NIPP1 is a ubiquitous nuclear protein that is required for spliceosome assembly. We report here that the phosphothreonine-binding Forkhead-associated domain of NIPP1 interacts with the cell cycle-regulated protein Ser/Thr kinase MELK (maternal embryonic leucine zipper kinase). The NIPP1-MELK interaction was critically dependent on the phosphorylation of Thr-478 of MELK and was increased in lysates from mitotically arrested cells. Recombinant MELK was a potent inhibitor of an early step of spliceosome assembly in nuclear extracts. This splicing defect was also seen with a kinase-dead mutant but was absent after mutation (T478A) of the NIPP1 binding site of MELK, indicating a mediatory role for NIPP1. Our data suggest that NIPP1 has a role in the cell cycle-regulated control of pre-mRNA splicing.

The nuclear protein NIPP1$^1$ (39 kDa) was originally discovered as a potent and specific inhibitor of protein Ser/Thr phosphatase-1 (PP1), hence its name, nuclear inhibitor of PP1 (PPI) (1–7). More recently, we have demonstrated that NIPP1 is also implicated in transcription as well as in pre-mRNA splicing by mechanisms that do not involve PP1 (8, 9). In transient transfection experiments, NIPP1 acted as a transcriptional repressor, which may be accounted for by the binding of the central and C-terminal domains of NIPP1 to the Polycomb protein, EED (embryonic ectoderm development) (9). The latter promotes transcriptional repression by the recruitment of a histone methyltransferase and histone deacetylases. NIPP1 also appears to be required for the assembly of spliceosomes, the protein-RNA complexes that catalyze pre-mRNA splicing (8). The splicingosomal function of NIPP1 requires its C-terminal domain as well as its N-terminal Forkhead-associated (FHA) domain, an established phosphothreonine-binding module. The FHA domain of NIPP1 mediates targeting to both the spliceosomes and the nuclear storage sites for splicing factors, known as “speekles” (8, 10). The targeting function of the FHA domain of NIPP1 is likely explained by its ability to bind to phospho-rylated forms of the essential splicing factors CDC5L (11) and SAP155 (12).

Here we show that the protein kinase MELK, which is structurally related to the AMP-activated protein kinases, also interacts in a phosphorylation-dependent manner with the FHA domain of NIPP1 and that this interaction is increased during mitosis. Furthermore, we demonstrate that recombinant MELK blocks spliceosome assembly by a mechanism that involves NIPP1. Our data suggest a novel link between pre-mRNA processing and cell cycle progression.

**EXPERIMENTAL PROCEDURES**

**Yeast Two-hybrid Screening—**NIPP1 (1–142), cloned into the pEG202 vector in-frame with the LexA DNA-binding domain, was used as bait for the screening of a HeLa cell cDNA library (11). In this library, the cDNAs are subcloned behind a galactose-inducible promoter in the pG4–5 vector in-frame with the B42 activation domain. Inter-acting proteins were identified by growth of the yeast strain EGY158 in a -leucine/+-galactose medium. The use of a plasmid-borne LacZ reporter gene (pSH18–34) with upstream LexA operators enabled a second, independent screening involving the expression of β-galactosidase in the presence of galactose. For the mapping of the NIPP1-MELK interaction site, MELK (1–651), MELK-(1–291/590–651), and 10 different Thr to Ala mutants of MELK-(326–651) were subcloned in the pG4–5 plasmid. MELK-(1–651), kindly provided as clone KIAA0175 by Dr. Nagase (Kazusa DNA Research Institute, Chiba, Japan), was subcloned by PCR with primers CTGGAATTCATGAAAGATTATGAT-GAATTCA and TCTGCTCCGATTTAATTTTCGCGCTGATAG-TAGG into the EcolR/XhoI sites of pG4–5. MELK-(1–291/590–651) was obtained by cutting pJG4–5-MELK-(1–651) with Bsp1407I and religation. All constructs were verified by DNA sequence analysis. The interaction between NIPP1 (1–142) and the MELK variants was quantified by measuring β-galactosidase activity in a liquid culture assay using 2-nitrophenyl-β-D-galactopyranoside as substrate (Clontech yeast protocols handbook).

**Antibodies—**Bacterially expressed polyhistidine-tagged MELK was used to raise antibodies in rabbits. The MELK antibodies were affinity-purified on His-MELK linked to CNBr-activated Sepharose 4B (Amer- sham Biosciences). Anti-GST antibodies (sc-459), Anti-His antibodies (sc-803), and Anti-LexA antibodies (Sc-7544) were obtained from Santa Cruz Biotechnology. Swine anti-rabbit and rabbit anti-mouse antibod- ies were purchased from Dako. Anti-FLAG antibodies were obtained from Stratagene. Mouse monoclonal anti-HA antibodies (12CA5) were delivered by Roche Diagnostics.

**Preparation of Recombinant MELK (Mutants)—**Wild-type MELK and MELK-(326–651) were cloned into the pET16b vector in-frame with a polyhistidine tag. The His-tagged proteins were purified by chromatography on Ni$^{2+}$-agarose-Sepharose (AffiLyn). For the expression of FLAG-tagged MELK (mutants) in COS-1 cells the cDNAs of human MELK-wild-type, MELK-T478A, MELK-D150A, and MELK-1–266 were cloned as BamHI fragments into the pSG5-Flag vector. The His-tagged proteins were purified on His-MELK linked to CNBr-activated Sepharose 4B (Amer- sham Biosciences). Anti-GST antibodies (sc-459), Anti-His antibodies (sc-803), and Anti-LexA antibodies (Sc-7544) were obtained from Santa Cruz Biotechnology. Swine anti-rabbit and rabbit anti-mouse antibod- ies were purchased from Dako. Anti-FLAG antibodies were obtained from Stratagene. Mouse monoclonal anti-HA antibodies (12CA5) were delivered by Roche Diagnostics.
grown as monolayer cultures at 27 °C in Express Five serum-free medium (Invitrogen). The cells were infected with the recombinant viruses at a multiplicity of infection of 10 and harvested after 48 h by low-speed centrifugation. As most of MELK and its mutants were expressed in insoluble inclusion bodies, we followed the procedure described by Berndt and Cohen (13) to dissolve and renature the insoluble species.

**Cell Cultures**—COS-1 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 100 units/ml of both penicillin and streptomycin. Cells were blocked in mitosis by the addition of colcemid (0.14 μg/ml) 20–24 h before lysis. 48 h after transfection, the cells were washed twice with ice-cold phosphate-buffered saline and lysed in 50 mM Tris/HCl, pH 7.5, 0.3 M NaCl, 0.5% Triton X-100, 0.5 mM diethiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM benzamidine, and 5 μM leupeptin. After sonication, the lysates were cleared by centrifugation (10 min at 16,000 × g), and the supernatants were used for GST pull-down assays.

**GST Pull-down Assays**—For the expression of NIPP1 as a GST fusion in mammalian cells, the cDNA of wild-type NIPP1 was cloned into the EcoRV-NotI sites of the pGMEK-T1 vector. Glutathione-agarose beads were pre-blocked with Tris-buffered saline plus bovine serum albumin (1 mg/ml) and 0.5% Triton X-100. After washing with Tris-buffered saline plus 0.1% Nonidet P-40, the beads were incubated with COS-1 cell lysates (Fig. 6) for 2 h at 10 °C. Subsequently, the beads were sedimented (30 s at 1000 × g) and washed twice with Tris-buffered saline plus 0.1% Nonidet P-40 and twice with Tris-buffered saline, and finally the associated proteins were analyzed by Tricine-SDS-polyacrylamide gel electrophoresis and immunoblotting. GST pull-down experiments with recombinant proteins and HeLa cell nuclear extracts were performed as described previously (11).

**Pre-mRNA Splicing in Nuclear Extracts**—A capped β-globin pre-mRNA fragment, comprising exon 1 through the BamHI site in exon 2, was synthesized in the presence of [α-32P]GTP. This primary transcript was used as a substrate for splicing in HeLa cell nuclear extracts (8). Spliceosomal complexes were separated by native gel electrophoresis as described previously (8).
RESULTS

**MELK Is a Novel Interactor of NIPP1**—A yeast two-hybrid screening of a HeLa cell library with the N-terminal third of NIPP1 (residues 1–142) as bait yielded, in addition to clones encoding CDC5L (11) and SAP155 fragments (12), 16 prey clones that all encoded the C-terminal half of the poorly characterized protein kinase MELK (for maternal embryonic leucine zipper kinase) (14) (Fig. 1). Compared with this MELK fragment, the full-length protein was a much better interactor with NIPP1 in two-hybrid assays (Fig. 1B). However, a deletion mutant of MELK, lacking the TP-rich domain, did not interact with NIPP1-(1–142). The MELK-NIPP1 interaction was lost by mutation (S68A/R69A/V70A/H71A) of the phosphate-binding loop of the FHA domain of NIPP1 (Fig. 1B), indicating that MELK interacted with the FHA domain of NIPP1 via a phosphorylated residue(s). Fig. 1C shows that all bait and prey proteins were well expressed, further validating the two-hybrid data.

We have previously demonstrated that CDC5L (11) and SAP155 (12) bind to the FHA domain of NIPP1 via threonine-phosphorylated TP dipeptide motifs. The fragment of MELK that binds to NIPP1 also contains numerous TP motifs (Fig. 1A). To examine whether one or several of these TP motifs are involved in the interaction with NIPP1, we performed two-hybrid assays after mutation (Thr to Ala) of each of the 10 TP motifs in the NIPP1-interaction domain of MELK (Fig. 2). All mutants were expressed well (Fig. 2B). However, four mutants showed a significantly reduced interaction with NIPP1 in the two-hybrid assays. The T478A mutant in particular was severely impaired in binding NIPP1. These data imply some TP motifs of MELK, in particular the TP motif comprising Thr-478 and Pro-479, in the interaction with NIPP1.

**Interaction of Purified Components**—To further explore whether MELK and NIPP1 interact directly and in a phosphorylation-dependent manner, we expressed the NIPP1-interacting C-terminal domain of MELK (residues 326–651) as a His-tagged protein in bacteria. Purified recombinant MELK-(326–651) did not sediment with GST-tagged NIPP1-(1–142) (Fig. 3). However, we noted that a preincubation of MELK-(326–651) with a HeLa cell nuclear extract in the presence of Mg-ATP enabled its interaction with GST-NIPP1-(1–142). Importantly, a co-sedimentation with GST-NIPP1-(1–142) was not seen after a preincubation of the T478A mutant with nuclear extracts under phosphorylation conditions (Fig. 3). The above data showed that MELK interacts directly with the FHA domain of NIPP1 and that this interaction is largely mediated by phosphorylated Thr-478. In further agreement with this interpretation, we found that the synthetic phosphopeptide MELK-(470–487)-phospho-Thr-478 could be used as a competitor to disrupt the interaction between GST-NIPP1-(1–142) and its FHA ligand, CDC5L, in nuclear extracts (Fig. 3B). By contrast, the nonphosphorylated MELK peptide did not have this effect.

**MELK Is an Inhibitor of Spliceosome Assembly**—To explore the role of the NIPP1-MELK interaction, we prepared purified bacterially expressed NIPP1 and baculovirus-expressed MELK. In addition to wild-type MELK we generated a NIPP1-binding mutant (MELK-T478A) as well as a kinase-dead mutant (MELK-D150A). The latter is mutated in the essential "DFG" triplet in kinase subdomain VII, which is required for the binding of Mg-ATP (15). All three proteins were expressed in Sf9 insect cells as insoluble inclusion bodies and were inactive. However, the inclusion bodies could be dissolved in 6 M guanidinium chloride, and MELK could be renatured by extensive and rapid dilution with a buffer containing Mn2+, as described previously for the catalytic subunit of PP1 (13). The renatured wild-type MELK was capable of phosphorylating exogenous substrates such as myelin basic protein (Fig. 4) as well as histones H1, 2A, and 3 (not shown). In addition, autophosphorylation of MELK was detected (Fig. 4). As expected, the D150A mutant was inactive, but surprisingly, at equal protein concentrations the T478A mutant was much more active than the wild-type protein.

Recombinant NIPP1 did not affect the ability of baculovirus-expressed MELK to autophosphorylate or to phosphorylate myelin basic protein (not shown). Because NIPP1 is an established pre-mRNA splicing factor, we subsequently examined whether the interactor MELK also has a role in pre-mRNA splicing. Fig. 5A shows that the addition of 1 μM wild-type MELK completely blocked the splicing of a fragment of BG-globin pre-mRNA by HeLa cell nuclear extracts. Interestingly, the kinase-dead mutant of MELK had the same effect, indicating that the splicing inhibition was independent of phosphorylation.

---

**Fig. 2.** Role of the TP dipeptide motifs of MELK in the interaction with NIPP1. A, two-hybrid interaction of MELK-(326–651) and the indicated point mutants with NIPP1-(1–142). The results are expressed as units ± S.E. for five observations. B, immunoblot with anti-HA antibodies showing expression of the MELK variants.

**Fig. 3.** Interaction of purified components. The enrichment of recombinant NIPP1 and baculovirus-expressed MELK in the FHA domain of NIPP1 was determined in a two-hybrid assay. The MELK-NIPP1 interaction was lost by mutation (S68A/R69A/V70A/H71A) of the phosphate-binding loop of the FHA domain of NIPP1 (Fig. 1B), indicating that MELK interacted with the FHA domain of NIPP1 via a phosphorylated residue(s).
Inhibition of Pre-mRNA Splicing by Protein Kinase MELK

A. GST pull-down

![Diagram of GST pull-down experiment]

B. Competition

![Diagram of competition experiment]

**Fig. 3. Phosphorylation-dependence of the MELK-NIPP1 interaction.** A, bacterially expressed MELK-(326–651) or MELK-(326–651)-T478A (0.12 mg/ml) was incubated for 30 min at 30 °C with HeLa cell nuclear extracts in the absence (−) or presence (+) of MgCl₂ (2 mM) and ATP (0.1 mM). The figure is an immunoblot with His antibodies showing the amount of the recombinant MELK fragments that co-sedimented with exogenous GST-NIPP1 (1–142) after preincubation with the nuclear extracts. B, pull-down with GST-NIPP1 (1–142) of CDC5L from HeLa cell nuclear extracts after preincubation of the extracts for 30 min with MgCl₂ (4 mM) and ATP (1 mM). The figure also shows the effect of the addition of 750 μM H9262 B with the nuclear extracts for 30 min with MgCl₂ (4 mM) and ATP (1 mM). The figure is an immunoblot with His antibodies showing the amount of the recombinant MELK fragments that co-sedimented with exogenous GST-NIPP1 (1–142) after preincubation with the nuclear extracts. C, pull-down with GST-NIPP1 (1–142) of CDC5L from HeLa cell nuclear extracts after preincubation of the extracts for 30 min with MgCl₂ (4 mM) and ATP (1 mM). The figure also shows the effect of the addition of 750 μM H9262 B with the nuclear extracts for 30 min with MgCl₂ (4 mM) and ATP (1 mM). The figure is an immunoblot with His antibodies showing the amount of the recombinant MELK fragments that co-sedimented with exogenous GST-NIPP1 (1–142) after preincubation with the nuclear extracts.

**Fig. 4. Catalytic activity of baculovirus-expressed MELK.** Full-length MELK and the indicated point mutants (T478A, loss of binding to NIPP1; D150A, kinase dead) were generated as described under “Experimental Procedures.” The figure shows the ability of the MELK variants to autophosphorylate (top panel) and to phosphorylate myelin basic protein (MBP) (middle panel). The MELK variants were used at an equal concentration as detected by immunoblot analysis with MELK antibodies (bottom panel).

**DISCUSSION**

The MELK-NIPP1 Interaction—We have shown here that MELK is an interactor of the FHA domain of NIPP1 and that this interaction involves the binding of a threonine-phosphorylated TP motif to the phosphate binding loop of the FHA domain. The TP motif of MELK appears to be the most critical for the interaction with NIPP1 in yeast cells, in COS-1 cells (Fig. 6), and in vitro with recombinant proteins (Fig. 3) comprises Thr-478 and Pro-479. However, as we have previously demonstrated for SAP155 (12), it is possible that other TP motifs of MELK, when phosphorylated, can also mediate binding to NIPP1. It will therefore be important to identify the protein kinase(s) that phosphorylates the TP motifs of MELK and to examine whether the activation of this kinase(s) results in an increased interaction with NIPP1. Interestingly, all three established ligands of the FHA domain of NIPP1, i.e. CDC5L (19), SAP155 (12), and MELK (17, 18), are hyperphosphorylated during mitosis, and at least in the case of SAP155 (12) and MELK (this work), this phosphorylation includes threonine residues that bind directly to the FHA domain of NIPP1. Thus, one or more mitotic kinases promote the interaction
The inhibition of spliceosome assembly by recombinant MELK. A, splicing of a radioactively labeled β-globin pre-mRNA fragment by a HeLa cell nuclear extract was carried out at 30°C in the absence or presence of the indicated MELK mutants at 1 μM (see legend to Fig. 4). After 120 min the RNA was phenol-extracted and subjected to denaturing PAGE (9%). The splicing products are indicated schematically. B, splicing of the β-globin pre-mRNA fragment was stopped at the indicated time points by the addition of heparin, and the spliceosomal complexes were separated by native gel electrophoresis. Radioactively labeled RNA was detected by autoradiography. The position of the H + E complexes and A + B + C complexes are indicated. H refers to nonspecific pre-ribonucleoprotein complexes.

The interaction of the FHA domain of NIPP1 with its ligands. A, FLAG-tagged MELK (mutants) and GST-tagged NIPP1 were co-expressed in COS-1 cells. 20–24 h before lysis the cells were either kept dividing asynchronously or were mitotically arrested by the addition of colcemid. GST-NIPP1 was pulled down from the cell lysates with glutathione-agarose, and the co-sedimented MELK (mutants) were visualized by immunoblot analysis with anti-MELK antibodies. GST-NIPP1 in the pellet (B) was visualized with GST antibodies. The expression of the MELK variants in the cell lysates was detected by immunoblot analysis with anti-FLAG antibodies (C). The arrows indicate the positions of MELK variants.

Increased SAP155-NIPP1 and MELK-NIPP1 interactions contribute to the splicing arrest during mitosis or rather are implicated in the re-initiation of splicing in the early G1 phase. It should be pointed out that other splicing factors have also been implicated in connecting RNA splicing with cell cycle progression (21). For example, it has recently been demonstrated that the SR protein SRp35 is required for splicing repression during mitosis (22).

The mitotic kinase that phosphorylates MELK on Thr-478 is unlikely to be MELK itself, since a kinase-dead mutant still showed an increased interaction with NIPP1 during mitosis (Fig. 6). We also found that recombinant MELK is not an in vitro substrate for cyclin-B/Cdk1 (not shown), indicating that Thr-478 is phosphorylated by a distinct mitotic kinase. It should also be pointed out that the increased phosphorylation of MELK on Thr-478 during mitosis is not necessarily the result of an increased MELK kinase activity but could also be accounted for by a reduced MELK phosphatase activity.

NIPP1 and Pre-mRNA Splicing—The inhibition of spliceosome assembly by MELK does not require a functional catalytic site but depends on Thr-478 (Fig. 5), which is essential for the binding of NIPP1. One possible explanation for this inhibition of spliceosome assembly is that MELK competes with other splicing factors for binding to the FHA domain and thereby prevents the recruitment of NIPP1 to the spliceosomes. We have previously demonstrated that the C-terminal third of NIPP1 is required for a late step (B → C transition) of spliceosome assembly and that the formation of the B-complex proceeds normally in the presence of NIPP1-(1–142) (8). Our observation that wild-type MELK, but not the NIPP1-binding mutant, blocks an early step of spliceosome assembly (Fig. 5) could therefore suggest that NIPP1-(1–142) is needed for an early step of spliceosome assembly. Inhibition of spliceosome assembly by MELK in splicing extracts did not require a functional catalytic domain. However, this does not rule out the possibility that MELK also affects pre-mRNA processing in vivo by protein phosphorylation. For example, we have noted that CDC5L and SAP155, as well as NIPP1, are in vitro substrates for phosphorylation by MELK, which could contribute to the regulation of splicing by MELK.

The interaction of the FHA domain of NIPP1 with its ligands is correlated with both splicing catalysis and splicing arrest. On the one hand, a functional FHA domain is required for spliceosome assembly (8), and ligands of the FHA domain, such as...
MELK, competitively block spliceosome assembly (Fig. 5). On the other hand, the FHA domain of NIPP1 is also required for the targeting of NIPP1 to the nuclear storage sites of splicing factors (10), and NIPP1 shows an increased interaction with SAP155 (12) and MELK (Fig. 6) during mitosis, when splicing is arrested. One explanation for these paradoxical findings is that the binding of NIPP1 to some FHA ligands is associated with splicing arrest, whereas the association with other ligands promotes spliceosome assembly. Alternatively, the FHA-mediated targeting of NIPP1 to the nuclear speckles during interphase and to the ceosome assembly. Alternatively, the FHA-mediated targeting of NIPP1 to the nuclear speckles during interphase and to the mitotic storage sites of splicing factors serves as a “priming” mechanism that enables a rapid resumption of pre-mRNA splicing by the recruitment of pre-assembled complexes of splicing factors during spliceosome assembly. Nevertheless, the tight regulation of the interaction between NIPP1 and its ligands by reversible phosphorylation and the fact that NIPP1 is associated with both a protein kinase (MELK) and a protein phosphatase (PP1) suggest a key “integrator” function for NIPP1 in signal transduction to splicing.

Regulation of MELK—Based on the structural conservation of its catalytic domain (23), MELK belongs to the family of CAMK-like protein kinases that also includes protein kinases MARK (24), PASK (25), as well as the AMP-activated protein kinases (26). It is currently not known how the activity of MELK is controlled but this is likely to be complex. MELK is capable of autophosphorylation (Fig. 4), but it is not yet clear whether this also affects its activity. Thr-167 of MELK corresponds to a phosphorylatable Thr within the activation loop of related protein kinases (26, 27). For example, activation of the AMP-activated kinase requires phosphorylation of this Thr in the activation loop by the tumor suppressor LKB1 (27). It also seems likely that the large C-terminal domain of MELK, which includes the TP-rich NIPP1-binding domain, has a regulatory function. Our preliminary data indicate that this domain is inhibitory because the T478A mutant is more active than wild-type MELK (Fig. 3) and because the recombinant C-terminal half of MELK is a strong inhibitor of the kinase activity of MELK. A further exploration of the mechanisms that control the activity of MELK will be essential to elucidate the signaling pathways that impinge on MELK and its interactor NIPP1.

Acknowledgments—We thank Valère Feytons, Annemie Hoogmartens, and Nicole Sente for expert technical assistance.

REFERENCES
1. Beullens, M., Van Eynde, A., Stalmans, W., and Bollen, M. (1992) J. Biol. Chem. 267, 16538–16544
2. Beullens, M., Van Eynde, A., Bollen, M., and Stalmans, W. (1993) J. Biol. Chem. 268, 13172–13177
3. Van Eynde, A., Beullens, M., Stalmans, W., and Bollen, M. (1994) Biochem. J. 297, 447–449
4. Van Eynde, A., Wera, S., Beullens, M., Torrekens, S., Van Leuven, F., Stalmans, W., and Bollen, M. (1995) J. Biol. Chem. 270, 28068–28074
5. Vulsteke, V., Beullens, M., Waelkens, E., Stalmans, W., and Bollen, M. (1997) J. Biol. Chem. 272, 32972–32978
6. Beullens, M., Van Eynde, A., Vulsteke, V., Connor, J., Shenolikar, S., Stalmans, W., and Bollen, M. (1999) J. Biol. Chem. 274, 14053–14061
7. Beullens, M., Vulsteke, V., Van Eynde, A., Jugiello, I., Stalmans, W., and Bollen, M. (2000) Biochem. J. 352, 651–658
8. Beullens, M., and Bollen, M. (2002) J. Biol. Chem. 277, 19855–19860
9. Jin, Q., Van Eynde, A., Beullens, M., Roy, N., Thié, G., Stalmans, W., and Bollen, M. (2003) J. Biol. Chem. 278, 30677–30685
10. Jugiello, I., Van Eynde, A., Vulsteke, V., Beullens, M., Boudrez, A., Keppens, S., Stalmans, W., and Bollen, M. (2000) J. Cell Sci. 113, 5761–5764
11. Boudrez, A., Beullens, M., Groenen, P., Van Eynde, A., Vulsteke, V., Jugiello, I., Murray, M., Kraimer, A. R., Stalmans, W., and Bollen, M. (2000) J. Biol. Chem. 275, 25411–25417
12. Boudrez, A., Beullens, M., Waalvens, E., Stalmans, W., and Bollen, M. (2002) J. Biol. Chem. 277, 31834–31841
13. Berndt, N., and Cohen P. T. W. (1990) Eur. J. Biochem. 190, 291–297
14. Heyer, B. S., Kochanowski, H., and Solter, D. (1999) Dev. Dyn. 215, 344–351
15. Hands, S. P., Hunter, T. (1995) PNAS J. 9, 576–596
16. Reed, R. (2000) Curr. Opin. Cell Biol. 12, 340–345
17. Blot, J., Chartrain, I., Roghi, C., Philippe, M., and Tassan, J.–P. (2002) Dev. Biol. 241, 327–338
18. Davezac, N., Baldis, V., Blot, J., Ducommen, B., and Tassan, J.–P. (2002) Oncogene 21, 7630–7641
19. Stukenberg, P. T., Lustig, K. D., McGarry, T. J., King, R. W., Kuang, J., and Kirschner, M. W. (1997) Curr. Biol. 7, 338–348
20. Bernstein, H. S., and Coughlin, S. R. (1998) J. Biol. Chem. 273, 4666–4671
21. Blencowe, B. J. (2003) Cell 111, 407–417
22. Manning, G., Whyte, D. B., Martin, R., Hunter, T., and Sudarsanam, S. (2002) Science 298, 1912–1943
23. Drees, G., Ebrat, A., Preuss, U., Mandelkow, E.-M., and Mandelkow, E. (1997) Cell 89, 297–308
24. Rutter, J., Michoff, C. H., Harper, S. M., Gardner, K. H., and McKnight, S. L. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 8991–8996
25. Hardie, D. G., Carling, D., and Carlson, M. (1998) Annu. Rev. Biochem. 67, 821–855
26. Hawley, S. A., Bourdeau, J., Reid, J. L., Mustard, K. J., Udd, L., Makela, T. P., Alessi, D. R., and Hardie, D. G. (2003) J. Biol. 2, 28