Stu2, the Budding Yeast XMAP215/Dis1 Homolog, Promotes Assembly of Yeast Microtubules by Increasing Growth Rate and Decreasing Catastrophe Frequency*

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**Background:** The reported inhibition of microtubule growth by Stu2 is difficult to reconcile with its cellular phenotypes.

**Results:** Using microscopy assays, we found that Stu2 increases the growth rate and decreases the catastrophe frequency of yeast microtubules.

**Conclusion:** Stu2 promotes microtubule growth, with considerably higher activity on budding yeast microtubules.

**Significance:** The biochemical properties of Stu2 reported here account for the mitotic phenotypes observed in cells.

Stu2 is the budding yeast member of the XMAP215/Dis1 family of microtubule polymerases. It is essential in cell division, allowing proper spindle orientation and metaphase chromosome alignment, as well as spindle elongation during anaphase. Despite Stu2 having a phenotype that suggests it promotes microtubule growth, like the other members of the XMAP215/Dis1 family, previous studies with purified Stu2 indicate only that it antagonizes microtubule growth. One potential explanation for these contradictory findings is that the assays were performed with mammalian brain tubulin, which may not be the right substrate to test the activity of Stu2 given that yeast and brain tubulins are quite divergent in sequence and that the vertebrate tubulins are subject to many post-translational modifications. To test this possibility, we reconstituted the activity of Stu2 with purified budding yeast tubulin. We found that Stu2 accelerated microtubule growth in yeast tubulin by severalfold, similar to the acceleration reported for XMAP215 in porcine brain tubulin. Furthermore, Stu2 accelerated polymerization in yeast tubulin to a much greater extent than in porcine brain tubulin, and the concentration of Stu2 required to reach 50% maximum activity in yeast tubulin was nearly 2 orders of magnitude lower than that in porcine brain tubulin. We conclude that Stu2 is a microtubule polymerase, like its relatives, and that its activity is considerably higher in yeast tubulin compared with mammalian brain tubulin. The biochemical properties of Stu2 reported here account for many of the phenotypes of Stu2 observed in cells.

Microtubules, cytoskeletal filaments composed of α,β-tubulin heterodimers, serve as tracks for molecular motors and provide structure to organelles such as mitotic spindles and axonemes (1, 2). The growth and shrinkage of microtubules are regulated by microtubule-associated proteins (MAPs)2 that can increase or decrease the rate of polymerization or depolymerization or that can regulate the switching between growth and shrinkage (3–5). This study addresses the protein Stu2, a member of the XMAP215/Dis1 family of microtubule polymerases, which, unexpectedly and unlike other members of the family, has been reported to inhibit, rather than accelerate, polymerization.

Members of the XMAP215/Dis1 family promote microtubule growth both in cells and in biochemical assays with purified proteins (6). The founding member of this family, XMAP215, is required for rapid microtubule growth in Xenopus laevis egg extracts and accelerates elongation of the fast-growing plus ends of microtubules by up to 8-fold in the presence of purified bovine brain tubulin (7). Tea1, the Aspergillus nidulans homolog of XMAP215, accelerates the growth of porcine brain microtubules to a similar extent as XMAP215 (8). Stu2, the budding yeast homolog of this family, is required for elongation of the mitotic spindle during anaphase (9), as well as for spindle orientation and metaphase chromosome alignment (10). Mutants lacking the TOG1 domain of Stu2 exhibit shorter cytoplasmic microtubules and shorter spindles (11), consistent with Stu2 promoting microtubule growth. However, studies with purified Stu2 have shown that it slows, rather than accelerates, elongation of microtubules grown in brain tubulin (12). Furthermore, these authors reported that Stu2 also promotes catastrophe, the transition of growing microtubules to shrinking ones, which often correlates with slower growth rates. One interpretation of these results is that not all members of the XMAP215/Dis1 family are polymerases and that the shared protein domains, notably the TOG domains (13, 14), may have divergent activities.

An alternative explanation for the difference in Stu2 and XMAP215 activities is that mammalian brain tubulin may not be the right substrate to test the activity of Stu2 given that yeast...
and brain tubulins are quite divergent in sequence and that the vertebrate tubulins are subject to many post-translational modifications (see “Discussion”). To test this possibility, we reconstituted the polymerization activity of Stu2 with budding yeast tubulin.

**EXPERIMENTAL PROCEDURES**

**Protein Expression, Purification, and Labeling**—We used two Stu2 constructs: the N-terminally His$_6$-tagged full-length protein (Stu2) and the same protein with C-terminal spacer (AAEFM) followed by enhanced GFP (Stu2-GFP). SF cells were transfected with the baculovirus encoding Stu2 or Stu2-GFP and harvested by centrifugation at 1300 rpm for 15 min. The pellets were dissolved in cold lysis buffer (50 mM HEPES (pH 7.5), 150 mM NaCl, 5% glycerol, 0.1% Tween 20, 1.5 mM MgCl$_2$, 1 mM EGTA, 10% glycerol, 0.2% Triton X-100, 100 mM KCl, and 0.5 mM MgATP, and protease inhibitors) and then used to measure the parameters of microtubule dynamics.

**RESULTS**

**Stu2 Is a Weak Polymerase on Porcine Brain Tubulin**—To investigate the activity of Stu2, we first performed experiments using porcine brain tubulin. Stable microtubules were grown from porcine brain tubulin, 25% of which was labeled with rhodamine, in the presence of GMP-CPP, a slowly hydrolyzable GTP analog (21). These stable microtubules were bound to the surface of a coverslip using anti-rhodamine antibodies and imaged by total internal reflection fluorescence microscopy (Fig. 1A). To investigate the effect of Stu2 on microtubule growth, 7 µM 10% Alexa Fluor 488-labeled porcine brain tubulin and various concentrations of unlabeled Stu2 were added. Microtubule growth and the transition between the growing and shrinking phases were quantified from the kymographs (Fig. 1B). Increasing the concentration of Stu2 increased the microtubule growth rate at the plus end by 2.4-fold from 0.448 ± 0.010 µm/min (mean ± S.E., n = 91) in control experiments with tubulin to only 1.071 ± 0.034 µm/min (mean ± S.E., n = 121) with 500 nM Stu2 (Fig. 1C, left panel). Approximately 200 nM Stu2 was required to reach 50% of this increase in growth rate. A similar acceleration of growth was observed at the minus end: Stu2 increased the growth rate from 0.007 ± 0.007 µm/min (mean ± S.E., n = 22) with tubulin alone to 0.342 ± 0.011 µm/min with 500 nM Stu2 (mean ± S.E., n = 79). Thus, Stu2 is a microtubule polymerase, like its Xenopus relative XMAP215 and its *Aspergillus* relative Tea1, although the acceleration of growth by Stu2 is only ~2-fold less than the 8-fold increase observed for the other proteins.

Stu2 had a small effect on catastrophe (Fig. 1C, right panel). In the absence of Stu2, the catastrophe rate at the plus end was 0.119 ± 0.013 min$^{-1}$ (mean ± S.E., n = 79). This rate increased slightly but significantly at intermediate Stu2 concentrations.
**Stu2 Is a Microtubule Polymerase**

We used a GFP-tagged construct to directly observe Stu2 interactions with microtubules. Stu2-GFP has a weak polymerase activity with porcine brain tubulin. The addition of 100 nM Stu2-GFP caused a modest increase in microtubule growth rate in 10 μM tubulin from 0.386 ± 0.050 μm/min (mean ± S.D., n = 9) to 0.485 ± 0.081 μm/min (mean ± S.D., n = 20). This rate increase was similar to that induced by the same concentration of unlabeled Stu2 (Fig. 1C). During dynamic assays in the presence of unlabeled tubulin, Stu2-GFP bound to the plus end of growing microtubules with higher affinity than the seed or the extension (Fig. 2A). At low concentrations of Stu2 (<1 nM), single molecules of Stu2-GFP were observed to bind to and diffuse on the microtubule surface; other Stu2 molecules were observed to bind stably near both microtubule ends (Fig. 2B). At lower salt concentrations, Stu2-GFP bound much more strongly to the microtubule lattice (Fig. 2C). In BRB80 without extra KCl, GMP-CPP-stabilized microtubules were decorated with Stu2-GFP along their whole length; furthermore, under these conditions, Stu2-GFP accelerated the depolymerization of these stable microtubules by 10-fold from the spontaneous rate of 0.0046 ± 0.0023 μm/min (mean ± S.D., n = 18) to 0.058 ± 0.014 μm/min (mean ± S.D., n = 35; p < 0.0001 by a t test).

At 7 μM porcine brain tubulin, no rescues were observed; in the presence of 500 nM Stu2, the rescue rate increased to 0.54 ± 0.09 min⁻¹ (mean ± S.E., n = 36 rescues). However, rescues were still relatively infrequent. At 500 nM Stu2, only 46% of the...

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**FIGURE 1. Stu2 is a weak polymerase on porcine brain tubulin.** A, schematic of the experimental design. Alexa Fluor 488-labeled porcine brain tubulin (green) in solution polymerizes onto the ends of rhodamine-labeled GMP-CPP-stabilized microtubules (red) bound to the coverslip with anti-rhodamine antibodies (blue). The surface of the flow chamber is imaged using total internal reflection (TIR) fluorescence microscopy, which minimizes background from the fluorescent tubulin in solution. B, kymographs in the absence (left) and presence (right) of Stu2 (300 nM) show that Stu2 accelerates growth at both ends (7 μM tubulin, 35 °C, BRB80 + 50 mM KCl). C, dependence of rates of microtubule growth (left) and catastrophe (right) on Stu2 concentration. ●, plus end; ○, minus end. Data are means ± S.D. of 33–121 microtubules for the growth rate and means ± S.E. for the catastrophe rate, where the S.E./mean was set equal to the reciprocal of the square root of the number of catastrophes (between 33 and 78 for the various concentrations).

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**FIGURE 2. Interaction of Stu2-GFP with porcine brain microtubules.** A, Stu2-GFP preferentially binds to the plus end of a growing microtubule (1.5 nM Stu2-GFP, 10 μM unlabeled tubulin, 35 °C, BRB80); B, Stu2-GFP (73 pm) can bind to the lattice and diffuse (arrow), or it can stably bind near one or both ends (arrowhead) (0 μM added tubulin, 35 °C, BRB80 + 110 mM KCl); C, Stu2-GFP depolymerizes GMP-CPP-stabilized microtubules in the absence of free tubulin in solution. In the absence of Stu2-GFP, the spontaneous depolymerization was not detectable over 30 min (left panel); in the presence of GFP-Stu2, the microtubules depolymerized (right panel) (58 pm Stu2-GFP, 0 μM added tubulin, 35 °C, BRB80).
shrinking plus ends rescued; this is expected to increase the microtubule length at catastrophe by a factor of \(<2\).

**Stu2 Is a Strong Polymerase on Budding Yeast Tubulin**—After characterizing the interaction of Stu2 with porcine brain tubulin, we studied its interaction with the conspecific budding yeast tubulin purified from strain BY11011 (see “Experimental Procedures”). Because the yield was low (18), we had insufficient protein for fluorescent labeling. We therefore used DIC microscopy to visualize the microtubules extending from budding yeast microtubules (DIC microscopy, 5 μm (125 nM Stu2), mean ± S.E., n = 28) to 11.5 ± 3.3 μm (125 nM budding Stu2, mean ± S.E., n = 12). Thus, Stu2 promotes the growth of yeast microtubules, increasing mean length by 10-fold through its combination of polymerase and anti-catastrophe activities. No rescues were observed.

**XMAP215 Is a Strong Polymerase of Yeast Tubulin**—Having found that Stu2 is a strong polymerase of its conspecific tubulin, we asked whether XMAP215 is also a stronger polymerase of vertebrate tubulin over yeast tubulin. To the contrary, we found that XMAP215 is also a strong polymerase of yeast tubulin. When we introduced increasing amounts of XMAP215 and 5 μM budding yeast tubulin to GMP-CPP-stabilized seeds and visualized the formation of dynamic microtubules using DIC microscopy at 28 °C (Fig. 4, C and D), we observed a large increase in yeast microtubule growth rates. Namely, the growth rate increased by 13-fold from 0.26 ± 0.07 μm/min (mean ± S.E., n = 198) observed with yeast tubulin alone to 3.45 ± 0.87 μm/min (mean ± S.D., n = 157) in the presence of 125 nM XMAP215. Consistent with previous reports (22, 24), XMAP215 caused an 9-fold increase in porcine microtubule growth rates, increasing the growth rate from 0.32 ± 0.08 μm/min (mean ± S.D., n = 106) in the absence of XMAP215 to 2.9 ± 0.2 μm/min (mean ± S.E., n = 13) in the presence of 125 nM XMAP215.
2.87 ± 1.25 μm/min (mean ± S.D., n = 18) in the presence of 125 nM XMAP215. These results show that XMAP215 increases the polymerization rates of both yeast and porcine tubulins to similar extents. However, the Michaelis-Menten fits to these curves reveal different $K_{\text{m}}$ values, indicating that XMAP215 is more active on porcine tubulin than on yeast tubulin ($K_{\text{m}}$ for porcine brain tubulin = 2.13 ± 0.56 nM, which is considerably lower than that for yeast tubulin, $K_{\text{m}}$ = 17.35 ± 3.22 nM).

XMAP215 doubled the catastrophe frequency of porcine microtubules from 0.192 ± 0.033 min⁻¹ (mean ± S.E., n = 33) in the control experiment to 0.377 ± 0.047 min⁻¹ (mean ± S.E., n = 66) in the presence of 125 nM XMAP215 (Fig. 4C). In contrast, when yeast tubulin was used, the addition of 125 nM XMAP215 halved the catastrophe frequency from 0.375 ± 0.050 min⁻¹ (mean ± S.E., n = 56) to 0.165 ± 0.029 min⁻¹ (mean ± S.E., n = 32). No rescues were observed with or without XMAP215.

**DISCUSSION**

We found that Stu2 stimulates the growth of microtubules polymerizing in the presence of budding yeast tubulin. The maximum stimulation of 5-fold is similar to that of the evolutionarily related proteins XMAP215 and Tea1. Thus, Stu2 is a microtubule polymerase, like the other members of the XMAP215/Dis1 family. The polymerase activity of purified Stu2 occurs at concentrations similar to those found in cells (80 nM) (27) and accounts for some of the observed phenotypes during mitosis. For example, anaphase microtubule elongation is impaired in both Stu2-depleted and Stu2-DTOG1 mutant cells (9–11), as expected if polymerase activity is decreased. The effects of Stu2 depletion on the dynamics of cytoplasmic microtubules (10) are not easily reconciled with our in vitro results, suggesting that interactions of Stu2 with other MAPs may be involved.

We could not confirm earlier results showing that Stu2 inhibits, rather than promotes, elongation of mammalian brain microtubules (12). Although Stu2 is a weak polymerase in the presence of porcine brain tubulin and requires high Stu2 concentrations, it nevertheless promoted microtubule growth under all conditions that we tested.

A key finding was that Stu2 has higher polymerase activity on budding yeast tubulin than on porcine brain tubulin. Although the α- and β-tubulins are highly evolutionarily conserved families of proteins, there are considerable differences between budding yeast and mammalian tubulins at both the gene and protein levels that might lead to this difference in Stu2 activity. Budding yeast has two α-tubulin genes and one β-tubulin gene (28, 29), whereas vertebrates have six α-tubulin genes and seven β-tubulin genes (30). Within α- and β-tubulins, the sequence identity between yeast and mammalian proteins is only 70–75%, with large differences at their C termini. Post-translational modifications of vertebrate tubulins lead to additional tubulin complexity at the protein level (31). These post-translational modifications occur mostly on the C termini of α- and β-tubulins, sites that are recognized by many regulators of microtubule dynamics (31, 32), including XMAP215 (24). In the case of bovine brain tubulin, these different gene products and post-translational modifications give rise to up to 21 different tubulin species that are distinguishable on an isoelectric focusing gel (33, 34). Thus, there are many potential molecular mechanisms that could give rise to the differences in activity of Stu2 on the different sources of tubulin.

XMAP215 also differentiated between tubulins: although the maximum enhancement of growth was in the same range for

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**FIGURE 4. Effects of XMAP215 on the dynamics of porcine and yeast tubulins.** A, kymographs showing the effect of XMAP215 on porcine and yeast microtubules (DIC microscopy, 5 μm yeast tubulin and 9 μm porcine tubulin with 125 nM XMAP215, 28 °C, BRB80 + 50 mM KCl). B, XMAP215 promotes the polymerization of budding yeast tubulin (green squares) to a similar extent as porcine brain tubulin (black circles). Data points are means ± S.E. For porcine tubulin, n = 106 microtubules in the absence of XMAP215, and n = 14–16 microtubules in the presence of XMAP215. For yeast tubulin, n = 198 microtubules in the absence of XMAP215 and n = 9–123 microtubules in the presence of XMAP215. Black and green lines are the fits to the Michaelis-Menten equation ($V_{\text{max}} = 2.88 ± 0.56$ and 3.64 ± 0.20 μm/min for porcine and yeast tubulins, respectively (mean ± S.E.); $V_{\text{max}} = 0.32 ± 0.08$ and 0.26 ± 0.07 μm/min for porcine and yeast tubulins, respectively (mean ± S.D.)). C, XMAP215 decreases the catastrophe frequency of yeast tubulin but increases the catastrophe of porcine tubulin. Data points are means ± S.E., where the S.E. was calculated as the mean divided by the square root of the number of catastrophes, which ranged from 19 to 66 for pig tubulin experiments and from 11 to 119 for yeast tubulin experiments.
yeast and porcine tubulins, less XMAP215 was needed to give a half-maximal promotion on porcine tubulin compared with yeast tubulin. This provides additional evidence for differences between yeast and brain tubulins. The lower concentration of XMAP215 required to accelerate growth may reflect a higher affinity of XMAP215 for the end of porcine microtubules; once at the end, however, the growth acceleration is similar. Interestingly, under the conditions that we tested, XMAP215 was a stronger polymerase than Stu2 on both tubulins, enhancing the growth rates of yeast and porcine tubulins by 13- and 9-fold, respectively, compared with enhancements of 5- and 2-fold by Stu2.

Stu2 decreased the catastrophe frequency of yeast microtubules but had little effect on the catastrophe frequency of porcine microtubules. Interestingly, XMAP215 also decreased catastrophe in yeast tubulin but increased catastrophe in porcine tubulin. This is another difference in regulation by MAPs between yeast and porcine tubulins. It is possible that these effects reflect the inherent differences in polymerization dynamics of these two tubulin types.

Stu2 shares many properties with XMAP215. Both accelerate microtubule growth and inhibit catastrophe, both preferentially bind microtubule ends over lattice, both diffuse on the lattice, and both accelerate depolymerization of stable microtubules when there is no free tubulin in solution. There are, however, some differences. As reported above, Stu2 can bind to the minus end, where it accelerates microtubule growth, which is not observed for XMAP215 (24). Another possible difference between Stu2 and XMAP215 is that the former increased the rate of transition from shrinking to growing (rescue). By contrast, rescues have not been reported for XMAP215 (and we also saw no rescues under our experimental conditions).

In summary, our results demonstrate that differences between yeast and mammalian tubulins can give rise to differential activities of interacting proteins, such as previously reported for kinesin (35) and dynein (36). In this study, we reported significant differences in protein activity when using tubulins from different sources, showing the importance of using conspecific tubulin when studying MAPs.

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REFERENCES
1. Howard, J. (2001) Mechanics of Motor Proteins and the Cytoskeleton, Sinauer Associates, Inc., Publishers, Sunderland, MA
2. Cassimeris, L., and Tran, P. T. (eds) (2010) Microtubules: In Vivo, 1st Ed., Academic Press, New York
3. Howard, J., and Hyman, A. A. (2007) Microtubule polymerases and depolymerases. Curr. Opin. Cell Biol. 19, 31–35
4. Akhmanova, A., and Steinmetz, M. O. (2008) Tracking the ends: a dynamic protein network controls the fate of microtubule tips. Nat. Rev. Mol. Cell Biol. 9, 309–322
5. van der Vaart, B., Akhmanova, A., and Straube, A. (2009) Regulation of microtubule dynamic instability. Biochem. Soc. Trans. 37, 1007–1013
6. Al-Bassam, J., and Chang, F. (2011) Regulation of microtubule dynamics by TOG-domain proteins XMAP215/Dis1 and CLASP. Trends Cell Biol. 21, 604–614
7. Gard, D. L., and Kirschner, M. W. (1987) A microtubule-associated protein from Xenopus eggs that specifically promotes assembly at the plus end. J. Cell Biol. 105, 2203–2215
8. Takeshita, N., Mania, D., Herrero, S., Ishitsuka, Y., Nienhaus, G. U., Podolski, M., Howard, J., and Fischer, R. (2013) The cell-end marker TeaA and the microtubule polymerase AlpA contribute to microtubule guidance at the hyphal tip cortex of Aspergillus nidulans to provide polarity maintenance. J. Cell Sci. 126, 5400–5411
9. Severin, F., Habermann, B., Hufferer, T., and Hyman, T. (2001) Stu2 promotes mitotic spindle elongation in anaphase. J. Cell Biol. 153, 435–442
10. Kosco, K. A., Pearson, C. G., Maddox, P. S., Wang, P. J., Adams, I. R., Salmon, E. D., Bloom, K., and Hufferer, T. C. (2001) Control of microtubule dynamics by Stu2p is essential for spindle orientation and metaphase chromosome alignment in yeast. Mol. Biol. Cell 12, 2870–2880
11. Al-Bassam, J., van Breugel, M., Harrison, S. C., and Hyman, A. (2006) Stu2p binds tubulin and undergoes an open-to-closed conformational change. J. Cell Biol. 172, 1009–1022
12. van Breugel, M., Drechsel, D., and Hyman, A. (2003) Stu2p, the budding yeast member of the conserved Dis1/XMAP215 family of microtubule-associated proteins is a plus end-binding microtubule destabilizer. J. Cell Biol. 161, 359–369
13. Ayaz, P., Ye, X., Huddlestone, P., Brautigam, C. A., and Rice, L. M. (2012) A TOG:αβ-tubulin complex structure reveals conformation-based mechanisms for a microtubule polymerase. Science 337, 857–860
14. Widlund, P. O., Steer, J. H., Pozniakovsky, A., Zanic, M., Reber, S., Brouhard, G. J., Hyman, A. A., and Howard, J. (2011) XMAP215 polymerase activity is built by combining multiple tubulin-binding TOG domains and a basic lattice-binding region. Proc. Natl. Acad. Sci. U.S.A. 108, 2741–2746
15. Gell, C., Friel, C. T., Borgonoivo, B., Drechsel, D. N., Hyman, A. A., and Howard, J. (2011) Purification of tubulin from porcine brain. Methods Mol. Biol. 777, 15–28
16. Hyman, A. A., Drechsel, D., Kellogg, D., Salser, S., Sawin, K., Steffen, P., Wordeman, L., and Mitchison, T. (1991) Preparation of modified tubulins. Methods Enzymol. 196, 478–485
17. Hunter, A. W., Caplow, M., Coy, D. L., Hancock, W. O., Diez, S., Wordeman, L., and Howard, J. (2003) The kinesin-related protein MCAK is a microtubule depolymerase that forms an ATP-hydrolyzing complex at microtubule ends. Mol. Cell 11, 445–457
18. Widlund, P. O., Podolski, M., Reber, S., Alper, J., Storch, M., Hyman, A. A., Howard, J., and Drechsel, D. N. (2012) One-step purification of assembly-competent tubulin from diverse eukaryotic sources. Mol. Biol. Cell 23, 4393–4401
19. Gell, C., Bormuth, V., Brouhard, G. J., Cohen, D. N., Diez, S., Friel, C. T., Helenius, J., Nitzsche, B., Petzold, H., Ribbe, J., Schäffer, E., Steer, J. H., Trushko, A., Varga, V., Widlund, P. O., Zanic, M., and Howard, J. (2010) Microtubule dynamics reconstituted in vitro and imaged by single-molecule fluorescence microscopy. Methods Cell Biol. 95, 221–245
20. Bormuth, V., Howard, J., and Schäffer, E. (2007) LED illumination for video-enhanced DIC imaging of single microtubules. J. Microsc. 226, 1–5
21. Hyman, A. A., Salser, S., Drechsel, D. N., Unwin, N., and Mitchison, T. J. (1992) Role of GTP hydrolysis in microtubule dynamics: information from a slowly hydrolyzable analogue, GMP-CPP. Mol. Biol. Cell 3, 1155–1167
22. Zanic, M., Widlund, P. O., Hyman, A. A., and Howard, J. (2013) Synergy between XMAP215 and EB1 increases microtubule growth rates to physiological levels. Nat. Cell Biol. 15, 688–693
23. Verde, F., Dogterom, M., Stelzer, E., Karsenti, E., and Leibler, S. (1992) Control of microtubule dynamics and length by cdc4- and cdc7-dependent kinases in Xenopus egg extracts. J. Cell Biol. 118, 1097–1108
24. Brouhard, G. J., Steer, J. H., Noetzl, T. L., Al-Bassam, J., Kinoshita, K., Harrison, S. C., Howard, J., and Hyman, A. A. (2008) XMAP215 is a processive microtubule polymerase. Cell 132, 79–88
25. Davis, A., Sage, C. R., Wilson, L., and Farrell, K. W. (1993) Purification and biochemical characterization of tubulin from the budding yeast Saccharomyces cerevisiae. Biochemistry 32, 8823–8835
26. Barnes, G., Louie, K. A., and Botstein, D. (1992) Yeast proteins associated...
with microtubules in vitro and in vivo. Mol. Biol. Cell 3, 29–47
27. Ghaemmaghami, S., Huh, W.-K., Bower, K., Howson, R. W., Belle, A., Dephoure, N., O’Shea, E. K., and Weissman, J. S. (2003) Global analysis of protein expression in yeast. Nature 425, 737–741
28. Neff, N. F., Thomas, J. H., Grisafi, P., and Botstein, D. (1983) Isolation of the β-tubulin gene from yeast and demonstration of its essential function in vivo. Cell 33, 211–219
29. Schatz, P. J., Pillus, L., Grisafi, P., Solomon, F., and Botstein, D. (1986) Two functional α-tubulin genes of the yeast Saccharomyces cerevisiae encode divergent proteins. Mol. Cell Biol. 6, 3711–3721
30. Ludueña, R. F. (1998) Multiple forms of tubulin: different gene products and covalent modifications. Int. Rev. Cytol. 178, 207–275
31. Janke, C., and Bulinski, J. C. (2011) Post-translational regulation of the microtubule cytoskeleton: mechanisms and functions. Nat. Rev. Mol. Cell Biol. 12, 773–786
32. Sirajuddin, M., Rice, L. M., and Vale, R. D. (2014) Regulation of microtubule motors by tubulin isotypes and post-translational modifications. Nat. Cell Biol. 16, 335–344
33. Williams, R. C., Jr., Shah, C., and Sackett, D. (1999) Separation of tubulin isoforms by isoelectric focusing in immobilized pH gradient gels. Anal. Biochem. 275, 265–267
34. Field, D. J., Collins, R. A., and Lee, J. C. (1984) Heterogeneity of vertebrate brain tubulins. Proc. Natl. Acad. Sci. U.S.A. 81, 4041–4045
35. Alonso, M. C., Drummond, D. R., Kain, S., Hoeng, J., Amos, L., and Cross, R. A. (2007) An ATP gate controls tubulin binding by the tethered head of kinesin-1. Science 316, 120–123
36. Alper, J. D., Tovar, M., and Howard, J. (2013) Displacement-weighted velocity analysis of gliding assays reveals that Chlamydomonas axonomal dynein preferentially moves conspecific microtubules. Biophys. J. 104, 1989–1998