, 1981; Vandre and Borisy, 1989). The association of cytocentrin with the poles follows a similar pattern, suggesting that the alteration responsible for binding of cytocentrin to the centrosome is its phosphorylation. In this respect, immunoblotting of purified cytocentrin with MPM-2 has produced inconclusive results: while a cross-reaction was observed (Fig. 4), the intensity of staining was rather weak. We cannot however exclude that only a small fraction of the total cytocentrin present in the cells’ cytosol becomes phosphorylated at mitosis and consequently associates with the centrosome. This is clearly an issue that will require a more detailed investigation in the future. It should be noted however that the ability of the mAb RK7 to recognize cytocentrin (or keratin 19) certainly does not depend on antigen phosphorylation, because this antibody was successfully used to identify a cDNA fragment coding for the 116 carboxy-terminal amino acids of keratin 19 from a lambda gt11 expression library (Chandler et al., 1991).

Cytocentrin localizes with the centrosome prior to the appearance of the asteres and was found associated with the multiple foci of MT growth in mitotic cells recovering from nocodazole treatment (Fig. 9), suggesting that this protein may play an important role in nucleating and/or stabilizing mitotic MTs. Other observations are however not consistent with such a function: (i) it could not be detected at the center of a significant fraction of miniasters present in taxol-treated cells (Fig. 7B); (ii) in cells recovering from nocodazole the multiple foci of tubulin polymerization appeared to contain markedly different amounts of cytocentrin (Fig. 9B,b), and (iii) in some mitotic cells fully recovered after nocodazole removal multipolar spindles were sometimes observed in which one of the poles lacked RK7 staining (not shown).

Another possible function, emphasizing cytocentrin’s cytoplasmic localization during interphase, its activation and binding to the centrosome in very early stages of mitosis, and its preferential association with 1-2 foci/cell in cells treated with taxol or nocodazole (presumably representing the centriole-containing centrosomes) is as a regulator of the centrosomal cycle. Studies employing enucleated sea urchin eggs have suggested that reproduction of the centrosomes at each cell division cycle is regulated by cytosolic rather than nuclear components (Sluder et al., 1986), and participation in this process may be the primary function of cytocentrin. Future experiments, utilizing microinjection of cytocentrin antibodies into interphase or mitotic cells, should be able to distinguish among these or other possible functions of this mitotic centrosomal protein.

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