### Casein Kinase 1 Delta Phosphorylates Tau and Disrupts Its Binding to Microtubules*

Received for publication, December 23, 2003, and in revised form, January 26, 2004  
Published, JBC Papers in Press, February 2, 2004, DOI 10.1074/jbc.M314116200

Guibin Li, Haishan Yin, and Jeff Kuret

From the ‡Center for Molecular Neurobiology, §Ohio State Biochemistry Program, and the ¶Department of Molecular and Cellular Biochemistry, Ohio State University College of Medicine and Public Health, Columbus, Ohio 43210

Tau hyperphosphorylation precedes neuritic lesion formation in Alzheimer’s disease, suggesting it participates in the tau fibrillation reaction pathway. Candidate tau protein kinases include members of the casein kinase 1 (CK1) family of phosphotransferases, which are highly overexpressed in Alzheimer’s disease brain and colocalize with neuritic and granulovacuolar lesions. Here we characterized the contribution of one CK1 isoform, CK1δ, to the phosphorylation of tau at residues Ser202/Thr205 and Ser396/Ser404 in human embryonic kidney 293 cells using immunodetection and fluorescence microscopy. Treatment of cells with membrane permeable CK1 inhibitor 3-(2,3,6-trimethoxyphenyl)methylenyl-indolin-2-one (IC261) lowered occupancy of Ser202/Thr205 phosphorylation sites by >70% at saturation, suggesting that endogenous CK1 was the major source of basal phosphorylation activity at these sites. Overexpression of CK1δ increased CK1 enzyme activity and further raised tau phosphorylation at residues Ser202/Thr205 and Ser396/Ser404 in situ. Inhibitor IC261 reversed tau hyperphosphorylation induced by CK1δ overexpression. Co-immunoprecipitation assays showed direct association of tau and CK1δ in situ, consistent with tau being a CK1δ substrate. CK1δ overexpression also produced a decrease in the fraction of bulk tau bound to detergent-insoluble microtubules. These results suggest that CK1δ phosphorylates tau at sites that modulate tau/microtubule binding, and that the expression pattern of CK1δ in Alzheimer’s disease is consistent with it playing an important role in tau aggregation.

---

**AD** is a progressive neurodegenerative disease characterized in part by the appearance of neurofibrillary tangles, neuritic plaques, and neurophil threads (1). Each manifestation of neurotic pathology is comprised of tau protein aggregated into filaments. Because the spatial distribution of tau fibrillation appears stereotypically, correlates with neuronal cell loss, and parallels cognitive decline, it is a useful marker of and may contribute to degeneration in AD and other dementias.

---

* This work was supported by National Institutes of Health Grant AG14452 (to J. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

© 2004 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in U.S.A.
was digested 30 h at 4 °C with thrombin, prior to repetition of immobilized metal affinity chromatography and gel filtration chromatography. Human brain ChiK cDNA served as template for PCR amplification of C-terminal-truncated ChiK (ChiK-Δ317). The sense primer (5'-ACCG-GATCCCATATGGAGCTGAGAGTC) incorporated an NdeI site at the start codon, whereas the downstream primer (5'-CTCGAGGATG- GTCTCCAGCAGGATCC) contained a TTA stop codon downstream of the codon for His117 followed by an XhoI site. The amplified PCR product was subcloned directly into TOPO2.1 (Invitrogen, Carlsbad, CA) and sequenced in both directions for errors. The ChiK-Δ317/TOPO2.1 cDNA was digested with NdeI and XhoI, the approximate 0.9-kg ChiK-Δ317 fragment was gel purified and subcloned into E. coli expression vector pET7C (28). Recombinant ChiK-Δ317 was purified by immobilized metal affinity chromatography and gel filtration chromatography as described previously for tau protein (28).

Cell Culture, Transfection, and Treatment—DNAs encoding the longest human tau isoform (htau40), human ChiK, or p25 (an activator of Cdk5) were cloned into the mammalian cytomegalovirus expression vector pCMV3.1 (Invitrogen). HEK-293 cells, which were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% bovine serum, 100 units/ml penicillin, G 250 ng/ml amphotericin B, and 100 μg/ml streptomycin (37 °C with 5% CO2), were transiently transfected with these plasmids by Lipofectamine and then subjected along with 100,000 × g supernatants (S1) to SDSPAGE and immunoblot analysis as described above.

Microtubule Binding Assay—To assay the ability of tau to bind microtubules in situ, HEK-293 cells transfected with pcDNA3.1/tau or Cki927 were for 24 h were harvested in 500 μl of Microtubule-stabilizing buffer (80 mM PIPES, pH 6.8, 1 mM GTP, 1 mM MgCl2, 1 mM EDTA, 0.5% Triton X-100, 30% glycerol, and 1× complete protease inhibitors) containing 10 μM Taxol at 37 °C for 10 min. After cell debris was removed by centrifugation (10,000 × g for 10 min) at 37 °C, supernatant fractions were collected and centrifuged again at 100,000 × g for 1 h at 35 °C (5). The resultant pellets (P1) were washed twice with and resuspended (500 μl) in Microtubule-stabilizing buffer, sonicated for 10 s, and then subjected along with 100,000 × g supernatants (S1) to SDSPAGE and the distribution of tau protein was examined by immunoblot analysis using monoclonal antibody Tau5.

Cell Extraction and Immunofluorescence Staining—Wild-type HEK-293 cells were cultured on coverslips for 24 h and transfected with Cki927/tau or pcDNA3.1/tau for 24 h as described above. To extract soluble proteins, cells were washed with phosphate-buffered saline and incubated with Microtubule-stabilizing buffer containing 10 μM Taxol for 15 min. The cells were then washed in Microtubule-stabilizing buffer without detergent. Both the extracted cells and non-extracted cells were fixed with methanol. Following the phosphate-buffered saline washes, cells were sequentially incubated with phosphate-buffered saline containing 3% bovine serum albumin for 1 h, Tau5 antibody (1:1000) overnight at 4 °C, and fluorescein-labeled goat anti-mouse IgG secondary antibody (1:1000) for 1 h. Immunostaining was visualized by fluorescence microscopy (Eclipse 800, Nikon, Japan), captured with a SPOT II digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI), and processed with Metamorph imaging software (Universal Imaging Corp., Downingtown, PA).

Analytical Methods—Hyperbolic inhibition curves were fit to the rectangular hyperbola: y = x/(x + IC50). Immuno-reactivity was determined at inhibitor concentration x, and constant b corresponds to x at 50% y (i.e. IC50).

RESULTS
ChiK Phosphorylates Tau in Vitro—To determine whether ChiK could directly phosphorylate tau, a truncated form of the human enzyme (ChiK-Δ317) was expressed and purified as a HiRes fusion protein and used to phosphorylate tau protein in vitro. Transcuded enzyme was used because it was easily prepared after heterologous expression in E. coli while retaining the protein kinase activity of full-length protein (33). Incubation of htau40 with purified ChiK-Δ317 in the presence of radioactive nucleotide substrate led to detectable phosphate incorporation within 30 min (Fig. 1A). Detection of total tau protein on Western blots using monoclonal antibody Tau5, the reactivity of which is not phosphorylation dependent (28), revealed a substantial electrostatic mobility shift by 4 h incubation (Fig. 1B). These slowly migrating tau species were strongly labeled by monoclonal antibodies AT8 and PHF1, which selectively bind phospho-tau residues Ser202,Thr205 (34) and Ser396/Ser404 (35), respectively. These data show that the
CK1 isoform Cκi could directly phosphorylate tau protein, and that monoclonal antibodies AT8 and PHF1 could be used as probes for Cκi-mediated phosphorylation reactions.

**Cki Phosphorylates Tau in Situ**—To determine whether Cκi could modulate tau phosphorylation in situ, HEK-293 cells were transiently transfected with plasmids encoding htau40 and Cκi, p25 (an activator of Cdk5), or empty pcDNA vector. Transfection times of 24 h were employed because cells remained viable during this time frame in all studies reported herein (data not shown). Cdk5 activator p25 was included because of its ability to activate endogenous Cdk5 in situ (36) and therefore serve as an alternate source of tau hyperphosphorylation activity. Total tau protein was detected with monoclonal antibody Tau5, whereas phospho-tau was detected with antibodies AT8 and PHF1. All cells transfected with htau40 constructs contained detectable levels of tau protein (Fig. 2A). When these cells were co-transfected with empty vector (i.e. in the absence of exogenous protein kinase), a portion of total tau was PHF1 and AT8 positive, reflecting basal levels of tau phosphorylation. Basal levels of Cκi also were substantial in these cells as demonstrated by Western analysis using monoclonal antibody 128A. However, cotransfection with DNA constructs encoding Cκi significantly increased the amounts of both Cκi and phospho-tau detectable by both the AT8 and PHF1 antibodies (Fig. 1A). These data recapitulate the pattern seen in vitro (Fig. 1A) and suggest that Cκi overexpression significantly increased tau phosphorylation in situ (Fig. 2B). Expression of p25 also induced a large increase in tau phosphorylation, primarily because of the absence of endogenous p25 in non-transfected cells (Fig. 2B).

To confirm that the increased levels of Cκi observed upon overexpression were accompanied by increases in phosphotransferase activity, Cκi was immunoprecipitated and subjected to in vitro kinase assays using htau40 and [γ-32P]ATP as substrates 24 h after transient transfection with Cκi/tau or pcDNA3.1/tau constructs. Radioactive assay reaction products were separated by SDS-PAGE, stained with Coomassie Blue, and visualized on a phosphorimager. Although Cκi activity was found in both the Cκi/tau overexpressing and pcDNA3.1/tau control cells (Fig. 3A), quantitation of the data showed that Cκi overexpression led to ~2-fold increases in recoverable phosphotransferase activity (Fig. 3B). These data confirmed that Cκi overexpression lead to increased Cκi activity within HEK-293 cells, but also showed that significant levels of Cκi activity were present under basal conditions.

**Cκi and Tau Directly Interact in Situ**—The Cκi-mediated tau phosphorylation observed in HEK-293 cells could be direct or arise from an indirect protein phosphorylation cascade. To distinguish these alternatives, tau and Cκi were separately immunoprecipitated from stable tau cells and subjected to Western analysis using monoclonal antibodies 128A or Tau5. Results showed that tau and Cκi coimmunoprecipitated in both experimental paradigms (Fig. 4, A and D). These data suggest that tau associates with Cκi in situ, and therefore potentially serves as a direct substrate for Cκi-mediated phosphorylation reactions.

To determine the region of Cκi involved in binding tau protein, htau40 (non-His6 tagged) was incubated with or without the truncation mutant CκiΔ317 in vitro and immunoprecipitated with antibody 128A. Subsequent Western analysis with antibodies 128A and Tau5 confirmed direct association...
between truncated Ckiδ and htau40 (Fig. 4B). These data suggest that at least a portion of the amino acid sequences mediating direct binding of Ckiδ to tau protein are located in the protein kinase catalytic domain.

**CK1 Inhibitor IC261 Reverses Tau Hyperphosphorylation Induced by Ckiδ Overexpression**—IC261 is a small molecule, membrane-permeable, ATP-competitive inhibitor of CK1 isoforms including Ckiδ (14, 37). To determine whether inhibitor treatment could selectively reverse Ckiδ-mediated tau hyperphosphorylation, HEK-293 cells were co-transfected with Ckiδ or P25/tau, P25/tau, or pcDNA3.1/tau for 24 h, treated with and without IC261 (10 μM) for 30 min, and then probed for phospho and total tau levels with antibodies PHF1, AT8, and Tau5. The resultant Western blots showed that IC261 inhibited tau phosphophorylation induced by Ckiδ overexpression without affecting expression levels of Ckiδ (Fig. 5A). Inhibition was selective for CK1 as shown by the failure of IC261 to significantly modulate tau phosphorylation induced by p25 overexpression (Fig. 5B). These findings are consistent with the inhibitory selectivity of IC261 determined both in vitro and in situ (37, 38), and confirm the utility of IC261 for inhibition of CK1 activity in culture. Moreover, they suggest that Ckiδ-mediated tau phosphorylation is opposed by endogenous phosphoprotein phosphatase activity that can decrease occupancy of phosphorylation sites in response to IC261 over time courses as short as 30 min.

**Endogenous CK1 Contributes to Basal Levels of Tau Phosphorylation**—The above data showed that basal levels of both Ckiδ and phospho-tau were substantial in HEK-293 cells expressing tau protein, and were consistent with previous studies showing that phosphorylation sites Ser396 and Ser202 were partially occupied in both stable and transiently transfected 3T3, Chinese hamster ovary, and SH-SY5Y cells (39–41). To assess the contribution of endogenous Ckiδ activity to basal levels of tau phosphorylation, an HEK-293 cell line stably expressing htau40 was generated and employed in two experimental approaches. First, the dependence of tau phosphorylation on activity contributed by all CK1 isoforms was estimated by treating stable tau-HEK-293 cells with varying concentrations of IC261. In the absence of inhibitor, significant amounts of tau were detectable in this cell line, with a portion being PHF1 reactive (Fig. 6A). Although addition of IC261 for 30 min did not change total tau levels (i.e., detectable with antibody Tau5), it did result in large decreases in levels of phospho-tau detected by antibody PHF1 (Fig. 6A). Inhibition of tau phosphorylation was dose dependent, with an IC50 of 1.5 ± 0.5 μM and up to 71.9 ± 7.5% of PHF1 reactivity at saturation (Fig. 6B). In vitro, IC261 has been shown to inhibit purified recombinant Ckiδ with an IC50 of 1.1 ± 0.1 μM and 98.1 ± 3.2% of activity at saturation (Fig. 6B; data replotted from Fig. 1 of Ref. 37). These data suggest that CK1 activity makes a major contribution to basal levels of tau phosphorylation in HEK-293 cells.

As a second approach, the specific contribution of CK1 isoforms Ckiδ/ε to basal tau phosphorylation was assessed by siRNA-mediated down-regulation conducted 48 h after transfection. RNA interference promotes hydrolysis of targeted mRNA in a sequence-specific reaction (42, 43). The levels of total tau, phosphorylated tau, and Ckiδ were then detected by immunoblot analysis as a negative control, siRNA oligonucleotides selective for luciferase (GL2) were employed. Compared with GL2 treatment, Ckiδ/ε siRNA lowered Ckiδ levels ~30% 48 h after transfection and decreased phosphorylated tau about 25% as well (Fig. 7B). RNA interference did not change the expression levels of total tau (Fig. 7A). These data indicate that either down-regulating Ckiδ activity or its protein level de-represses tau phosphorylation, and suggests that Ckiδ/ε contrib-
tau decreased in response to phosphorylation induced by Cki.

HEK-293 cells transiently transfected (24 h) with tau/p25, tau/Cki, or empty vector/tau were treated with either 10 µM IC261 (IC), or vehicle control (Con) for an additional 30 min. Cell lysates were then prepared and subjected to SDS-PAGE (30 µg/lane). A, representative immunoblots using monoclonal antibodies AT8, PHF1 Tau5, 128A, and C-19 show that IC261 inhibited basal and Cki-induced tau phosphorylation without modulating the levels of total tau or Cki. In contrast, neither p25 levels nor p25-induced tau phosphorylation was significantly affected by IC261. B, phospho-tau levels were quantified by densitometry from three independent experiments, normalized to levels of total tau, and plotted as percent phospho-tau in untreated, empty vector/tau-transfected cells. **, p < 0.01 compared with untreated empty vector/tau-transfected cells. ##, p < 0.01 compared with untreated Cki/tau-transfected cells.

To confirm these findings, HEK-293 cells transiently cotransfected with Cki/tau or pcDNA/tau for 24 h were detergent extracted, fractionated into soluble (S1) and particulate (P1) fractions (the latter containing microtubules), and subjected to Western analysis using antibodies Tau5 and PHF1.

Phospho-tau (detected by PHF1) was found exclusively in the soluble fraction, with greater amounts recovered from cells transfected with Cki/tau than with pcDNA/tau (Fig. 8B). In contrast, significant amounts of total tau (detected by Tau5) were retrieved in both particulate and soluble fractions (Fig. 8B). However, the subcellular distribution of total tau shifted toward the soluble pool in cells transfected with Cki/tau compared with pcDNA/tau cells (Fig. 8C).

Together these data suggest that the microtubule binding activity of tau decreases in response to Cki-mediated phosphorylation. To test this hypothesis, soluble tau was prepared from each of the cell populations described above (fraction S1) was subjected to in vitro microtubule binding assays. Results showed that a portion of soluble tau from pcDNA/tau cells retained an ability to associate with synthetic microtubules (Fig. 8D), consistent with tau overexpression leading to saturation of endogenous microtubules. In contrast, soluble tau prepared from Cki/tau cells was almost devoid of microtubule binding activity (Fig. 8D), despite accumulating to higher levels than in control extracts (Fig. 8, C and D). These data suggest that the accumulation of soluble tau observed in situ upon Cki overexpression results from phosphorylation of tau protein at sites that directly modulate microtubule binding.

**DISCUSSION**

**Cki Isozymes and Tau Phosphorylation**—Because of its natively unfolded structure and high complement of hydroxylamino acids, tau is an efficient substrate for many protein kinases in vitro (44), including tissue-derived Cki activity (13, 16). Here it was found that Cki serves as a tau protein kinase in situ as well. First, studies with the selective inhibitor IC261 showed that over 70% of basal tau phosphorylation at Ser396/398 and Ser404 sites in HEK-293 cells stems from Cki activity. Supporting this conclusion, the IC50 for IC261 in situ was only slightly higher than the value determined in vitro with purified
Ciō. Moreover, the IC261 inhibition isotherm was consistent with a single class of binding site. Because IC261 inhibits most CK1 isoforms with similar potency (37), basal levels of tau phosphorylation could potentially result from the activity of multiple CK1 isoforms. But the decrease in phospho-tau accompanying down-regulation of Ciō using RNA interference suggests that these specific isoforms compose at least a portion of IC261-sensitive tau phosphorylation activity under basal conditions. Indeed, Ciō and tau could be co-immunoprecipitated from stable tau cells containing only basal levels of Ciō. These data demonstrate that tau and Ciō directly associate in situ, and are consistent with their colocalization to microtubules (16).

Second, overexpression of Ciō increased tau phosphorylation at sites it phosphorylated in vitro. Again, IC261 totally reversed the tau hyperphosphorylation induced by Ciō overexpression. However, the recognition sequence mediating the substrate selectivity of Ciō is not understood in the context of tau phosphorylation. On the basis of studies with short peptides, CK1 isoforms are phosphotrophic kinases that recognize the motif S/T(P)XXS/T (45, 46). Nonetheless, priming of substrates with phosphate is not a strict requirement, and motifs consisting of acidic residues N- or C-terminal to the phosphorylatable residue also are phosphorylated by CK1 (20, 47, 48). Although our in vitro data showing that Ciō phosphorylated recombinant tau at Ser202/Thr205 and Ser396/Ser404 is consistent with a previous study showing that tissue-derived CK1 activity phosphorylates tau at Thr231, Ser396/Ser404 (17), the relationship between these sites and the motifs summarized above is weakly apparent only for Ser396/Ser404. It appears that recognition of full-length tau as substrate differs from that of short peptides. In any event, phosphorylation of tau at Ser202/Thr205 and Ser396/Ser404 by Ciō does not require priming by other protein kinases.

Consequences of CK1-mediated Tau Phosphorylation—Tau protein binds and stabilizes microtubules (49). However, this microtubule stabilizing function is regulated by its phosphorylation state. For example, hyperphosphorylated tau binds to microtubules weakly, but strong binding can be restored after dephosphorylation (4, 50, 51). Thus, the stability of the axonal microtubule-tau association is thought to be regulated by the posttranslational modification state of phospho-tau. This notion has been based on the observation that dephosphorylated tau associates with microtubules weakly, whereas phospho-tau binds tightly (49). However, this relationship between these sites and the motifs summarized above is weakly apparent only for Ser396/Ser404. It appears that recognition of full-length tau as substrate differs from that of short peptides. In any event, phosphorylation of tau at Ser202/Thr205 and Ser396/Ser404 by Ciō does not require priming by other protein kinases.

Consequences of CK1-mediated Tau Phosphorylation—Tau protein binds and stabilizes microtubules (49). However, this microtubule stabilizing function is regulated by its phosphorylation state. For example, hyperphosphorylated tau binds to microtubules weakly, but strong binding can be restored after dephosphorylation (4, 50, 51). Thus, the stability of the axonal microtubule-tau association is thought to be regulated by the posttranslational modification state of phospho-tau. This notion has been based on the observation that dephosphorylated tau associates with microtubules weakly, whereas phospho-tau binds tightly (49). However, this relationship between these sites and the motifs summarized above is weakly apparent only for Ser396/Ser404. It appears that recognition of full-length tau as substrate differs from that of short peptides. In any event, phosphorylation of tau at Ser202/Thr205 and Ser396/Ser404 by Ciō does not require priming by other protein kinases.

Consequences of CK1-mediated Tau Phosphorylation—Tau protein binds and stabilizes microtubules (49). However, this microtubule stabilizing function is regulated by its phosphorylation state. For example, hyperphosphorylated tau binds to microtubules weakly, but strong binding can be restored after dephosphorylation (4, 50, 51). Thus, the stability of the axonal microtubule-tau association is thought to be regulated by the posttranslational modification state of phospho-tau. This notion has been based on the observation that dephosphorylated tau associates with microtubules weakly, whereas phospho-tau binds tightly (49). However, this relationship between these sites and the motifs summarized above is weakly apparent only for Ser396/Ser404. It appears that recognition of full-length tau as substrate differs from that of short peptides. In any event, phosphorylation of tau at Ser202/Thr205 and Ser396/Ser404 by Ciō does not require priming by other protein kinases.
cytoskeleton may be influenced the equilibrium between tau phosphorylation and dephosphorylation. Phosphorylation sites that mediate this activity include Ser^{202}, Ser^{235}, Ser^{356}, and Ser^{404} (55), whereas Cdk5/p25 has been shown to phosphorylate tau at Thr^{181}, Ser^{202}, Thr^{235}, Thr^{237}, Ser^{396}, and Ser^{403} (54, 67, 68). Thus GSK3β and Cdk5/p25 potentially join CK1 in modulating tau/microtubule equilibrium (69). Although the potential interplay among these enzymes is unknown, it is interesting to note that GSK3β functions primarily as a phosphate-directed protein kinase dependent on priming of substrates by other protein kinases (70). Because of its strong contribution to basal tau phosphorylation state and inducibility in response to stress, CK1 could potentially provide priming activity as it does with β-catenin phosphorylation (71). Yet CK1 also recognizes primed substrates as in the case of p35 (72), and in this substrate recognition mode could potentially further amplify disease-associated phosphorylation of sites found tightly clustered around the microtubule repeat region (2, 3).

Finally, we note that CK1 phosphorylates proteins in addition to tau in vivo. In the cases of p35 (72), β-catenin (73), multidrug transporter Pdr5 (74), Hedgehog signaling effector Cubitus interruptus (75), and proteins involved in circadian rhythm control (76), CK1-mediated phosphorylation modulates protein turnover. This function may be part of the neuronal response to stress, accounting for both the induction of CK1 in AD and its presence in granulovacuolar degeneration bodies. Thus CK1-mediated tau phosphorylation may result from an attempt by the cell to modulate protein turnover in response to cellular distress.

Acknowledgments—We thank Dr. Rick Dobrowsky, University of Kansas, for providing the p25-pCDNA construct, Dr. Peter Davies, Albert Einstein College of Medicine, for providing PHF1 antibody, and Dr. Lester I. Binder for providing monoclonal antibody Tau5.

REFERENCES

1. Buee, L., Bussiere, T., Buee-Scherrer, V., Delacourte, A., and Hof, P. F. (2000) Brain Res. Brain Res. Rev. 33, 95–130
2. Hanger, D. P., Bettis, J. C., Loviyy, T. L., Blackstock, W. P., and Anderton, B. H. (1998) J. Neurochem. 71, 2465–2476
3. Morishima-Kawashima, M., Hasegawa, M., Takio, K., Suzuki, M., Yoshida, H., Watanabe, A., Titani, K, and Ihara, Y. (1999) Neurobiol. Aging 16, 386–380
4. Bramlett, C. T., Goedert, M., Jakes, R., Merrick, S. R., Trojanowski, J. Q., and Lee, V. M (1993) Neuron 10, 1098–1099
5. Wagner, U., Utton, M., Galle, J., and Miller, C. (1996) J. Cell. Sci. 109, 1527–1543
6. Fish, K. J., Cegielska, A., Getman, M. E., Lands, G. M., and Virshup, D. M. (1995) J. Biol. Chem. 270, 14875–14883
7. Graves, P., Haas, D., Hagen, M.M., DePaoli-Roach, A., and Roach, P. (1993) J. Biol. Chem. 268, 6394–6401
8. Rowles, J., Slaughter, C., Moonaw, C., Hsu, J., and Cobb, M. H. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 8548–8552
9. Zhai, L., Graves, P. R., Robinson, L. C., Italiano, M., Colbert, M. R., Rowles, J., Cobb, M. H., DePaoli-Roach, A. A., and Roach, P. J. (1995) J. Biol. Chem. 270, 12717–12724
10. Kitabayashi, A. N., Kusuda, J., Hira, M., and Hashimoto, K. (1997) Genomics 46, 133–137
11. Xu, R. M., Carmel, G., Sweet, R. M., Kuret, J., and Cheng, X. (1995) EMBO J. 14, 1015–1023
12. Simkowski, K., and Tao, M. (1980) J. Biol. Chem. 255, 6456–6461
13. Singh, T., Grundke-Iqbal, I., and Iqbal, K. (1995) J. Neurochem. 64, 1420–1423
14. Behrend, L., Milne, D. M., Sotter, M., Deppert, W., Campbell, L. E., Meek, D. W., and Knippschild, U. (2000) Oncogene 19, 5301–5313
15. Okochi, M., Walter, J., Kayama, A., Nakajo, S., Baba, M., Iwatsubo, T., Meier, M., Kohle, P. J., and Haass, C. (2000) J. Biol. Chem. 275, 390–397
16. Behrend, L., Sotter, M., Kurth, M., Rutter, G., Heukeshoven, J., Deppert, W., and Knippschild, U. (2000) Eur. J. Cell Biol. 79, 240–251
17. Singh, T. J., Zaidi, T., Grundke-Iqbal, I., and Iqbal, K. (1996) Mol. Cell. Biochem. 154, 143–151
18. Cegielska, A., Gietzen, K. F., Rivers, A., and Virshup, D. M. (1998) J. Biol. Chem. 273, 1857–1864
19. Knippschild, U., Milne, D. M., Campbell, L. E., DeMaggio, A. J., Christenson, D. W., and Knippschild, U. (2000) J. Neurochem. 77, 1727–1736
20. Marini, O., Bastis, V. H., Cesario, L., Meglio, F., Pagano, M. A., Antonelli, M., Allerhand, C. A., Pinna, L. A., and Allerhand, J. E. (2003) Proc. Natl. Acad. Sci. U.S.A. 100, 10193–10200
21. Gross, S., Simery, C., Schatten, G., and Anderson, R. (1997) J. Cell Sci. 110, 3833–3890
22. Ghoshal, N., Smiley, J. F., DeMaggio, A. J., Hoeckstra, M. F., Cochran, E. J., Binder, L. I., and Kuret, J. (1999) Am. J. Pathol. 155, 1163–1172
23. Yasojima, K., Kuret, J., DeMaggio, A. J., McGee, E., and McGee, P. L. (2000) Brain Res. 865, 116–129
24. Hu, J. H., Chernoff, K., Pelech, S., and Krieger, C. (2003) J. Neurochem. 85, 422–431
