Direct Regulation of Microtubule Dynamics by Protein Kinase CK2*

 Anthony C. B. Lim‡, Sock-Yeen Tiu‡, Qing Li‡, and Robert Z. Qi§

From the 1Institute of Molecular and Cell Biology, 30 Medical Drive, Singapore 117609 and the 2Department of Biochemistry, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China

Microtubule dynamics is essential for many vital cellular processes such as morphogenesis and motility. Protein kinase CK2 is a ubiquitous protein kinase that is involved in diverse cellular functions. CK2 holoenzyme is composed of two catalytic α or α′ subunits and two regulatory β subunits. We show that the α subunit of CK2 binds directly to both microtubules and tubulin heterodimers. CK2 holoenzyme but neither of its individual subunits exhibited a potent effect of inducing microtubule assembly and bundling. Moreover, the polymerized microtubules were strongly stabilized by CK2 against cold-induced depolymerization. Interestingly, the kinase activity of CK2 is not required for its microtubule-assembling and stabilizing function because a kinase-inactive mutant of CK2 displayed the same microtubule-assembling activity as the wild-type protein. Knockdown of CK2αα′ in cultured cells by RNA interference dramatically destabilized their microtubule networks, and the destabilized microtubules were readily destructed by colchicine at a very low concentration. Further, over-expression of chicken CK2α or its kinase-inactive mutant in the endogenous CK2αα′-depleted cells fully restored the microtubule resistance to the low dose of colchicine. Taken together, CK2 is a microtubule-associated protein that confers microtubule stability in a phosphorylation-independent manner.

Protein kinase CK2 (formerly known as casein kinase 2) is ubiquitously expressed and highly conserved in eukaryotic cells (1–4). It comprises two catalytic α or α′ subunits and two regulatory β subunits to form a heterotetrameric structure in which the two β subunits dimerize to link the two α or α′ subunits (5). As a protein serine/threonine kinase, CK2 has a very broad phosphorylation spectrum, and over 300 protein substrates of CK2 have been identified to date (6). A number of studies have indicated that CK2 is involved in a wide variety of cellular processes including cell cycle, apoptosis, transcriptional regulation, and signal transduction (1, 3, 6). CK2 is instrumental and necessary for promoting cell survival (3, 7). Disruption of genes encoding both of the catalytic subunits of CK2 is synthetic lethal in fission yeast (9). Similarly, it is embryonic lethal when CK2α is knocked down in Caenorhabditis elegans by RNA interference or in mice by gene disruption, respectively (8, 10). Hence, production of both the α and β subunits of CK2 appears to be mandatory for cell viability.

A few lines of evidence have lead to implication that CK2 might be involved in the regulation of microtubule cytoskeleton reorganization (12–14). CK2 was localized to microtubule structures such as the mitotic spindle of dividing cells and was found to associate with the cold-stable fraction of microtubules from the rat brain (14, 15). More recently, the α and α′ subunits were shown to bind tubulin in a far Western assay (16). Further, CK2 is able to phosphorylate a number of microtubule elements, including MAP1B and a neuron-specific β-tubulin isotype (6). The phosphorylation of MAP1B was proposed to facilitate the microtubule association of MAP1B and thereby microtubule assembly, whereas the physiological role of the β-tubulin isotype phosphorylation is still unclear (12, 17). Despite these findings, the direct correlation of CK2 and microtubule stability has not been established.

In the present study, we have investigated the physical association of CK2 with microtubules and the direct effect of CK2 on microtubule dynamics. Our results show that CK2 is a microtubule-associated protein (MAP)1 that induces microtubule assembly and bundling in vitro. CK2-polymerized microtubules appear stable under cold treatment. In cultured cells, knockdown of CK2αα′ has a severe effect on microtubule stability, which implies that CK2 mediates microtubule integrity in vivo. Moreover, a kinase-inactive mutant of CK2 displayed the same microtubule polymerizing and stabilizing activity in vitro and in vivo. Thus, the microtubule assembling and stabilizing action of CK2 is independent of its kinase function.

EXPERIMENTAL PROCEDURES

Plasmid Constructions—The coding sequences of chicken CK2α and its kinase-inactive mutant (CK2αaK68A) were subcloned into pGEX4T (Amersham Biosciences), pET32 (Novagen), and pIsee-Myc (18). The full-length sequence of human CK2β was cloned by a reverse transcription polymerase chain reaction and inserted into pQE30 (Qiagen).

Protein Binding Assay—Proteins tagged with GST or His6 were bacterially expressed and prepared as described previously (19). To test tubulin binding, GSH-Sepharose beads (Amersham Biosciences) pre-bound with GST, GST-CK2α, or the complex of GST-CK2αHis-CK2β were incubated with purified tubulin (99% pure and MAP-free, cytoskeleton) for 1 h at 4 °C. After being extensively washed with binding buffer (20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 20 mM MgCl2, 1 mM dithiothreitol, and 0.1% Nonidet P-40), the beads were boiled in SDS-PAGE sample buffer and analyzed by immunoblotting. Antibodies against α- and β-tubulin were from Sigma. The binding of His-tagged proteins with tubulin was performed with nickel-nitrotriacetic acid beads (Ni-NTA, Qiagen) in binding buffer without dithiothreitol. In the microtubule binding assay, microtubules, which were pre-assembled using taxol in PEM buffer (80 mM PIPES, pH 6.8, 1 mM MgCl2, 1 mM EGTA) supplemented with 1 mM GTP, were incubated with the indicated proteins. The samples were subsequently loaded onto a buffered cushion (50% glycerol in PEM buffer) and centrifuged to spin down the...
microtubules and associated proteins. The pellet and the supernatant were analyzed by immunoblotting.

**Microtubule Assembly**—Microtubules were assembled in vitro from the purified MAP-free tubulin at 2 mg/ml in PEM buffer supplemented with 1 mM GTP at 35 °C, and the turbidity of the solutions was monitored at 340 nm (20). CK2 was added at various amounts as indicated to promote the assembly. To visualize assembled microtubules, tubulin and fluorescently-labeled tubulin (Cytochrome) at the ratio of 7:1 were used in the polymerization (21). Microtubules were fixed with 0.1% paraformaldehyde and then permeabilized in PBS containing 4% paraformaldehyde and then permeabilized in PBS containing 1% Triton X-100, 0.1% Triton X-100, 10 mM CaCl2, and Roche protease inhibitor mixture, which was prewarmed to 35 °C, to extract cytosolic soluble tubulin heterodimers and preserve microtubules (assembled insoluble tubulin polymers). The extract was clarified by centrifugation and the supernatant designated as the free tubulin fraction. After a brief washing with the microtubule-stabilizing buffer, the pellet was extracted with the microtubule-stabilizing buffer (80 mM PIPES, pH 6.8, 1 mM MgCl2, 1 mM EGTA, 0.5% Triton X-100, 10% glycerol, and Roche protease inhibitor mixture). The extract was clarified by centrifugation and the supernatant designated as the free tubulin fraction. After a brief washing with the microtubule-stabilizing buffer, the pellet was extracted with the microtubule-stabilizing buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 10 mM CaCl2, and Roche protease inhibitor mixture). The extract was clarified by centrifugation to yield the polymerized tubulin fraction. Both fractions were analyzed by immunoblotting, and each band on the blots was quantitated using a Bio-Rad GS-700 imaging densitometer and analyzed with the Multi-Analyst version 1.0.1, program (Bio-Rad).

**Cell Culture, Transfection, and Immunofluorescence**—COS-7, HeLa, and 293T cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. The siRNA sequence designed for human CK2α/α′ is 5′-CCAGCGUGUGAAGUCAUCUUGU-3′, which has a few discrepancies with the corresponding sequence of chicken CK2α/α′. 20 μl CK2α/α′ siRNA or a scrambled siRNA sequence was applied into the transfection using a TransIT-TKO transfection reagent (Mirus). Simultaneous transfection of siRNA and plasmid DNA was done using TransIT-TKO and LipofectAMINE (Invitrogen) concurrently. After transfection, the cells were cultured for 24 h before treatment with 0.2 μM colchicine (Sigma) for 3 h. The cells were subjected to differential extraction of free and polymerized tubulin or to immunostaining. For immunofluorescence, the cells were fixed in PBS containing 4% paraformaldehyde and then permeabilized in PBS containing 0.2% Triton X-100. After a blocking wash with 10% goat serum and 0.1% Triton X-100 in PBS, immunostaining was performed with antibodies as indicated. CK2α- and CK2β-specific antibodies were from Santa Cruz Biotechnology. The secondary antibodies are Fluor594 goat anti-mouse IgG and Fluor488 donkey anti-goat IgG (Molecular Probes). The cells were then washed in PBS, mounted, and photographed on an MRC-1024 laser scanning confocal microscope (Bio-Rad).

**RESULTS**

**CK2 Forms a Direct Complex with Microtubules**—The direct association of CK2 and microtubules was probed by a series of binding assays using recombinant CK2 and purified MAP-free tubulin as well as pre-assembled microtubules. The α/β heterodimer of tubulin was found to associate with the catalytic α subunit as well as the holoenzyme of CK2 (Fig. 1A). CK2β alone did not result in the pull-down of any tubulin (Fig. 1B), which is in agreement with a previous observation using far Western blotting (16). To verify the microtubule association of CK2, taxol-assembled microtubules were incubated with CK2 holoenzyme or its individual subunit proteins. The microtubules were then spun down to test whether these proteins co-purified with the microtubules. Consistently, CK2α and the holoenzyme of CK2 were found to associate with the microtubule pellet, whereas CK2β and GST, as a control protein, failed to co-purify with the microtubules (Fig. 1C), indicating that the CK2 holoenzyme associates with microtubules at a high affinity through CK2α.

Cellular localization of CK2 to microtubule networks was revealed by immunofluorescent staining of cultured COS-7 cells. Microscopic imaging of endogenous CK2α and CK2β displayed a clearly defined positioning with the microtubule network, particularly in the cell periphery (Fig. 2A). As confirmation, pools of tubulin existing as free heterodimers or polymers (microtubules) were differentially extracted from the cultured cells to examine the distribution of CK2 (22). Both CK2α and CK2β appeared in the microtubule fraction as well as the fraction of free tubulin heterodimers, although there appeared to be more CK2β in the microtubule fraction (Fig. 2B). Taken together with the results from the in vitro binding assays, this provides evidence of the direct association of CK2 with cellular microtubules.

**CK2 Induces Microtubule Polymerization**—We next investigated whether CK2 has any effect on microtubule dynamics by using an in vitro assay of microtubule assembly from purified MAP-free tubulin (20). During the assay, the turbidity change of the solution was measured as tubulin polymerizes or depolymerizes. In the absence of CK2, there was minimal polymerization of tubulin even after a prolonged incubation (Fig. 3, A and B). The addition of CK2 at a ratio of 1:240 to tubulin resulted in substantial polymerization of tubulin into microtubules (Fig. 3, A and B). Clearly, both the rate and extent of polymerization were dramatically enhanced by CK2. When the amount of CK2 was increased, tubulin polymerization was increased in a dose-dependent manner (Fig. 3, A and B). To verify the microtubule formation, rhodamine-labeled tubulin was applied into the polymerization experiments for direct visualization of the assembled microtubules by fluorescence microscopy (21). As shown in Fig. 3C, microtubule filaments and bundles were readily observed with the CK2-incubated tubulin, whereas the incubation of tubulin without CK2 showed no obvious microtubule formation. Therefore, we have found that CK2, in addition to showing high affinity binding to tubulin and microtubules, induces the assembly of tubulin into microtubules. Moreover, CK2 appeared to cause microtubule bundling, suggesting a strong stabilizing effect on the microtubules.

CK2 holoenzyme is a tetrameric complex of two α or α′ subunits and two β subunits (5). Given that observation that CK2α of the holoenzyme interacts with microtubules, we explored whether the microtubule assembly function of CK2 is restricted to the holoenzyme by applying the α and β subunits of CK2 individually into the microtubule assembly assay. In contrast to the holoenzyme, when either the α or β subunit was tested, there was minimal polymerization of tubulin even after a prolonged incubation (Fig. 4). The CK2α- and CK2β-polymerized samples had no marked difference from the background tubulin polymerization, which was shown in the GST-incubated sample. Thus, only CK2 holoenzyme, but not each of the individual subunits, has the ability to induce microtubule assembly even though CK2α has shown microtubule binding activity.

CK2 has been known to catalyze phosphorylation of a neural isoform of β-tubulin and some of the MAPs, raising the possibility that it may affect microtubule dynamics through a kinase reaction (12, 17). Although ATP was not present in the in vitro microtubule assembly assay, CK2 is capable of utilizing either ATP or GTP as the phosphate donor in its phosphorylating reactions (23). We designed an experiment to assess the role of CK2 kinase activity in microtubule assembly. A kinase-inactive holoenzyme of CK2, in which CK2α was replaced with the kinase-inactive mutant CK2αk68A, was tested in the microtubule assembly assay. Fig. 5 shows that the kinase-inactive CK2 conferred the same microtubule polymerizing activity as the wild-type enzyme, indicating that the microtubule assembly entity of CK2 is independent of its kinase activity and phosphorylation of any microtubule proteins.

Microtubules from brains can be separated into two pools, namely “cold labile” and “cold stable,” according to whether...
they are resistant to cold treatment for microtubule disassembly (24). It has been found that CK2 is enriched in the cold-stable fraction of the microtubule preparation from rat brain (14). This observation, together with our findings that CK2 associates with microtubules to promote microtubule assembly, prompted us to explore the possibility that CK2 may contribute to the cold stability of microtubules. To test this likelihood, CK2-polymerized microtubules were incubated on ice, and the
turbidity change was monitored. As a comparison, tau-polymerized microtubules were treated under the same condition, given the fact that tau does not confer cold stability to microtubules (25). As expected, the tau-polymerized sample was depolymerized almost completely within a few minutes (Fig. 6). However, the turbidity of the CK2-polymerized sample was only marginally reduced even after a prolonged cold incubation (Fig. 6), indicating that CK2 functions to stabilize microtubules against cold-induced disassembly.

**CK2 Stabilizes Microtubules in Vivo**—To evaluate the role of CK2 in microtubule dynamics in vivo, we knocked down CK2α/α’ in HeLa cells by gene silencing using a siRNA duplex derived from the human sequence of CK2α/α’ (26, 27). As shown by the CK2α immunoblot, the introduction of CK2α/α’ siRNA into the cells led to a dramatic decrease of the CK2α/α’ proteins to a minimal cellular level (Fig. 7A). To assess the knockdown effect on microtubules, the amount of cellular microtubules (assembled insoluble tubulin polymers) was determined using the differential extraction method and immunoblotting (22). In addition, the integrity of the cellular microtubule network was examined by immunofluorescent staining and confocal microscopy. The knockdown of CK2α/α’ significantly reduced the cellular content of microtubules (Fig. 7, A and B), suggesting CK2 as one of the factors in stabilizing microtubules in vivo. We further assessed microtubule stability using colchicine, which is a microtubule-disrupting agent. When colchicine was applied at a low concentration (0.2 μM) onto the cells that were transfected with a scrambled siRNA sequence, most of the microtubule structure remained intact (Fig. 7, A and B). However, such a low dose of colchicine caused severe disruption of the microtubule structure in the CK2α/α’-depleted cells where the microtubule networks were collapsing toward the perinuclear membrane (Fig. 7B); almost negligible amount of microtubules was extracted from these cells (Fig.,
7). Apparently, the removal of CK2α had a strong effect on cellular microtubule architecture, rendering it very unstable. As a result, it was readily destructed by colchicine at a very low concentration.

To further substantiate the microtubule stabilizing function of CK2, we tested whether microtubule stability could be restored by expression of chicken CK2α in endogenous CK2α-depleted cells. As observed with the HeLa cells, knockdown of CK2α in cultured human 293T fibroblasts using siRNA strongly destabilized the microtubule network, resulting in almost complete disruption of the microtubules by colchicine at 0.2 μM (Fig. 7C). When chicken CK2α was expressed in the 293T cells in which endogenous CK2α was knocked down, the cellular microtubules completely retained their integrity against the colchicine-induced disruption (Fig. 7C). More interestingly, when the expression was performed using the kinase-inactive mutant CK2αK68A, it exhibited the same effect as wild-type CK2α in rescuing microtubules from colchicine treatment (Fig. 7C). These data demonstrate that CK2 is an important mediator of cellular microtubule stability and exerts its effect in a phosphorylation-independent manner.

**DISCUSSION**

Microtubules are a major cytoskeletal constituent in all eukaryotes. In living cells, the microtubule architecture is stabilized by structural MAPs, which associate with microtubules...
and promote in vitro microtubule assembly (28, 29). The evidence presented here identifies CK2 as a structural MAP that mediates microtubule dynamics. We have conducted experiments showing that CK2 is localized to and co-extracted with microtubules. The in vitro binding assays demonstrate the direct interaction of CK2 with microtubules as well as tubulin heterodimers, and the binding affinity is comparable with that of known MAPs. Microtubule binding sequences are often found in MAPs as repeated sequence stretches rich in basic amino acids. Although the sequence of CK2α contains some basic regions, it is not found to have any typical microtubule-binding motif in CK2α. Thus, the microtubule association of CK2α may suggest new microtubule-binding domains.

Structural MAPs such as MAP2 and tau are known to stimulate microtubule assembly from tubulin heterodimers. In our microtubule assembly assays, CK2 exhibited a potent activity of inducing microtubule assembly and bundling from purified tubulin. The physical association of CK2 to microtubules and tubulin heterodimers stimulates both the rate and the extent of microtubule growth. Although CK2α can bind microtubules, the microtubule assembling and stabilizing function is solely a property of the holoenzyme. In addition, CK2-polymerized microtubules display stability against cold treatment, suggesting that CK2 is a strong stabilizer of microtubules. Taken together with the observation that a substantial amount of CK2 exists in the cold-stable microtubules of rat brain (14), our findings suggest that CK2 is a new factor endowing the cold stability of microtubules. To date, the STOP proteins, double-cortin and BPAG1n3, are the only known MAPs that confer cold stability on microtubules (30–34).

Structural MAPs are known to contribute to microtubule stability and distribution within cells (35). The finding of CK2 as a structural MAP stimulated our interest in evaluating the regulatory role of CK2 in vivo in microtubule cytoskeleton. The knockdown of CK2α from cells has a strong destabilizing effect on the microtubule architecture. As a result, the microtubule network is very vulnerable and can be readily destroyed by colchicine insult at 0.2 μM, whereas such a low concentration of colchicine does not have any significant effect on microtubules of cells with intact CK2. Thus, CK2 has an indispensable role in stabilizing cellular microtubules. This is substantiated by the introduction of chicken CK2α into the cells to compensate for the loss of endogenous CK2α. The microtubule instability caused by the deficit of CK2α can be rectified completely by the expression of chicken CK2α, which assures that CK2 is a vital structural MAP conferring microtubule stability in vivo. It is noteworthy that the removal of CK2α did not cause severe microtubule disruption in the cells, possibly because of the existence of multiple MAPs other than CK2 in the cells, to support the microtubule network.

CK2 is a Ser/Thr protein kinase with a broad substrate spectrum that includes MAP1B and a neural-specific isoform of amounts of microtubules extracted from the cells as compared with the control, which is the sample transfected with the scrambled siRNA sequence and treated without colchicine. The data are representative of three separate experiments. B, cells in the experiments described in A were fixed and stained with the β-tubulin antibody for confocal microscopic imaging. C, expression of the wild-type or the kinase-inactive mutant of chicken CK2α restored microtubule stability against colchicine treatment in CK2α-depleted cells. Prior to treatment with colchicine (0.2 μM), 293T cells were double transfected with siRNA of human CK2α and one of the following expression constructs: chicken CK2α, the kinase-inactive mutant of chicken CK2α (CK2αK68A), or the empty vector. Expression of Myc-tagged chicken CK2α and CK2αK68A was detected by anti-Myc immunoblotting of the cell lysates. Microtubules were extracted using the differential extraction method (see “Experimental Procedures”) for anti-β-tubulin immunoblotting. Representative results of three separate experiments are shown.
CK2 Mediates Microtubule Dynamics

β-tubulin. We examined whether the kinase activity of CK2 is involved in the microtubule assembly stimulated by CK2. Our in vitro assays of microtubule assembly using the kinase-inactive mutant of CK2 indicate that the microtubule-assembling activity of CK2 is independent of its kinase activity. This was corroborated by the experiment of expressing the kinase-inactive mutant of chicken CK2α in the CK2α/α'-knock-down cells, which completely compensated for the lost of endogenous CK2α/α', rendering the microtubules resistant to colchicine attack. With these results, it becomes clear that CK2 imparts a direct regulation of microtubule organization through its physiological action. As a multifunctional enzyme, CK2 has been demonstrated to execute its functions through its phosphorylation of a wide range of substrates. The results presented here reveal a novel CK2 function that is dissociated from its intrinsic kinase property.

It has been proposed that CK2 plays an important role in the maintenance of cell morphology and polarity. Depletion of the catalytic subunits of CK2 in neuroblastoma cells using an antisense approach blocks neuritogenesis (27, 36). Pertinent observations also came from yeasts, of which the temperature-sensitive mutants of CK2α and CK2β demonstrated their importance in cell morphogenesis (9, 37, 38). The function described here for CK2 in microtubule dynamics may provide a mechanistic explanation of its role in cell shape control.

Acknowledgments—We thank Dr. Claude Cochet for the chicken CK2α and CK2αK68A constructs, Dr. Michel Goedert for the tau construct, Dr. B. L. Tang for the pDeo-Myc vector, Dr. Walter Hunziker for critical reading of the manuscript, Dr. Jerry H. Wang for invaluable comments on the manuscript, and Dr. Alice Tay for support of this work.

REFERENCES

1. Allende, J. E., and Allende, C. C. (1995) FASEB J. 9, 313–323
2. Blankset, P. R. (2000) Prog. Neurobiol. 60, 211–246
3. Litchfield, D. W. (2003) Biochem. J. 369, 1–15
4. Pinna, L. A., and Meggio, F. (1997) Prog. Cell Cycle Res. 3, 77–97
5. Niefed, K., Guerra, B., Ermakov, I., and Issinger, O. G. (2001) EMBO J. 20, 5329–5331
6. Meggio, F., and Pinna, L. A. (2003) FASEB J. 17, 349–368
7. Ahmed, K., Gerber, D. A., and Cochet, C. (2002) Trends Cell Biol. 12, 226–230
8. Padmanabha, R., Chen-Wu, J. L., Hanna, D. E., and Glover, C. V. (1990) Mol. Cell Biol. 10, 4899–4909
9. Rethinaswamy, A., Birnbaum, M. J., and Glover, C. V. (1998) J. Biol. Chem. 273, 5869–5877
10. Busch, T., Vernet, M., Blond, O., Jensen, H. H., Pointu, H., Olsen, B. B., Cochet, C., Issinger, O. G., and Boldyreff, B. (2003) Mol. Cell Biol. 23, 908–915
11. Fraser, A. G., Camath, R. S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M., and Ahringer, J. (2000) Nature 408, 325–330
12. Diaz-Nido, J., Serrano, L., Mendez, E., and Avila, J. (1988) J. Cell Biol. 106, 2057–2065
13. Serrano, L., Diaz-Nido, J., Woroscil, F., and Avila, J. (1987) J. Cell Biol. 105, 1731–1739
14. Serrano, L., Hernandez, M. A., Diaz-Nido, J., and Avila, J. (1989) Exp. Cell Res. 181, 263–272
15. Diaz-Nido, J., and Avila, J. (1992) Second Messengers Phosphoproteins 14, 39-53
16. Faust, M., Schuster, N., and Montenarh, M. (1999) FEBS Lett. 462, 51–56
17. Diaz-Nido, J., Serrano, L., Lopez-Otin, C., Vandekerckhove, J., and Avila, J. (1990) J. Biol. Chem. 265, 13949–13954
18. Tang, H. L., Ony, Y. S., Huang, B., Wei, S., Wong, E. T., Qi, R., Horstmann, H., and Hong, W. (2003) J. Biol. Chem. 278, 40008–40017
19. Qu, D., Li, Q., Lin, H. Y., Cheung, N. S., Li, R., Wang, J. H., and Qi, R. Z. (2002) J. Biol. Chem. 277, 7324–7332
20. Gaskin, F. (1982) Methods Enzymol. 85, 433–439
21. Belmont, L. D., Hyman, A. A., Sawin, K. E., and Mitchison, T. J. (1990) Cell 62, 579–589
22. Lieuvin, A., Labbe, P. C., Doree, M., and Job, D. (1994) J. Cell Biol. 124, 855–866
23. Niefed, K., Putter, M., Guerra, B., Issinger, O. G., and Schomburg, D. (1999) Nat. Struct. Biol. 6, 1100–1103
24. Webb, B. C., and Wilson, L. (1980) Biochemistry 19, 1999–2001
25. Basu, P. W., Pankowski, T. P., Cimbalik, K. A., Toyma, K., Bakalis, S., Ashford, P. J., and Kosik, K. S. (1994) J. Cell Sci. 107, 135–143
26. Sayed, M., Pelez, S., Wong, C., Marotta, A., and Salt, B. (2001) Oncogene 20, 6994–7005
27. Ullas, L., Diaz-Nido, J., and Avila, J. (1993) EMBO J. 12, 1633–1640
28. Desai, A., and Mitchison, T. J. (1997) Annu. Rev. Cell Dev. Biol. 13, 83–117
29. Mandelkow, E., and Mandelkow, E. M. (1995) Curr. Opin. Cell Biol. 7, 72–81
30. Denariere, E., Fourret-Lieuvin, A., Bas, C., Pirlot, F., Chapel, A., Margolis, R. L., and Job, D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6055–6060
31. Gleeson, J. G., Lin, P. T., Flanagan, L. A., and Walsh, C. A. (1999) Neuron 23, 257–271
32. Guillau, L., Bosc, C., Fourret-Lieuvin, A., Denariere, E., Pirlot, V., Lafanecere, L., and Job, D. (1998) J. Cell Biol. 142, 167–179
33. Horesh, D., Sapir, T., Francis, F., Wolf, S. G., Capi, M., Elbaum, M., Chelly, J., and Reiner, O. (1999) Hum. Mol. Genet. 8, 1599–1610
34. Yang, Y., Bauer, C., Strasser, G., Wollman, R., Julien, J. P., and Fuchs, E. (1999) Cell 98, 229–238
35. Feng, Y., and Walsh, C. A. (2001) Nat. Rev. Neurosci. 2, 408–416
36. Ullas, L., Diaz-Nido, J., and Avila, J. (1994) J. Cell Mol. Neurobiol. 14, 407–414
37. Roussou, I., and Draetta, G. (1994) Mol. Cell Biol. 14, 576–586
38. Snell, V., and Nurse, P. (1994) EMBO J. 13, 2066–2074
