Journal of Cell Science 109, 561-567 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JCS7047

Katanin, the microtubule-severing ATPase, is concentrated at centrosomes

Francis J. McNally[1,3,*], Katsuya Okawa[2], Akihiro Iwamatsu[2] and Ronald D. Vale[3,4]

[1]Section of Molecular and Cellular Biology, 2310 Storer Hall, University of California at Davis, Davis, CA 95616, USA
[2]Kirin Brewery Co. Ltd., Central Laboratories for Key Technology, 1-13-5 Fukuura, Kanazawa-ku Yokahama, Kanagawa, Japan
[3]Department of Pharmacology and [4]Howard Hughes Medical Research Institute, University of California at San Francisco, San Francisco, CA 95616-0450, USA

*Author for correspondence at address 1

SUMMARY

The assembly and function of the mitotic spindle involve specific changes in the dynamic properties of microtubules. One such change results in the poleward flux of tubulin in which spindle microtubules polymerize at their kinetochore-attached plus ends while they shorten at their centrosome-attached minus ends. Since free microtubule minus ends do not depolymerize in vivo, the poleward flux of tubulin suggests that spindle microtubules are actively disassembled at or near their centrosomal attachment points. The microtubule-severing ATPase, katanin, has the ability actively to sever and disassemble microtubules and is thus a candidate for the role of a protein mediating the poleward flux of tubulin. Here we determine the subcellular localization of katanin by immunofluorescence as a preliminary step in determining whether katanin mediates the poleward flux of tubulin. We find that katanin is highly concentrated at centrosomes throughout the cell cycle. Katanin's localization is different from that of γ-tubulin in that microtubules are required to maintain the centrosomal localization of katanin. Direct comparison of the localization of katanin and γ-tubulin reveals that katanin is localized in a region surrounding the γ-tubulin-containing pericentriolar region in detergent-extracted mitotic spindles. The centrosomal localization of katanin is consistent with the hypothesis that katanin mediates the disassembly of microtubule minus ends during poleward flux.

Key words: Microtubule, Katanin, Centrosome

INTRODUCTION

Katanin is a heterodimeric, ATP-dependent, microtubule-severing protein purified from sea urchin eggs. Katanin has a microtubule-stimulated ATPase activity and hydrolysis of ATP is required for the microtubule-severing reaction (McNally and Vale, 1993). Although two ATP-independent microtubule-severing proteins have been isolated from *Xenopus* eggs (Shiina et al., 1992, 1994), katanin is likely to be the predominant microtubule-severing protein in vivo, since all the microtubule-severing activity observed in crude extracts requires ATP (McNally and Vale, 1993; Shiina et al., 1992). Katanin is also the only protein known to disassemble stable microtubules to form polymerization-competent tubulin subunits (McNally and Vale, 1993).

Whereas progress has been made in the biochemical characterization of purified katanin, nothing is known about its function in vivo. One clue as to the function in vivo of microtubule severing may be provided by the post-translational regulation of the activity observed in *Xenopus* egg extracts. Experiments done by several groups (Vale, 1991; Shiina et al., 1992; Verde et al., 1992) indicate that microtubule-severing activity is activated post-translationally when cdc2-kinase is activated in *Xenopus* extracts, although there are no data indicating whether this effect is direct or indirect. The activation of microtubule-severing activity by cdc2-kinase has led to the hypotheses that it might play a specific role at the G_2 to M transition in either disassembling the interphase microtubule array (Vale, 1991; Karsenti, 1993) or mediating poleward flux of tubulin (Sawin and Mitchison, 1994). Poleward flux is a phenomenon in which microtubules of the mitotic spindle polymerize at their kinetochore-attached plus ends while they are disassembled at their centrosome-attached minus ends (Mitchison, 1989). Because free minus ends of microtubules are stable in vivo (Nicklas et al., 1989), the shortening of microtubule minus ends during poleward flux implies that microtubules are actively disassembled near their centrosomal attachment points. The idea that a microtubule-severing protein might be active at centrosomes is supported by observations of microtubule detachment from centrosomes in vitro (Belmont et al., 1990) and in vivo (Kitanishi-Yumura and Fukui, 1987). Like the microtubule-severing activity observed in *Xenopus* extracts, both the velocity of poleward flux (Mitchison and Salmon, 1992) and the frequency of microtubule detachment from centrosomes (Belmont et al., 1990) increase when cdc2-kinase is activated.

As a preliminary step in determining the in vivo function of katanin, we determined the subcellular localization of the protein by immunofluorescence with katanin-specific antibodies. Here we report that katanin exhibits a unique centrosomal localization in vivo that is distinct from that of γ-tubulin, the protein thought to nucleate microtubule growth from the

centrosome. This result supports the hypothesis that katanin mediates the active disassembly of microtubules near their centrosomal attachment points.

MATERIALS AND METHODS

Production of affinity-purified katanin-specific antibodies

Katanin was purified as previously described from sea urchin (*Strongylocentrotus purpuratus*) eggs (McNally and Vale, 1993), and the p81 subunit was resolved from the p60 subunit by SDS-PAGE. An acrylamide slice containing 25 μg of p81 was excised and used to immunize a rabbit via the popliteal lymph node. The animal was boosted by intramuscular injection at two-week intervals with 25 μg of p81 per boost. p81-specific antibodies were purified from serum by binding to SDS-PAGE-purified, nitrocellulose-immobilized p81 katanin and eluting successively with 0.2 M glycine (pH 2.4) and 4 M $MgCl_2$. This antibody preparation, anti-$p81^{aff}$, was further purified and concentrated by chromatography on Protein A-Sepharose.

In order to produce anti-peptide antibodies, internal peptide sequences were obtained for both p81 and p60 katanin. p81 and p60 were resolved by SDS-PAGE and blotted to PVDF membranes. Immobilized protein samples were subjected to protease digestion and resulting peptides were resolved by reversed phase HPLC and microsequenced. One p81 peptide sequence, DRNKPANLDMDAFLPPAHAQQAPRVNA (1097 peptide), obtained from overlapping trypsin and endoproteinase Asp-N peptides, and one p60 endoproteinase Asp-N peptide sequence, DPTRRSEPSKPANRAPG (1096 peptide), were synthesized with N-terminal cysteine residues and coupled separately to maleimide-activated cationized BSA (cBSA, Pierce) and to keyhole limpet hemocyanin (KLH) with glutaraldehyde. One rabbit was immunized with a mixture of cBSA-immobilized and KLH-immobilized p81 peptide, and a second rabbit was immunized with a mixture of cBSA-immobilized and KLH-immobilized p60 peptide. Serum from each rabbit was affinity-purified on columns of the appropriate peptide immobilized on Pierce SulfoLink Coupling Gel. The p81 peptide antibody is referred to as anti-$p81^{1097}$ and the p60 peptide antibody is referred to as anti-$p60^{1096}$.

Other primary antibodies

The anti-β-tubulin antibody used was mouse monoclonal E7 made by M. Klymkowski.

The anti-γ-tubulin antibody was an affinity-purified rabbit anti-peptide antibody made against the C terminus of *Xenopus laevis* γ-tubulin by Yixian Zheng and Bruce Alberts. This antibody specifically recognized a single polypeptide of 50 kDa in immunoblots of isolated *S. purpuratus* spindles (not shown).

Preparation of second antibodies

Fluorescent second antibodies were purchased from Jackson ImmunoResearch. All second antibodies were incubated with methanol-fixed, rehydrated *S. purpuratus* embryos and with methanol-fixed, rehydrated isolated spindles for 2 hours followed by removal of embryos and spindles by centrifugation. This preparation was essential for reducing background staining.

Sea urchins and immunofluorescence

S. purpuratus were obtained from Marinus (Venice, CA), North Coast Invertebrate Collectors (Bodega Bay, CA) and from Dan Buster (collected at Point Arena, CA). Release of gametes was induced by injection of 0.56 M KCl. Jelly coats were removed from eggs by passage through 150 μm Nitex (Tetko) screens. Eggs were then fertilized in seawater containing 10 mM *p*-aminobenzoic acid and further passaged through 150 μm Nitex to remove fertilization envelopes. Embryos were cultured in CFSW (436 mM NaCl, 9 mM KCl, 34 mM $MgCl_2$, 16 mM $MgSO_4$, 5 mM Tris-HCl, pH 8.2) in monolayers at 12°C for 2-12 hours. Shortly before fixation, embryos were immobilized on polylysine-coated coverslips, washed once in PMEG (100 mM K-PIPES, 1 mM $MgSO_4$, 1 mM EGTA, 10% glycerol, pH 6.8), then immersed in 90% methanol, 50 mM NaEGTA at –20°C for 1-72 hours. Embryos were rehydrated by immersing coverslips in TBS (150 mM NaCl, 50 mM Tris-HCl, pH 7.5) with 0.05% Triton X-100 and 0.1% $NaBH_4$ for 10 minutes. Reduction with $NaBH_4$ eliminated autofluorescence of chromatin, which was prevalent in some embryos. Coverslips were blocked with 4% BSA, 0.05% Triton X-100 in TBS for 10 minutes before incubation in primary antibody diluted in 4% BSA, 0.05% Triton X-100 in TBS for 1 hour. Concentrations of primary antibody ranged from 1 to 10 μg/ml IgG. Coverslips were washed with four consecutive washes of TBS, 0.05% Triton X-100, followed by incubation in fluorophore-labelled second antibody diluted in 4% BSA, 0.05% Triton X-100 in TBS, 1 μg/ml DAPI for 1 hour. Cy3-labelled second antibodies were used for rabbit primary antibodies and Cy5-labelled second antibodies were used for mouse primary antibodies. After washing four times in TBS, 0.05% Triton X-100, coverslips were mounted in Mowiol 4-88 (Calbiochem) containing 2.5% 1,4-diazobicyclo-[2.2.2]-octane or 1 mg/ml *p*-phenylenediamene in 80% glycerol.

For double-label experiments, sequential rather than simultaneous antibody incubations were carried out to reduce binding of anti-mouse second antibodies to rabbit primary antibodies and vice versa. No fluorescence signal was observed when either embryos stained with rabbit primary antibody were labelled with Cy5-conjugated anti-mouse antibody and vice versa or when Cy5-stained embryos were observed with the Cy3 filter/beamsplitter combination and vice versa.

Isolation of detergent-extracted spindles

Isolated spindles were prepared essentially as described by Salmon and Segall (1980). *S. purpuratus* eggs were dejellied, fertilized and cultured as described above. Synchronous first or second mitotic metaphase was determined by DIC microscopy. Batches of embryos with greater than 90% synchrony in metaphase were washed twice in PMEG by sequential centrifugation at 500 ***g*** and resuspension. Embryos were resuspended in 2 ml of PMEG, then rapidly mixed with 48 ml of 16 mM K-PIPES, 30% glycerol, 1 mM $MgSO_4$, 10 mM EGTA, 0.3% Triton X-100, pH 6.8. Embryo remnants were washed 3 times in this buffer by sequential centrifugation at 1000 ***g*** and resuspension by repeated pipetting with a Pasteur pipette, and then were finally suspended in 1 ml of 16 mM K-PIPES, 50% glycerol, 1 mM $MgSO_4$, 1 mM EGTA, pH 6.8. Samples of these isolated spindles were affixed to polylysine-coated coverslips and observed by DIC microscopy. Only those preparations with a high percentage of intact spindles with morphologically distinct chromosomes, microtubules and centrosomes were used for immunofluorescence. Coverslips coated with isolated spindles were fixed in 90% methanol, 50 mM EGTA at –20°C and processed as described above for embryos.

Microscopy

Confocal sections of labelled embryos and spindles were obtained with a Zeiss LSM laser scanning confocal microscope based on a Zeiss Axiovert 100 and utilizing both ×63 Plan Neofluar NA 1.25 and ×100 Plan Neofluar NA 1.3 objectives. Other images were captured with a SIT camera (DAGE-MTI SIT68) mounted on a Nikon Microphot SA microscope equipped with a ×60 Plan Apo NA 1.4 objective. These images were digitized with a Scion AG5 framegrabber and NIH Image software.

RESULTS

Antibodies specific for p81 and p60 katanin label the centrosomes of sea urchin embryonic cells

The subcellular distribution of katanin was analyzed by

immunofluorescence labelling of methanol-fixed *S. purpuratus* embryos. An affinity-purified antibody specific for the 81 kDa subunit of katanin (anti-p81^{aff}) labelled one or two bright spots in every cell and weakly labelled the entire cytosol in most cells (Fig. 1A and B). These spots were judged to be centrosomes because they coincided with the centers of microtubule asters (Fig. 1B and D) and with the poles of mitotic spindles (Fig. 1A and C) in cells double labelled with both anti-katanin and anti-β-tubulin antibodies. Diffuse staining by katanin-specific antibodies was sometimes observed in the vicinity of microtubules (e.g. see Fig. 1A); however, extensive colocalization of katanin and β-tubulin was not observed.

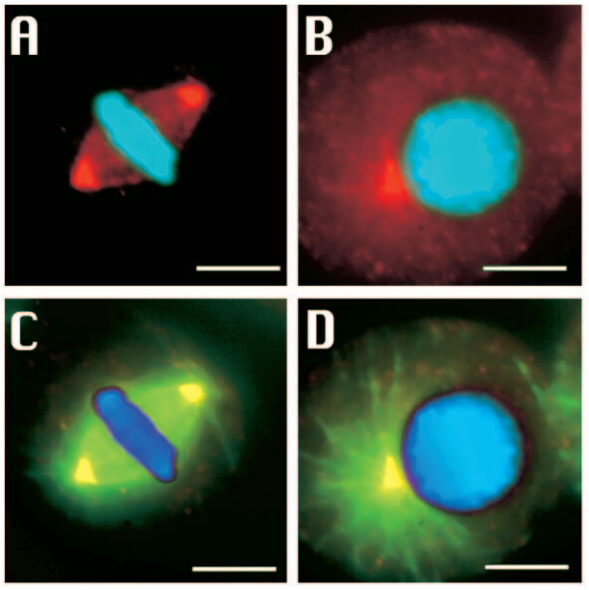

Fig. 1. Double-label immunofluorescence showing localization of p81 katanin (with anti-p81^{aff} antibody) at spindle poles (A and C) and at the center of a microtubule aster (B and D). Staining of p81 katanin and DNA is shown in A and B, with katanin staining in red and DNA staining in blue. In the triple-labelled images (C and D), β-tubulin staining is shown in green and the overlap of β-tubulin staining with katanin staining is shown in yellow. The two cells shown were from methanol-fixed *S. purpuratus* embryos at the 64-celled stage. Images of each fluorophore were captured separately with a SIT camera, pseudocolored and overlaid using Adobe Photoshop software. Bar, 5 μm.

Several criteria were used to establish the specificity of the centrosomal staining. First, two different affinity-purified antibodies specific for the 81 kDa subunit of katanin (anti-p81^{aff} and anti-p81^{1097}) and one affinity-purified antibody specific for the 60 kDa subunit of katanin (anti-p60^{1096}) yielded identical staining patterns in methanol-fixed cells (Fig. 2, and results not shown). Second, centrosome staining by all three antibodies was competed by purified antigen. Two of the antibodies (anti-p81^{1097} and anti-p60^{1096}) were anti-peptide antibodies and were competed by 10 μM of peptide antigen but not by 10 μM of a control peptide (Fig. 2, and results not shown). Staining by the anti-p81^{aff} antibody was competed by 15 μg/ml of purified katanin (Fig. 2). Third, each of the three katanin-specific antibodies recognized single polypeptide bands in immunoblots of crude blastula embryos (Fig. 3). Fourth, neither preimmune sera nor fluorescent second antibodies alone stained centrosomes under the conditions used to label with the katanin-specific antibodies. Finally, centrosomal staining was observed in embryos fixed under different conditions (methanol/EGTA or glutaraldehyde/formaldehyde, data not shown). Taken together, these results confirm that both p81 and p60 katanin are indeed highly concentrated in the centrosomal region of sea urchin embryonic cells.

Katanin is localized at centrosomes throughout the cell cycle and throughout development

Staining of centrosomes by katanin-specific antibodies was observed at all cell-cycle stages (Fig. 4). In interphase cells, either one or two foci of katanin staining were always observed in a perinuclear position. These foci often appeared flattened against the nucleus. During both metaphase and anaphase, katanin staining at the spindle poles was often half-moon- or crescent-shaped (e.g. see Fig. 4D). Similar staining patterns were observed in cells of embryos fixed at all stages of development through the blastula stage (data not shown).

Katanin localization in isolated spindles reveals a unique 'hollow ball' structure

In order to examine the morphology of katanin's localization at spindle poles in more detail, immunofluorescence localization

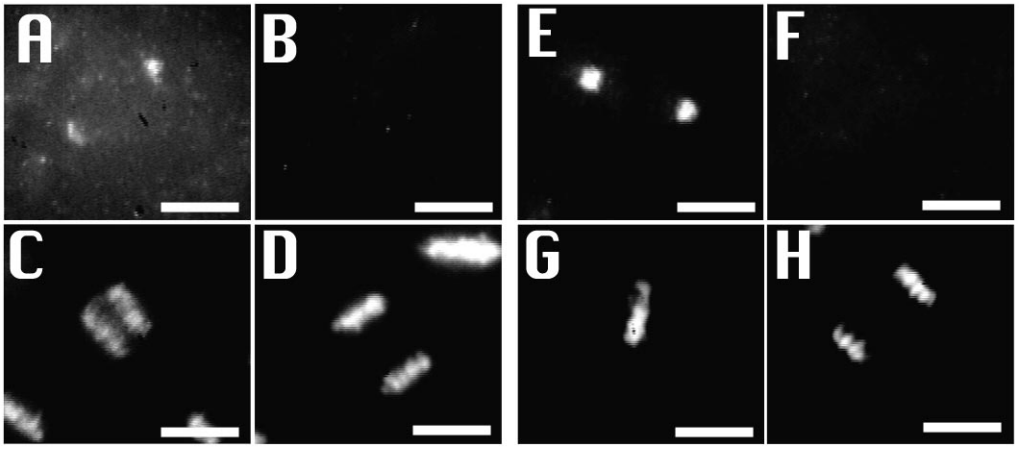

Fig. 2. Demonstration that centrosome staining by two different katanin-specific antibodies is specifically competed by antigen. The corresponding DAPI stain of DNA (C, D, G and H) is shown below each katanin-stained image (A, B, E and F). Staining by the p60 katanin-specific antibody anti-p60^{1096} was not competed by 10 μM of a control peptide (A) but was competed by 10 μM of peptide antigen (B). Staining by the anti-p81^{aff} antibody (E) was specifically competed by 15 μg/ml MonoQ-purified katanin in the presence of 4 mg/ml BSA (F). Cells shown are from methanol-fixed 32-celled embryos. Images were captured with a SIT camera. Bar, 10 μm.

was carried out on isolated spindles. When sea urchin embryos at the first or second mitotic metaphase are extracted with detergent in low ionic strength buffers, the cortex dissolves leaving an isolated spindle (Salmon and Segall, 1980). Immunofluorescence localization of katanin in these detergent-extracted spindles revealed that katanin remained highly concentrated at the centrosomes (Fig. 5), suggesting that katanin is not a membrane-associated protein. Katanin antibodies reproducibly labelled a hollow ball at each spindle pole in isolated spindles (Fig. 5). The hollow balls were generally half-moon- or crescent-shaped as had been the case for the localization in methanol-fixed cells. Since antibody penetration and imaging were far superior in isolated spindles than in methanol-fixed first division embryos, it was not possible to ascertain definitively whether the hollow ball staining occurred in methanol-fixed cells. However, some suggestion of the hollow ball staining is seen in the methanol-fixed second division metaphase spindle in Fig. 4C and D. Katanin-specific antibodies weakly labelled microtubules in detergent-extracted spindles; however, this labelling was far less intense than staining of centrosomes.

Katanin localization is distinct from that of γ-tubulin

The hollow ball staining by katanin antibodies in isolated spindles suggested that katanin localization might be spatially distinct from that of known centrosomal proteins like γ-tubulin. We therefore compared the localization of katanin and γ-tubulin at the poles of isolated spindles. Katanin antibodies labelled in a hollow ball pattern in over 90% of isolated spindles from one preparation while γ-tubulin antibodies labelled in a solid ball pattern in over 90% of isolated spindles from the same preparation (Fig. 5). The high frequency of solid ball labelling of spindle poles by the γ-tubulin antibody indicated that the hollow ball staining by katanin antibody was not due to failure of antibodies to penetrate to the center of the centrosome. These results indicated that katanin localization was spatially distinct from γ-tubulin localization in isolated spindles, with the katanin surrounding the γ-tubulin.

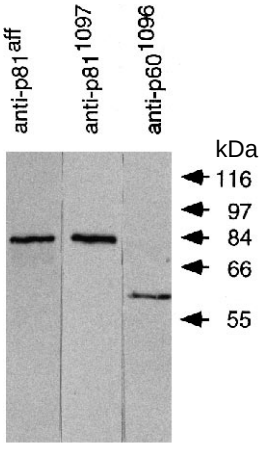

Fig. 3. Western blots showing the specificity of the three katanin-specific antibodies, anti-p81^{aff}, anti-p81^{1097} and anti-p60^{1096}. *S. purpuratus* blastula embryos were concentrated by centrifugation and boiled in 5% SDS before proteins were separated by SDS-PAGE, blotted to nitrocellulose and probed with each antibody. Peroxidase-conjugated second antibody and chemiluminescence detection were used.

Maintenance of katanin localization requires microtubules

Comparison of the morphology of katanin localization and γ-tubulin localization indicated that these proteins were associated with distinct structures. In an independent approach to this question, the dependence of katanin's localization on microtubules was examined. One feature of pericentriolar proteins like γ-tubulin and pericentrin is that their localization is unaffected when microtubules are depolymerized with the drug nocodazole (Stearns et al., 1991; Doxsey et al., 1994). When microtubules in *S. purpuratus* embryos were depolymerized with 20 μM nocodazole for 5 minutes before methanol fixation, centrosomal localization of katanin was abolished and only diffuse cytoplasmic staining was observed (Fig. 6). To determine whether the effect of nocodazole was due to a lack of microtubules or to a perturbation of microtubule dynamics, embryos were treated with taxol, a drug that stabilizes microtubules. Treatment of embryos with 20 μM taxol did not result in the dispersal of discrete staining of centrosomes by katanin-specific antibodies (Fig. 6). The results obtained with nocodazole and with taxol treatments indicated that intact microtubules rather than normal microtubule dynamics are required for maintenance of katanin's localization at the centrosome.

DISCUSSION

Immunofluorescence labelling with katanin-specific antibodies has indicated that katanin is highly concentrated at the centrosomes throughout the cell cycle in sea urchin embryonic cells.

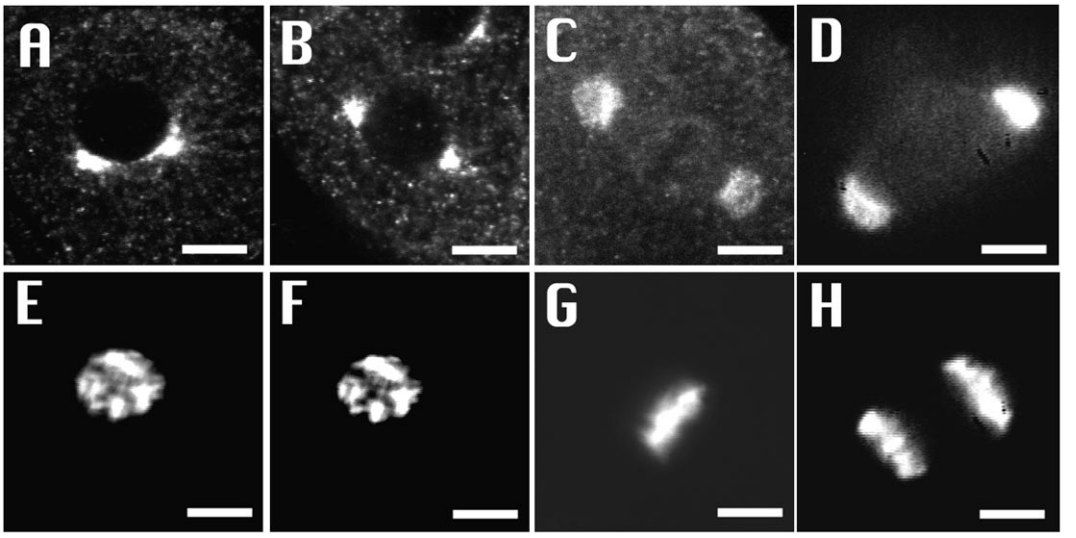

Fig. 4. Images showing that the p81-specific antibody, anti-p81^{aff}, labels two centrosomes throughout the cell cycle (A, B, C and D). The corresponding DAPI stain of DNA is shown in the bottom row (E, F, G and H). The interphase (A) and prophase (B) cells were from 4-celled embryos, whereas the metaphase (C) and anaphase (D) cells were from second-division embryos. A, B, C, E, F and G are confocal images while D and H were captured with a SIT camera. Bar, 5 μm.

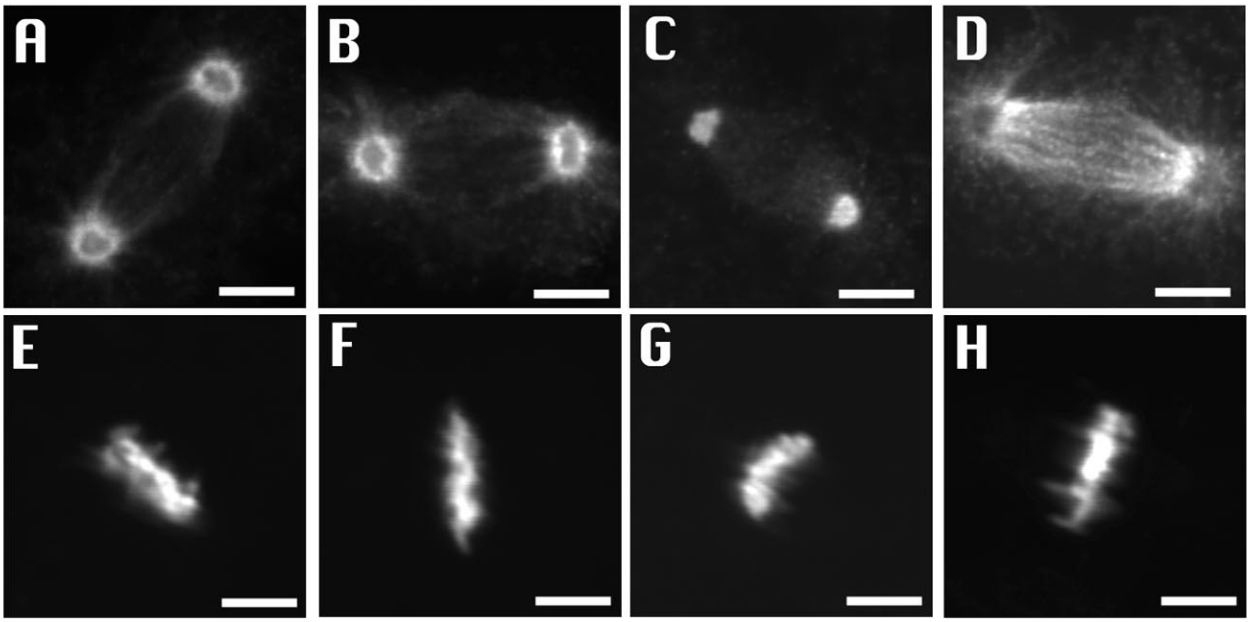

Fig. 5. Confocal images of immunofluorescence of detergent-extracted second metaphase spindles. The corresponding DAPI stain of DNA (E, F, G and H) is shown below each antibody-labelled image (A, B, C and D). The p81 katanin-specific antibody, anti-p81^{aff} (A), and the p60 katanin-specific antibody, anti-p60^{1096} (B), each labelled a hollow ball around the outside of each spindle pole. A γ-tubulin-specific antibody labelled smaller solid balls at each spindle pole (C). Staining with a β-tubulin antibody is shown for comparison (D). Bar, 5 μm.

Three major criteria were used to demonstrate that this staining was not coincidental or artifactual. First, two different antibodies specific for one subunit (p81) and, one antibody specific for the other subunit of katanin (p60), all labelled the same structure. Second, staining by each antibody was specifically competed by soluble antigen. Third, each antibody was completely specific for a polypeptide of the appropriate molecular mass in western blots of whole embryos. Since centrosomal staining was observed in cells fixed with methanol without previous detergent extraction, the centrosomal localization of katanin cannot be due to spurious binding of katanin to centrosomes during detergent extraction. These results indicate that katanin is indeed concentrated at centrosomes in vivo.

The centrosomal localization of katanin was not predictable from earlier work on the protein. Since katanin was purified as a soluble protein from unfertilized egg extracts (McNally and Vale, 1993), it might be expected that katanin would be dispersed throughout the cytoplasm. This appears to be the case in unfertilized eggs (data not shown); however, katanin appears to associate with the centrosome assembled onto the sperm basal body sometime before the first mitotic prophase (the earliest point at which katanin localization has been clearly imaged). In earlier work katanin was found to bind to microtubules (McNally and Vale, 1993), suggesting that it might be bound to microtubules in vivo. However, stable binding of katanin to microtubules is sensitive to ionic strength (McNally and Vale, 1993) and katanin does not cosediment with microtubules polymerized by addition of taxol to sea urchin egg extracts (data not shown). The apparently low affinity of katanin for microtubules may explain the absence of extensive colocalization of katanin with microtubules in vivo.

Whereas katanin is not bound along the length of microtubules, microtubules are required to maintain katanin's centrosomal localization as indicated by the dispersal of katanin-specific centrosome staining by nocodazole treatment. This sensitivity to nocodazole differs from the nocodazole-resistant localization of the integral pericentriolar proteins γ-tubulin (Stearns et al., 1991) and pericentrin (Doxsey et al., 1994), and suggests that katanin is not an integral component of the pericentriolar material. This conclusion is supported by the finding that katanin localizes in a hollow ball pattern that extends

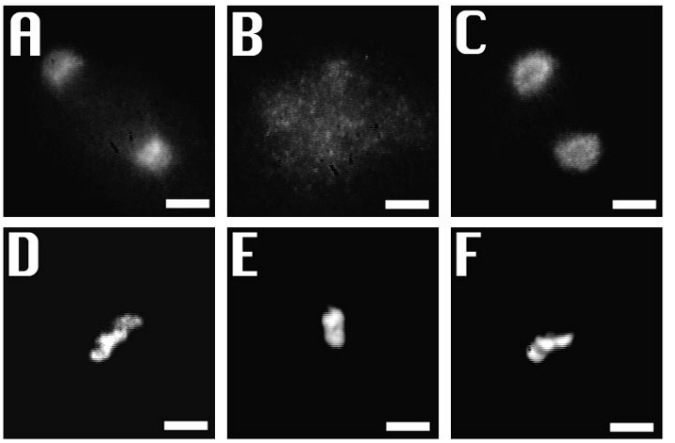

Fig. 6. Immunofluorescence showing the sensitivity of katanin's centrosomal localization to drugs that perturb microtubules. Four-celled *S. purpuratus* embryos were treated for 5 minutes with 20 μM of each drug in CFSW before methanol fixation and staining with anti-p81^{aff} antibody. The corresponding DAPI stain of DNA (D, E and F) is shown below each katanin-stained image (A, B and C). Nocodazole treatment (B) completely dispersed the katanin localization observed in untreated embryos (A), whereas taxol did not change the localization of katanin (C). Images were captured with a SIT camera. Bar, 5 μm.

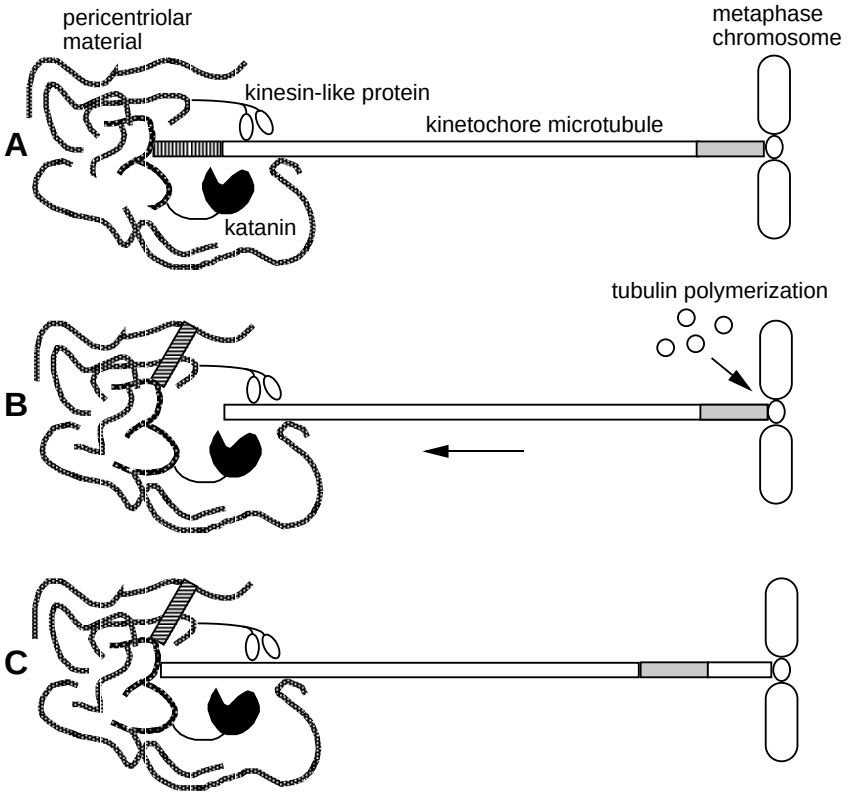

Fig. 7. Model demonstrating how katanin might act during the poleward flux of tubulin in the mitotic spindle. Katanin and a kinesin-like protein are shown attached to a hypothetical matrix within the centrosome. Severing of the kinetochore microtubule by katanin releases a short microtubule fragment and allows the kinesin-like protein to pull the kinetochore microtubule toward the centrosomal matrix. Simultaneous polymerization of tubulin at the kinetochore-attached end of the microtubule results in the movement of marked tubulin subunits within the microtubule (shaded bar) toward the centrosome. The short microtubule generated in this reaction might be disassembled to tubulin dimers by katanin or it might nucleate growth of a new microtubule.

beyond the diameter of γ-tubulin staining in isolated spindles. Because available γ-tubulin antibodies did not stain centrosomes in methanol-fixed sea urchin cells, it was not possible to compare directly the morphology of the katanin-containing structure and the pericentriolar material in unextracted cells. Thus it remains unclear whether the katanin-containing structure actually surrounds the pericentriolar material in vivo or if the katanin-containing structure is simply more sensitive to osmotic swelling during detergent extraction. In either case, katanin is bound to a structure distinct from the γ-tubulin-containing pericentriolar material. One possibility is that katanin is a component of the same nocodazole-sensitive pericentrosomal structure that is labelled by antibodies specific for the NuMa protein during mitosis (Tousson et al., 1991).

Regardless of the molecular nature of katanin's localization at the centrosome, the concentration of katanin at the centrosome places katanin in the perfect position to sever and disassemble microtubules near their minus ends in the pericentriolar material. Katanin would be properly positioned to sever microtubules whether its localization surrounds or overlaps with the pericentriolar material, since microtubule minus ends are distributed throughout the volume of the pericentriolar material (Moritz et al., 1995). Katanin-mediated severing and disassembly of microtubules at the centrosome in conjunction with the pulling force of a plus-end-directed, kinesin-like motor protein attached to the centrosome could drive the shortening of spindle microtubules required for the poleward flux of tubulin in the metaphase spindle (Sawin and Mitchison, 1994) (Fig. 7). The existence of a mechanism that severs microtubules from their centrosomal attachment points is supported by two observations. First, microtubule release from the centrosome has been observed in vivo (Kitanishi-Yumura and Fukui, 1987) and in vitro (Belmont et al., 1990). Second, the distribution of microtubule minus ends throughout the mammalian spindle apparatus (Mastronarde et al., 1993) suggests that these microtubules were released from the centrosome where all microtubule growth is believed to originate. Further experiments blocking katanin's activity in vivo will be required to elucidate the details of katanin's role at the centrosome.

The authors are grateful to Yixian Zheng and Bruce Alberts for the anti-γ-tubulin antibody and Diana Myles for the use of her confocal microscope.

F.J.M. was supported by a fellowship from the American Cancer Society, California Division, Inc. This work was supported by an NIH grant to R.D.V. and by support from the University of California for F.J.M.

REFERENCES

Belmont, L., Hyman, A., Sawin, K. and Mitchison, T. (1990). Real-time visualization of cell cycle-dependent changes in microtubule dynamics in cytoplasmic extracts. *Cell* **62**, 579-589.

Doxsey, S. J., Stein, P., Evans, L., Calarco, P. D. and Kirschner, M. (1994). Pericentrin, a highly conserved centrosome protein involved in microtubule organization. *Cell* **76**, 639-650.

Karsenti, E. (1993). Severing microtubules in mitosis. *Curr. Biol.* **3**, 208-210.

Kitanishi-Yumura, T. and Fukui, Y. (1987). Reorganization of microtubules during mitosis in *Dictyostelium*: dissociation from MTOC and selective assembly/disassembly in situ. *Cell Motil. Cytoskel.* **8**, 106-117.

Mastronarde, D., McDonald, K., Ding, R. and McIntosh, J. (1993). Interpolar spindle microtubules in PtK cells. *J. Cell Biol.* **123**, 1475-1489.

McNally, F. and Vale, R. (1993). Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* **75**, 419-429.

Mitchison, T. (1989). Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J. Cell Biol.* **109**, 637-652.

Mitchison, T. and Salmon, E. (1992). Poleward kinetochore fiber movement

occurs during both metaphase and anaphase-A in newt lung cell mitosis. *J. Cell Biol.* **119**, 569-582.

Moritz, M., Braunfeld, B. B., Fung, J. C. Sedat, J. W., Alberts, B. M. and Agard, D. A. (1995). Three-dimensional structural characterization of centrosomes from early *Drosophila* embryos. *J. Cell Biol.* **130**, 1149-1159.

Nicklas, R. B., Lee, G., Rieder, C. and Rupp, G. (1989). Mechanically cut mitotic spindles: clean cuts and stable microtubules. *J. Cell Sci.* **94**, 415-423.

Salmon, E. D. and Segall, R. R. (1980). Calcium-labile mitotic spindles isolated from sea urchin eggs (*Lytechinus variegatus*). *J. Cell Biol.* **86**, 355-365.

Sawin, K. and Mitchison, T. (1994). Microtubule flux in mitosis is independent of chromosomes, centrosomes, and antiparallel microtubules. *Mol. Biol. Cell* **5**:217-226.

Shiina, N., Gotoh, Y. and Nishida, E. (1992). A novel homo-oligomeric protein responsible for an MPF-dependent microtubule-severing activity. *EMBO J.* **11**, 4723-4731.

Shiina, N., Gotoh, Y., Kubomura, N., Iwamatsu, A. and Nishida, E. (1994). Microtubule severing by elongation factor 1 alpha. *Science* **266**, 282-285.

Stearns, T., Evans, L. and Kirschner, M. (1991). γ-tubulin is a highly conserved component of the centrosome. *Cell* **65**, 825-836.

Tousson, A., Zeng, C., Brinkley, B. and Valdivia, M. (1991). Centrophilin: a novel mitotic spindle protein involved in microtubule nucleation. *J. Cell Biol.* **112**, 427-440.

Vale, R. D. (1991). Severing of stable microtubules by a mitotically activated protein in *Xenopus* egg extracts. *Cell* **64**, 827-839.

Verde, F., Dogterom, M., Steizer, E., Karsenti, E. and Leibler, S. (1992). Control of microtubule dynamics and length by cyclin A and cyclin B dependent kinases in *Xenopus* egg extracts. *J. Cell Biol.* **118**, 1097-1108.

Zhai, Y., Kronebusch, P. J. and Borisy, G. G. (1995). Kinetochore microtubule dynamics and the metaphase-anaphase transition. *J. Cell Biol.* **131**, 721-734.

(Received 3 October 1995 - Accepted 3 December 1995)