Feedback Regulation of Raf-1 and Mitogen-activated Protein Kinase (MAP) Kinase Kinases 1 and 2 by MAP Kinase Phosphatase-1 (MKP-1)*

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Inactivation of growth factor-regulated mitogen-activated protein (MAP) kinases (ERK1 and ERK2) has been proposed to occur in part through dephosphorylation by the dual specificity MAP kinase phosphatase-1 (MKP-1), an immediate early gene that is induced by mitogenic signaling. In this study, we examined the effect of MKP-1 on signaling components upstream of ERK1 and ERK2. Coexpression of MKK1 or MKK2 with MKP-1 resulted in 7–10-fold activation of mitogen-activated protein kinase kinase (MKK), which required the presence of a protease-sensitive phosphorylation site. Endogenous MKK1 and MKK2 were also activated upon MKP-1 expression. Raf-1, a direct regulator of MKK1 and MKK2, was activated under these conditions, and a synergistic activation of MKK was observed upon coexpression of Raf-1 and MKP-1. This effect did not appear to involve synthesis of autocrine growth factors or the inhibition of basal extracellular signal-regulated kinase (ERK) activity but was inhibited by a dominant negative Ras mutant, indicating that MKP-1 enhances Ras-dependent activation of Raf-1 in a cell autonomous manner. This study demonstrates positive feedback regulation of Raf-1 and MKK by the MKP-1 immediate early gene and a potential mechanism for activating Raf-1/MKK signaling pathways alternative to those involving ERK.

The mitogen-activated protein (MAP)* kinase cascade has emerged as a key signaling pathway regulating factor-dependent cell growth and differentiation through intracellular phosphorylation (1, 2). Growth factor regulation of this pathway involves the phosphorylation and activation of MAP kinases, ERK1 and ERK2, by MAP kinase kinases, MKK1 and MKK2 (3–6). ERKs 1 and 2 phosphorylate various targets including upstream and downstream protein kinases, cell surface receptors, and nuclear transcription factors (1, 7). MKKs 1 and 2, in turn, can be activated through phosphorylation by members of the Raf protein kinase family, including Raf-1, which is ubiquitously expressed (8, 9). Receptor-dependent activation of Raf-1 involves its interaction with p21 Ras, through a mechanism that is not completely defined, but appears to involve Raf-1 dimerization and phosphorylation by heterologous protein kinases (10–13).

The product of the immediate early gene, MAP kinase phosphatase (MKP-1), is able to dephosphorylate phosphoserine/threonine as well as phosphotyrosine residues, and shows selectivity for ERKs 1 and 2 in vitro, with lower activity toward other MAP kinases such as JNK and p38 MAP kinase (14, 15). MKP-1 inactivates ERK following growth factor stimulation in intact cells and also suppresses signaling downstream of ERK at the level of gene transcription and proliferation (15–17), most likely through its inhibitory effects on MAP kinase. Interestingly, MKP-1 is transcriptionally induced by stress- regulated pathways such as those occurring in response to UV treatment or activation of stress-regulated protein kinases (18, 19), suggesting that MKP-1 may function as a mediator of cross-regulatory pathways between mammalian protein kinase cascades. A similar cross-regulation is observed between osmotic stress- and pheromone-activated protein kinase cascades in yeast involving transcriptional induction of protein-tyrosine phosphatases (20–23).

To obtain a more complete understanding of how MKP-1 regulates cell signaling, we examined the effects of this enzyme on other components in the MAP kinase pathway in NIH 3T3 cells. Our studies demonstrate the novel finding that MKP-1 positively regulates Raf-1 and MKK components upstream of ERK in an ERK-independent manner, providing a means by which different pathways downstream of Raf-1 might be differentially controlled.

MATERIALS AND METHODS

Cell Culture—NIH 3T3 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with penicillin (100 units/ml), streptomycin (100 µg/ml), and 10% fetal bovine serum (FBS, Life Technologies, Inc.).

Transfections—Cells were seeded at 2 × 10^5 cells per well into six-well plates (35 mm diameter) and transfected at 50–80% confluence using 5 µl of LipofectAMINE (Life Technologies, Inc.) according to manufacturer’s instructions and 1 µg of cDNA unless otherwise noted. Transfection efficiency was estimated as 20–30%, based on fluorescence of cells transfected in parallel with a construct (pK7-GFP) expressing green fluorescent protein (a gift of Dr. Ian Macara). The cDNA constructs for expression of wild-type and mutant hemagglutinin (HA)-tagged MKKs 1 and 2 in pMCI have been described previously (24–26). Constructs for expression of Myc-tagged wild-type MKP-1 and MKP-1 (C258S) in a pCEP4 vector were a gift of Drs. Hong Sun and Nicholas Tonks (15). A construct for expression of Flag-tagged Raf-1 was a gift of Dr. Roger Davis (27). Dominant negative Raf-1 (S621A) construct was a gift of Dr. Deborah Morrison (28). Dominant negative H-Ras (S17N) construct was a gift of Dr. Melanie Cobb.

Immunoprecipitations and Phosphorylation Assays—Cells were harvested 40–48 h following transfection by washing twice in cold phos-
phosphate-buffered saline and extraction in 350 μl of tissue lysis buffer (20 mM Tris, pH 7.4, 137 mM NaCl, 2 mM EDTA, 1% Triton X-100, 25 mM β-glycerophosphate, 2 mM sodium pyrophosphate, 10% glycerol, 1 mM sodium orthovanadate, 1.0 mM phenylmethylsulfonyl fluoride, and 1 mM benzamidine) followed by centrifugation at 15,000 rpm for 10 min to clarify lysates. Antibodies (0.3 μg recognizing cases, Antibodies (0.3 μg recognizing cases, Anti-MKK2 (C-12, Santa Cruz), hemagglutinin tag (12CA5, BabCo), or Raf-1 (C-12, Santa Cruz) were added to separate aliquots of lysates, which were allowed to incubate on ice for 1 h. Immune complexes were then incubated end-over-end at 4 °C with 20 μl of protein A- or protein G-Sepharose (Pharmacia Biotech Inc.) for 1 h. The immune complexes were washed twice with kinase buffer (25 mM Hepes, pH 7.4, 25 mM β-glycerophosphate, 25 mM MgCl₂, 2 mM dithiothreitol, and 1.0 mM sodium orthovanadate) and incubated with 30 μl of kinase buffer containing 10 μCi of [γ-³²P]ATP, 30 μM cold ATP, and substrate for 10 min at 30 °C. Substrates used were 5 μg of myelin basic protein for measurement of ERK activity, 1 μg of catalytically inactive ERK2 (K52R) for MKK activity, or 1 μg of catalytically inactive MKK1 (R97 m) for Raf-1 activity. Reactions were quenched with Laemmli sample buffer and resolved by SDS-PAGE, followed by autoradiography.

**Transcription Assays—**AP-1 promoter-driven transcription was assayed by transfecting cells with a TRE-CAT reporter construct (pTREAS-N) (a gift of Drs. Ami Aronheim and Michael Karin) and v-Raf-3611-MSV (a gift of Dr. Peter Shaw), constitutively active MKK1-G1C (ΔN4/S218E/S222D) (26), or constitutively active MKK2-KW +71 (ΔN4/S224/S226D) (25) in the presence or absence of MKP-1 cDNA. Forty-eight hours after transfection, cells were harvested and chloroenzyme acetyltransferase (CAT) activity was measured in 20 μl of cell extract by incorporation of tritium from [3H]acetyl coenzyme A into chloroenzyme A in 3 min.

**Western Blotting—**ERK, MKK, Raf-1, or Ras guanine nucleotide exchange factor (SOS) were detected by probing proteins using SDS-PAGE followed by semidy transfer (Eliard Instruments) onto Immobilon P (Millipore). Blots were blocked for 1 h with Tris-buffered saline (TBS, 50 mM Tris, pH 8, 0.15 M NaCl). Tween 20 (0.1%), nonfat dry milk (5%), followed by incubation with antibodies (listed above, IM2 for the Flag epitope on Raf-1 (Kodak), C-23 for SOS (Santa Cruz)) in TBS/Tween 20 (1:1000 dilution in all cases). The blots were washed four times with TBS/Tween 20, then incubated with horseradish peroxidase secondary antibodies or alkaline phosphatase secondary antibodies (1:5000). Protein detection was performed using enhanced chemiluminescence (ECL, Amer sham) with horseradish peroxidase secondary antibodies or nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate with alkaline phosphatase secondary antibodies. In some cases, anti-HA and antiERK primary antibodies were incubated together to detect HA-MKK and ERK proteins simultaneously.

**RESULTS**

**MKP-1 Expression Activates MKK1 and MKK2—**Cells were transiently transfected with wild type MKK1 or MKK2 and MKP-1, and the activity of expressed MKK was measured. As shown in Fig. 1A, coexpression with wild type MKK1 resulted in 7–10-fold enhancement of the activity of expressed MKK1 or MKK2. Controls from one experiment showed comparable size of MKP-1 or the MKP1 (C258S) mutant was present in transfected cells (Fig. 3A). As expected, MKP-1 suppressed ERK activity in response to constitutively active MKK2-KW +71, evident by the disappearance of the gel retarded form of ERK2 (Fig. 3B). Similarly, MKP-1 suppressed ERK2 activation by constitutively active MKK1-G1C, PDGF, or serum (data not shown). Inhibition of signaling downstream of ERK was tested by coexpressing an AP1 promoter-driven CAT

**Fig. 1. Activation of expressed MKK1 and 2 by MKP-1.** Cells were transiently transfected with HA-tagged wild type MKK1 or 2 cDNA (1 μg) in the presence or absence of MKP-1 (1 μg). HA-MKK catalytic activity was measured by immunocomplex assays using catalytically inactive ERK2(K52R) as a substrate. A, activities of MKK1 or MKK2 in the presence of MKP-1 or inactive MKP-1(C258S), normalized to their respective activities in the absence of MKP-1. The number in parentheses indicates the number of separate transfection experiments in which the mean and standard errors were derived. B, Western blots of immunoprecipitates of HA-tagged MKK, developed with 12CA5 monoclonal antibody, from one experiment indicate comparable loading. C, relative ERK2 activity in cells transfected with MKK1, plus or minus MKP-1. ERK2 activity from PDGF-stimulated cells is shown for comparison. D, Western blot of ERK2 shows slower migrating ERK2 in PDGF-stimulated cells but no difference in MKK-transfected cells in the presence or absence of MKP-1.

In parallel experiments, endogenous MKK1 and MKK2 activities were enhanced 2-fold upon expression of MKP-1, demonstrating that these effects were not limited to overexpressed MKK (data not shown). This activation is submaximal, since the transfection efficiency of these cells was approximately 20–30%. In comparison, PDGF (25 ng/ml) treatment of cells resulted in 8–10-fold activation of endogenous MKK1 (data not shown). The activation of expressed MKK by MKP-1 was also observed in cells that were serum-depleted for 18 h prior to cell lysis (Fig. 2A). Under serum-starved conditions, ERK activation was not detected in the absence of growth factor stimulation (Fig. 2B), suggesting that the effect of MKP-1 on MKK was not due to removal of ERK activity. In three separate serum starvation experiments, the average MKK1 activation by MKP-1 was approximately 10-fold (Fig. 2C). Therefore, these effects of MKP-1 were observed with expressed as well as endogenous MKK under serum-starved and serum-fed conditions.

Expression of MKP-1 was confirmed by metabolically labeling cells with [³⁵S]methionine and immunoprecipitating the Myc-tagged MKP1. A 40-kDa protein corresponding to the reported size of MKP-1 or the MKP1 (C258S) mutant was present in transfected cells (Fig. 3A). As expected, MKP-1 suppressed ERK activation in response to constitutively active MKK2-KW +71, evident by the disappearance of the gel retarded form of ERK2 (Fig. 3B). Similarly, MKP-1 suppressed ERK2 activation by constitutively active MKK1-G1C, PDGF, or serum (data not shown). Inhibition of signaling downstream of ERK was tested by coexpressing an AP1 promoter-driven CAT
MKK1 Activation Requires Regulatory Serine Phosphorylation Sites—MKK1 is activated by phosphorylation at Ser-218 and Ser-222 (29, 30). The dependence of MKK1 activation by MKP-1 on these phosphorylation sites was examined by coexpressing MKP-1 with a series of MKK1 mutants with varying specific activities. MKK1(S218E/S222D), which substitutes regulatory phosphorylation sites with acidic amino acids, MKK1(DN4), which deletes amino acid residues 44–51 but leaves Ser-218 and Ser-222 intact, and MKK1(ΔN4/S218A/S222A), which substitutes regulatory phosphorylation sites with acidic amino acids, MKK1(ΔN4), which deletes amino acid residues 44–51 but leaves Ser-218 and Ser-222 intact, and MKK1(ΔN4/S218A/S222A), which substitutes regulatory phosphorylation sites with Ala in the ΔN4 background, are constitutively active mutants with 65-fold, 80-fold, and 40-fold greater activity than basal wild type MKK1, respectively (26). MKK1(ΔN4/S218A/S222A) has activity comparable to basal wild type MKK1 (24). MKP-1 expression resulted in activation of wild type MKK1 as well as MKK1(ΔN4) (Fig. 4A). In contrast, MKP-1 had no effect on any of the MKK1 mutants containing Ser-218 or Ser-222 substitutions. Controls demonstrated comparable protein levels of each MKK1 in the immune complex assays (Fig. 4B). These data suggest that an upstream activator that targets Ser-218 and Ser-222 is required for MKP-1-dependent activation of MKK.

Involvement of Raf-1 in MKK1 Activation in Cells Expressing MKP-1—Raf-1 has been shown to be a major upstream activator of MKK1 and MKK2 (8, 9). The dependence of Raf-1 activity on MKP-1 was explored by transfecting cells with HA-tagged MKK1 and varying amounts of Raf-1 cDNA in the presence or absence of MKP-1. A small amount of Raf-1 expression significantly enhanced the MKK activation by MKP-1 (Fig. 5A). Increasing the amount of overexpressed Raf-1 did not further augment MKK1 activation by MKP-1 (Fig. 5A). Western blots probing the Flag epitope showed increased expressed Raf-1 levels in transfected cells, but no variation due to MKP-1 (data not shown). Endogenous MKK activity was also elevated with
increasing Raf-1 levels in the presence of MKP-1 compared with MKK activity observed in the absence of MKP-1 (data not shown).

Raf-1 immunoprecipitated from the same cell extracts showed corresponding activation by MKP-1 (Fig. 5 B). In the absence of expressed Raf-1, MKP-1 caused a 7-fold activation of endogenous Raf-1. Expression of Raf-1 resulted in up to 50-fold greater Raf activity in immunoprecipitates, which was further augmented by 3–5-fold by coexpression of MKP-1. When the same cells were treated with PDGF, Raf-1 activity was elevated by 10–20-fold, comparable to the activities seen in the presence of MKP-1. Thus, MKP-1 activates Raf-1 in unstimulated cells to levels comparable to PDGF-treated conditions.

Raf-1 protein from PDGF-treated cells showed a characteristic gel mobility retardation due to phosphorylation, whereas Raf-1 from MKP-1-expressing cells showed a migration similar to Raf-1 from unstimulated cells (data not shown). This suggests that Raf-1 regulation by MKP-1 may occur by a mechanism distinct from PDGF-induced Raf-1 stimulation. Furthermore, MKP-1 failed to activate the mutant, Raf-1(S621A) (data not shown). Although expression of Raf-1 (S621A) did not inhibit the activation of expressed MKK by MKP-1, mutant Raf-1 led to no further enhancement of MKK-1 activation by MKP-1 (data not shown). Taken together, these data provide evidence that the activation of MKK by MKP-1 occurs through elevation of Raf-1 catalytic activity.

**Involvement of Ras in MKK and Raf-1 Activation by MKP-1**

Involvement of Ras was further investigated using a dominant negative H-Ras mutant (S17N), which inhibits growth factor-dependent activation of ERK. Coexpression of Ras (S17N) with MKP-1 and wild type MKK1 showed partial inhibition of MKK activation by MKP-1 (Fig. 6A) under conditions of comparable loading of MKK immunoprecipitates. Parallel controls confirmed the dominant interfering effect of Ras (S17N) on Ras signaling by showing a complete inhibition of PDGF-stimulated ERK activity (Fig. 6B). The data strongly suggest that Raf-1 activation by MKP-1 occurs through a Ras-dependent mechanism.

A key mechanism for Raf-1 activation involves interaction of Raf-1 with Ras-GTP following GDP/GTP exchange catalyzed by the SOS exchange factor (30, 31, 32). Phosphorylation of SOS by ERK- and MKK-dependent pathways reportedly prevents Ras/Raf-1 association and Raf-1 activation under conditions correlating with a reduced mobility of SOS on SDS-PAGE (33–35). We examined SOS phosphorylation under conditions where MKK was activated by MKP-1 and endogenous ERK phosphorylation was inhibited by MKP-1. Under basal conditions, no gel mobility shift of SOS was observed (data not shown). Growth factor treatment of cells results in retardation of SOS gel mobility previously attributed to Ser/Thr phosphorylation and correlated with SOS inactivation (33, 34). In addition, no discernible difference in the gel retardation was observed in cells transfected with MKP-1, suggesting that SOS phosphorylation response to PDGF is not altered by MKP-1 (data not shown).

To test whether the activation of Raf-1 and MKK by MKP-1 occurred through MKP-1 induction of secreted growth factors, conditioned medium was removed from cells transfected with MKP-1 and added onto cells transfected only with HA-MKK1. Under these conditions, no activation of expressed MKK1 was observed (data not shown). Furthermore, cells were transfected
Activation of Raf-1 and MKK by MKP-1

The growth factor-stimulated MAP kinase cascade has been shown to be inactivated at the level of ERK1 and ERK2 by the dual specificity phosphatase, MKP1 (14–17, 36–38). Previous studies proposed that MKP-1 might function as a feedback regulator of ERK signaling. Originally, MKP-1 was identified through its transcripational induction following growth factor stimulation of cells and was described as an immediate early gene whose message is rapidly induced in a manner independent of protein synthesis (39). The kinetics of MKP-1 induction are thus consistent with its potential role in catalyzing ERK dephosphorylation, which occurs during a phase of inactivation observed in response to many types of stimuli. However, the induction of MKP-1 and dephosphorylation of ERK are uncorrelated in some studies, suggesting that this may not occur in all cells. For example, the inactivation of ERK in PC12 cells that occurs several minutes following epidermal growth factor-dependent activation is unaffected by cycloheximide, conditions that suppress MKP-1 induction (40, 41). These findings suggest that alternative functions of MKP-1 exist that have yet to be described.

In this study, we show for the first time that MKP-1 positively regulates enzymes upstream of ERK, namely MKK1, MKK2, and Raf-1, thus providing an additional pathway for feedback regulation of Raf-1 by immediate early gene expression. This effect appears to occur through a cell autonomous mechanism and is partially blocked by dominant negative Ras, indicating that Ras-mediated activation of Raf-1 is most likely involved.

Several mechanisms for negative feedback regulation of upstream signaling targets by ERK, including cell surface receptors (epidermal growth factor receptor), Ras guanine nucleotide exchange factor (SOS), or Raf-1 have been proposed (33–35, 42, 43). In particular, SOS phosphorylation by ERK has been shown to down-regulate signaling through dissociation of Grb2-SOS (33–35). Furthermore, others have reported Raf-1 activation in unstimulated cells treated with the MKK1 inhibitor, PD98059 (60). It is possible that part of the stimulatory effect we observe with MKP-1 can be accounted for by relief of signal repression by ERK. However, it is unlikely that ERK inhibition accounts for all of the effects because our data were taken in cells treated under basal conditions and reproduced under serum-starved conditions where ERK activity is low or undetectable. Consistent with this, under basal or serum-starved conditions, we found no evidence for retardation in SOS gel mobility, which has been attributed to feedback phosphorylation by ERK- or MKK-dependent kinases (33, 34, 44). In addition, PDGF-induced SOS phosphorylation and gel mobility retardation were unaffected by MKP-1 expression, suggesting that altered SOS activity due to phosphorylation is not involved. The available data suggest the possibility that MKP-1 activates Raf-1 through Ras, involving a mechanism that may not involve inactivation of ERK.

The incomplete effect of Ras (S17N) on MKP-1 activation of MKK suggests that Ras-independent mechanisms for Raf-1 activation might also be regulated by MKP-1. Ras-independent means of Raf-1 activation might occur through Raf dimerization or through direct phosphorylation by tyrosine kinases, protein kinase C, ceramide-activated protein kinase, or kinase suppressor of ras, KSR (11–13, 45–53). Raf-1 inhibition may involve phosphorylation of Ser-43 or Ser-621 by protein kinase A (54–56). Alternatively, Raf-1 may be regulated by interactions with other signaling components such as 14-3-3 proteins (57–59). It is conceivable that alternative mechanisms for Raf-1 activation may be regulated indirectly through dephosphorylation of cellular targets by MKP-1. A significant implication of our study is that MKP-1 may alter the flux through different Raf-1-dependent pathways, thus providing a means of uncoupling different regulatory pathways downstream of Ras. For example, Raf-1 has been shown to phosphorylate other proteins, including IkB and the proapoptotic Bad protein (61–63), suggesting that it targets other substrates and downstream pathways in addition to MKK1 and MKK2 and the ERK pathway. Under some conditions, signaling through Raf-1 may also activate p70 S6 kinase through an ERK-independent mechanism (64). Our results would predict that induction of MKP-1 (e.g. through immediate early gene expression or activation of stress-activated protein kinase pathways) might enhance signaling through alternative Raf-1 targets under conditions where ERK signaling is suppressed. Another possibility that has yet to be demonstrated is that MKK targets other cellular

![Graph](image-url)
Activation of Raf-1 and MKK by MKP-1

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substrates in addition to ERK, in which case phosphorylation of these substrates might also be enhanced by MKP-1.

In summary, this study reveals the ability of MKP-1 to serve as a positive regulator of Raf-1 signaling and presents an important consideration for studies utilizing MKP-1 as an inhibitor of ERK signaling. The results suggest a novel approach to address Raf-1 regulation by both Ras-independent and de-inhibitor of ERK signaling. The results suggest a novel approach important consideration for studies utilizing MKP-1 as an in-

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REFERENCES

1. Lewis, T. L., Shapiro, P. S., and Ahn, N. G. (1998) Adv. Cancer Res. 74, in press

2. Hunter, T. (1995) Cell 80, 225–236

3. Seger, R., Ahn, N. G., Posada, J., Munar, E. S., Jensen, A. M., Cooper, J. A., Cobb, M. H., and Krebs, E. G. (1992) J. Biol. Chem. 267, 14373–14383

4. Wu, J., Harrison, J. K., Dent, P., Lynch, K. R., Weber, M. J., and Sturgill, T. W. (1993) Mol. Cell. Biol. 13, 4539–4548

5. Crews, C. M., Alesandrini, A., and Eriksson, R. L. (1992) Science 258, 478–480

6. Zheng, C. F., and Guan, K. L. (1993) J. Biol. Chem. 268, 11435–11439

7. Treisman, R. (1996) Curr. Opin. Cell Biol. 8, 205–215

8. Kyriakis, J. M., App, H., Zhang, X., Banerjee, P., Brautigan, D. L., Rapp, U. R., and Avruch, J. (1992) Nature 358, 417–421

9. Dent, P., Haser, W., Haystead, T. A. J., Vincent, L. A., Roberts, T. M., and Sturgill, T. W. (1992) Science 257, 1404–1407

10. Avruch, J., Zhang, Z. F., and Kyriakis, J. M. (1994) Trends Biol. Sci. 19, 271–274

11. Marais, R., Light, Y., Paterson, H. F., and Marshall, C. J. (1995) EMBO J. 14, 3136–3145

12. Farrar, M. A., Alberola-Ila, J., and Perlmutter, R. M. (1996) Nature 383, 178–181

13. Luo, Z., Teirion, G., Belshaw, P. F., Varras, D., Marshall, M., and Avruch, J. (1996) Nature 383, 181–185

14. Franklin, C. C., and Kraft, A. S. (1995) Cell 80, 225–236

15. Sun, H., Charles, C. H., Lau, L. F., and Tonks, N. K. (1993) Science 257, 1187–1192

16. Brondello, J. M., McKenzie, F. R., Sun, H., Tonks, N. K., and Pouyssegur, J. (1995) Oncogene 10, 1895–1904

17. Charles, C. H., Sun, H., Lau, L. F., and Tonks, N. K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5292–5296

18. Alesi, D. R., Gomez, N., Mclrea, G., Lewis, T., Keyse, S. M., and Cohen, P. (1995) Curr. Biol. 5, 283–289

19. Wu, J., Lai, L. F., and Sturgill, T. W. (1994) FEBS Lett. 353, 9–12

20. Anderson, N. G., Li, P., Marsden, L. A., Williams, N., Roberts, T. M., and Sturgill, T. W. (1991) Cell Biochem. 7, 573–576

21. Takishima, K., Griswold-Prenner, J., Ingebritsen, T., and Rosner, M. R. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2520–2524

22. Holt, K., Kasson, B. G., and Pessin, J. E. (1996) Mol. Cell. Biol. 16, 577–583

23. McCarthy, S. A., Samuels, M. L., Pritchard, C. A., Abraham, J. A., and McMahon, M. (1995) Genes Dev. 9, 1995–1996

24. Kolch, W., Heidecker, G., Kocks, G., Hummel, R., Vahidi, H., Mischak, H., Finkenstaller, G., Marme, D., and Rapp, U. R. (1995) Nature 364, 249–252

25. To, J. R., Dvor, I. O., and Morrison, D. K. (1995) Mol. Cell. Biol. 15, 7170–7179

26. Jelinek, T., Dent, P., Sturgill, T. W., and Weber, M. J. (1996) Mol. Cell. Biol. 16, 2701–2709

27. Yao, B., Zhang, Y., Delikat, S., Mathias, S., Basu, S., and Kolesnick, R. (1995) Nature 376, 307–310

28. Sundaram, M., and Han, M. (1995) Cell 83, 889–901

29. Therrien, M., Chang, H. C., Solomon, N. H., Karin, P. D., Wasserman, D. A., and Rubin, G. M. (1995) Cell 83, 879–888

30. Kornfeld, K., Horn, D. B., and Horvitz, H. R. (1995) Cell 83, 903–914

31. Irie, K., Gotoh, Y., Yashar, B. M., Errede, B., Nishida, E., and Matsumoto, K. (1994) Oncogene 13, 16116–16119

32. Hafer, S., Adler, H. S., Mischak, H., Janossch, P. Heidecker, G., Wolfman, A., Pippig, S., Lohse, M., Ueffing, M., and Kolch, W. (1994) Mol. Cell. Biol. 14, 6696–6703

33. Mischak, H., Seitz, T., Janossch, P. Ulitz, M., Strein, H., Schellerer, M., Philipp, A., and Kolch, W. (1996) Mol. Cell. Biol. 16, 5409–5418

34. Wu, J., Dent, P., Jelinek, T., Wolfman, A., Weber, M. J., and Sturgill, T. W. (1993) Science 262, 1065–1069

35. Freed, E., Symons, M., Maclouf, S. G., McCormick, F., and Raggioli, R. (1998) Science 265, 1713–1715

36. Fanti, W. J., Muslin, A. J., Kikuchi, A., Martin, J. A., McNichol, M. A., Gross, R. W., and Williams, L. T. (1994) Nature 371, 612–614

37. Irie, K., Gotoh, Y., Yashar, B. M., Errede, B., Nishida, E., and Matsumoto, K. (1994) Science 265, 1716–1719

38. Alesi, D. R., Cuesta, A., Cohen, P., Dudley, D. T., and Saltiel, A. R. (1995) J. Biol. Chem. 270, 27489–27494

39. Wang, H.-G., Rapp, U. A., and Reed, J. C. (1996) Cell 87, 629–638

40. Li, S., and Sedy, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9247–9251

41. Galaktionov, K., Jesus, C., and Beach, D. (1995) Genes Dev. 9, 1046–1055

42. Lenormand, P., McMahon, M., and Pouyssegur, J. (1996) J. Biol. Chem. 271, 15762–15768