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The Nuclear-Mitotic Apparatus Protein is Important in the Establishment and Maintenance of the Bipolar Mitotic Spindle Apparatus

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The formation and maintenance of the bipolar mitotic spindle apparatus require a complex and balanced interplay of several mechanisms, including the stabilization and separation of polar microtubules and the action of various microtubule motors. Nonmicrotubule elements are also present throughout the spindle apparatus and have been proposed to provide a structural support for the spindle. The Nuclear-Mitotic Apparatus protein (NuMA) is an abundant 240 kD protein that is present in the nucleus of interphase cells and concentrates in the polar regions of the spindle apparatus during mitosis. Sequence analysis indicates that NuMA possesses an unusually long α-helical central region characteristic of many filament forming proteins. In this report we demonstrate that microinjection of anti-NuMA antibodies into interphase and prophase cells results in a failure to form a mitotic spindle apparatus. Furthermore, injection of metaphase cells results in the collapse of the spindle apparatus into a monopolar microtubule array. These results identify for the first time a nontubulin component important for both the establishment and stabilization of the mitotic spindle apparatus in multicellular organisms. We suggest that nonmicrotubule structural components may be important for these processes.

INTRODUCTION

The mitotic spindle apparatus is a biochemically complex assembly responsible for segregation of chromosomes at mitosis (for review see McIntosh and Koonce, 1989; McIntosh and Hering, 1991; Gorbsky, 1992). How the spindle apparatus is formed and maintained is not well understood. A great deal of emphasis has been placed on the study of microtubules, which are the most prominent elements in the apparatus and clearly participate in chromosome segregation and separation of the poles. During prophase, the centrosomes, from which the microtubules emanate, divide and move to the opposite sides of the nucleus. After nuclear envelope breakdown, the released chromosomes interact with spindle microtubules and move to align together at the center of the metaphase spindle apparatus in prelude to anaphase.

Several mechanisms are believed to be important in chromosome movement along spindle microtubules during prometaphase. These include the generation of force by the action of various microtubule motors that are thought to be located at the kinetochores and along spindle fibers (Leslie *et al.*, 1987; Pfarr *et al.*, 1990; Steuer *et al.*, 1990; Hoyt *et al.*, 1992; Roof *et al.*, 1992), and also possibly by forces exerted from the assembly and disassembly of microtubules themselves (Rieder *et al.*, 1986; Spurck and Pickett-Heaps, 1987; Koshland *et al.*, 1988). Although this chromosome movement pauses during metaphase, the observation of microtubule flux during this stage suggests that the apparent stability of the metaphase spindle apparatus may in fact represent a complex but balanced interplay of the several mechanisms acting along microtubules (Gorbsky and Borisy, 1989; Mitchison, 1989).

In addition to microtubules, other less well-defined elements of the spindle have been identified, and these components may be important in the function of the spindle apparatus. Isolated sea urchin spindles, which have been extracted with free calcium to remove almost all of their tubulin, retain a spindle shaped matrix composed mainly of a protein that may be related to intermediate filament proteins (Leslie *et al.*, 1987). Moreover, other nonmicrotubule filaments composed of tektin (Steffen and Linck, 1992), and spoke (Paddy and Chelsky, 1991) proteins have been shown to be present throughout the spindle apparatus. Although the func-

tion of these components has not been determined, nonmicrotubule elements have been proposed to provide a structural support for the spindle (Pickett-Heaps *et al.*, 1984).

The Nuclear-Mitotic Apparatus protein (NuMA) is an abundant 240-kD protein that is present in the nucleus of interphase cells and concentrates in the polar regions of the spindle apparatus during mitosis (Lyderson and Pettijohn, 1980). Sequence analysis indicates that NuMA possesses an unusually long coiled-coil central region similar to many filament forming proteins (Yang *et al.*, 1992; Compton *et al.*, 1992). Furthermore, NuMA is resistant to high salt, detergent and nuclease extractions, indicating that it is a component of the nucleoskeleton (Lyderson and Pettijohn, 1980). The function of NuMA in the nucleus and during mitosis is not known. In this report we have investigated the role of NuMA in vivo by microinjection of anti-NuMA antibodies into living cells.

MATERIALS AND METHODS

Generation and Affinity Purification of Antibodies

Rabbit anti-NuMA serum r240-C was generated against the glutathione-S-transferase fusion protein, GST::2, containing 510 amino acids of the NuMA helical region, as previously described (Yang *et al.*, 1992). Anti-NuMA antibodies were affinity-purified by chromatography on GST::2-cross-linked Sepharose (Pharmacia, Piscataway, NJ). Preimmune IgG was purified by chromatography on protein A-agarose (Pierce Rockford, IL), and anti-GST antibodies were affinity-purified from an antiserum generated against a glutathione-S-transferase fusion protein unrelated to the NuMA protein by chromatography on GST protein-cross-linked Sepharose. All purified antibody preparations were dialyzed against 0.5 × Phosphate-buffered saline (PBS) (67 mM NaCl, 1.4 mM KCl, 2.2 mM Na_2HPO_4, 0.73 mM KH_2PO_4; pH 7.3), and concentrated with Centricon microconcentrators (Amicon, Danvers, MA) to 5 mg/ml.

Preparation of HeLa Extracts

HeLa proteins were prepared by lysing cells directly in culture dishes with 2× sample buffer (125 mM tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 6.8; 10% β-mercaptoehanol; 4% Sodium dodecyl sulfate (SDS); 20% glycerol; and 0.004% bromphenol blue) and by boiling for 5 min.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblot Analysis

Cellular proteins were separated on 7% polyacrylamide gels as described in Laemmli (1970) and blotted onto nitrocellulose (Burnette, 1981). The blots were blocked in Tris-buffered saline (TBS), containing 20% (wt/vol) Fetal calf serum (FCS), 10% nonfat dry milk, 0.2% sodium azide, for 4 h at room temperature, then incubated with human autoantibodies or affinity-purified rabbit sera (all diluted 1:1000 in the blocking solution) overnight at 4°. Immunoreactive polypeptides were detected by incubation with ^{125}I-protein A (>30 mCi/mg; Amershm Chemical, Arlington Heights, IL, diluted in TBS, 0.1% [wt/vol] bovine serum albumin [BSA]).

Microinjection Assays

CV-1 cells were plated at low density on coverslips 2 d before microinjection and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, in a 7% CO_2 37° humidified incubator. CV-1 cells were chosen because they are relatively flat and adhere to the substrate throughout the cell cycle; these features make them both readily amenable to injection and useful for following mitotic stages. Cells were pressure-injected (Narashige IM-200) with ~0.1 pl of antibody solution and immediately returned to the incubator. Thereafter, cells were removed only for observations and fixation. Metaphase cells were identified by the presence of a bipolar spindle apparatus by phase-contrast microscopy and the alignment of condensed chromosomes at the center of the spindle (for example, see Figure 3A, time 0).

Immunofluorescence

Cells were fixed in a microtubule stabilizing buffer (MTSB: 100 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), pH 6.8; 2 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA); 2 mM $MgCl_2$) containing 4% formaldehyde and stained as previously described (Yang *et al.*, 1992). To localize the injected antibodies, the cells were stained with biotinylated anti-rabbit antibodies, and then with fluorescein-conjugated streptavidin (Zymed, South San Francisco, CA). Cells were also stained with DM-1A, a mouse anti-tubulin monoclonal antibody (Sigma, St. Louis, MO), or an independent mouse-derived anti-NuMA serum (m240-A; [Yang *et al.*, 1992]) and visualized with rhodamine-conjugated goat anti-mouse antibodies (Cappel, Duram, NC). Cells were examined on a Zeiss Axiovert (Thornwood, NY) and Leitz Aristoplan microscopes equipped with epifluorescence optics.

RESULTS

Specificity of Affinity-Purified Anti-NuMA Antibodies

Anti-NuMA rabbit polyclonal serum r240-C was generated against a glutathione-S-transferase (GST) fusion protein containing ~510 residues of the central portion of the NuMA helical domain, as previously described (Yang *et al.*, 1992). Immunofluorescence experiments with this serum yielded the cell cycle-dependent immunostaining pattern indistinguishable from that of the original anti-NuMA human autoimmune serum (see Yang *et al.*, 1992, Figures 10 and 11). Immunoblot analysis revealed that r240-C anti-NuMA antibodies react strongly with NuMA::TRPE fusion protein whereas preimmune serum does not (see Yang *et al.*, 1992). Affinity-purified anti-NuMA antibodies, anti-GST antibodies, and preimmune IgG were prepared from rabbit sera as described in MATERIALS AND METHODS. Immunoblot analysis of total cellular protein isolated from HeLa cells demonstrated that the affinity-purified anti-NuMA antibodies react with a single band ~240 kD in molecular mass that is identical in size to the NuMA protein recognized by human serum (Figure 1). Long exposure failed to reveal any other bands recognized by the rabbit serum. No polypeptides were rec-

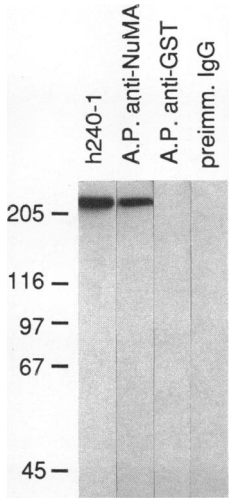

Figure 1. Immunoblot of total HeLa proteins with affinity-purified anti-NuMA antibodies recognize a single 240 kD polypeptide indentical in size to the NuMA protein. Total HeLa proteins were separated on a 7% SDS-polyacrylamide gel, transferred to Immobilon membrane, and probed with (h240-1), the human autoimmune serum h240-1 originally used to identify NuMA (Yang *et al.*, 1992); (A.P. anti-NuMA), affinity-purified anti-NuMA antibodies from rabbit serum r240-C (see MATERIALS AND METHODS); (A.P. anti-GST), affinity-purified anti-glutathione-S-transferase antibodies (see MATERIALS AND METHODS); and (preimmune IgG), total IgG purified from preimmune serum of rabbit used for r240-C.

ognized by the affinity-purified anti-GST serum or preimmune serum.

Immunofluorescence experiments with HeLa cells and CV1 cells reveals that in both cell types, the affinity-purified anti-NuMA antibodies react strongly with the nucleus of interphase cells and the polar regions of the spindle apparatus in mitotic cells (see Yang *et al.*, 1992 for results with CV1 cells). No staining was observed with either affinity-purified anti-GST antibodies or preimmune serum.

Microinjection of Anti-NuMA Antibodies During Interphase and Early Mitosis Prevents the Formation of a Bipolar Spindle Apparatus

To study the function of the NuMA protein in vivo, affinity-purified anti-NuMA antibodies were first microinjected into the cytoplasm of asynchronous interphase African Green Monkey Kidney CV-1 cells. As expected, because the antibody cannot enter the nucleus where NuMA is located, antibody staining was diffuse throughout the cytoplasm; no significant localization of antibody was apparent at the centrosomes, as assayed by indirect immunofluorescence with anti-rabbit (to localize the injected antibodies) and anti-tubulin antibodies. Moreover, an extensive cytoplasmic microtubule array was visible and appeared to be unaffected by the antibodies. Of 29 cells injected with anti-NuMA antibodies, 10 cells entered mitosis during the ensuing 8-h observation period. All 10 cells became round and arrested in a prometaphase-like configuration (see below); these cells ultimately dislodged from the coverslip surface 2 to 4 h after entering mitosis.

To further investigate the possible role of NuMA during mitosis, we next examined the effect of microinjecting anti-NuMA antibodies into CV-1 cells at various mitotic stages. In uninjected cells, after nuclear envelope breakdown NuMA usually concentrates at the two spindle poles, which have separated and are in the process of forming a bipolar spindle apparatus (Lyderson and Pettijohn, 1980; Compton *et al.*, 1992; Yang *et al.*, 1992). Forty-seven cells in prophase or prometaphase were injected with anti-NuMA antibodies. One to two hours following injection, forty (85%) became round and arrested at a state resembling prometaphase (Figure 2A). Thirty of the arrested cells were fixed 60–120 min after injection and stained with anti-tubulin antibodies; each contained a single microtubule array that was surrounded by chromosomes (Figure 2B). The microtubules appeared nonuniform and somewhat disorganized. Interestingly, in all thirty cells the injected NuMA antibodies localized as a single focus (in 90% of the cells) or as two adjacent or overlapping foci (10%) at the presumed centrosomal region (i.e., the center of the microtubule array). Thus the anti-NuMA antibodies block formation of a bipolar spindle apparatus.

Microinjection of Anti-NuMA Antibodies at Metaphase Causes the Collapse of an Extant Bipolar Spindle Apparatus

During metaphase, NuMA normally assumes a crescent-shaped distribution around each spindle pole. When metaphase cells were injected with anti-NuMA antibodies, 88% (57/65) did not continue through cell division. Of the 57 cells that arrested at mitosis, 4 remained at metaphase with the chromosomes aligned at the center of the spindle. Surprisingly however, the remaining 53 cells appeared to regress from metaphase after injection (Figure 3A); the bipolar spindle apparatus that had been visible by phase-contrast microscopy at the time of injection disappeared within 15–30 min after injection. Furthermore, chromosomes that had been organized at the metaphase plate at injection redistributed into a prometaphase-like formation. Immunofluorescence experiments with 30 arrested cells fixed ~1–2 h after injection revealed that each cell contained a single aberrant microtubule array surrounded by condensed chromosomes (Figure 3B). Similar to prophase-injected cells, NuMA antibodies were localized as a single focus at the center of the microtubule array. Double staining experiments of five cells with anti-rabbit antibodies (to visualize the location of the NuMA antibodies) and serum 5051, an centrosomal antibody (Tuffanelli *et al.*, 1983), confirmed that the anti-NuMA antibodies surrounded the centrosome. Thus in total 83% (93/112) of cells that were injected before anaphase (prophase and metaphase) failed to form or maintain a spindle apparatus.

To follow the regression of the spindle apparatus more closely, metaphase-injected cells were fixed and immunostained 5, 15, 20–30, and 40–60 min after antibody injection (Figure 4). Five cells were analyzed at each timepoint. In cells fixed after 5 min, the NuMA antibodies had localized to the polar regions of the

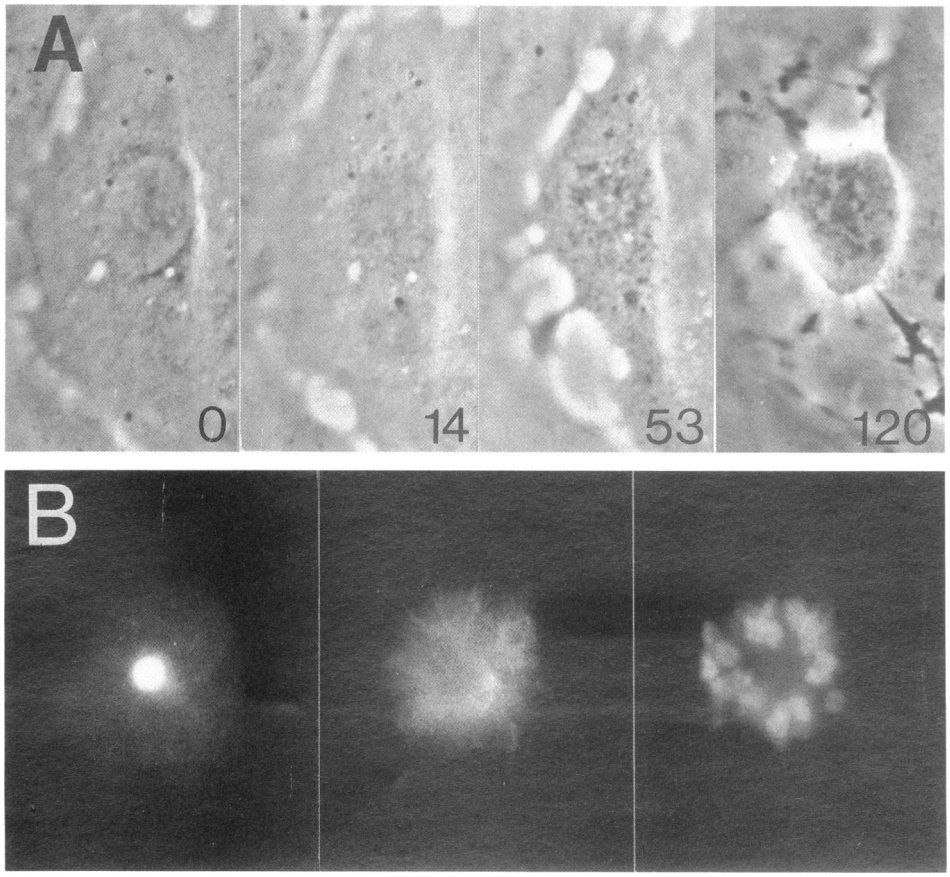

Figure 2. Anti-NuMA antibodies injected into the cytoplasm of interphase or early mitotic cells block the formation of a bipolar mitotic spindle apparatus. A. A prophase cell just before complete nuclear envelope breakdown, was injected with anti-NuMA antibodies and observed over time. Numbers indicate minutes following injection. B, After the final observation at 120 min, the same cell was fixed and stained for double immunofluorescence. The cell was stained with (left) anti-rabbit antibodies, to localize the injected anti-NuMA antibodies; (center) DM-1A, an anti-tubulin mAb; and (right) Hoechst 33258, to visualize DNA.

spindle apparatus (Figure 4A). However, in contrast to the normal crescent-shaped distribution about the spindle poles observed in uninjected cells, in injected cells NuMA was tightly concentrated in the immediate vicinity of the spindle poles. The spindle apparatus appeared normal in these cells. Cells fixed 15 min or later after injection contained aberrant spindles (Figure 4B–D). At 15 min the spindle was partially collapsed, and the microtubule arrays appeared disheveled (Figure 4B). At 20–30 min the "half spindles" had almost merged, and the arrays appeared even more disheveled (Figure 4C). For cells fixed 40–60 min after injection, only a single abnormal microtubule array was present and NuMA antibodies were localized as a focus at the putative centrosome region (Figure 4D). Thus these results also indicate that anti-NuMA antibodies cause a collapse of the mitotic spindle apparatus.

Microinjection of Anti-NuMA Antibodies at Anaphase Does not Inhibit Mitosis

In contrast to the results from prophase and metaphase cells, 97% (32/33) of cells that were injected during anaphase successfully completed mitosis and cell division. Anti-NuMA antibodies that were injected immediately after the onset of anaphase did not appear to impede chromosome segregation. For cells fixed at late anaphase, the spindle microtubules appeared to be unaffected even though NuMA antibodies were localized to the polar regions of the spindle (Figure 5A). Five cells that completed cytokinesis were fixed and immunostained with anti-tubulin antibodies; these cells appeared to possess normal cytoplasmic microtubule arrays (Figure 5B). Interestingly, the anaphase-injected NuMA antibodies were localized as foci at the putative centrosomal positions of the daughter cells. Little or no NuMA antibody localization was seen over the nucleus. Immunofluorescence with a mouse anti-NuMA serum of three cells that completed cell division confirmed that NuMA remained at the centrosome, with little or no antigen present in the nucleus (Figure 5C). These results suggest that NuMA by itself is not required for nuclear reformation at telophase (however, see below).

Microinjection of Control Antibodies do not Cause a Mitotic Defect

Control injection experiments were also performed. All thirty cells injected with preimmune IgG (10/10 at prophase-prometaphase, 10/10 at metaphase, and 10/10 at anaphase), and 29 of 30 cells injected with anti-GST antibodies (10/10 at prophase-prometaphase, 9/10 at

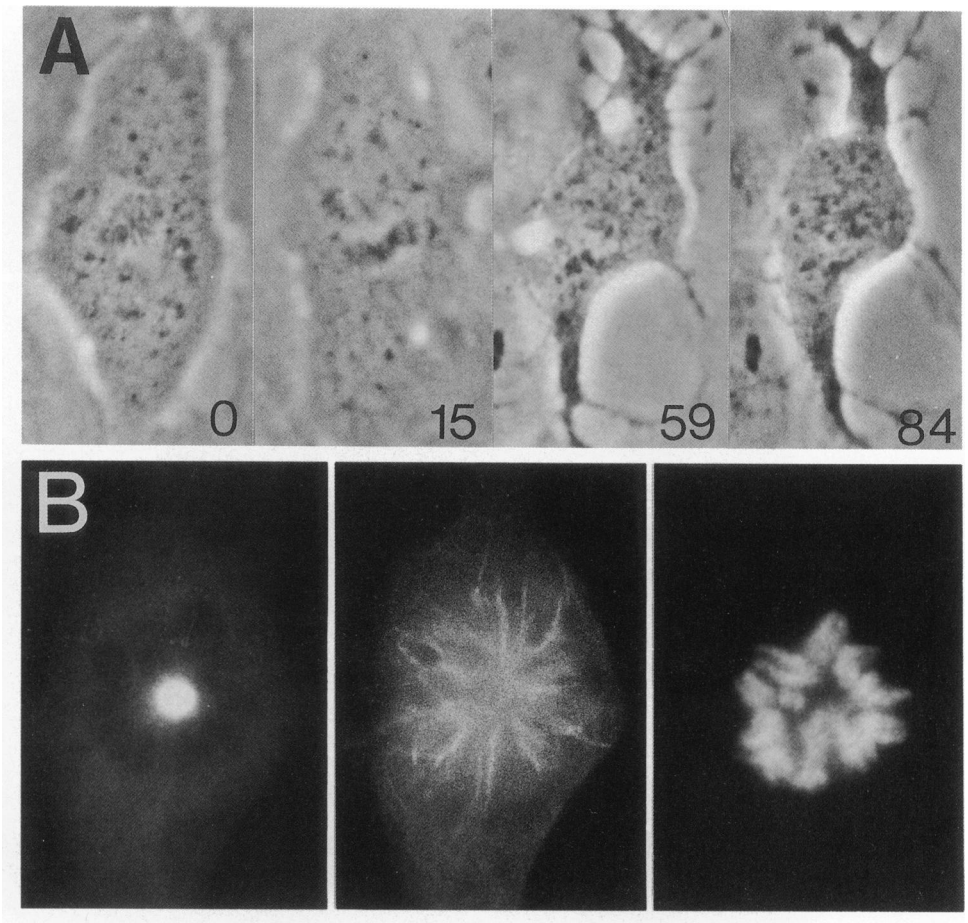

Figure 3. Anti-NuMA antibodies injected into the cytoplasm of metaphase cells cause the collapse of the bipolar mitotic spindle apparatus. A, A metaphase cell was injected with anti-NuMA antibodies and observed over time. Numbers indicate the time of observation, in minutes, after antibody injection. B, After the final observation at 84 minutes post-injection, the same cell was fixed and immunostained with (left) anti-rabbit antibodies, to visualize the injected antibodies; (center) DM-1A, an anti-tubulin mAb; and (right) Hoechst 33258, to visualize DNA. An examination of all focal planes revealed that the anti-NuMA antibodies were apparent only at the single focus, as shown.

metaphase, and 10/10 at anaphase) successfully completed mitosis and cell division.

DISCUSSION

The microinjection results presented above indicate that NuMA is important for the establishment of the mammalian spindle apparatus at prophase and its maintenance at metaphase. There are several possible mechanisms by which the NuMA antibodies might disrupt spindle formation. First, because NuMA is a large coiled-coil protein, it may form filaments (Yang *et al.*, 1992). The putative NuMA fibers may serve as a structural lattice that helps stabilize and maintain the spindle apparatus and/or as a support that allows sliding of microtubules (discussed below). Injection of anti-NuMA antibodies may prevent the formation of such fibers, or block NuMA interactions with other nonmicrotubule structures of the spindle apparatus and consequently lead to the eventual destabilization of the spindle apparatus. Consistent with this interpretation, the antibodies initially appear to have a greater effect on NuMA distribution than on microtubules; after injection, the cell loses its normal crescent-shaped distribution of NuMA before the spindle microtubule array appears disrupted (Figure 4A).

Another mechanism by which anti-NuMA antibodies might disrupt spindle formation is by directly preventing NuMA (or NuMA filaments) from binding to the microtubules. This possibility appears unlikely, because the NuMA protein can concentrate at the spindle pole in cells injected before the complete dissolution of the nuclear envelope. Because NuMA was previously shown to require the presence of intact microtubules to localize at the poles (Price and Pettijohn, 1986), it appears that the presence of anti-NuMA antibodies does not inhibit microtubule binding (regardless of whether the microtubule binding is direct or indirect).

A third possible mechanism of inhibition of spindle formation by anti-NuMA antibodies, is that the antibodies congregate in the polar regions and nonspecifically interfere with events that occur at those sites (e.g., microtubule nucleation). Nonspecific inhibition remains a formal possibility with any antibody injection experiment and could explain our results obtained with injections into prophase cells. Other investigators have identified antibodies specific to other components of the spindle apparatus that cause a metaphase-arrest after

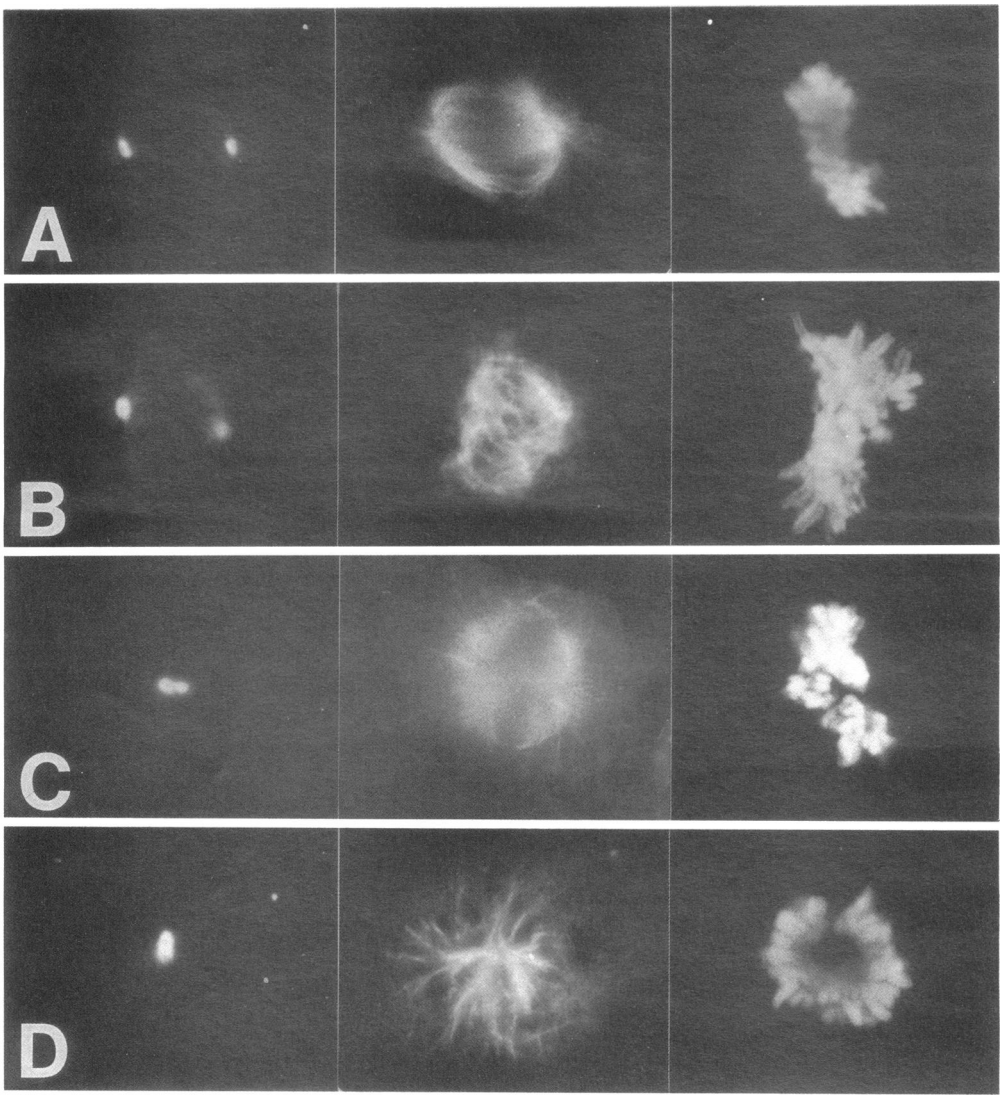

Figure 4. Injection of cells at metaphase with anti-NuMA antibodies causes a gradual collapse of the spindle apparatus over time. Metaphase CV-1 cells were injected with anti-NuMA antibodies, and fixed at various times postinjection for double immunofluorescence experiments (A–D). The cells were stained with (left) anti-rabbit antibodies, to visualize the localization of injected antibodies; (center) DM-1A, an anti-tubulin mAb; and (right) Hoechst 33258, to visualize DNA. Cells were injected, and fixed for staining after (A) 5 min; (B) 15 min; (C) 24 min, and (D) 45 min.

microinjection (Izant *et al.*, 1983; Dinsmore and Sloboda, 1989; Nislow *et al.*, 1990; Joshi *et al.*, 1992). Furthermore, injection of antibodies to the N-2B5 antigen (Izant *et al.*, 1983) or γ-tubulin (Joshi *et al.*, 1992) into metaphase cells caused a reduction in the number of spindle microtubules; however, the spindle poles remained separated and the chromosomes were positioned between the spindle poles. In contrast, after injection of anti-NuMA antibodies, the bipolar spindle apparatus collapsed into a monopolar microtubule array with the concomitant displacement of chromosomes from the metaphase plate. Our results suggest that the injected anti-NuMA antibodies caused the specific loss of a function involved in maintaining the separation of the spindle poles, while not interfering with those mechanisms associated with the subsequent collapse of the spindle. Thus it is unlikely that this collapse is due to a simple nonspecific inhibition event; if anti-NuMA antibodies merely "gum up" the spindle, they might be expected to interfere with both opposing forces, and hence cause a metaphase arrest.

A final possibility is that the antibodies cause a nonspecific general effect not related to the spindle (e.g., loss of cell viability) that ultimately results in failure to form or maintain the spindle apparatus. Although this is also possible with any microinjection experiments, we note that the anti-NuMA antibodies only block formation/maintenance of the apparatus at prophase or

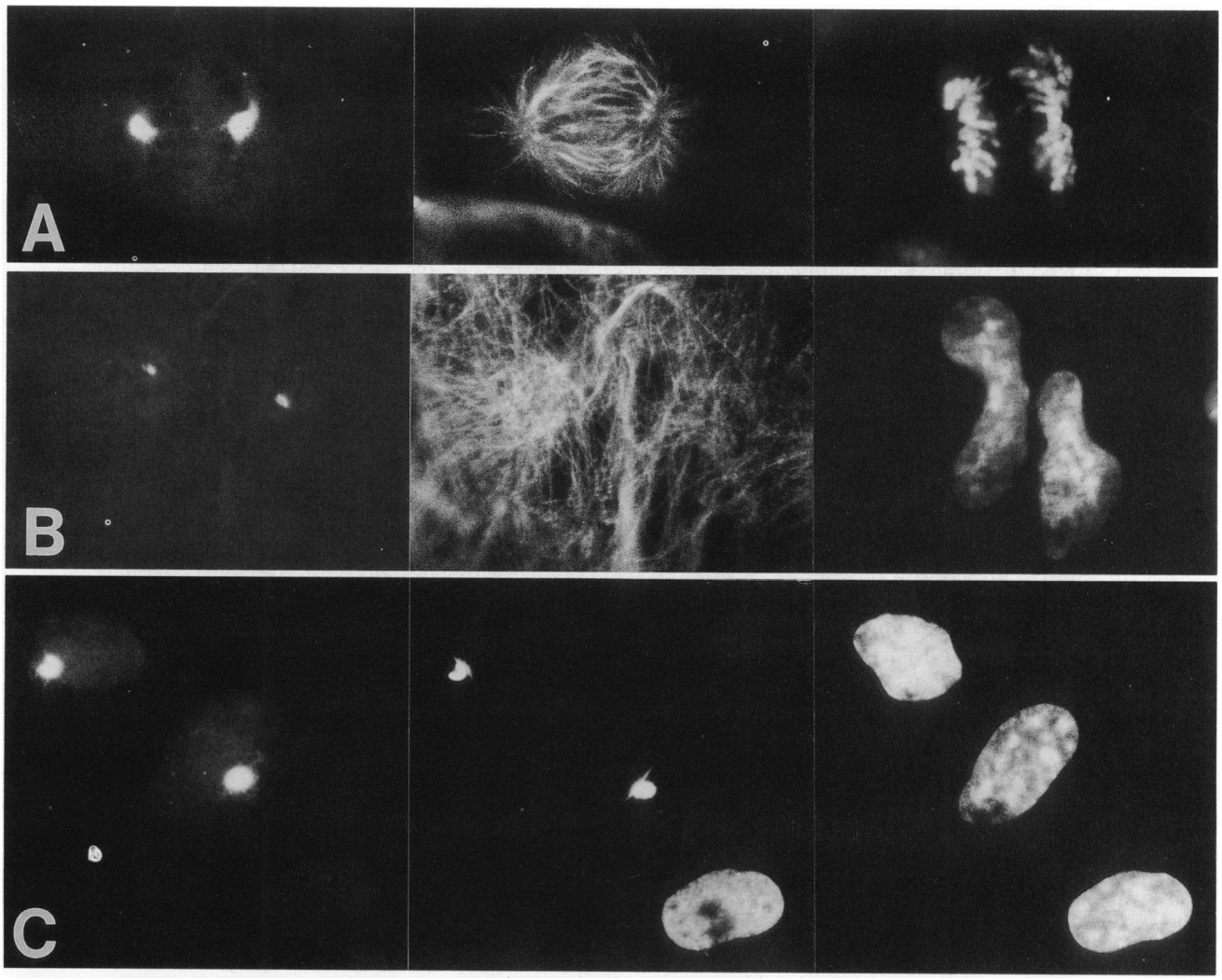

Figure 5. Injection of anti-NuMA antibodies into cells during anaphase does not inhibit chromosome segregation or subsequent completion of cell division. CV-1 cells were injected with anti-NuMA antibodies immediately after the onset of anaphase. The injected cells were fixed for immunofluorescence (A) at late anaphase, 15 minutes post-injection, or (B, C) following the completion of cytokinesis, ~1–2 h postinjection. Cells were immunostained with (A, B) (left) anti-rabbit antibodies, to identify the localization of injected anti-NuMA antibodies; (center) DM-1A, an anti-tubulin mAb; and (right) Hoechst 33258, to visualize DNA. (C) Cells were stained with (left) anti-rabbit antibodies; (center) a mouse derived anti-NuMA serum (Yang *et al.*, 1992); and (right) Hoechst 33258. An uninjected cell with a normal interphase nuclear distribution of NuMA protein (as shown with mouse anti-NuMA antibody staining) is depicted in the lower right corner.

metaphase; they do not interfere with chromosome segregation function during anaphase or nuclear reformation at telophase, implying a relatively specific inhibition. Our data are most consistent with the hypothesis that NuMA is directly involved in the establishment and maintenance of a bipolar spindle apparatus, and we further suggest that nonmicrotubule structural components might be important for this process.

Previously, Kallajoki *et al.* (1991) microinjected monoclonal antibodies against a NuMA-like antigen called SPN into HeLa cells and found that one of seven monoclonal antibodies caused a block in mitosis. Based on molecular weight and minor subcellular distribution differences from that of NuMA, the authors argued that the SPN antigen is not NuMA, although a counter argument has been proposed (Compton *et al.*, 1992). In any event no evidence was presented that would indicate that the SPN antigen functions to establish or maintain the bipolar spindle apparatus. To our knowledge, the only other nontubulin components that have been shown to be required for the establishment and stabilization of the mitotic apparatus are kinesin homologs, which are required for these processes in several fungi (Enos and Morris, 1990; Hagan and Yanagida, 1990; Hoyt *et al.*, 1992; Roof *et al.*, 1992). The kinesin homologs are thought to cause the separation of the

poles by sliding interdigitating microtubules of the two half-spindles. An alternative possibility, however, is that in mammalian cells, kinesin might function by the attachment of one end with NuMA elements and the other to microtubules; the motor might slide microtubules past stationary NuMA elements. Consistent with this possibility, Rodionov *et al.* (1991) reported recently that anti-kinesin antibodies microinjected into PtK1 cells at the prophase-prometaphase transition resulted in a mitotic arrest in which spindle poles came together into a monopolar array with the chromosomes arranged around the poles.

The forces that cause spindle collapse when metaphase cells are injected with anti-NuMA antibodies are not known. However, when five metaphase cells were injected with anti-NuMA antibodies and subsequently treated with nocodazole, a microtubule depolymerizing drug (Yang and Snyder, unpublished data), the spindle poles remained separated indicating that spindle collapse is mediated via microtubule-based mechanisms. Because injection of anti-NuMA antibodies at anaphase also did not cause the collapse of the mitotic spindle apparatus, the force(s) that pulled the poles together in prophase and metaphase-injected cells is either not present, or is much weaker during anaphase. Likely candidates for generating the spindle collapsing force(s) are the minus end motors located at the kinetochores of each sister chromatid (Pfarr *et al.*, 1990; Steuer *et al.*, 1990). These motors are expected to cause poleward-directed tension on kinetochore microtubules, particularly while the sister chromatids remain attached. The separation of sister chromatids at anaphase should then release this tension, at least in part, as the chromosomes segregate to the poles. Therefore, NuMA containing structures would be expected to be more important in balancing the poleward forces earlier in mitosis when sister chromatid remain attached, rather than at anaphase when they have separated. Consistent with the possibility that kinetochore forces cause collapse of the spindle apparatus in metaphase cells injected with anti-NuMA antibodies, the rate of collapse (20–30 min for a ~10-μm half spindle) is similar, albeit slightly slower, to that expected for chromosome movements observed during anaphase (~0.5-2 μm/min; see MacIntosh and Pfarr, 1991 and references therein).

The early association of NuMA with the reforming nucleus at telophase suggested a possible role of NuMA in nuclear reformation (Price and Pettijohn, 1986; Yang et al., 1992). Although injection of antibodies in anaphase cells did not block nuclear reformation (and indicated that NuMA remained at the poles), it is still possible that NuMA participates in this process. Other evidence from our laboratory indicates that a protein immunologically related to NUF1, a large yeast nuclear coiled-coil protein, is present in the nucleus of mammalian cells (Mirzayan *et al.*, 1992). It is possible that both NuMA and the mammalian NUF1 or some other protein serve as structural components that participate in nuclear reformation at telophase and that the inhibition of both is required to block nuclear reformation. Additional studies are required to test this possibility.

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