γ-Tubulin is required for nucleation and polarized organization of microtubules in vivo. The mechanism of microtubule nucleation by γ-tubulin and the role of associated proteins is not understood. Here we show that in vitro translated monomeric γ-tubulin nucleates microtubules by lowering the size of the nucleus from seven to three tubulin subunits. In capping the minus end with high affinity (10¹⁰ M⁻¹) and a binding stoichiometry of one molecule of γ-tubulin/microtubule, γ-tubulin establishes the critical concentration of the plus end in the medium and prevents minus end growth. γ-Tubulin interacts strongly with β-tubulin. A structural model accounts for these results.

Microtubules are highly dynamic polymers of the cytoskeleton that play a crucial role in the organization of intracellular space and in cell division. In an interphase cell, the microtubule network is organized in a radial fashion from the microtubule organizing center, which in animal cells consists of a pair of centrioles surrounded by an electron-dense pericentriolar material. Microtubules are nucleated at the pericentriolar material by their minus end (1). The control of the polarity of the microtubule array is essential in cell function and is mediated by γ-tubulin. γ-Tubulin, a member of the tubulin superfamily first identified in Aspergillus nidulans (2), then found in all eukaryotes (3), is required for microtubule nucleation at the centrosome (4–9) and duplication of the centrioles (10), but the mechanism by which it establishes microtubule assembly is not understood. Although γ-tubulin is mainly localized in the pericentriolar material, it is also found in cytoplasmic complexes (11). A large γ-tubulin-ring complex (γ-TuRC)¹ was first identified in the Xenopus egg (12). It was also found in mammalian cells (see Refs. 13, 14, and 15 for a recent review) and at the centrosome in the Drosophila embryo (16), together with a smaller (280 kDa) complex (17). The small complex comprises two molecules of γ-tubulin and one molecule each of γ-tubulin complex proteins GCP2 and GCP3 (18, 19). Homologs of the Saccharomyces cerevisiae Spc97p and Spc98p, which are associated with γ-tubulin in the yeast cytoplasmic γ-tubulin complex and at the spindle pole bodies (20, 21). The γ-TuRC derives from the smaller complex by condensation and association with other proteins. The γ-TuRC nucleates microtubule assembly in vitro (12). Two models were proposed for microtubule nucleation by the γ-TuRC. In one model (12), the γ-TuRC acts as a template in which the 13 γ-tubulin subunits forming the ring interact end-on with the terminal α-tubulin subunits at the minus end of the nascent microtubule. In the other model (22), the ring opens and extends into a protofilament interacting laterally with αβ-tubulin units to seed a bidimensional microtubule lattice. The smaller γ-tubulin complex is a poorer nucleator in vitro (18); however, the fact that it has some nucleating activity has to be considered regarding the molecular mechanism of microtubule nucleation and calls for a detailed investigation of the interaction of γ-tubulin itself with microtubule ends. In vitro translated γ-tubulin is in part monomeric (23). The bulk in vitro translated γ-tubulin has been shown to bind microtubule minus ends with high affinity (10¹⁰ M⁻¹) and a stoichiometry of 13 ± 2 γ-tubulin/microtubule (24). In the present work, experiments were performed to analyze the thermodynamic and kinetic effects of γ-tubulin, isolated as a monomer, in microtubule assembly in vitro, with the ultimate goal to understand the specific roles of γ-tubulin and of the associated proteins in the nucleation of microtubules at the centrosome.

MATERIALS AND METHODS

In Vitro Translation and Semipurification of Monomeric 35S-labeled γ-Tubulin—35S-Radiolabeled human γ-tubulin was synthesized in vitro using the rabbit reticulocyte lysate system. A coupled translation-transcription system (TNT™ Coupled Reticulocyte Lysate Systems, Promega Corp., Madison, WI) was used, in which T7 polymerase of the recombinant pET plasmid containing the cDNA encoding human γ-tubulin was incubated in the presence of 50 μCi of [35S]methionine (PROMIX in vitro cell labeling, Amersham Pharmacia Biotech). The translation product was purified by phosphocellulose chromatography and gel filtration on Superose 6 HR (Amersham Pharmacia Biotech) in S buffer (80 mM MES-KOH, pH 6.9, 1 mM EGTA, 1 mM MgCl₂, 1 mM dithiothreitol, 1.4 mM glycerol). The 35S elution profile showed a minor 900-kDa peak (attributed to complexes of γ-tubulin with cytoplasmic chaperonin), followed by a major narrow peak corresponding to the overexpressed monomeric 50-kDa γ-tubulin (23). Fractions containing monomeric γ-tubulin were equilibrated in P buffer (60 mM MES-KOH, pH 6.9, 0.75 mM EGTA, 0.75 mM MgCl₂, 1 mM dithiothreitol, 1.4 mM glycerol, 1 mM GTP), rapidly frozen on liquid nitrogen, and stored at −80 °C. The concentration of monomeric γ-tubulin was determined by quantitative immunoblotting using a polyclonal antibody against γ-tubulin raised in the laboratory and the ECL chemiluminescent detection (Amersham Pharmacia Biotech) and comparison with standards of bacterially expressed γ-tubulin. Routinely, 10 ± 3 pmol of radiochemically pure γ-tubulin were obtained (Fig. 1). A control sample was prepared in exactly the same way except for the absence of cDNA encoding γ-tubulin in the reticulocyte lysate.

Immunodepletion of γ-tubulin from the semipurified preparation was performed as follows. In vitro translated 35S-labeled monomeric γ-tubulin eluted from the Superose 6 HR column (0.22 pmol) was incubated in P buffer at 4 °C for 90 min with 7.5 μg affinity-purified anti-γ-tubulin antibody in phosphate-buffered saline buffer (depleted sample) or with phosphate-buffered saline buffer alone (mock depleted control).

Polymerization Measurements—Tubulin purified from pig brain by three polymerization cycles followed by phosphocellulose chromatography...
phy (see Ref. 25 for all standard procedures in tubulin handling) was used in all experiments. Polymerization assays were carried out in PMg buffer (P buffer containing 6 mM MgCl₂) as described (25). Polymerization was monitored turbidimetrically at 350 nm, 37 °C, with a 0.4-cm path through a spectrophotometer (Cary 1, Varian spectrophotometer). Experiments were performed in a range of concentrations where it was checked that the turbidity change was linearly correlated with the amount of assembled microtubules. The lag time τ was defined as the time at which the linear semilogarithmic plot of the time course of turbidity change intercepts the turbidity line at zero time. Microtubule elongation assays were performed using preassembled microtubule seed solution (30 μM tubulin) that was 10-fold diluted into the cuvette containing prewarmed tubulin at a series of concentrations in PMg buffer. All pipettings were done gently to avoid microtubule breakage and using truncated warm pipettes tips. Initial rates of elongation (26) were derived from the turbidity recordings using the ChemStation software (Hewlett-Packard).

Electron Microscopy—Histograms of microtubule length distributions were derived from electron micrographs of negatively stained samples of microtubules assembled from tubulin (5 μM) in the absence or presence of 0.7 mM γ-tubulin. Observations were made in a Philips EM 410 microscope at a 12,470-fold magnification. Analysis was performed using Optimas software.

Sedimentation Assays—Microtubules containing or not 35S-labeled γ-tubulin were sedimented at 200,000 × g at 37 °C for 10 min in a TL 100 Topes Beckman ultracentrifuge. The amount of tubulin in the supernatant was determined by SDS-polyacrylamide gel electrophoresis. The Coomassie Blue-stained bands were scanned (Arcus, NIH Image software) and compared with standards that were co-electrophoresed on the same gel. The amounts of γ-tubulin free and bound to microtubules were determined also by SDS-polyacrylamide gel electrophoresis of the pellets and supernatants, followed by autoradiography (PhosphorImager, Molecular Dynamic) with comparison with standards of 35S-labeled γ-tubulin.

GTP Hydrolysis Measurements—The rate of hydrolysis of GTP bound to tubulin during the nucleation of microtubules was measured as described (25). The [γ-32P]GTP-tubulin 1:1 complex was separated from free GTP by Sephadex G-25 gel filtration (PD10, Amersham Pharmacia Biotech) and incubated at 37 °C in PMg buffer, at a concentration of 6 μM in the presence or absence of γ-tubulin. Aliquots were removed at time intervals during the nucleation period before microtubules were assembled, acid quenched, and processed for PI extraction. Turbidity measurements were made to verify that no microtubule assembly occurred during the measurements.

Blot Overlay Assay with 35S-Labeled γ-Tubulin—SDS-polyacrylamide gel electrophoresis of pure tubulin was done at pH 9.5 under conditions (27) suitable for maximum separation of α- and β-subunits. For brain tubulin, it has been established that β is the faster migrating subunit (28, 29). Following transfer on nitrocellulose and visualization of the α- and β-subunits using Ponceau Red, the membrane was submitted to Western immunoblotting (ECL, Amersham Pharmacia Biotech) using the DM1A anti-α-tubulin monoclonal antibody (ICN) at a 1:4000 dilution. α-Tubulin was verified to be the slower migrating polypeptide. The nitrocellulose sheet was then dehybridized following the ECL protocol provided by Amersham Pharmacia Biotech and washed extensively in buffer S6 containing 30 mg/ml bovine serum albumin. The sheet was incubated for 2 h at room temperature in the presence of gel-filtered 35S-radiolabeled γ-tubulin (0.6 nM, 25 Ci/mmol), washed extensively five times, dried, and autoradiographed using Hyperfilm βmax (Amersham Pharmacia Biotech).

RESULTS

Monomeric γ-Tubulin Facilitates Nucleation of Microtubules in Vitro—In vivo translated 35S-labeled γ-tubulin was translated in vitro using the reticulocyte lysate system, partially purified by phosphocellulose chromatography and isolated as a 50-kDa polypeptide by Superose 6 chromatography (see “Materials and Methods” and Fig. 1). The effect of monomeric γ-tubulin on the time course of spontaneous polymerization of pure tubulin was analyzed. γ-Tubulin caused a dose-dependent decrease in the nucleation lag time that precedes microtubule growth (Fig. 2A). Typically, at 12 μM tubulin, the lag time decreased from 12 to 5 min in the presence of 0.6 nM γ-tubulin. The decrease in lag time showed a saturation behavior, and the lag reached a low limit value upon increasing the concentration of γ-tubulin. No effect was observed with a material purified through the same steps from reticulocyte lysates that did not express γ-tubulin. That control testified that putative 50-kDa polypeptides coming from the reticulocyte lysate and contaminating the monomeric γ-tubulin fraction were not responsible for the observed nucleating effect. Immunodepletion of γ-tubulin from the preparation using IgG-coated Dynabeads coupled to anti-γ-tubulin antibody abolished the effect (Fig. 2B). Because γ-tubulin was isolated as a 50-kDa protein, it is not possible to imagine that the immunodepletion removed a protein associated with γ-tubulin that could be responsible for the observed effect. In conclusion, monomeric γ-tubulin facilitates microtubule nucleation in vitro.

Short linear oligomers of tubulin are formed in the premucleation stages of tubulin polymerization. The nucleus is the linear oligomer that has reached equal probabilities of lateral or longitudinal interaction with an αβ-tubulin dimer (30–33). The size of the nucleus is equal to 2m – 1, where m is the value of the slope of the log/log plot of the lag time versus the concentration of αβ-tubulin (34). The value of m was lower in the presence of γ-tubulin (Fig. 2C). The size of the nucleus decreased from 7 to 3 tubulin subunits at a saturating concentration of γ-tubulin. Accordingly, the apparent rate constant for microtubule growth increased with γ-tubulin, consistent with a higher number of microtubules. Histograms of length distribution of microtubules (Fig. 2D) showed average lengths of 5.0 and 3.7 μM for microtubules assembled in the absence or presence of 0.6 nM γ-tubulin, respectively.

Monomeric γ-Tubulin Lowers the Critical Concentration for Microtubule Assembly—The effect of γ-tubulin on the critical concentration for microtubule assembly was measured turbidimetrically and in sedimentation assays (Fig. 3A). The critical concentration decreased in a saturation fashion with γ-tubulin from 2.5 μM to a lower limit of 1.3 μM, reached at 0.8 nM γ-tubulin (Fig. 3A, inset). The binding of γ-tubulin to sedi-
γ-Tubulin Nucleates Microtubules

**Monomeric γ-Tubulin Caps Microtubule Minus Ends**—Further evidence for capping of the minus end of microtubules by γ-tubulin was derived from measurements of the rate of microtubule growth at different concentrations of dimeric tubulin (Fig. 4). This kinetic assay allows the determination of the association rate constant of tubulin to microtubule ends, derived from the slope of the J(c) plot, and of the critical concentration, derived from the intercept on the concentration axis (26). In the absence of γ-tubulin, the plot reflects the contribution of both ends to microtubule growth. A value of $7.5 \mu M^{-1} s^{-1}$ was found for the sum of the association rate constants at the plus and minus ends. The critical concentration was 1.5 μM, which is intermediate between the critical concentrations at the plus and minus ends. In the presence of 0.8 nm γ-tubulin, the J(c) plot displayed a lower slope, consistent with a value of the association rate constant of $4.4 \mu M^{-1} s^{-1}$, and extrapolated to a lower value, 0.8 μM, of the critical concentration. This behavior is consistent with growth of microtubules from the plus end only, in the presence of γ-tubulin. The value of the...
ever the strong binding of g-tubulin and used as seeds of assembly by diluting them in a pre-warmed solution of tubulin at the indicated concentrations with (a) or without (c) 0.8 nM g-tubulin, respectively. The initial rate of turbidity increase was measured. Open circles refer to the rate of growth at the two ends measured in the absence of g-tubulin; closed triangles refer to rates of growth at the plus end (+), measured in the presence of g-tubulin. The rates were normalized to the same concentration (0.4 nM) of microtubules by measuring the length distribution in each microtubule seed solution. The thin line represents the Jo(c) plot at the minus end (−) obtained by subtracting the rates measured in the absence or presence of g-tubulin.

association rate constant of tubulin to the minus end (2.7 μM−1 s−1) and the critical concentration for assembly at the minus end (2.3 μM) were derived by subtraction of the two plots. According to these results, the rate constant for association of tubulin to microtubules is 1.6-fold lower at the minus end than at the plus end, in good agreement with previous electron microscopy measurements (35, 38, 39). In conclusion, the present kinetic measurements are in good agreement with previous studies in which g-tubulin was localized at the minus end of microtubules (24) and show evidence for the function of g-tubulin as a minus end capper.

Monomeric γ-Tubulin Inhibits GTP Hydrolysis Linked to Destabilization of Prenuclei Oligomers—Nucleation of microtubules is accompanied by GTP hydrolysis, which destabilizes the nuclei by preventing lateral interaction of prenuclei oligomers with tubulin (25). Hydrolysis of GTP is thought to be linked to the curling of linear oligomers into rings, which is the favored conformation when GDP is bound to tubulin (40). In the absence of 0.7 nM γ-tubulin the hydrolysis of GTP during the nucleation phase was decreased by 2-fold, whereas more nuclei were formed (data not shown). This result suggests that γ-tubulin enhances nucleation by inhibiting the hydrolysis of GTP on oligomers, thus preventing their subsequent destabilization.

γ-Tubulin Interacts with β-Tubulin in Blot Overlay Assays—To determine how γ-tubulin interacts with tubulin at the minus end of microtubules, a blot overlay assay was carried out (Fig. 5) using in vitro translated monomeric 35S-radiolabeled γ-tubulin and nitrocellulose-transferred αβ-tubulin electrophoresed under conditions that maximize the separation of α and β subunits (27–29). α-Tubulin was identified as the slower migrating subunit. γ-Tubulin bound the faster migrating β-tubulin exclusively. No binding was observed using thermodenatured 35S-labeled γ-tubulin. This result provides biochemical evidence for the strong interaction between β- and γ-tubulin that was expected from genetic studies (2). The demonstration of a strong interaction of γ-tubulin with β-tubulin does not rule out its interaction with α-tubulin, which is also expected given the fact that α-tubulin is exposed at the minus end (47). However the strong binding of γ-tubulin to β-tubulin imposes con-straints on the possible structural models for minus end capping.

FIG. 4. Effect of γ-tubulin on the rate of microtubule growth. Microtubules were assembled in the presence or absence of 0.8 nM γ-tubulin and used as seeds of assembly by diluting them in a pre-warmed solution of tubulin at the indicated concentrations with (a) or without (c) 0.8 nM γ-tubulin, respectively. The initial rate of turbidity increase was measured. Open circles refer to the rate of growth at the two ends measured in the absence of γ-tubulin; closed triangles refer to rates of growth at the plus end (+), measured in the presence of γ-tubulin. The rates were normalized to the same concentration (0.4 nM) of microtubules by measuring the length distribution in each microtubule seed solution. The thin line represents the Jo(c) plot at the minus end (−) obtained by subtracting the rates measured in the absence or presence of γ-tubulin.

Monomeric γ-tubulin, in the absence of associated proteins, is able to nucleate microtubules in vitro and to cap microtubule minus end. Although the γ-tubulin used here is only partially purified from reticulocyte lysates, control experiments unambiguously establish that the nucleation activity is because of monomeric γ-tubulin. This result is in partial agreement with Vassilev et al. (41) and with Li and Joshi (24) who measured the binding of γ-tubulin to microtubules. However a binding stoichiometry of 12–15 (13 on average) γ-tubulin/microtubule end was determined by Li and Joshi (24), whereas here we find that a single molecule of monomeric γ-tubulin is sufficient to nucleate and cap the minus end. The partial discrepancy between the two estimates may be because of the difference in the material and in the methods used to quantitate the number of microtubules. The in vitro translated γ-tubulin used by Li and Joshi (24) was not isolated as monomeric γ-tubulin by a sizing column. We relied on length distribution measurements of negatively stained microtubules observed in the electron microscope, whereas Li and Joshi (24) used fluorescence optical microscopy of rhodamine-labeled microtubules and found a saturating amount of 130 pm γ-tubulin bound/microtubule, in a solution that contained 0.2 μM polymerized tubulin and 20 μM Taxol, which should correspond to 10 pm microtubules assuming a stoichiometry of 13 γ-tubulin/microtubule. The authors measured that the number of microtubules was increased 1.5-fold by 8–10 passages through a 26-gauge needle. Different results found in the literature about the average length of sheared microtubules using the same technique (23, 42–45) provide numbers in the range of 1–2 μm, the lower range corresponding to Taxol-stabilized microtubules that do not rapidly redistribute in length. If the microtubules in Li and Joshi’s (24) experiment were 1.5-fold longer before shearing, they must have been 1.5–3 μm long, which corresponds to a concentration of 80–40 pm. This microtubule concentration would in turn correspond to a stoichiometry of 1.6–3.2 γ-tubulin bound/microtubule. On the other hand, the estimate coming from measurements of the number of microtubules in the fluorescence microscope corresponds to an average length of 200,000/ (1650 × 10) = 12.1 μm before shearing, and 12.1/1.5 = 8 μm after shearing, a value much too high to be compatible with the shearing assay. We conclude that there may have been some artifacts in the fluorescence assay, like the lack of resolution of small microtubules or the lack of appreciation of aggregated
microtubules, that may have led to an underestimated value of the concentration of microtubule ends, hence an overestimated value of the stoichiometry of bound γ-tubulin/microtubule end.

In most cell extracts γ-tubulin is found in small and large γ-tubulin complexes, which have nucleating properties. The fact that the intrinsic properties of monomeric γ-tubulin enable it to act as a nucleator and to block minus end growth with high affinity and at a molar ratio of only one molecule of γ-tubulin/microtubule suggests that γ-tubulin in those complexes must interact in the same fashion with the minus end of microtubules. A simple model accounting for our results is presented in Fig. 6. This model incorporates the present findings in classical models proposed for microtubule nucleation from pure tubulin (30, 31). In those models, nucleation of microtubules is driven by the lateral association of αβ-tubulin with linear oligomers of tubulin in which the subunits interact longitudinally. The lateral association generates a bidimensional microtubule lattice. The longitudinal and lateral growths of the microtubular sheet are determined by the free energy of longitudinal and lateral interactions. In line with this concept and with the model proposed by Erickson and Stoffler (22), we propose that γ-tubulin facilitates nucleation by binding laterally to β-tubulin with a much higher affinity than αβ-tubulin itself. As a result, γ-tubulin initiates the growth of a bidimensional microtubule wall from a linear oligomer of αβ-tubulin that contains a lower number of subunits than the one which an αβ-tubulin molecule would have a chance to interact with. This feature accounts for the decrease in the nucleation lag time, for the increase in microtubule number and corresponding decrease in average length, for the lower cooperativity in the kinetics of polymerization in the presence of γ-tubulin, and for the lower GTP hydrolysis during the nucleation phase. The model of lateral association of γ-tubulin to polymerized β-tubulin is in good agreement with the conclusions derived from the identification of the peptides of γ-tubulin interacting with αβ-tubulin, which indicate that γ-tubulin and αβ-tubulin associate through lateral interactions (46). A single γ-tubulin appears able to cap the microtubule minus end, preventing growth from that end. To account for this result, the model proposed here stipulates that whereas the plus end-directed face of γ-tubulin is able to interact longitudinally with the minus end of an αβ-tubulin subunit along a protofilament, it cannot interact with tubulin at its other end. Further, the strong capping suggests that no lateral association of αβ-tubulin can take place with the lateral face of γ-tubulin opposite the one that interacts strongly with a β-tubulin, making γ-tubulin a perfect plug.

The high affinity of γ-tubulin for microtubule ends and the absence of measurable interaction in solution between dimeric αβ-tubulin and γ-tubulin is very puzzling. Such a large difference in affinity suggests that the regions of αβ-tubulin that are recognized by γ-tubulin are hidden in dimeric tubulin and exposed upon tubulin assembly. It is also possible that the strong binding of γ-tubulin to the minus end involves its interaction with two tubulin molecules, a lateral interaction with β-tubulin and a longitudinal interaction with α-tubulin, a situation that can occur with the appropriate geometry at the minus end only.

How do the present results relate to the nucleation of microtubules in vivo by small and large complexes? It is highly likely that one of the γ-tubulin subunits in the complexes interacts with the minus end in the same way as monomeric γ-tubulin. The role of the associated proteins GCP2 and GCP3 may be to enhance the nucleating/capping properties by stabilizing the bonds between the γ-tubulin subunits in the oligomer. Within the template open ring model, the γ-tubulin at one end of the ring would interact laterally with β-tubulin, whereas association with the GCPs would enhance the longitudinal interaction between γ-tubulin and the α-subunits at the minus end. Within the protofilament oligomer model, the longitudinal interactions between γ-tubulin subunits maintained by GCP2 and GCP3 would tighten the lateral association of the γ-tubulin oligomer to the protofilament. In conclusion, the present work may help understand the structural and thermodynamic implications of the function of γ-tubulin complexes in vivo.

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REFERENCES
1. Kellogg, D. R., Moritz, M., and Alberts, B. M. (1994) Annu. Rev. Biochem. 63, 639–674
2. Oakley, C. R., and Oakley, B. R. (1999) Nature 338, 662–664
3. Joshi, H. C. (1994) Curr. Opin. Cell Biol. 6, 54–62
4. Oakley, B. R., Oakley, C. E., Yoon, Y., and Jung, M. K. (1990) Cell 61, 1289–1301
5. Horio, T., Uzawa, S., Jung, M. K., Oakley, B. R., Tanaka, K., and Yanagida, M. (1991) J. Cell Sci. 99, 693–705
6. Joshi, H. C., Palacios, M. J., McNamara, L., and Cleveland, D. W. (1992) Nature 356, 80–85
7. Felix, M. A., Antony, C., Wright, M., and Maro, B. (1994) J. Cell Biol. 124, 19–31
8. Sohle, S. G., and Snyder, M. (1995) J. Cell Biol. 131, 1775–1788
9. Sunkel, C. E., Gomes, R., Sampiano, P., Perdigao, J., and Gonzalez, C. (1995) EMBO J. 14, 28–36
10. Ruiz, P., Beisson, J., Rossier, J., and Dupuis-Williams, P. (1999) Curr. Biol. 9, 48–46
11. Stearns, T., and Kirschner, M. W. (1994) Cell 76, 623–638
12. Zheng, Y., Wong, M. L., Alberts, B. M., and Mitchison, T. J. (1995) Nature 378, 578–583
13. Meads, T., and Schroer, T. A. (1995) Cell Motil. Cytoskeleton 32, 273–288
14. Detraves, C., Mazarguil, H., Lajoie-Mazenc, I., Julian, M., Raynaud-Messina, B., and Wright, M. (1995) Cell Motil. Cytoskeleton 36, 179–189
15. Jeng, R., and Stearns, T. (1999) Trends Cell Biol. 9, 339–342
16. Moritz, M., Braunfeld, M. B., Sedat, J. W., Alberts, B. M., and Agard, D. A. (1995) Nature 378, 638–640
17. Moritz, M., Zheng, Y., Alberts, B. M., and Oegema, K. (1996) J. Cell Biol. 142, 1–12
18. Oegema, K., Wiese, C., Martin, O. C., Milligan, R. A., Iwamatsu, A., Mitchison, T. J., and Zheng, Y. (1999) J. Cell Biol. 144, 721–733
19. Murphy, S. M., Urbani, L., and Stearns, T. (1998) J. Cell Biol. 141, 663–674
20. Knop, M., Pereira, G., Giessler, S., Grein, K., and Schiebel, E. (1997) EMBO J. 16, 1550–1564
21. Knop, M., and Schiebel, E. (1997) EMBO J. 16, 6985–6995
22. Erickson, H. P., and Stoffler, D. (1996) J. Cell Biol. 135, 5–8
23. Melki, R., Vainberg, I., Chow, R., and Cowan, N. (1999) J. Cell Biol. 122, 1301–1310
24. Li, Q., and Joshi, H. C. (1995) *J. Cell Biol.* **131**, 207–214
25. Carlier, M.-F., Didry, D., and Pantaloni, D. (1997) *Biophys. J.* **73**, 418–427
26. Carlier, M.-F., Hill, T. L., and Chen, Y. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 771–775
27. Best, D., Warr, P. J., and Gull, K. (1981) *Anat. Biochem.* **114**, 281–284
28. Bryan, J., and Wilson, L. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68**, 1762–1766
29. Clayton, L., Quinlan, R. A., Roobol, A., Pogson, C. I., and Gull, K. (1980) *FEBS Lett.* **115**, 301–305
30. Erickson, H. P., and Pantaloni, D. (1981) *Biophys. J.* **34**, 295–308
31. Voter, W. A., and Erickson, H. P. (1984) *J. Biol. Chem.* **259**, 10430–10438
32. Kuchnir-Fygenson, D., Flyvbjerg, H., Sneppen, K., Libchaber, A., and Leibler, S. (1995) *Phys. Rev. Lett.* **51**, 5058–5063
33. Flyvbjerg, H., Jobs, E., and Leibler, S. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 5975–5979
34. Oosawa, F., and Asakura, S. (1975) *Thermodynamics of the Polymerization of Protein*, pp. 41–55, Academic Press, New York
35. Bergen, L. G., and Borisy, G. G. (1980) *J. Cell Biol.* **84**, 141–150
36. Margolis, R. L., and Wilson, L. (1978) *Cell* **13**, 1–8
37. Rodionov, V. I., and Borisy, G. G. (1998) *Science* **275**, 215–218
38. Mitchison, T. J., and Kirschner, M. W. (1984) *Nature* **312**, 232–237
39. Horio, T., and Hotani, H. (1986) *Nature* **321**, 605–607
40. Howard, W. D., and Timasheff, S. N. (1986) *Biochemistry* **25**, 8292–8300
41. Vassilev, A., Kimble, M., Silflow, C. D., LaVoie, M., and Kurumaya, R. (1995) *J. Cell Biol.* **106**, 1083–1092
42. Farrell, K. W., Jordan, M. A., Miller, H. P., and Wilson, L. (1987) *J. Cell Biol.* **104**, 1035–1046
43. Keates, R. A., and Hallett, F. R. (1988) *Science* **241**, 1642–1645
44. Williams, R. C., Jr., and Bone, L. A. (1989) *J. Biol. Chem.* **264**, 1663–1670
45. Yamauchi, P. S., Flynn, G. C., Marsh, R. L., and Purich, D. L. (1993) *J. Neurochem.* **60**, 817–826
46. Llanos, R., Chevrier, V., Ronjat, M., Meurer-Grob, P., Martinez, P., Frank, R., Bornens, M., Wade, R. H., Wehland, J., and Job, D. (1999) *Biochemistry* **38**, 15712–15720
47. Mitchison, T. J. (1993) *Science* **261**, 1044–1047