Distribution of a Matrix Component of the Midbody during the Cell Cycle in Chinese Hamster Ovary Cells

Caterina Sellitto and Ryoko Kuriyama

Department of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, Minnesota 55455

Abstract. Monoclonal antibodies were raised against isolated spindles of CHO (Chinese hamster ovary) cells to probe for molecular components specific to the mitotic apparatus. One of the antibodies, CHO1, recognized an antigen localized to the midbody during mitosis. Immunofluorescence staining of metaphase cells showed that although the total spindle area was labeled faintly, the antigen corresponding to CHO1 was preferentially localized in the equatorial region of the spindle. With the progression of mitosis, the antigen was further organized into discrete short lines along the spindle axis, and eventually condensed into a bright fluorescent dot at the midzone of the intercellular bridge between two daughter cells. Parallel immunostaining of tubulin showed that the CHO1-stained area corresponded to the dark region where microtubules are entrapped by the amorphous dense matrix components and possibly blocked from binding to tubulin antibody. Immunoblot analysis indicated that CHO1 recognized two polypeptides of mol wt 95,000 and 105,000. The immunoreaction was always stronger in preparations of isolated midbodies than in mitotic spindle fractions. The protein doublet was retained in the particulate matrix fraction after Sarkosyl extraction (Mullins, J. M., and J. R. McIntosh. 1982. *J. Cell Biol.* 94:654–661), suggesting that CHO1 antigen is indeed a component of the dense matrix. In addition to the equatorial region of spindles and midbodies, CHO1 also stained interphase centrosomes, and nuclei in a speckled pattern that was cell cycle–dependent. Thus, the midbody appears to share either common molecular component(s) or a similar epitope with interphase centrosomes and nuclei.

Mitotic spindles are the structures responsible for equidistribution of the genetic material into each daughter cell. The spindle disappears after the completion of chromosome movement and the remnant structure remains as the midbody. Details of the process of midbody formation during mitosis have been described by electron microscopy (3, 5, 8, 37, 40, 43). An amorphous matrix appears at late anaphase in the midzone of the mitotic spindle surrounding the overlapping microtubules. With the progress of cleavage furrow formation, this region is packed together into a dense structure that can be easily identified by light microscopy (18). Microinjection experiments show that molecules such as dyes or immunoglobulins can pass across the intercellular bridge of two living daughter cells (48). Sealing of the midbody after completion of mitosis takes from a few minutes in fertilized sea urchin eggs (46) to a few hours in mammalian cells (46, 48). Based on these observations, it has been suggested that the midbody may have a role in the maintenance of cytoplasmic continuity between two daughter cells. However, the function of midbodies in the mechanism of cell division has yet to be clarified.

The molecular composition of midbodies should be elucidated for a clear understanding of their role in mitosis and cytokinesis. To a first approximation, the polypeptide composition of isolated midbodies has been analyzed for cultured mammalian cells (28, 41). Alpha and beta tubulin are the major polypeptides of the midbody. In mouse L929 cells, the midbody fraction was also shown to include varying amounts of plasma membrane proteins and a polypeptide with apparent mol wt 42,000, which was distinguished from actin by differences in isoelectric point (28). Mullins and McIntosh have found the protein composition of midbodies isolated from Chinese hamster ovary (CHO)[1] cells to be very similar to that of spindles (41). Over 35 minor bands were counted on gels, and a mol wt 115,000 doublet was suggested to be a component of the midbody matrix (41). The biochemical approach, although effective in identifying the major constituents of midbodies, does not seem to be sensitive enough to identify minor components that may play important roles in cell division. Furthermore, fractionation experiments provide no way to distinguish bona fide midbody proteins from nonspecific contaminants.

Immunocytochemical approaches, on the other hand, have

1. *Abbreviations used in this paper*: CHO, Chinese hamster ovary; MAP1, microtubule-associated protein 1.

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made it possible to identify several specific proteins localized in the region of cleavage furrow. They include microtubule-associated proteins (6, 10, 11, 23, 51), myosin (1, 19), actin (1, 7), actin-binding proteins (1, 20, 42), and calmodulin (55). None of these components, however, seem specific to the midbody and no evidence has yet been provided concerning the organization and/or function of this organelle.

As part of an effort to identify molecular factors controlling mitotic events, we isolated and characterized a monoclonal antibody (CHO1) that recognizes an antigen specifically localized to midbodies in cultured mammalian cells. Here we report that this antigen is a component of the midbody matrix, and that its appearance in cells is cell cycle–dependent. The antibody was also found to cross-react with interphase nuclei and centrosomes, suggesting that they might share common molecular components or a similar epitope with the midbody. Using CHO1 antibody, we provide information about the distribution of the midbody matrix during mitosis and discuss its possible roles in the mechanism of mitosis.

Materials and Methods

Cell Culture and Synchronization

CHO cells were grown as monolayers in Ham's F-10 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% FBS (HyClone Laboratories, Logan, UT), antibiotics, and 15 mM Hepes at pH 7.2 in a humid atmosphere with 10% CO_2 at 37°C.

Mitotic cells were prepared as described earlier (29). Briefly, cells from an exponentially growing culture were blocked at S phase and at the boundary between G1 and S phase by adding thymidine to a final concentration of 2–5 mM. After 12–16 h, the monolayers were washed free of thymidine and returned to fresh medium. Cells were cultured for an additional 4–5 h, then exposed to 0.1 μg/ml nocodazole for 4.5–5 h. Rounded cells in mitotic stages were gently shaken off and collected by centrifugation in a tabletop centrifuge.

Isolation of Spindles and Midbodies

Mitotic spindles and midbodies were isolated in a taxol-containing medium according to the procedure described previously (31). Mitotic cells, collected as above, were released from nocodazole treatment by washing twice with fresh F-10 medium. After incubating at 37°C for 15 min (in the case of spindle isolation) or 30–35 min (for midbody isolation), taxol (provided by Dr. M. Suffness, National Cancer Institute, Bethesda, MD) was added to a final concentration of 10 μg/ml for 0.5–1 min to stabilize the spindle microtubules in vivo. The sample was sedimented in a conical tube and carefully washed with distilled water. The wash liquid was removed and the cells suspended in an isolation medium containing 2 mM Pipes at pH 6.9, 0.25% Nonidet P40, and 10 μg/ml taxol. Released spindles and midbodies were then collected by centrifugation at 1,000 *g* for 20 min.

Midbody extraction was performed according to Mullins and McIntosh (41). Midbody pellets were chilled on ice and washed with 50 mM 2-(*N*-morpholino)ethane sulfonic acid (MES) at pH 6.3 to remove excess isolation medium. The sample was then resuspended in the same buffer containing 0.35% Sarcosyl LN-30 (CIBA-Geigy Corp., Greensboro, NC), which solubilizes the midbody microtubules while leaving the central zone of dense matrix intact (41). The extraction process was monitored by phase-contrast microscopy and, after 30–60 min, the phase-dense matrix zones were pelleted at 14,000 *g* for 15 min at 4°C.

Preparation of mAbs

Female BALB/c mice were used for immunization with CHO mitotic spindle fraction. Pellets of the isolated spindles were resuspended in SDS-sample buffer (10% glycerol, 5% 2-mercaptoethanol, 3% SDS, 62.5 mM Tris-HCl, pH 6.8) and boiled for 3–5 min. The denatured antigens were adsorbed on nitrocellulose strips (1 × 1 cm, 0.45 μm pore size) and surgically implanted under the skin of mice (25). Mice were then boosted twice intravenously at 2-wk intervals with sonicated native antigen. On the third day after the last boost, immunized spleens were removed for fusion with NS-1 mouse myeloma cells (26). Hybridoma supernatants were screened by indirect immunofluorescence microscopy of methanol-fixed whole CHO cells on coverslips as described (30). Positive hybridomas were subcloned twice by limiting dilution and ascitic fluid was prepared to obtain large quantities of antibody. The immunoglobulin class was determined with the Ouchterlony immunodiffusion test.

Immunofluorescence Microscopy

CHO cells grown on polylysine-coated glass coverslips were washed with warm PBS and fixed with absolute methanol at −20°C for at least 5 min. Cells at different stages of mitosis, isolated spindles and midbodies, and the dense matrix fraction prepared from isolated midbodies by Sarkosyl treatment were attached to polylysine-coated coverslips and fixed with methanol as above. After rehydration with PBS, the coverslips were incubated with CHO1 (1/500 dilution in PBS) for 30–60 min at 37°C in a moist chamber, rinsed thoroughly with PBS and stained with fluorescein-conjugated secondary antibody (Cooper Biomedical, Inc., Malvern, PA) for 30–60 min at 37°C.

Double staining was performed by incubation of coverslips in a mixture of CHO1 and (*a*) a rabbit polyclonal anti-tubulin antibody (Polysciences Inc., Warrington, PA) that was visualized by labeling with an L-rhodamine-goat-anti-rabbit serum (Tago Inc., Burlingame, CA); (*b*) a human autoimmune serum specific to the pericentriolar material (No. 5051, provided by Drs. T. Mitchison and M. Kirschner, University of California, San Francisco, Ca) (21); (*c*) a human autoimmune serum containing an antibody specific to centromeres (provided by Dr. N. Meryhew, University of Minnesota, Minneapolis, MN). Human sera were visualized with a fluoresceine isothiocyanate-goat-anti-human antibody (Cooper Biomedical, Inc.). Microscopic observation was made on an Olympus BH-2 microscope with epifluorescence optics. Photographs were taken on Tri-X film.

Electrophoresis and Immunoblotting

The electrophoretic analysis of proteins was carried out as described by Laemmli (32). Pellets of isolated CHO mitotic spindles and midbodies were resuspended and boiled in SDS-sample buffer and run on 7.5% SDS-polyacrylamide gels. Proteins on gels were stained with Coomassie Brilliant Blue R or electrophoretically transferred onto nitrocellulose paper (0.45 μm pore size, Schleicher & Schuell Inc., Keene, NH) (53) in transfer buffer (25 mM Tris-HCl at pH 8.3, 192 mM glycine, 20% methanol) with 0.1% SDS overnight, followed by transfer into the same buffer without detergent for 4–5 h (30).

For immunostaining, nitrocellulose strips were first incubated with 3% BSA in Tris-buffered saline (TBS: 10 mM Tris-HCl at pH 7.4, 150 mM NaCl) for 2 h at room temperature, then overnight with CHO1 mAb diluted in TBS containing 1% FBS and 0.05% Tween-20. A mouse-peroxidase-anti-peroxidase complex (ICN Biochemicals, Cleveland, OH) was used to visualize immunoreactive bands after color reaction with 2,3-diaminobenzidine as the enzyme substrate.

Results

CHO1 mAb Recognizes an Antigen Localized to the Midbody in Whole Mitotic Cells

mAb CHO1 was obtained by immunizing mice with mitotic spindles isolated from synchronized CHO cells in a taxol-containing medium (31). Ascitic fluid was prepared to obtain large quantities of antibody (class IgM). All of the experiments in this report were performed using the ascitic fluid.

The antigen recognized by CHO1 was localized by indirect immunofluorescence microscopy of whole mitotic CHO cells. Fig. 1 shows micrographs of CHO cells at different stages of mitosis immunostained with both monoclonal CHO1 (*A'*–*E'*) and polyclonal anti-tubulin (*A''*–*E''*) antibodies. At 15 min of recovery from the mitotic block, cells were already in metaphase (*A*, *A''*). At this stage, the distribution of CHO1 antigen was diffused among the spindle fibers, with a slightly higher concentration around the metaphase plate (*A'*). At later times, CHO1 antigen was localized into discrete

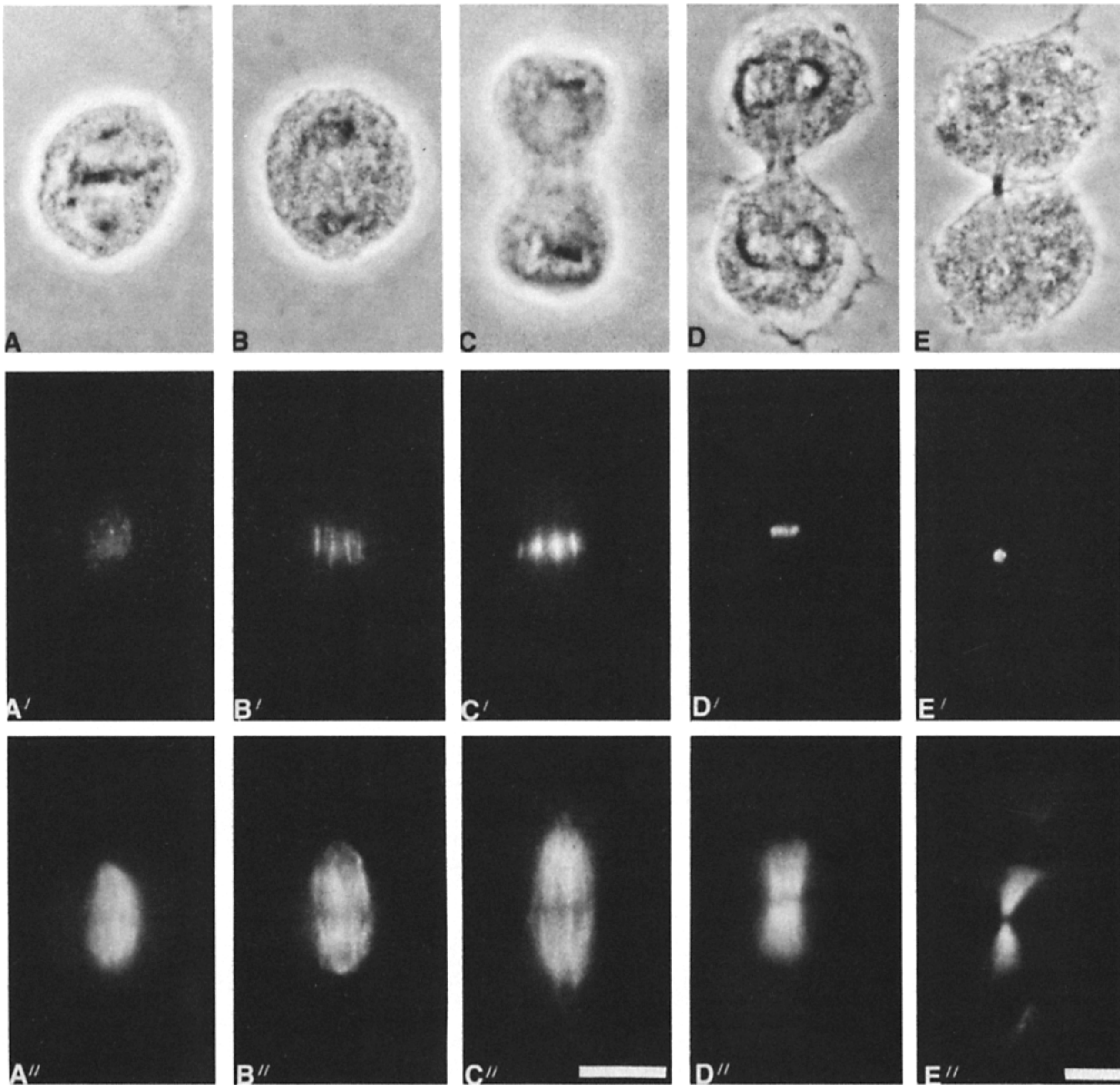

Figure 1. Localization of CHO1 antigen in whole CHO mitotic cells at different stages of mitosis. The same cells are seen by phase-contrast (*A–E*) and immunofluorescence microscopy after double-staining with CHO1 (*A′–E′*) and anti-tubulin (*A″–E″*) antibodies. All micrographs are at the same magnification except *C–C″*. Bar, 5 μm.

short lines at the interzonal region of the spindle (*B′*). This position was maintained during the remainder of mitosis. With the progress of cytokinesis (*C–C″*), the fibers labeled with CHO1 condensed into brighter bands in the furrowing region. The fibers gradually shortened (*D′*) and coalesced into a bright fluorescent dot in the center of the thin intercellular bridge between the two daughter cells (*E′*). This CHO1-stained spot corresponded to the dark band of the midbody seen in phase-contrast microscopy (*E*) and to the dark area in the middle of the intercellular bridge shown by the anti-tubulin staining (*E″*). The cells at this stage were already in interphase and the centrosomes had started to reorganize the network of cytoplasmic microtubules (*E″*).

The immunofluorescence staining of the spindle and midbody was sensitive to trypsin treatment, but not to treatment with low ionic strength medium or digestion with RNase and DNase. Alkaline phosphatase treatment failed to abolish the staining, indicating that the epitope to CHO1 was not phosphorylated (data not shown). CHO1 antibody cross-reacted with the spindle/midbody component in all the mammalian cell types tested so far, including HeLa, 3T3, PtK_1, LLC-PK_1, gerbil fibroma, and Indian muntjac skin cells (data not shown).

CHO1 Antibody Stains the Midzone of Isolated Mitotic Spindles and Midbodies

Mitotic spindles and midbodies were isolated as described in Materials and Methods and double-stained with CHO1

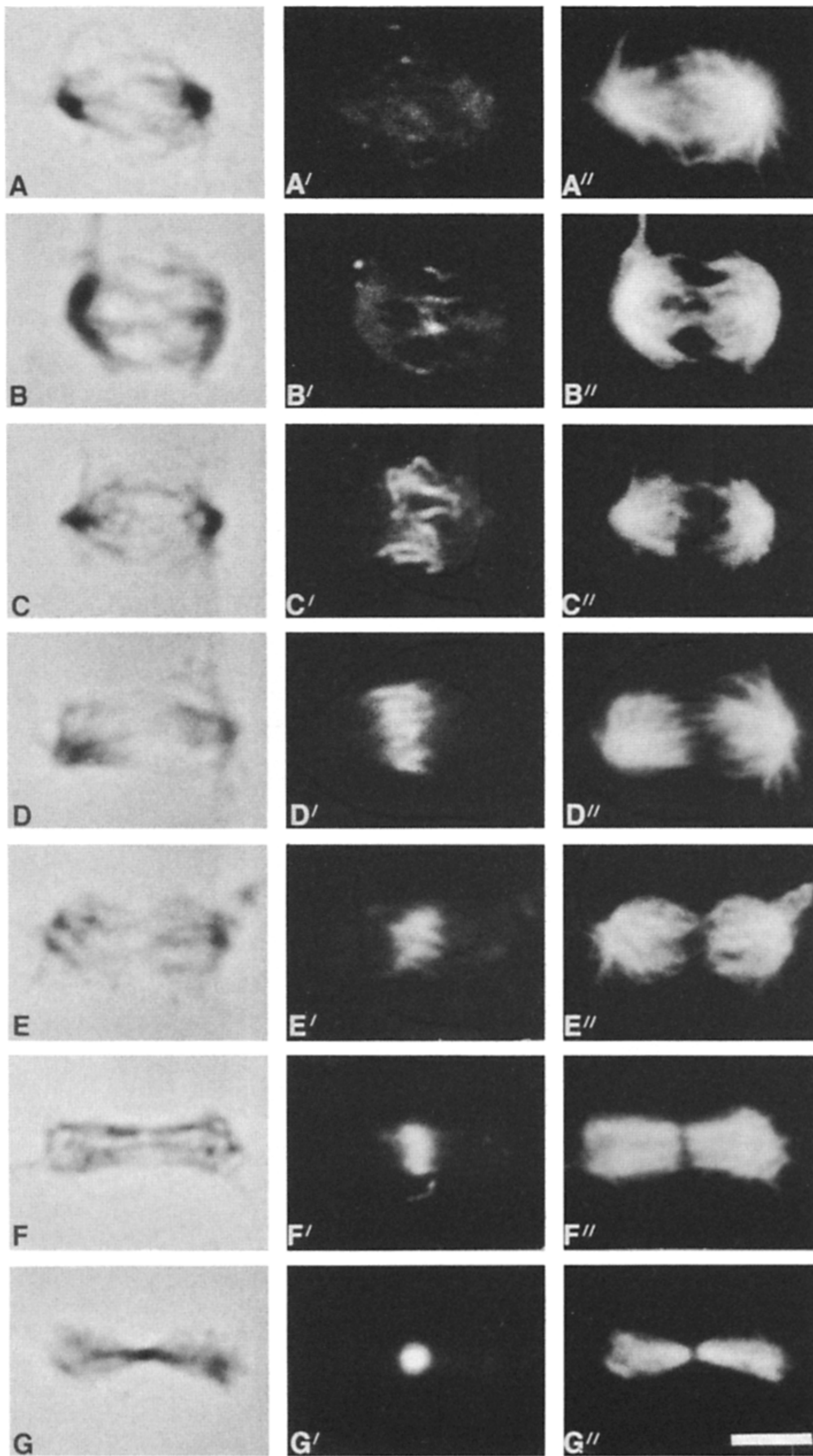

Figure 2. Localization of CHO1 antigen in isolated mitotic spindles and midbodies. The same isolated mitotic structures are seen by phase-contrast (*A–G*) and immunofluorescence microscopy after double-staining with CHO1 (*A′–G′*) and anti-tubulin (*A″–G″*) antibodies. Bar, 5 μm.

and anti-tubulin antibodies. Fig. 2 shows details of the spatial relationship between CHO1 antigen (*A′* to *G′*) and microtubules (*A″* to *G″*) in spindles and midbodies arranged in the order of mitotic process. At metaphase or early anaphase (*A–A″*), CHO1 staining was mostly diffused throughout the spindle region, although there was some accumulation of antigenic material in the midzone area (*A′*). As mitosis progressed, spindles changed their shape (*B–E*) and CHO1 anti-

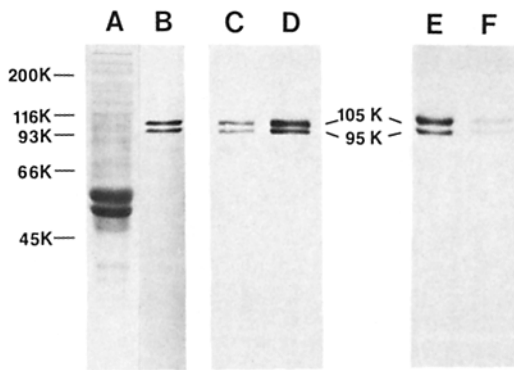

Figure 3. Identification of CHO1 antigen by immunoblot analysis of isolated mitotic spindles and midbodies. (Lane *A*) 7.5% polyacrylamide gel stained with Coomassie Blue. (Lanes *B–F*) Immunoreaction of nitrocellulose blots with CHO1. (*A* and *B*) Isolated fraction containing both spindles and midbodies; (*C*) isolated spindle fraction; (*D*) isolated midbody fraction; (*E*) insoluble and (*F*) soluble fraction of midbodies after treatment with 0.35% Sarkosyl LN-30 for 55 min at 0°C. Numbers on left indicate the position of molecular weight markers: myosin (200 kD), β-galactosidase (116 kD), phosphorylase B (93 kD), bovine serum albumin (66 kD), and ovalbumin (45 kD).

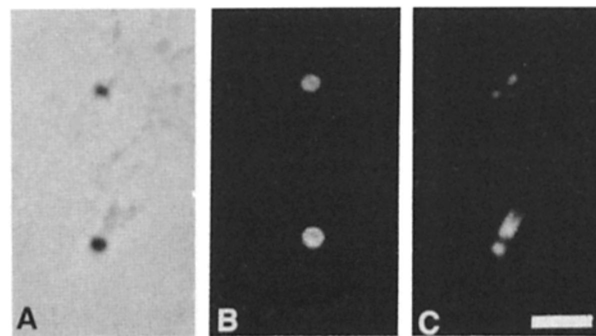

Figure 4. Sarkosyl-extraction of isolated midbodies. The phase-contrast micrograph (*A*) shows remnants of midbodies after extraction with 0.35% Sarcosyl LN-30 for 55 min at 0°C. Double-immunofluorescence staining with CHO1 (*B*) and anti-tubulin (*C*) antibodies demonstrates that CHO1 antigen is retained in the Sarkosyl-insoluble particulate fraction along with short residual microtubules. Bar, 2.5 μm.

gen was redistributed to fibers that shortened (*C'* to *F'*) and coalesced into the midbody (*G'*). These results indicate that the CHO1 antigen was not lost during the isolation procedure, and that the antibody staining pattern remained essentially unaltered from that in whole mitotic cells. The tubulin staining (*A"–G"*) was excluded from the area where CHO1 antigen localized, although electron microscopic observation has clearly shown the presence of microtubules in that region (5, 15, 37).

CHO1 Antigen Is a Matrix Component with Apparent Molecular Masses of 95 and 105 kD

Because CHO1 antigen is one of the components located at the central region of the spindle and midbody, we examined the molecular specificity of the CHO1 antibody by separating protein fractions on one-dimensional polyacrylamide gels (Fig. 3). Lane *A* shows the Coomassie Blue staining pattern of an isolated fraction containing both spindles and midbodies. A number of minor polypeptides are present along with the major proteins, alpha and beta tubulin. The corresponding CHO1 immunoblot shows two bands of 95 and 105 kD (lane *B*) which are not at all prominent on the stained protein gel (lane *A*).

Immunoreaction was also performed with two different protein preparations. One preparation was obtained 15 min after removal of nocodazole and included metaphase spindles as a major component (Fig. 2 *A*). The other fraction was prepared from the same number of mitotic cells 30–35 min after recovery from nocodazole treatment and was enriched in midbodies (Fig. 2 *G*). Although the Coomassie Blue staining pattern of these two preparations did not show any remarkable difference in total protein composition (data not shown), a much stronger signal for the 95 and 105 kD bands appeared in midbody fractions on immunoblots (compare lanes *C* and *D* in Fig. 3). These results suggest that the epitope to CHO1 antibody is more concentrated in midbodies than in mitotic spindles.

To further localize the CHO1 antigen within the midbody structure, we have fractionated isolated midbodies by treatment with Sarkosyl, which is known to selectively solubilize midbody microtubules leaving the matrix portion unaltered (41). Residual structures of midbodies after treatment with 0.35% Sarkosyl for 55 min at 0°C are shown in Fig. 4 with phase-contrast (*A*) and immunofluorescence microscopy after double-staining with CHO1 (*B*) and anti-tubulin (*C*) antibodies. Clearly the CHO1 antigen is associated with these particulate structures along with short remnants of microtubules. After separation of the particulate from the Sarkosyl-soluble fraction by centrifugation, distribution of CHO1 antigen was examined on immunoblots. As shown in Fig. 3, *E* and *F*, the majority of the 95 and 105 kD antigens were not released in the Sarkosyl-soluble fraction (*F*) but were retained in the Sarkosyl-insoluble matrix fraction (*E*). Based on these results, we conclude that 95 and 105 kD polypeptides labeled by CHO1 antibody are components of the midbody matrix.

CHO1 Antigen Appears within Cells in a Cell Cycle–dependent Manner

In addition to staining the midbody at mitosis, CHO1 antibody was also found to label different structures in interphase cells. Fig. 5, *B* and *E* show immunofluorescence micrographs of nonsynchronized, exponentially growing CHO cells labeled with CHO1 antibody. Some of the interphase nuclei were stained in a speckled pattern that varied in extent and intensity from one cell to another. CHO1 also labeled small spots next to the interphase nuclei. These spots were not always evident, especially when nuclei included many fluorescent dots.

To compare the intranuclear structure reactive to CHO1 with centromeres, we have double-stained the same cells with CHO1 as well as with an anticentromere autoimmune serum (Fig. 5 *C*). Labeling of the centromeres in nuclei could be detected in all cells (*C*), whereas the speckled pattern of nuclear staining with CHO1 was limited to certain interphase cells (*B*). It appeared, from a close observation that

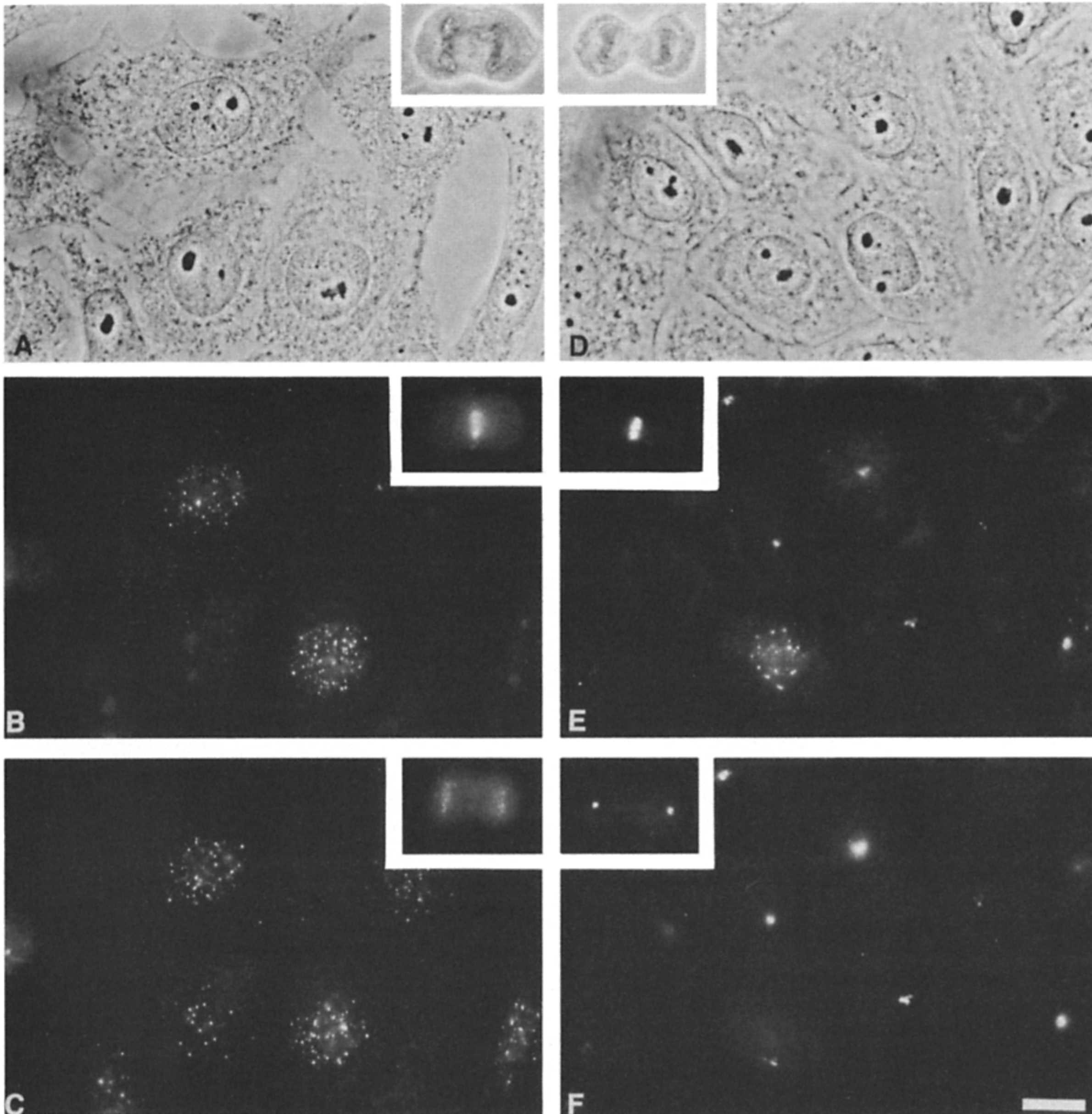

Figure 5. Cross-reactivity of CHO1 antibody with interphase nuclei and centrosomes. Exponentially growing CHO cells are seen in phase-contrast (*A* and *D*) and immunofluorescence microscopy after double-staining with CHO1 (*B* and *E*) and human autoimmune sera specific to centromeres (*C*) and centrosomes (*F*), respectively. Cells in mitosis are shown in the insets. Bar, 10 μm.

the nuclear dots visualized by CHO1 antibody did not colocalize with centromeres. The mitotic cell in the inset of Fig. 5, *B* and *C* reveals the presence of a CHO1-positive matrix component at the midzone (*B*) distinct from the small dots stained on the chromosomes with the anti-centromere serum (*C*).

In contrast to the pattern seen using the anti-centromere antibody, double-immunofluorescence staining of interphase cells with CHO1 antibody and anti-centrosome serum No. 5051 (21) led us to conclude that the CHO1-reactive spots next to the nuclei are centrosomes (Fig. 5, *E* and *F*). Mitotic cells, however, did not show any colocalization of CHO1 antigen at the centrosomes (insets in Fig. 5, *E* and *F*). Whereas CHO1-reactive material localized at the midzone (*E*), 5051 labeled bright dots at each mitotic pole (*F*). Centrosomal staining with CHO1 antibody is, therefore, restricted to interphase cells.

The appearance of CHO1 antigen at the midbody matrix of mitotic cells was further confirmed by experiments in which CHO cells were treated with cytochalasin B, a potent inhibitor of cytokinesis in mammalian cells (27). Cytochalasin B-treated mitotic cells went through mitosis, but failed to separate into two daughter cells, resulting in binucleate cells (Fig. 6 *A*). CHO1 antigen appeared in cytochalasin B-treated cells as in normal control cells, forming a band of short fibers at the central region between two reformed nuclei (Fig. 6 *B*). Because of the inhibition of cleavage furrow formation, the CHO1-stained fibers did not coalesce but

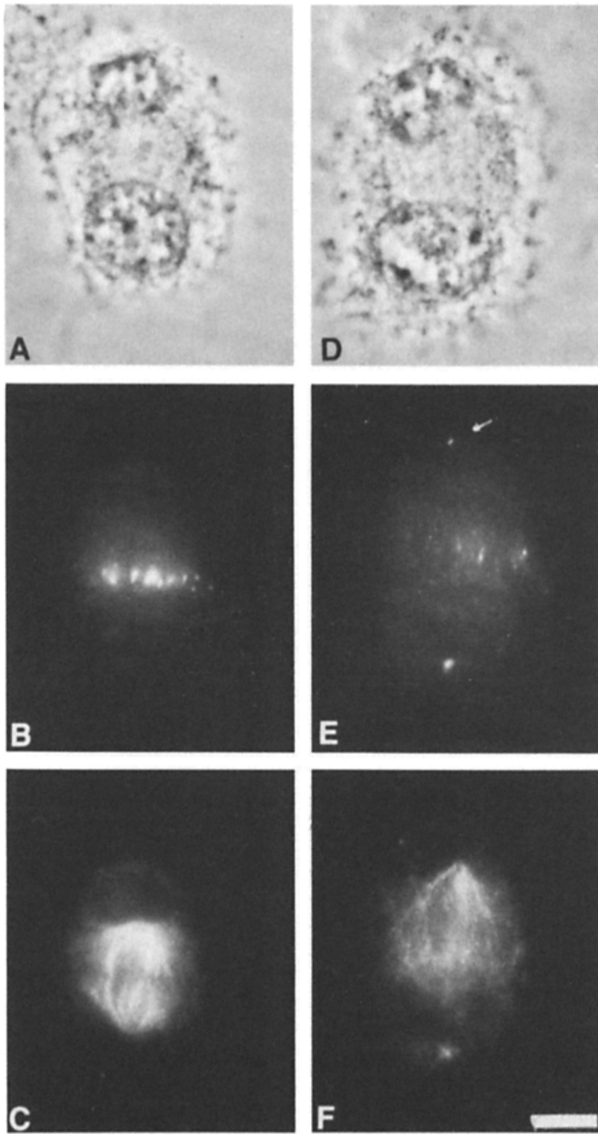

Figure 6. Disappearance of CHO1 antigen in cytochalasin B–treated cells. Mitotic cells were released from nocodazole treatment and further cultured with fresh medium. After 10 min, cytochalasin B was added to a final concentration of 10 μg/ml and cells were incubated for an additional 20 min. The same cells are seen by phase contrast (*A* and *D*) and immunofluorescence microscopy after double-staining with CHO1 (*B* and *E*) and anti-tubulin (*C* and *F*) antibodies. The treatment with cytochalasin B resulted in the formation of binucleate cells, due to inhibition of cleavage furrow formation. Arrows in *E* indicate the position of interphase centrosomes revealed by CHO1 staining. Bar, 5 μm.

remained scattered through the equatorial plane of division. As the cell cycle proceeded from mitosis to interphase, fluorescence intensity in those fibers gradually decreased, then disappeared from the interzonal region (*E*). Once the cell entered interphase, centrosomes became evident at the position corresponding to the former mitotic poles (Fig. 6 *E*, *arrows*). Fig. 6 *E* should be compared with Fig. 1 *E'*, in which the midbody matrix, once compartmentalized in the intercellular bridge and no longer surrounded by the bulk of interphase cytoplasmic environment, persists for a considerable period into the next interphase stage.

Discussion

Using isolated CHO spindles as immunogen, we have prepared a mAb specific to a matrix component of mammalian spindles and midbodies. The antigen was found to be diffusely distributed in the spindle region in metaphase cells, but reorganized into discrete short fibers aligned along the spindle axis during anaphase. The staining concentrated into a bright spot at the midzone of the intercellular bridge between two daughter cells at the end of mitosis. Isolated spindles and midbodies were also labeled with CHO1 antibody, indicating that the antigen is tightly integrated with these structures.

The distribution of CHO1 antigen shows a strong similarity with that of the midbody matrix reported by electron microscopic studies (3, 8, 15, 37, 40). The matrix has been described as an amorphous electron-dense material that appears in the interzone between separating chromosomes at anaphase and surrounds bundles of parallel arrays of overlapping microtubules. The double-staining pattern with anti-tubulin antibody and CHO1 strongly suggests that CHO1 antigen is specifically localized in the interzonal region of spindles and intercellular bridges, where the matrix entraps microtubules and may block their accessibility to the anti-tubulin antibody (48). Shortening of CHO1-stained bright fibers during successive stages of poleward chromosome movement could reflect the sliding of antiparallel microtubules originating from each half spindle and overlapping at the midzone.

The antigenic molecules recognized by CHO1 antibody have been identified as two polypeptides of 95 and 105 kD by immunoblotting. By gel analysis of isolated spindles and midbodies, Mullins and McIntosh have already suggested a 115 kD doublet to be a component of the midbody matrix (41). Do these two sets of polypeptides represent the same proteins? Although both the components are preferentially retained in the Sarkosyl-insoluble matrix fraction, the molecular weight of the 115-kD doublet may be slightly different from 95 and 105 kD. Moreover, the 115-kD doublet is abundant enough to be identified on protein stained gels, whereas 95 and 105 kD are not at all prominent and can be recognized only after immunoblots. The difference in protein yield, however, may arise from the different protocols employed for the isolation of spindles and midbodies. The most critical difference is that the 115-kD doublet is present in equal amounts in prometaphase–metaphase spindles as well as in midbodies (41). In contrast, the appearance of the 95 and 105 kD CHO1 antigen changes during mitotic progression. By immunoblot analysis the midbody fraction is enriched in the CHO1 antigen compared with the spindle preparations obtained from the same number of mitotic cells. It is not clear whether the total amount of CHO1 antigen is higher in midbodies than spindles, or whether the epitope to CHO1 is more exposed in midbodies than in spindles. Further studies will be required to evaluate the two sets of polypeptides in more detail.

The midbody is discarded after cell separation and eventually deteriorates (39); however, the midbody could be more than a remnant of the mitotic apparatus. The presence of the matrix component(s) in stages earlier than anaphase raises the possibility they may have other important roles in mitosis. The matrix is detected at the middle portion of spindles

where microtubules from opposing half-spindles interdigitate (15, 16, 52). The midbody microtubules are known for their relatively higher resistance to physical and/or chemical treatments which disrupt microtubules (37). The matrix may provide these properties by coating microtubules at the midplane; alternatively spindle microtubules, initiated at the pole and captured by the matrix, may be selectively stabilized to prevent their disassembly (36-38). The amorphous matrix components could therefore play an important role in the formation and maintenance of spindle structure during mitosis.

Recent progress in immunological approaches has made it possible to identify several factors located in the midzone of spindles and in midbodies where microtubules interdigitate. They include kinesin (56), chromosome scaffold proteins (14), a polypeptide of mol wt 112,000 reactive to a human autoimmune serum (2), high-molecular weight phosphoproteins recognized by mAbs raised against mitotic HeLa cells (12, 54), isolated CHO spindles (49), and brain microtubule-associated protein 1 (MAP1) (13). Wordeman and Cande have recently reported a 205-kD phosphoprotein located in the area of overlap between antiparallel microtubules in elongating diatom spindles (57). The functional importance of this factor is implied by its phosphorylation in association with ATP-dependent spindle elongation in vitro. Because it is now generally accepted that two half-spindles are sliding apart at the microtubule-overlap region (22, 35, 36), matrix components, including CHO1 antigen, might be involved in the generation of forces necessary to move chromosomes to opposite poles during anaphase B.

The localization of CHO1 antigen within cells is cell cycle-dependent and any disturbance, such as treatment with cytochalasin B, can lead to the disappearance of the matrix antigen as the cell enters interphase. On the other hand, the same antigen, once it is compartmentalized in the intercellular bridge and is no longer surrounded by the bulk of interphase cytoplasmic environment, can retain its reactivity with CHO1 antibody. The disappearance of CHO1 antigen might be due to simple dispersion in cytochalasin B-treated cells. It is also possible that the interphase cytoplasm contains some transient factors that can cause the disappearance of CHO1 matrix antigen. CHO1 also reacts with nuclei and centrosomes at interphase. Unlike the centrosomal antibodies reported so far which stain centrosomes preferentially at mitosis (4, 9, 13, 17, 33, 44, 54) or constantly during the complete cell cycle (2, 21, 24, 34, 45, 47, 50, 56), CHO1 reacts only with interphase centrosomes and not with mitotic centrosomes. As far as we are aware, this is the first antibody specific to the interphase centrosome. It is not clear whether similar components are actually present in nuclei, centrosomes, and midbodies, or whether CHO1 antibody cross-reacts with unrelated proteins that coincidentally share a similar antigenic determinant. It is interesting to note that antibodies to scaffold proteins (14), mitotic HeLa cell (54), isolated CHO spindles (49), and MAP1 (13) are all able to stain not only the matrix zone of spindles and midbodies but also interphase nuclei. The CHO1-reactive nuclear component, as well as the centrosomal component, might be a precursor for the matrix that is released into the cytoplasm after nuclear envelope breakdown and eventually transferred to the middle portion of the spindle.

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