Identification of a Human Brain-specific Isoform of Mammalian STE20-like Kinase 3 That Is Regulated by cAMP-dependent Protein Kinase*

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A novel isoform of mammalian STE20-like kinase 3 (MST3) with a different 5′ coding region from MST3, termed MST3b, was identified by searching through expressed sequence tag data base and obtained by rapid amplification of cDNA 5′-ends. MST3b was assigned to the long arm of human chromosome 13, D13S159–D13S280, by use of the National Center for Biotechnology Information sequence-tagged sites data base. Reverse transcriptase-polymerase chain reaction and Northern blot analysis with a probe derived from 5′ distinct sequence of MST3b revealed that the expression of MST3b mRNA is restricted to the brain, in contrast to ubiquitous distribution of MST3 transcript. Western analysis confirmed the brain-specific expression of MST3b protein. In situ hybridization of rat brain sections with a MST3b-specific probe indicated that MST3b is widely expressed in different brain regions, with especially high expression in hippocampus and cerebral cortex. When expressed in human embryonic kidney 293 (HEK293) cells, MST3b effectively phosphorylated myelin basic protein, as well as undergoing autophosphorylation. Interestingly, expression of MST3, but not MST3b, in HEK293 cells was able to activate the endogenous p42/44 mitogen-activated protein kinase (MAPK) up to 4-fold, whereas neither isoform activated p38 MAPK under the same conditions. Further experiments demonstrated that MST3b, but not MST3, was effectively phosphorylated by activation of cyclic AMP-dependent protein kinase (PKA) in both in vivo and in vitro assays. The mutation of Thr-18 into Ala in MST3b (T18A), a putative PKA phosphorylation site that is absent in MST3, abolished its phosphorylation by PKA. Consequently, expression of the T18A mutant in HEK293 cells led to partial activation of p42/44 MAPK, indicating that MST3b is under the regulation of PKA. Taken together, our data provide evidence that the two isoforms of STE20-like kinase 3 are differentially distributed and regulated.

Accumulating evidence has shown that the common, high evolutionarily conserved intracellular signaling cascades known as mitogen-activated protein kinase (MAPK) modules play an essential role in many cellular processes, such as growth, differentiation, and stress-related response (1, 2). In budding yeast, STE20 functions upstream of the MAPK pathway as a link to heterotrimeric G-protein (3–5) and phosphorylates STE11, which acts as a MAPK kinase kinase for STE7 (6). STE7 in turn acts as a MAPK kinase for FUS and KSS1 MAPK (5). Recently, several mammalian STE20-like kinases, such as p21Rac/Cdc42-activated kinases (PAKs) (6) and germline kinase (GCK) (7), have also been characterized as potential upstream kinases for MAPK pathways (8, 9). Kinases related to STE20 can be divided into two subfamilies based on their structure and regulation. The first subfamily, termed the PAK subfamily, contains a carboxyl-terminal catalytic domain and an amino-terminal regulatory domain with a p21Rac/Cdc42-binding domain and, in some cases, a pleckstrin homology domain as well. Overexpression of PAKs can activate both p38 MAPK and c-Jun amino-terminal kinase (JNK) but not p42/44 MAPK in a number of cell types (9–11). The second subfamily, the so-called GCK subfamily, has a catalytic domain at the amino terminus and lacks a recognizable PBD. Among this subfamily, GCK (7, 12), hematopoietic progenitor kinase kinase 1 (13, 14), kinase homologous to STE20/Sps1p kinase (15), GCK-like kinase (16), Nck-interacting kinase (17), and hematopoietic progenitor kinase/GCK-like kinase (18), which possess extensive homology with each other in their carboxyl termini, make one subgroup, whereas STE20/oxidant stress responsive kinase-1 (19), mammalian STE20-like kinases (MST 1, 2, and 3) (20–22), and lymphocyte-oriented kinase (23) make another subgroup. The former subgroup activates the JNK pathway specifically but not p38 MAPK pathway (12–18). The latter subgroup is not reported to directly activate the known MAPK pathways (19–23). Furthermore, little is known about how STE20-like kinases and their signaling pathways are regulated by other cell signaling pathways, such as cyclic AMP-dependent protein kinase (PKA) and protein kinase C.

Recently, considerable convergence as well as cross-talk has been demonstrated between PKA pathway and MAPK pathway. PKA is a mediator of the actions of hormones and neuro-
transmitters that activate adenylyl cyclase and increase intracellular cyclic AMP via the heterotrimeric GTP-binding protein (24, 25). PKA can phosphorylate a series of specific target proteins and play central roles in the regulation of many fundamentally important physiologic processes (24, 25). PKA down-regulates the activity of Raf-1 kinase by phosphorylation of Ser-621 in Raf (26), in contrast to the activation of p42/44 MAPK and the transcription factor Elk-1 by PKA through a B-Raf and Rap1-dependent pathway (27). In addition, it has been reported recently that PKA can activate p42/44 MAPK effectively through phosphorylation of Ser-23 in hematopoietic protein tyrosine phosphatase (28). However, it is unclear whether PKA can regulate STE20-like kinases, which are up-stream kinases of MAPK pathways. In this report, we have demonstrated that MST3b, a human brain-specific STE20-like kinase, can be negatively regulated by PKA in its functional activation of p42/44 MAPK signaling pathway.

EXPERIMENTAL PROCEDURES

Bioinformatics and Cloning of MST3b—The expressed sequence tag data base of GenBankTM was searched with the conservative catalytic sequence (base number M94719, amino acids 620–876) as probe by BLAST (basic local alignment search tool) at the Web site of the National Center for Biotechnology Information (29, 30). The 5′ rapid amplification of cDNA ends (5′ RACE) was then performed using human brain Marathon cDNA library (CLONTECH) with two gene-specific primers (5′-GAGTCGACACTGAATCCAG-3′ and 5′-GAAGCTTCAACAA-GAATTCTCA-3′) according to the manufacturer’s protocol. Two polymerase chain reaction (PCR) bands obtained were gel-purified and ligated into pGEM-T vector (Promega). After the bands were examined with DNA sequencing using deoxy-methylated chain-termination and reverse transcription (RT)-PCR, two distinct clones of MST3 with different 5′ end were identified. For genome mapping, the entire sequence of the two cDNA clones was used as a query in BLAST search and electronic PCR analysis (31) in the National Center for Biotechnology Information sequence-tagged site (STS) data base.

RT-PCR and Northern Blot Analysis of MST3b—Total RNA from human various tissues was isolated with TRIzol reagent (Life Technologies, Inc.) according to the manufacturer’s protocol. Lysates from HEK293 cells (48 h posttransfection) were made in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer containing 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, 2% SDS, 1% 2-mercaptoethanol. Aliquots containing 50 μg of protein form HEK293 cells or 100 μg of protein from rat tissue were subjected to 8–10% SDS-PAGE and then electroblotted onto nitrocellulose membranes. The membranes were probed with primary and peroxidase conjugated secondary antibodies. The immune complexes were visualized using enhanced chemiluminescence detection (Amersham Pharmacia Biotech) according to the manufacturer’s protocol. The results were quantified by densitometric scanning and represented as mean ± S.E. from at least three independent experiments.

Identification of p42/44 MAPK Phosphorylation and Activity—p42/44 MAPK phosphorylation was measured with Western blot analysis using anti-phospho-specific p42/44 MAPK polyclonal antibodies, and quantification of p42 MAPK bands was used to represent p42/44 MAPK phosphorylation. The p42/44 MAPK activity to phosphorylate myelin basic protein (MBP) was measured as described previously (37). In brief, HEK293 cells transfected with plasmids indicated were lysed in SDS sample buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM dithiothreitol, 2 μg/ml leupeptin, 4 μg/ml aprotinin, 1 μg/ml pepstatin A, 20 μM NaF, 0.1 mM phenylmethylsulfonyl fluoride, 1% Triton X-100). After incubation on ice for 20 min and centrifugation at 13,000 × g at 15 min at 4 °C, total-p42/44 MAPK antibody was added to the supernatant and incubated at 4 °C for 2 h. The immune complexes formed were captured by protein A-agarose, followed by washing three times with lysis buffer and twice with kinase buffer (40 mM HEPES, pH 7.5, 5 mM magnesium acetate, 1 mM EDTA, 2 mM dithiothreitol). The captured complexes were then suspended in 50 μl of kinase buffer supplemented with 25 μg of MBP, 50 μM ATP, 2 μCi of [γ-32P]ATP and incubated for 30 min at 30 °C. The reactions were terminated by addition of SDS-PAGE sample buffer, and samples were incubated at 95 °C. After resolution, the gels were dried and exposed to x-ray films.

Measurement of MST3b Activity and Phosphorylation (19, 22)—After transient transfection, the cells were lysed in cold lysis buffer (137 mM NaCl, 25 mM Tris-HCl, pH 7.4, 2 mM KCl, 0.1% Tween-20, 1 mM dithiothreitol, 2 μg/ml leupeptin, 4 μg/ml aprotinin, 1 μg/ml pepstatin A, 20 μM NaF, 0.2 mM phenylmethylsulfonyl fluoride) with repeated additions of high ionic strength syringes. The cells were then centrifuged at 13000 × g for 30 min, and the cell extracts were exposed to anti-HA monoclonal antibody for 3 h. The immune complexes were collected with protein A-Sepharose and washed three times with lysis buffer and another three times with kinase buffer (50 mM Tris-HCl, pH 7.4, 10 mM MgCl2, 1 mM EGTA). MBF phosphorylation by MST3b and MST3b-KR was started by the addition of 500 μg/ml MBP and 100 μM ATP con-
taining 5 μCi of [γ-32P]ATP in the kinase buffer. MST3b phosphorylation by PKA were started by the addition of 100 units/ml purified catalytic unit of PKA (Sigma) and 100 μM ATP containing 5 μCi of [γ-32P]ATP. Following incubation at 30 °C for 30 min, the reactions were stopped by adding SDS-PAGE sample buffer. After SDS-PAGE and autoradiography, the results were quantified by densitometric scanning of x-ray films.

The assay to measure in vivo phosphorylation of MST3b was performed basically as described (38). HEK293 cells transfected were pre-labeled with [32P]orthophosphate (100 μCi/ml, Amersham Pharmacia Biotech) in phosphate-free Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.). The cells were treated with or without forskolin (10 μM) or pretreated with or without H89 (4.8 μM) for 30 min at 37 °C. After stimulation, the cells were lysed by addition of 1 ml of RIPA buffer containing 150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 1% (v/v) Nonidet P-40, 0.5% (w/v) deoxycholate, 10 mM NaF, 0.2 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 0.1% (w/v) SDS. Then, the supernatant from centrifugation of 80,000 g for 15 min at 4 °C was absorbed through incubation with 100 μg/ml protein A-Sepha-

RESULTS

Cloning of MST3b—After searching against the nonredu-
dant expressed sequence tag data base of GenBankTM, a group of human expressed sequence tags, the deduced amino acid sequence of which was similar to STE20 catalytic domain, were identified (data not shown). The contig analysis showed that these expressed sequence tags constructed a novel STE20-like kinase, the 5’ sequence of which was unknown. 5’ RACE was then performed to obtain the full-length sequence of this novel kinase transcript, and two PCR bands were obtained (Fig. 1A). Sequencing of multiple independent clones from the two 5’ RACE products revealed that there were two cDNAs coded for two similar human STE20-like kinases with a divergent amino terminus. One of kinases turned out to be MST3 as earlier reported by Schinkmann and Blenis (22). The other was a novel isoform of MST3 kinase, and named by us as MST3b (AF083420) with an identical sequence (Fig. 1B, underlined) to MST3 after nucleotide 223. Sequencing of the two full-length cDNAs obtained by RT-PCR demonstrated the two isoforms with a divergent 5’ end (data not shown). The analysis of MST3b sequence showed that one ATG translation initiation codon (Fig. 1B, asterisk) was in the context of Kozak consensus sequence (39), and there were three stop codons (nucleotides 8, 32, and 116) 5’ to this ATG in frame. The 3’ untranslated region contained a putative polyadenylation signal, AATAAA, located 15 nucleotides 5’ from the poly(A) tail (data not shown). MST3b exhibited to encode a protein of 443 residues with a molecular mass of approximately 49 kDa. After BLAST analysis of STS data base of GenBankTM with the full-length sequence of MST3b as a query, one STS (WI-12444, GenBankTM accession number G13373) was identified (data not shown). This STS was mapped to human chromosome 13 between D13S159 and D13S280 (81.5–87.5 cm) (40, 41). The electronic PCR analysis also confirmed the result (data not shown).

Brain-specific Distribution of MST3b—Expression pattern of MST3b in different tissues was investigated using the specific primers or probes designed from the 5’ divergent region, which can distinguish MST3b and MST3. RT-PCR with the specific primers revealed that MST3b transcript was restricted to brain and was not detectable in other tissues such as heart, liver, kidney, spleen, lung, stomach, pancreas, or muscle etc. (Fig. 2A). In contrast, MST3 transcript appeared in all of tissues detected (Fig. 2A), agreeing well with the results previously reported (22). Northern blot analysis using MST3b-specific cDNA probe confirmed that an approximately 2.5-kilobase transcript was present only in brain (Fig. 2B). Brain-specific expression of MST3b protein was confirmed by Western blotting with the polyclonal antibodies recognizing a peptide sequence conserved in MST3b and MST3 (Fig. 2C). A level of MST3b protein that was possibly higher than expected in brain may result from the material for Western analysis, which was mainly isolated from cerebral cortex and hippocampus.

In situ hybridization experiments with a MST3b-specific and digoxigenin-labeled antisense cRNA probe were performed to study the detailed distribution of MST3b in brain. MST3b transcript was widely expressed in different brain regions, with high levels in hippocampus, cerebral cortex, and hypothalamus and moderate levels in geniculate nucleus and thalamic nucleus (Fig. 2D). MST3b was present generally in neuronal cells (data not shown). MST3b expression in Purkinje cells appeared greatly reduced in the cerebellum as compared those in the forebrain regions. As control, the hybridization with a MST3b sense cRNA probe did not yield detectable signal (data not shown).
over, the autophosphorylation of MST3b resulted in a higher kinase activity to phosphorylate MBP (Fig. 3C), implying a regulatory role of MST3b autophosphorylation. In addition, MST3b activity toward MBP was not affected by stimulation with H$_2$O$_2$, forskolin, menadione, or serum starvation (data not shown), which is similar to the case of MST3 (22).

**Differential Regulation of p42/44 MAPK Pathways by MST3 and MST3b**—Because STE20-like kinases have been predicted to function as upstream of MAPK pathways (3–5), the potential activation of p42/44 and p38 MAPK by MST3b expressed in HEK293 cells was tested. Data showed that overexpression of MST3, but not of MST3b, resulted in significant increase of endogenous p42/44 MAPK phosphorylation (about 4-fold, $p < 0.05$) determined by Western blot analysis (Fig. 4, A and B). Parallel to the elevated MAPK phosphorylation, the p42/44 MAPK activity toward MBP was also increased in response to overexpression of MST3, but not to that of MST3b, as detected by immunoprecipitation kinase assays (Fig. 4C). The expression levels of the total p42/44 MAPK were not changed by overexpression of MST3b or MST3 (Fig. 4A). Under the same conditions, the overexpression of either kinase failed to stimulate endogenous p38 MAPK and JNK (data not shown). These data indicated that MST3 and MST3b could differentially regulate p42/44 MAPK phosphorylation and activity.

The only structural difference between MST3 and MST3b lies in their amino terminus (Fig. 5A), in which MST3b, but not MST3, possesses a typical consensus sequence for PKA phosphorylation at residues 14 to 18 (Lys-Arg-Arg-Ala-Thr) (42). Therefore, we further tested whether this likely PKA phosphorylation site is involved in the differential regulation of p42/44 MAPK by MST3b and MST3. Fascinatingly, the single point mutation of Thr-18 to Ala of MST3b enabled its expression to elevate both phosphorylation (Fig. 5, B and C) and activity (Fig. 5D) of p42/44 MAPK (2.5-fold, $p < 0.05$). This indicated that MST3b is likely subjected to PKA regulation and the regulation site by PKA seems located at its amino terminus.

**Phosphorylation of MST3b by PKA**—Further experiments were carried out to examine whether MST3b is directly phosphorylated by PKA, using in vivo metabolic labeling and in
vitrō phosphorylation assays. HEK293 cells were transfected with pcDNA3, MST3b, and MST3. Phosphorylation of endogenous p42/44 MAPK was detected by using phospho-specific p42/44 MAPK antibody. The blots were reprobed with total p42/44 MAPK antibody or anti-HA antibody. B, the phosphorylation of p42/44 MAPK was quantified by densitometry and is presented as mean ± S.E. from three independent experiments. *, p < 0.05 versus pcDNA3. C, the lysates of the transfected cells were subjected to immunoprecipitation with total p42/44 MAPK antibody followed by in vitrō kinase assay using MBP as substrate of p42/44 MAPK. The results are representative of at least three independent experiments.

Fig. 4. Differential regulation of p42/44 MAPK phosphorylation and activity by MST3 and MST3b. A, HEK293 cells were transfected with pcDNA3, MST3b, and MST3. Phosphorylation of endogenous p42/44 MAPK was detected by using phospho-specific p42/44 MAPK antibody. The blots were reprobed with total p42/44 MAPK antibody or anti-HA antibody. B, the phosphorylation of p42/44 MAPK was quantified by densitometry and is presented as mean ± S.E. from three independent experiments. *, p < 0.05 versus pcDNA3. C, the lysates of the transfected cells were subjected to immunoprecipitation with total p42/44 MAPK antibody followed by in vitrō kinase assay using MBP as substrate of p42/44 MAPK. The results are representative of at least three independent experiments.

Fig. 5. Up-regulation of p42/44 MAPK phosphorylation and activity by the mutation of Thr-18 to Ala of MST3b. A, schematic amino acid sequence of N termini of MST3b and MST3. In MST3b, a putative PKA phosphorylation sequence is indicated by underlining, and Thr-18, which was mutated to Ala is indicated by boldface. B, HEK293 cells were transfected with pcDNA3, T18A, MST3b, and MST3. Phosphorylation of endogenous p42/44 MAPK was detected by using phospho-specific p42/44 MAPK antibody. The blots were reprobed with total p42/44 MAPK antibody or anti-HA antibody. C, the phosphorylation of p42/44 MAPK was quantified by densitometry and is presented as mean ± S.E. from three independent experiments. *, p < 0.05 versus pcDNA3. D, the lysates of the transfected cells were subjected to immunoprecipitation with total p42/44 MAPK antibody followed by in vitrō kinase assay using MBP as substrate of p42/44 MAPK. The results are representative of at least three independent experiments.

We have already demonstrated that, besides MST3b phosphorylation by PKA, mutation of PKA phosphorylation site in MST3b (Thr-18) significantly enhanced its activity to up-regulate MAPK pathway (in Fig. 5). However, MST3b from MST3b-transfected HEK293 cells treated with forskolin and MST3b treated with PKA after isolation revealed no apparent change of its activity against MBP in vitrō (data not shown). There are at least two possible explanations for this negative result. First, MBP, as a general pseudosubstrate for many serine/threonine protein kinases, is not the natural substrate of MST3b (its downstream molecules remain unknown), and therefore the MBP assay could not detect the PKA regulation of MST3b. Second, PKA may indirectly regulate the activity of MST3b via modulation of its translocalization, cellular distri-
bution, and kinase/substrate interaction as in the case of a PKA-regulated protein tyrosine phosphatase (28). Study to identify endogenous substrates of this kinase will facilitate elucidation of the physiological significance of MST3b phosphorylation by PKA.

DISCUSSION

In the present study, we have cloned two isoforms of mammalian STE20-like kinase 3 by means of bioinformatics, and one was identified as a brain-specific kinase, MST3b, with a divergent amino terminus from the other, MST3 (22). This diversity in 5′ coding region of some protein kinases has been observed. For example, three variants of murine Cβ-gene of PKA, from the alternative first exon that is spliced, have been shown to have three different N termini with distinct biochemical characteristics and discrete expression pattern (43). We hypothesize that the two isoforms in this study, MST3 and MST3b, also derive from a 5′ alternative splicing exon, because RT-PCR and sequencing analyses revealed that the two isoforms contained the identical cDNA sequence after nucleotide 223 and one common STS (WI-12444) that has been mapped to chromosome 13. In addition, there is one standard exon/intron (between nucleotides 223 and 224 in MST3b, data not shown) boundary with canonical consensus splice site (44), further supporting hypothesis of the alternative splicing. Furthermore, searches at the Web site of the Columbia Genome Center found that one yeast artificial chromosomes (827 h9) from Center d’Etudes Polymorphism Humain in France was positive in PCR analysis for WI-12444, and two cosmids of Columbia Genome Center in Columbia University (53D1 and 102D5) were positive in hybridization analysis for the STS (41). These data would be useful to further characterize the genomic structure of MST3b and MST3.

It has been demonstrated that STE20-like kinase family functions as upstream of stress-activated kinase (p38 MAPK and JNK) pathways through interaction with small G-proteins (Rac1, Cdc42, and Rab8) (6, 45) that activate the downstream MAPK pathways (9, 11). A recent report reveals that PAK1, a member of STE20-like kinase family, not only activates p42/44 MAPK via Rac/Cdc42 but also directly phosphorylates MEK1 on Ser-298, which in turn activates p42/44 MAPK (46). The current study further added the evidence that another member of STE20-like kinases, MST3/MST3b, also allow activation of p42/44 MAPK, at least under the condition of overexpression. However, the pathway that leads to activation of p42/44 MAPK by MST3/MST3b remains to be elucidated. In addition, amino acid sequence analysis showed that both MST3 and MST3b possess a conservative Gβ-binding motif of STE20 kinase family (47), suggesting that the β subunits of heterotrimeric G proteins may also mediate MST3/MST3b signaling.

Through different methodologies (RT-PCR, Northern analysis, Western analysis, and in situ hybridization), the present study established that the MST3b transcript is specifically expressed in brain, in contrast to ubiquitous distribution of
MST3. Less is known so far about how the alternative splicing of a protein kinase leads to the specific tissue distribution, especially in the STE20-like family. One possibility could be due to exist of the brain-specific splicing enzyme/splicingosome (48). The brain-specific isoform MST3b may play a distinct functional role from MST3. It has been reported that the expression pattern of a few members of STE20 kinase family is relatively restricted to a certain tissue: PAK3 in brain (49, 50), hematopoietic progenitor kinase 1 in hematopoietic cells (13, 14), and lymphocyte-oriented kinase in lymphocytes (23). This expression pattern may give some clues as to its possible functional and physiological roles. For instance, a point mutation of Pak3, which highly expresses in cerebral cortex and hippocampus, has been linked to a multiple pedigree with X-linked form of nonsyndromic mental retardation (51). The apparent high expression of MST3b in hippocampus and cerebral cortex may imply its potential functions involved in some important neurobiological activities.

It has been shown in this study that MST3b and MST3 can regulate differentially and specifically the p42/44 MAPK pathway but not p38 MAPK or JNK. The differential regulation of MAPK by the two isoforms has been further demonstrated to come from the differential regulation of these two kinases by PKA. Apparently, PKA negatively regulates MST3b via phosphorylation of Thr-18 at the amino terminus of MST3b, which is not present in MST3. To our knowledge, this is the first evidence that a mammalian STE20-like kinase can be directly phosphorylated and functionally regulated by PKA. Our data also provide a novel mechanism for PKA to modulate MAPK pathways, besides the known mechanisms through c-Raf-1 kinase (26), B-Raf/Rap-1 pathways (27), or hematopoietic protein tyrosine phosphatase (28). In addition, it is reported that lacking the CBF1 catalytic subunit of PKA, which is most highly expressed in the cerebral cortex and hippocampus (43), exhibit a decrease in hippocampal long term potentiation and the defects of both long term depression and depotentiation in the Schaffer collateral-CA1 synapse (52). Considering the similar expression pattern of the CBF1 catalytic subunit of PKA as that of MST3b, one could reasonably speculate that this reported regulation of MST3b by PKA would be of critical importance for neurobiological functions of MST3b as well as PKA in these regions.

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REFERENCES
1. Seger, R., and Krebs, E. G. (1995) FASEB J. 9, 726–735
2. Widmann, C., Gibson, S., Jarpe, M. B., and Johnson, G. L. (1999) Physiol. Rev. 79, 143–180
3. Leherer, R., Dignard, D., Harcus, D., Thomas, D. Y., and Whiteway, M. (1992) EMBO J. 11, 4815–4824
4. Ramer, S. W., and Davies, R. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 452–456
5. Herskowitz, I. (1995) Cell 80, 187–197
6. Manser, E., Leung, T., Salihuddin, H., Zhao, Z. S., and Lim, L. (1994) Nature 370, 40–46
7. Katz, P., Whalen, G., and Kehrl, J. H. (1994) J. Biol. Chem. 269, 16802–16809
8. Robinson, M. J., and Cobb, M. H. (1997) Curr. Opin. Cell Biol. 9, 180–186
9. Paul, A., Wilson, S., Belham, C. M., Robinson, C. J., Scott, P. H., Gould, G. W., and Plein, R. (1997) Cell. Signal. 9, 403–410
10. Kyriakis, J. M., and Avruch, J. (1996) J. Biol. Chem. 271, 24313–24316
11. Sells, M. A., and Chernoff, J. (1997) Trends. Cell Biol. 7, 162–167
12. Pombo, C. M., Kehrl, J. H., Sanchez, I., Katz, P., Avruch, J., Zen, L. L., Woodgett, J. R., Force, T., and Kyriakis, J. M. (1995) Nature 377, 750–754
13. Hu, M. C., Qiu, W. R., Wang, X., Meyer, C. F., and Tan, T. H. (1996) Genes Dev.