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Cloning and Characterization of hMAP126, a New Member of Mitotic Spindle-Associated Proteins

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One novel gene product, *hMAP126,* was demonstrated to interact with p29 in the yeast two-hybrid assay. The full-length cDNA of hMAP126 has been obtained and encodes a protein of 1120 amino acids. Multiple tissue Northern blot analysis showed that hMAP126 was abundantly expressed in the testis. Polyclonal antiserum against hMAP126 was raised and affinity-purification of anti-hMAP126 antibodies was performed. The subcellular distribution of hMAP126 was localized to the mitotic spindle. Furthermore, hMAP126 was identified to be post-translationally modified and phosphorylated by p34^{cdc2} kinase *in vitro.* Taken together, we have isolated a novel protein, hMAP126, which may be involved in the functional and dynamic regulation of mitotic spindles.

Key Words: **hMAP126; mitotic spindle; p29.**

During cell division, the duplicated chromosomes must be evenly separated into two daughter cells. The cellular structure responsible for this task is the mitotic spindle, a macromolecular complex consisting primarily of microtubules and associated proteins. Assembly of the mitotic spindle starts during late G_2 phase, after centrosome replication. The activation of cdc2 kinase triggers the dismantling of interphase microtubules and the nucleation of centrosomes, leading to an increase in microtubule dynamics. Microtubules are polar polymers of α-β tubulin dimers with a slow-growing minus end and a fast-growing plus end. The minus ends of microtubules are focused into two poles, while the plus ends are oriented toward chromosomes, creating the typical bipolar spindle (1–5). Microtubule-associated proteins have an important function in this self-organization process. They can be grouped into two categories: the kinesin superfamily, including both plus- and minus-end-directed motors; and cytoplasmic dynein, which, together with its activator dynactin, forms a large multiprotein assembly that moves toward microtubule minus ends (1–5).

Studies of the mitotic role of microtubule-associated proteins (MAPs) have identified several MAPs that localize to the mitotic spindle. XMAP230, XMAP4, XMAP215, and XMAP310 are microtubule stabilizing factors, promoting microtubule growth by reducing catastrophes and increasing the growth rate (6–9). In contrast, destabilizing factors, such as OP18/Stathmin, katanin, and XKCM1, a member of the Kin I kinesin family, facilitate the disassembly of microtubules (10–12). In addition, several Drosophila mutants, like Mast and Orbit, were demonstrated to participate in bipolar mitotic spindle organization (13, 14). TPX2, a newly discovered spindle component that accumulates in nuclei during interphase, has been shown to translocate to the spindle poles in a dynein-dependent manner. Depletion of TPX2 from mitotic *Xenopus* egg extracts resulted in spindles with a reduced density of microtubules and abnormally split poles. In addition, TPX2 is required for the accumulation of the plus-end-directed kinesin Xklp2 at spindle poles (15, 16).

Recent work has revealed that the small GTPase, Ran, which is essential during interphase for nuclear import, promotes microtubule polymerization in its active, GTP-bound form. Consistent with these observations is the fact that the Ran guanine nucleotide exchange factor, RCC1, is required for microtubule polymerization in mitotic extracts. RCC1 activates Ran by facilitating the exchange of bound GDP for GTP (17). Because RCC1 associates with chromatin, it has been proposed that chromatin-bound RCC1 induces a high concentration of Ran-GTP around mitotic chromosomes and locally promotes microtubule assembly. Current models show that the DNA-microtubule stabilization pathway starts with chromosomally localized RCC1, which generates RanGTP. RanGTP binds to the

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transport factor importin β, causing it to release cargoes, such as NuMA, that function to stabilize microtubules, and thereby promoting spindle assembly. RanGTP also binds to the import receptor importin β and the export receptor CAS and influences their association with importin α. The net result is the release of TPX2 from importin α. Free TPX2 can then function in microtubule assembly (18, 19).

We have recently isolated a human nuclear protein, p29, which was demonstrated to associate with GCIP, a cyclin-D interacting protein, in the yeast two-hybrid method and *in vitro* GST pull-down assay. The transient expression of HA- and GFP-tagged p29 in HeLa cells localized in the nucleus (20). To investigate the function of p29, we performed the yeast two-hybrid screening and one novel gene, named as hMAP126, was identified to interact with p29 (data not shown). The interaction between p29 and hMAP126 will be described by Chang *et al.* elsewhere. Here we present the cloning and characterization of a new human mitotic spindle associated protein, hMAP126.

EXPERIMENTAL PROCEDURES

Cloning of hMAP126. An oligo-(dT)-primed human testis cDNA library purchased from Clontech was screened by a probe corresponding to a.a. 610–1120 of hMAP126, which was the interaction region with p29. Positive clones were ligated into pBluescript. Nucleotide sequences were determined by automated sequencing.

Northern blot analysis. Multiple tissue poly(A^+) blots from 16 different human tissues were purchased from Clontech. The full-length of the hMAP126 cDNA probe was labeled with [α-^{32}P]dCTP with a random priming labeling kit (Amersham Pharmacia Biotech, Piscataway, NJ). The hybridization was performed according to the manufacturer's instructions. After hybridization and washing, the membranes were exposed to X-ray film for 24 h at −80°C.

Production and purification of His-tagged fusion protein. For the construction of 6× His-tagged hMAP126 fusion protein, the PCR product of hMAP126 was cloned into a pQE30 vector (Qiagen, Hilden, Germany). The pQE construct was transformed into *Escherichia coli* strain M15 (pREP4). The expression of His tag fusion protein was induced with 1 mM isopropyl-1-thio-D-galactopyranoside at 37°C for 3–5 h. Bacterial pellets were sonicated and solubilized in the lysis buffer (1.5% *N*-lauroylsarcosine, 1% Triton-X100, 150 mM NaCl, 10 mM Tris pH 8.0). The purification of His-tagged fusion protein was carried out according to manufacturer's instructions (Qiagen).

Antibodies. Anti-hMAP126 polyclonal antiserum was raised in rabbits against a truncated hMAP126 recombinant protein (a.a. 800–1120). The polyclonal antiserum was further purified by Affi-10 gel (Bio-Rad, Hercules, CA) coupled with His-tagged hMAP126. After extensive washing with 10 mM Tris, pH 7.5, and 500 mM NaCl, the antibodies were eluted with 100 mM glycine, pH 2.5, and 100 mM triethylamine, pH 11, and dialyzed with PBS for two days.

Synchronization and immunofluorescence. HeLa cells were incubated with nocodazole (0.1 μg/ml) for 16 h. Loosely attached metaphase cells were collected by gentle pipetting and replated in fresh medium without nocodazole. Cells were gently washed with PBS and fixed in cold methanol (−20°C) for 10 min. Affinity-purified anti-hMAP126 antibody was applied and incubated at 37°C for 1 h. After rinsing with PBS, cells were incubated with anti-rabbit antibodies conjugated with rhodamine (Jackson Immuno Research, West Grove, PA) at 37°C for 30 min. Subsequently, DNA was labeled with 4,6-diamidino-2-phenylindole (Sigma, St. Louis, MO) and coverslips were mounted using mounting media purchased from Sigma. Confocal images were recorded using a Leica confocal laser scanning microscope (Leica, Germany).

Western blotting analysis. HeLa cells were incubated with nocodazole (0.1 μg/ml) for 16 h. Metaphase arrested HeLa cells were collected by gentle pipetting and lysed in the lysis buffer. The rest of the attached cells were trypsinized and cell extracts were prepared. Western blot analysis was conducted using affinity-purified anti-hMAP126 antibodies and an ECL system (Amersham Pharmacia Biotech) to detect signals.

In vitro phosphorylation of hMAP126 by cdc2 kinase. Ten micrograms of affinity-purified hMAP126 recombinant protein was incubated with 10 units of p34^{cdc2}/cyclinB kinase (New England Biolabs, Beverly, MA) and 50 μCi of [γ-^{32}P]ATP at 30°C for 1 h in a total volume of 100 μl according to manufacturer's instructions. For the lambda protein phosphatase assay, 50 μl of p34^{cdc2} kinase phosphorylation product was incubated with 400 units of lambda protein phosphatase (New England Biolabs) at 30°C for 1 h. The reactions were terminated by adding SDS sample buffer, and proteins were separated by SDS–PAGE. The gel was dried and protein phosphorylation and dephosphorylation were detected by autoradiography.

RESULTS

Molecular Cloning of hMAP126

To obtain the full-length cDNA of this novel gene, an oligo-(dT)-primed human testis cDNA library was screened. The cDNA sequences of this novel gene were determined to be approximately 4.1 Kb, and the 3360-bp open-reading frame encoded a protein of 1120 amino acids with a predicted molecular mass of 126 kDa (Fig. 1A). Therefore, we designated this novel molecule as hMAP126 and deposited its sequence in the GenBank database under the Accession No. AF345347. To make sure that we had obtained the methionine initiation site, 5′-RACE PCR was performed using a human HeLa marathon-ready cDNA purchased from Clontech as the template. The result showed the same methionine initiation site as our cDNA library screening (data not shown).

Structural analysis revealed one potential cdc2 phosphorylation site (21), TPLR, located on its N-terminal end (a.a. 24–27). One potential bipartite nuclear localization signal (22), a.a. 537–556, was present in hMAP126, suggesting that hMAP126 may be a nuclear protein. Structural alignment revealed that hMAP126 shared a significant homology with the ubiquitous kinesin heavy chain (uKHC) (a.a. 657–866, Fig. 1B), which belongs to the KIN N subfamily, indicating that hMAP126 may be a new member of kinesin family.

Expression of Human hMAP126 in Normal Human Tissues

On Northern blot analysis, one major transcript of 4.1 Kb was abundantly expressed in testis cells, with a much weaker signal detected in placenta, liver, pancreas, thymus, and colon tissues (Fig. 2). Another tran-

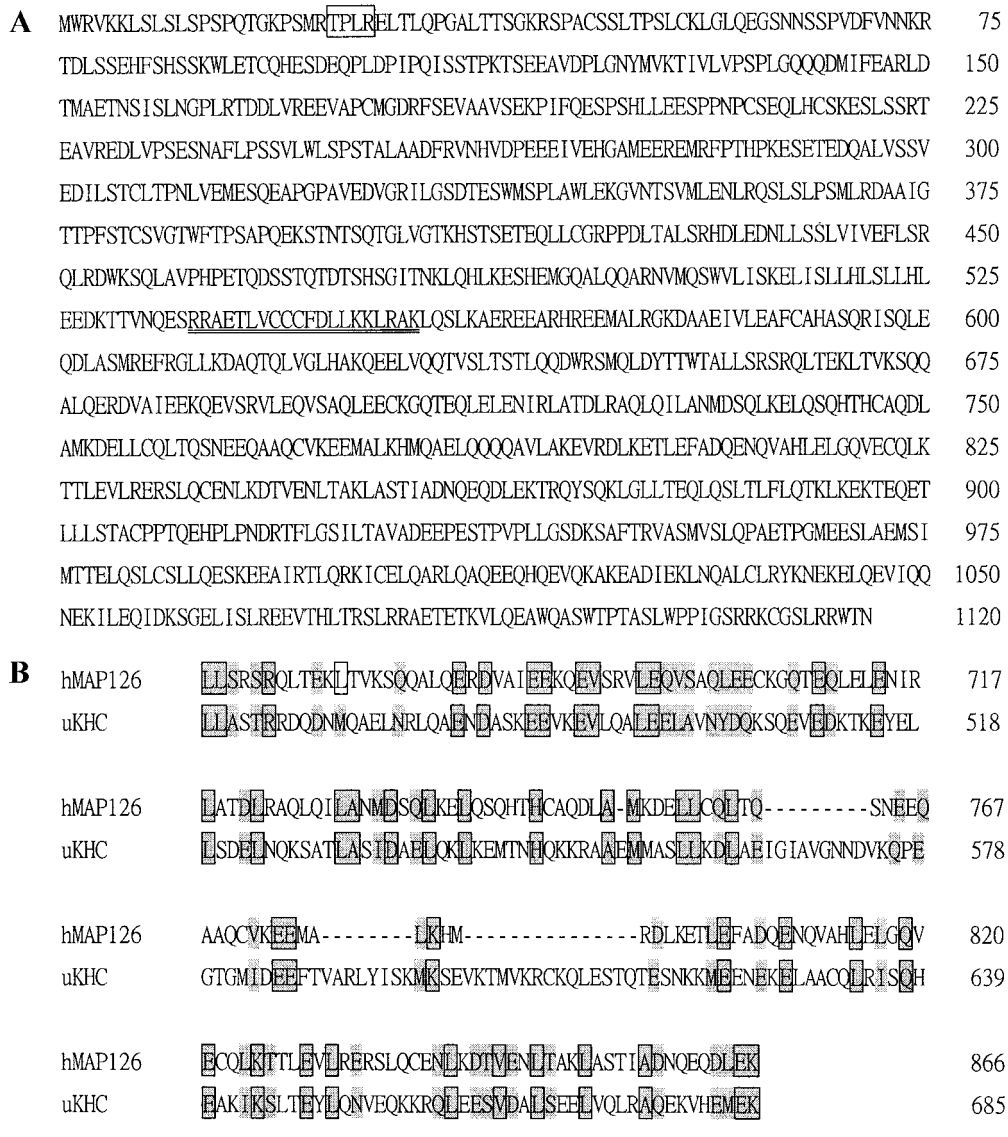

FIG. 1. (A) Deduced amino acid sequence of hMAP126. The $p34^{cdc2}$ kinase phosphorylation site is boxed and the potential nuclear localization signal is double-underlined. (B) Amino acid sequence alignment of hMAP126 with ubiquitous kinesin heavy chain (uKHC). Identical residues are boxed and shaded. Similar residues are shaded. Human ubiquitous kinesin heavy chain protein sequences were retrieved from GenBank database with the Accession No. P33176.

script of 6.0 Kb was found in kidney cells. Whether this higher band in the kidney was an alternatively spliced product remains to be investigated.

The Subcellular Localization of hMAP126

To address the subcellular localization of hMAP126, polyclonal antibody against hMAP126 was raised. Subsequently, affinity purification of anti-hMAP126 polyclonal antibodies was carried out. The specificity of affinity-purified anti-hMAP126 polyclonal antiserum was determined by Western blot analysis. One major band with an approximate molecular mass of 126 kDa was detected, which existed abundantly in cell lysates of metaphase arrested HeLa cells (Fig. 3A, lane 2). No obvious signal was detected in cell extracts of interphase cells (Fig. 3A, lane 1). Similarly, during interphase, a very weak staining pattern of hMAP126 by immunofluorescence was observed in the cytoplasm (Fig. 3B). Strikingly, during metaphase, a strong mitotic spindle staining pattern was obtained (Fig. 3B), indicating that hMAP126 may be a mitotic spindle associated protein and participate in the regulation of mitosis. In contrast, immunoneutralization using anti-hMAP126 antiserum incubated with 6× His-tagged hMAP126 recombinant protein did not detect any signal in the metaphase (data not shown). Furthermore, the HA-tagged hMAP126 construct also showed a mitotic spindle pattern in HeLa cells (data not shown),

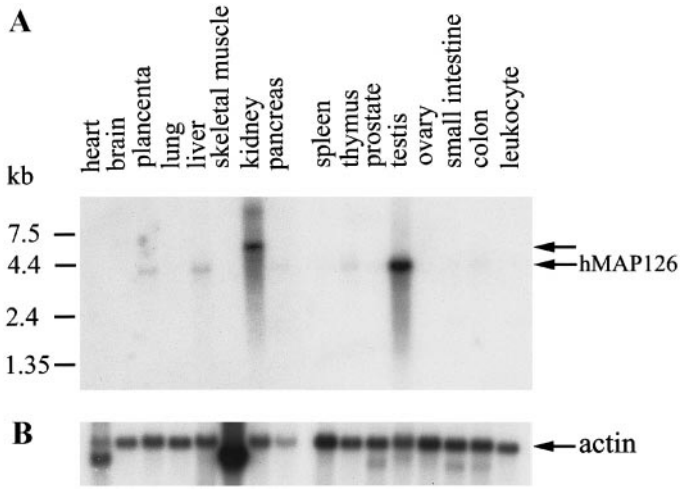

FIG. 2. Northern blot analysis of hMAP126 in different human adult tissues. Multiple tissue northern (MTN) blot membranes were purchased from Clontech. The open-reading frame of hMAP126 was eluted from PCR product, labeled with [α-^{32}P]dCTP, and hybridized with MTN membranes. β-actin was used as an internal control.

supporting that hMAP126 is a mitotic spindle associated protein.

hMAP126 Is Posttranslationally Modified and Phosphorylated by cdc2 Kinase

Since hMAP126 contains a consensus cdc2 kinase phosphorylation site, we set out to examine whether

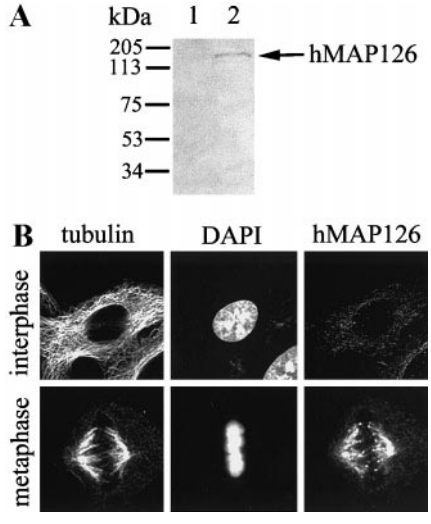

FIG. 3. Subcellular localization of hMAP126. (A) HeLa cells were incubated with nocodazole (0.1 μg/ml) for 16 h. Loosely attached metaphase cells were collected by gentle pipetting and lysed in the lysis buffer (lane 2). The rest of attached cells were trypsinized and cell extracts were prepared (lane 1). Western blot analysis was conducted using affinity-purified anti-hMAP126 polyclonal antibodies. (B) HeLa cells were grown on coverslips and incubated with nocodazole (0.1 μg/ml) for 16 h. Cells were fixed in cold methanol (−20°C) for immunofluorescence detection. Monoclonal anti-α-tubulin antibodies were purchased from Sigma. DAPI staining represents the position of DNA.

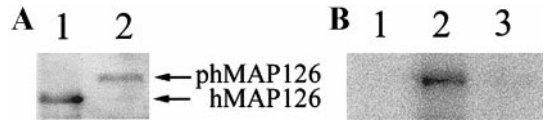

FIG. 4. Phosphorylation of hMAP126 by p34^{cdc2} in vitro. (A) Post-translational modification of hMAP126. Full-length hMAP126 ORF was cloned into *E. coli* His-tagged expression vector pQE30 and affinity purification of 6xHis-hMAP126 was carried out using Ni-NTA agarose (lane 1). HeLa cells were incubated with nocodazole (0.1 μg/ml) for 16 h. Metaphase arrested cells were collected and cell extracts were prepared (lane 2). Western blot analysis was performed using 10% SDS–PAGE gel. (B) Ten micrograms of affinity-purified 6× His-tagged hMAP126 was incubated with [γ-^{32}P]ATP alone (lane 1) or [γ-^{32}P]ATP in the presence of p34^{cdc2} (lane 2). The p34^{cdc2} phosphorylation product was incubated with 400 units of lambda protein phosphatase (lane 3). These reactions were terminated by adding SDS sample buffer and proteins were separated by SDS–PAGE and detected by autoradiography.

hMAP126 was post-translationally modified. Full-length ORF of hMAP126 was cloned into *E. coli* His-tagged expression vector pQE30 and affinity purification of 6×His-hMAP126 was conducted using Ni-NTA agarose (Fig. 4A, lane 1). Cell extracts of HeLa cells released from nocodazole incubation was also prepared (Fig. 4A, lane 2). Western blot analysis showed that the mobility of hMAP126 from cell extracts of HeLa cells arrested at metaphase was apparently higher than from those of *E. coli*, suggesting that hMAP126 was post-translationally modified.

To ensure that the mobility shift of p126 was caused by phosphorylation, most likely by p34^{cdc2} kinase, *in vitro* phosphorylation was conducted. The 6× His-tagged hMAP126 was purified from *E. coli* as the substrate and incubated with p34^{cdc2} kinase purchased from New England Biolabs. hMAP126 was found to be phosphorylated by p34^{cdc2} kinase (Fig. 4B, lane 2). Phosphate incorporation was not observed when hMAP126 was incubated with [γ-^{32}P]ATP alone (Fig. 4B, lane 1), demonstrating that the phosphorylation is not due to a contaminating kinase in the preparation of affinity-purified His-tagged hMAP126. The lambda protein phosphatase treatment significantly lessened phosphate incorporation (Fig. 4B, lane 3), suggesting that hMAP126 was phosphorylated by p34^{cdc2} kinase. Furthermore, a negative control using 6× His-tagged ERP28 as the substrate did not reveal any phosphorylation of 6× His-tagged ERP28 by p34^{cdc2} kinase (data not shown).

DISCUSSION

In this investigation, we isolated a novel protein, hMAP126, and the mRNA expression pattern of hMAP126 suggested that it might have an alternative-splicing product in the kidney. A search of the human genome database revealed that *hMAP126* gene is located on chromosome 17q11-12. This is the region in

which has been suggested that HER-2/neu protooncogene and peroxisome proliferator-activated receptor binding protein (PBP/PPARBP) are amplified and over-expressed in 25% of ovarian and breast tumors (23, 24). This raises the possibility that hMAP126 is potentially amplified and over-expressed in certain types of carcinomas.

The kinesin superfamily comprises a large and structurally diverse group of microtubule-based motor proteins that produce force-generating activities within cells. Kinesin-related proteins contain a structurally similar domain, the motor domain, composed of a conserved globular catalytic core and a neck region. The eight kinesin classes fall into three major groups, which correspond to motors that have their catalytic cores positioned either at N-terminus (KIN N), C-terminus (KIN C) or internal (KIN I) to the polypeptide chain (25). KIN N and KIN C motor domains usually generate opposing forces in the spindle. KIN N kinesins form plus end-directed bipolar tetramers to generate outward forces. In contrast, KIN C kinesins is a family of minus end-directed kinesins to provide inward forces (2, 3). Structural alignment showed that hMAP126 shared a significant homology with the ubiquitous kinesin heavy chain (uKHC). Phylogenetic sequence analysis revealed that uKHC belongs to the KIN N subfamily, indicating that hMAP126 may provide an outward force that pushes the spindle pole apart, which is largely in agreement with our immunofluorescence observations.

A variety of mitotic spindle-associated proteins have been shown to be phosphorylated by $p34^{cdc2}$, which apparently regulates their association with mitotic spindles. In Cut7 from *S. pombe*, the mutation of threonine 1011 (the potential $p34^{cdc2}$ phosphorylation site) to an alanine remained ineffectual on spindle assembly (26). Conversely, mutation of the threonine 927 in HsEg5 eliminated spindle association (27). Mutation of threonine 937 in XlEg5 also disrupted mitotic spindle association (28). Dynein light intermediate chain was also phosphorylated by cdc2 kinase, and Ser197 phosphorylation was dramatically reduced in metaphase extracts depleted of cdc2 kinase (29). Our data showed that hMAP126 was phosphorylated by $p34^{cdc2}$ *in vitro*. It seems reasonable to hypothesize that the cell cycle-regulated localization of hMAP126 and its association with mitotic spindles may in part result from a cell cycle-dependent phosphorylation by $p34^{cdc2}$ kinase. Although it is likely that the dramatic distribution of hMAP126 on the mitotic spindles is not just the result of the single cdc2 phosphorylation event described in this study, and other post-translational modification mechanisms may affect its distribution, it is still tempting to propose that $p34^{cdc2}$ kinase may regulate the temporal and spatial functions of hMAP126.

An important issue regarding why the mitotic spindles contain so many motors leads to the assumption that similar mitotic motors have functional redundancy. However, recent live-cell quantitative analyses reveal subtle but significant differences when these motors exert their influence on spindle pole separation (30). Thus, these similar motors perform complementary but not entirely overlapping functions. Also, KIN N and KIN C kinesins function antagonistically, indicating that cells use multiple mitotic motors in parallel to generate a delicate balance of complementary and antagonistic forces. The precise role of hMAP126 in the regulation of mitotic spindles and its targeting to the spindles remain to be defined. Nonetheless, with the completion of human genome project, more candidate genes involved in dynamic regulation of mitotic spindles will be identified and further investigation may solve some of the remaining puzzles.

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REFERENCES

1. Heck, M. (1999) Dr. Dolittle and the making of the mitotic spindle. *Bioessays* **21,** 985–990.
2. Heald, R. (2000) Motor function in the mitotic spindle. *Cell* **102,** 399–402.
3. Sharp, D. J., Rogers, G. C., and Scholey, J. M. (2000) Microtubule motors in mitosis. *Nature* **407,** 41–47.
4. Winey, M., and O'Toole, E. T. (2001) The spindle cycle in budding yeast. *Nat. Cell Biol.* **3,** E23–E27.
5. Wittmann, T., Hyman, A., and Desai, A. (2001) The spindle: A dynamic assembly of microtubules and motors. *Nat. Cell Biol.* **3,** E28–E34.
6. Andersen, S. S., Buendia, B., Dominguez, J. E., Sawyer, A., and Karsenti, E. (1994) Effect on microtubule dynamics of XMAP230, a microtubule-associated protein present in Xenopus laevis eggs and dividing cells. *J. Cell Biol.* **27,** 1289–1299.
7. Andersen, S. S., and Karsenti, E. (1997) XMAP310, a Xenopus rescue-promoting factor localized to the mitotic spindle. *J. Cell Biol.* **139,** 975–983.
8. Charrasse, S. M., Schroeder, M., Gauthier-Rouviere, C., Ango, F., Cassimeris, L., Gard, D. L., and Larroque, C. (1998) The TOGp protein is a new human microtubule-associated protein homologous to the Xenopus XMAP215. *J. Cell Biol.* **111,** 1371–1383.
9. Belmont, L., and Mitchison, T. J. (1996) Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. *Cell* **84,** 623–631.
10. McNally, F. J., Okawa, K., Iwamatsu, A., and Valve, R. D. (1996) Katanin, the microtubule-severing ATPase, is concentrated at centrosomes. *J. Cell Sci.* **109,** 561–567.
11. Walczak, C., Michison, T. J., and Desai, A. B. (1996) XKCM1: A Xenopus kinesin-related protein that regulates microtubule dynamic during mitotic spindle assembly. *Cell* **84,** 37–47.
12. Gard, D. L., and Kirschner, M. W. (1987) A microtubule-associate protein from Xenopus eggs that specifically promotes assembly at the plus-end. *J. Cell Biol.* **105,** 2203–2215.
13. Lemos, C. L., Sampaio, P., Maiato, H., Costa, M., Omel'yanchuk,

H. V., Liberal, V., and Sunkel, C. E. (2000) Mast, a conserved microtubule-associated protein required for bipolar mitotic spindle organization. *EMBO J.* **19,** 3668–3682.

14. Inoue, Y. H., do Carmo Avides, M., Shiraki, M., Deak, P., Yamaguchi, M., Nishimoto, Y., Matsukage, A., and Glover, D. M. (2000) Orbit, a novel microtubule-associated protein essential for mitosis in *Drosophila melanogaster*. *J. Cell Biol.* **149,** 153–165.
15. Wittmann, T., Boleti, H., Antony, C., Karsenti, E., and Vernos, I. (1998) Localization of the Kinesin-like protein Xklp2 to spindle poles requires a leucine zipper, a microtubule-associated protein, and dynein. *J. Cell Biol.* **143,** 673–685.
16. Wittmann, T., Wilm, M., Karsenti, E., and Vernos, I. (2000) TPX2, a novel Xenopus MAP involved in spindle pole organization. *J. Cell Biol.* **149,** 1405–1418.
17. Heald, R., and Weis, K. (2000) Spindles get the Ran around. *Trends Cell Biol.* **10,** 1–4.
18. Gruss, O. J., Carazo-Salas, R. E., Schatz, C. A., Guarguaglini, G., Kast, J., Wilm, M., Le Bot, N., Vernos, I., Karsenti, E., and Mattaj, I. W. (2001) Ran induces spindle assembly by reversing the inhibitory effect of importin α on TPX2 activity. *Cell* **104,** 83–93.
19. Nachury, M. V., Maresca, T. J., Salmon, W. C., Waterman-Storer, C. M., Heald, R., and Weis, K. (2001) Importin β is a mitotic target of the small GTPase Ran in spindle assembly. *Cell* **104,** 95–106.
20. Chang, M. S., Chang, C. L., Huang, C. J., and Yang, Y. C. (2000) p29, a novel GCIP-interacting protein, localizes in the nucleus. *Biochem. Biophys. Res. Commun.* **279,** 732–737.
21. Moreno, S., and Nurse, P. (1990) Substrates for p34cdc2: In vivo veritas? *Cell* **61,** 549–551.
22. Robbins, J., Dilworth, S. M., Laskey, R. A., and Dingwall, C. (1991) Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: Identification of a class of bipartite nuclear targeting sequence. *Cell* **64,** 615–623.
23. Slamon, D. J., Godolphin, W., Jones, L. A., Holt, J. A., Wong, S. G., Keith, D. E., Levin, W. J., Stuart, S. G., Udove, J., Ullrich, A., and Press, M. F. (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* **244,** 707–712.
24. Zhu, Y., Qi, C., Jain, S., Le Beau, M. M., Espinosa, III, R., Atkin, G. B., Lazar, M. A., Yeldandi, A. V., Rao, M. S., and Reddy, J. K. (1999) Amplification and overexpression of peroxisome proliferator-activated receptor binding protein (PBP/PPARBP) gene in breast cancer. *Proc. Natl. Acad. Sci. USA* **96,** 10848–10853.
25. Vale, R. D., and Fletterick, R. J. (1997) The design plan of kinesin motors. *Annu. Rev. Cell Dev. Biol.* **13,** 745–777.
26. Drummond, D. R., and Hagan, I. M. (1998) Mutations in the bimC box of Cut7 indicate divergence of regulation within the bimC family of kinesin related proteins. *J. Cell Sci.* **111,** 853–865.
27. Blangy, A., Lane, H. A., d'Herin, P., Harper, M., Kress, M., and Nigg, E. A. (1995) Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo. *Cell* **83,** 1159–1169.
28. Sawin, K. E., Mitchison, T. J. (1995) Mutations in the kinesin-like protein Eg5 disrupting localization to the mitotic spindle. *Proc. Natl. Acad. Sci. USA* **92,** 4289–4293.
29. Dell, K. R., Turck, C. W., and Vale, R. D. (2000) Mitotic phosphorylation of the dynein light intermediate chain is mediated by cdc2 kinase. *Traffic* **1,** 38–44.
30. Straight, A. F., Sedat, J. W., and Murray, J. W. (1998) Time-lapse microscopy reveals unique roles for kinesins during anaphase in budding yeast. *J. Cell Biol.* **143,** 687–694.