Nuclear Localization of Mitogen-activated Protein Kinase Kinase 1 (MKK1) Is Promoted by Serum Stimulation and G2-M Progression

REQUIREMENT FOR PHOSPHORYLATION AT THE ACTIVATION LIP AND SIGNALING DOWNSTREAM OF MKK

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Stimulation of mammalian cells results in subcellular relocalization of Ras pathway enzymes, in which extracellular signal-regulated protein kinases rapidly translocate to nuclei. In this study, we define conditions for nuclear localization of mitogen-activated protein kinase 1 (M KK1) by examining effects of perturbing the nuclear export signal (NES), the regulatory phosphorylation sites Ser218 and Ser222, and a regulatory domain at the N terminus. After disrupting the NES (Δ32–37), nuclear uptake of MKK was enhanced when quiescent cells were activated with serum-phorbol 12-myristate 13-acetate or BXB-Raf-1 cotransfection. Uptake was enhanced by mutation of Ser218 and Ser222 to Glu and Asp, respectively, and blocked by mutation of these residues to Ala, although mutation of Lys97 to Met, which renders MKK catalytically inactive, did not interfere with uptake. Therefore, nuclear uptake of MKK requires incorporation of phosphate or negatively charged residues at the activation lip but not enzyme activity. On the other hand, uptake of an active MKK mutant with disrupted NES (Δ32–51) was elevated in quiescent as well as stimulated cells, and pretreatment of cells with the MKK inhibitor 1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene blocked nuclear uptake. Thus, signaling downstream of MKK is also necessary for translocation. Finally, wild type MKK containing an intact NES translocates to nuclei during mitosis before envelope breakdown. Comparison of mutants with Ser to Glu and Asp or Ala substitutions indicates that Ser phosphorylation is also required for mitotic nuclear uptake of MKK.

Among the key signaling pathways regulating mammalian cell growth and differentiation is the MAP kinase cascade, comprising MAP kinases, ERKs 1 and 2, and MAP kinase kinases MKK1 and 2 (for review, see Refs. 1 and 2). This pathway is activated by many different extracellular stimuli through p21 Ras-coupled mechanisms. Enhancement of MKK or ERK activity in response to cell stimulation involves phosphorylation at residues located within the activation lip of each kinase. In the case of human MKK, phosphorylation at two serine residues (Ser218 and Ser222 in MKK1; Ser222 in MKK2) by upstream protein kinases, Raf-1, c-Mos, or MAP/ERK kinase kinase (MEKK) leads to maximal enzyme activation.

In addition to kinase activation, several studies have demonstrated that the components in this pathway undergo regulated subcellular relocalization. After cell stimulation, ERKs are taken up into nuclei within 5–30 min and are retained for several hours (3–5). This enables transmission of signaling to the nucleus, where an important end result is transcriptional control. The involvement of ERK in phosphorylation and regulation of a number of nuclear factors suggests that redistribution of ERK from cytosolic to nuclear compartments is necessary for signaling (for review, see Ref. 6). In addition, stable nuclear localization of ERK has been correlated with differentiation of PC12 cells, a process involving phosphorylation of nuclear transcription factors involved in neuronal gene expression (7–9).

Initial studies failed to show a similar translocation of MKK in response to signaling (5, 10). This was later modified by the discovery of a leucine-rich sequence located between residues 32 and 42 of MKK (ALQKKLEEL), which behaves as a functional nuclear export signal (NES) (11). Removal or mutation of the NES leads to constitutive nuclear localization of MKK, and cross-linking of peptides containing this sequence to heterologous proteins facilitates their nuclear export. Sequences of this type have been shown to be recognition sites for binding to Crm1/exportin 1, a nuclear protein that facilitates export of cellular components including PKIα, IκB, Rev, and Dsk1, in addition to MKK (12, 13). The fungal antibiotic leptomycin B binds to Crm1 and interferes with its association with NES-containing proteins, thus blocking their export (13–15). Accordingly, the distribution of MKK can be shifted from cytosolic to nuclear within 1 h of leptomycin B treatment in COS cells (14), suggesting that although nuclear localization of MKK is not stable, import is dynamic with rapid rates of influx and efflux.

Interestingly, nuclear import of MKK mutants lacking the NES appears to be promoted in response to serum treatment. In serum-starved COS or HEK293 cells, an MKK mutant with deletion of residues 32–51, which disrupts the NES, a K97A mutation rendering the enzyme inactive, and S218E/S222E mutations at the activation lip, was predominantly cytosolic but distributed into nuclei after 10 min of serum treatment (16). This suggests that mechanisms for nuclear import of MKK are regulatable, either through enhanced import or inhibition of export. Presumably, such mechanisms could require activation of signaling pathways, such that phosphorylation and/or
activation of MKK might be important conditions for stabilizing its nuclear distribution.

In this study, we examined the requirements for nuclear localization of MKK1 by first comparing the behavior of MKK mutants with respect to serum-stimulated nuclear import. Our results show that enhanced negative charge at the activation lip promotes import, although elevated kinase activity is not required, indicating that phosphorylation, not MKK activation, is a key event necessary for nuclear import. However, translocation is sensitive to a cell-permeable MKK inhibitor, indicating that signaling events downstream of MKK are also important for stimulation of import. We also show that wild type MKK redistributes to nuclei early in mitosis, before nuclear envelope breakdown, demonstrating that nuclear uptake of MKK containing a functional NES can be regulated normally during the cell cycle. Comparison of the mitotic nuclear localization of various MKK mutants corroborates the behavior seen on serum stimulation. We conclude that phosphorylation of MKK at the activation lip and activation of downstream targets are necessary events regulating nuclear translocation of wild type MKK.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—Mouse NIH 3T3 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum (Gemini). Cells were grown to 50% confluence and then transfected using LipofectAMINE (Life Technologies, Inc.) mixed with 1 μg of cDNA constructs driving expression of various human MKK1 mutants under the cytomegalovirus promoter (plasmid pMCL; Ref. 17), or constitutively active BXB-Raf-1. The MKK coding sequence was then excised from pRSET with mutations confirmed by dideoxy chain termination DNA sequencing. The MKK coding sequence was then excised from pRSET with BamHI and HindIII and ligated between the corresponding sites in pMCL (17).

**Immunofluorescence, Immunoblotting, and Protein Kinase Assays**—Affinity-purified primary antibodies used in immunohistological studies included anti-phospho-MEK1/2, a rabbit polyclonal antibody recognizing phosphorylated MKK1 and MKK2 (New England Biolabs); C-18, a rabbit polyclonal antibody recognizing the C terminus of MKK1 (Santa Cruz); anti-ACTIVE MAP kinase, a rabbit polyclonal antibody recognizing diphosphorylated ERK1 and ERK2 (Promega); C-14, a rabbit polyclonal antibody recognizing the C terminus of ERK2 (Santa Cruz); 12CA5, a mouse monoclonal antibody to hemagglutinin tag sequence (BabCo); and M-20, a goat polyclonal antibody to lamin B (Santa Cruz).

For immunofluorescence, cells were grown on glass coverslips and transfected as above. After transfection and treatment, coverslips were rapidly rinsed in cold (4 °C) phosphate-buffered saline, immediately fixed by addition of 0.1% glutaraldehyde and 2% formaldehyde in phosphate-buffered saline for 5 min, and then permeabilized with cold (4 °C) phosphate-buffered saline, immediately fixed by addition of 0.1% glutaraldehyde and 2% formaldehyde in phosphate-buffered saline for 5 min, and then permeabilized with cold (37 °C) 100% methanol for 5 min. Similar results were obtained when samples were fixed with 10% neutral buffered formalin for 5 min and then permeabilized with cold (–20 °C) 100% methanol for 5 min. After permeabilization, cells were incubated in Tris-buffered saline (50 mM Tris, pH 7.6, 0.15 M NaCl), 0.1% Tween 20, and 3% bovine serum albumin for 1 h. Coverslips were incubated with primary antibody for 1 h, washed four times with Tris-buffered saline and 0.1% Tween 20, incubated for 1 h with fluorescein isothiocyanate- or Texas Red-conjugated donkey anti-rabbit, anti-mouse, or anti-goat secondary antibody (0.8 μg/ml, Jackson Laboratories), washed four times with Tris-buffered saline and 0.1% Tween 20, and finally counterstained with DAPI (0.4 μg/ml in phosphate-buffered saline).

Primary antibodies were used at dilutions of 1:100, with the exception of 12CA5, which was used at 1:500. Controls for nonspecific staining of phosphorylated MKK were performed by preincubating anti-phospho-MEK1/2.
with 0.1 mg/ml diphosphorylated CSVSQILDS/P/MANS/P/FVGTTSY, synthesized by Macromolecular Resources (Pittsburg, CO). Controls for non-specific staining of HA-MKK1 were performed by preincubating 12CA5 antibody with 0.1 mg/ml YPYDVPIYA (a gift of James Goodrich). Cells were viewed and photographed using a Zeiss Axiosplan fluorescence microscope with a Photometrics SenSys digital charge-coupled device camera system, and images were manipulated using IPLAB Spectrum software.

For immunoblots, cells were grown in 60-mm dishes and treated as above. Cells were harvested by scraping into lysis buffer containing 50 mM 2-glycerol phosphate, 1 μM microcystin, 0.2 mM sodium orthovanadate, 1.5 mM EGTA, 1 mM benzamidine, 2 μg/ml pepstatin A, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM diithiothreitol, followed by centrifugation (17,000 × g, 10 min). Proteins in extract supernatants (20 μg/lane) were separated by SDS-polyacrylamide gel electrophoresis (15% acrylamide) and transferred to Immobilon P (Millipore). Blots were reacted for 1 h with primary antibody (1:1000 dilution for each antibody) followed by 0.8 μg/ml donkey anti-rabbit or anti-mouse IgG coupled to horseradish peroxidase (Jackson Laboratories) and visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech).

Kinase assays were performed as described previously (19). HA-tagged MKK1 was immunoprecipitated from extract supernatants with 12CA5 antibody, followed by phosphorylation of bacterially expressed recombinant ERK2-K52R (1 μM) (a gift of Melanie Cobb; Ref. 20) using 10 mM MgCl2 and 0.1 mM ATP.

RESULTS

HA-WT-MKK1 was overexpressed in NIH 3T3 cells and probed by indirect immunofluorescence using antibody 12CA5 to the HA tag. The localization of WT-MKK was cytosolic in starved cells and unaffected by stimulation with serum-PMA or coexpression with constitutively active BXB-Raf-I (Fig. 1, A–C). In contrast, disruption of the NES by deletion of residues 32–37 resulted in a mutant (ΔN6-MKK) that localized to the cytosol in starved cells but redistributed to nuclei in response to serum-PMA or coexpression with BXB-Raf (Fig. 1, D–F). Thus, regulated nuclear uptake of MKK can be observed with a mutant lacking the NES. This supports and extends previous reports using a ΔN3/K97M/S218E/S222D-MKK1 mutant (16) and establishes that deletion of the NES is sufficient to reveal serum-stimulated nuclear uptake of MKK. The fact that redistribution can be observed by coexpression with BXB-Raf in the absence of serum-PMA further indicates that the activation of the Raf-MKK-ERK pathway is a key part of the stimulatory signal for nuclear uptake.

Examination of the phosphorylation state of the ΔN6-MKK mutant by immunoblotting with anti-phospho-MEK1/2 antibodies showed that this mutant is phosphorylated in response to serum-PMA stimulation (Fig. 2A, lanes 1 and 2), conditions under which its specific activity is enhanced (Fig. 2F, lanes 1 and 2). We therefore asked whether the regulated nuclear import of this mutant is sensitive to the increased phosphorylation state at the activation lip versus the activation state of MKK. Two variants of ΔN6-MKK were designed to distinguish these possibilities. ΔN6/KM contains a mutation of Lys83 to Met and is inactive but phosphorylatable at the activation lip. ΔN6/AA contains mutations in Ser218 and Ser222 to Ala and is inactive and not phosphorylatable at the activation lip. A third variant, ΔN6/ED, contains S218E and S222D mutations and is not phosphorylatable but is constitutively activated due to the introduction of negative charge at the activation lip, with 55-fold greater activity than WT-MKK in vitro (18). In response to transfection with ΔN6/ED, MKK and ERK activity were elevated in serum-starved cells (Fig. 2C, C and F, lanes 7 and 8).

Each mutant was expressed and examined under serum-starved or serum-PMA-treated conditions. Like WT and ΔN6, both ΔN6/KM and ΔN6/AA were cytosolic in serum-starved cells (Fig. 3, A, C, E, and G). Like ΔN6, the ΔN6/KM mutant redistributed to nuclei in response to serum (Fig. 3, D and F), although under this condition ΔN6/KM was phosphorylated, but not activated (Fig. 2, A and F, lanes 3 and 4). In contrast, ΔN6/AA showed little nuclear uptake in response to serum-PMA (Fig. 3H, a condition under which it was unphosphorylated and inactive (Fig. 2, A and F, lanes 5 and 6). This suggests that the presence of phosphorylatable residues is needed for nuclear accumulation, although the activation of MKK by mutation is not sufficient for this process. Consistent with this hypothesis, the ΔN6/ED mutant was constitutively nuclear even in starved cells (Fig. 3, F and J). Thus, negatively charged residues at the activation lip appear to stabilize the nuclear localization of MKK.

The results with ΔN6-MKK were tested further by examining the nuclear uptake of MKK with residues 32–51 deleted (ΔN3-MKK), which both enhances basal activity as well as removes the NES (11, 17). Variants of ΔN3 were constructed, containing mutations of S218A/S222A (ΔN3/AA) or S218E/S222D (ΔN3/ED). As expected by removal of the NES, nuclear uptake of ΔN3 was significant in quiescent cells, although uptake was enhanced in the presence of serum-PMA (Fig. 3, K and L). Likewise, whereas ΔN3/ED was constitutively nuclear, ΔN3/AA was excluded in the presence or absence of stimulation.

![Fig. 2. Expression and activation of MKK mutants after transfection and cell stimulation.](image-url)
This corroborates results above with the ΔN6 mutants, suggesting that phosphorylation or incorporation of negative charge at the activation lip is critical for uptake. A final MKK mutant was tested, combining deletion of residues 44–51 (ΔN4) with S218E/S222D, which has 630-fold greater activity than WT-MKK (17) but retains the NES. This mutant is constitutively cytoplasmic even under stimulated conditions (Fig. 3, S and T), indicating that activation of MKK is not sufficient for nuclear import.

A possibility not excluded by these experiments is that facilitated nuclear uptake of MKK involves regulation of import or export factors targeted by signaling pathways. This is supported by evidence showing that the combinatorial mutant, ΔN3/KM/ED, is excluded in quiescent cells but nuclear in response to serum-PMA (16). We performed the same experiment and obtained similar results (Fig. 3, M and N). Although this mutant is negatively charged at the activation lip, it is inactive as a consequence of the K97M mutation. Thus, although phosphorylation at the activation lip may be a necessary condition for nuclear uptake, uptake may also require activation of downstream signaling targets. One possibility, consistent with the nuclear redistribution of MKK in response to BXB-Raf coexpression, is that such factors may be targeted by components downstream in the MKK-ERK pathway. Therefore, we tested the effect of blocking signaling downstream of MKK on its nuclear uptake.

U0126 is a phenylthiobutadiene compound recently described as a selective inhibitor of MKK1 and MKK2 (21). Pretreatment of NIH 3T3 cells with 20 μM U0126 blocked ERK activation ≥ 90% in response to serum-PMA (Fig. 4 A). Similar results were observed in cells transfected with ΔN6-MKK or ΔN3-MKK before inhibitor treatment (Fig. 4, B and C). Under these conditions, nuclear uptake of ΔN6-MKK in response to serum-PMA was inhibited (Fig. 5, A–D). Interestingly, nuclear uptake of ΔN3-MKK was also suppressed under conditions of serum starvation as well as serum-PMA treatment (Fig. 5, E–H). U0126 has previously been shown to inhibit the constitutively active mutant ΔN3/ED-MKK (21); therefore, it should also inhibit ΔN3-MKK. The resulting behavior indicates that suppressing the activity of the ΔN3 mutant interferes with its nuclear accumulation. We conclude that ERK signaling downstream of MKK is needed to promote nuclear uptake of MKK mutants in response to cell stimulation.

Although different forms of MKK show clear differences in acute nuclear uptake, all experiments were performed using mutant enzymes in which the NES is deleted. However, in cycling NIH 3T3 cells growing in 10% fetal bovine serum (as well as all other mammalian cell lines we have so far examined), we have observed that endogenous MKK becomes activated and localized to nuclei during mitosis, revealed by immunocytochemical staining with an antibody that recognizes phosphorylated MKK (Fig. 6, A–C; 34). We therefore examined the distribution of HA-tagged WT-MKK1 after expression in cycling NIH 3T3 cells and observed nuclear localization in mitotic cells, despite the fact that the NES was retained (Fig. 6, D–F). This relocalization occurs early in prophase, before nu-

![Fig. 3. Characteristics of nuclear translocation of various MKK mutants in response to serum-PMA.](image-url)
5% serum and 50 nM PMA for 2 h. Extracts were prepared, and aliquots were examined by Western blotting using primary antibodies: anti-ACTIVE MAP kinase (A) and a mixture of 12CA5 and anti-ACTIVE MAP kinase (B and C). Elution of expressed MKK1 and endogeneous diphosphorylated ERK1/2 are indicated.

clear envelope breakdown, as demonstrated by staining of the nuclear matrix using an antibody to lamin B (Fig. 6E). A similar distribution of endogeneous WT-MKK was observed when probing cells with antibody recognizing the C terminus of MKK1 (data not shown).

The importance of phosphorylation on the nuclear uptake was then tested by examining mitotic cells for distribution of expressed HA-tagged MKK mutants, all of which contained an intact NES sequence. As observed with WT-MKK, S218E/S222D and K97M mutants showed nuclear staining in prophase cells identified by DAPI staining of partially condensed chromatin (Fig. 7, D, F, J, and M). Under these conditions, significant phosphorylation of endogeneous and expressed MKK was observed in nuclei, revealed using the anti-phospho-MEK1/2 antibody (Fig. 7, E and K). In contrast, nuclear localization of the S218A/S222A mutant was reduced substantially compared with the other mutants, although nuclear staining of endogeneous phosphorylated MKK could still be observed (Fig. 7, G and H). This indicates that removal of phosphorylatable residues at the activation lip suppresses MKK uptake during mitosis in a manner similar to the behavior in response to acute stimulation by serum-PMA.

DISCUSSION

In this study, we present evidence demonstrating that, like ERK, nuclear uptake of MKK in mammalian cells occurs in a regulatable manner. Our findings demonstrate that the dependence of uptake on acute cell stimulation reflects a requirement for at least two events involved in the Raf-MKK-ERK pathway. Incorporation of negative charge at the activation lip of MKK is a necessary condition for nuclear uptake, demonstrated by the correlation of translocation with phosphorylation, the ability to mimic this effect by substituting the phosphorylatable serine residues with acidic amino acids, and the interference with uptake by disrupting these phosphorylatable residues with alanine.

Importantly, nuclear uptake of MKK is also observed with catalytically inactive mutants, indicating that the enzymatic activity of MKK is not important for signal-induced import. Nevertheless, the contrast in behavior of ΔN3/ED and ΔN3/KM/ED mutants, in which the inactive ΔN3/KM/ED mutant is cytosolic but imported in response to stimulation, whereas constitutively active ΔN3/ED is nuclear even under unstimulated conditions, suggests at least some dependence on MKK activity, perhaps through the activation of downstream signaling events. Consistent with this hypothesis, serum-PMA-stimulated nuclear import of MKK was blocked by the MKK inhibitor U0126 under conditions in which ERK activation was strongly inhibited. This block in uptake was observed with ΔN3-MKK as well as ΔN6-MKK, indicating that nuclear localization of the constitutively active ΔN3 and ΔN3/ED under serum-starved conditions is most likely attributable to elevated ERK activity in cells expressing these mutants.

Mechanisms involved in ERK translocation are instructive toward understanding events regulating MKK translocation. A nuclear localization signal is not apparent in the primary sequence of ERK, and the available evidence indicates that elevated nuclear localization of ERK occurs after its overexpression, suggesting that ERK import may occur through passive diffusion (5). In addition, ERK import is blocked by overexpression of MKK, whereas MKK mutants that are unable to form stable MKK-ERK interactions have no effect, suggesting that ERK translocation occurs after dissociation of an MKK-ERK complex (23). On the other hand, recent studies demonstrate that ERK import requires phosphorylation at the activation lip, an event that promotes homodimerization, and ERK mutants deficient in dimerization are not retained efficiently in nuclei (24). Nuclear localization of ERK may be stabilized through dimerization, which occludes a potential NES located at the dimerization interface, or through new interactions with nuclear targets.

Taken together, a working hypothesis for translocation
events after cell stimulation can be developed, based on previous work and the findings of our study. In unstimulated cells, MKK and ERK exist in the cytosolic compartment bound within low affinity complexes. Cell stimulation leads to phosphorylation and activation of MKK and ERK, concomitant with disruption of MKK-ERK interactions. ERK is then imported by a mechanism that is still unknown, possibly involving passive diffusion or facilitated uptake. Dimerization of ERK or complex formation with nuclear targets may then suppress the rate of export, enabling stable nuclear localization for several hours. Phosphorylation of MKK enhances its uptake through mechanisms that depend on its phosphorylation as well as activation of signaling components downstream, including ERK. Because acute uptake is observed only with MKK mutants disrupted in the export recognition sequence, the regulation by MKK phosphorylation and ERK activation most likely occurs at the level of import. Furthermore, the import rate after stimulation must still be slow relative to the rate of export, which presumably involves interaction of MKK with export factors, including Crm1 and Ran-GTP. Thus under normal conditions, the rate of import must occur during mitosis, thus stabilizing nuclear pools of MKK in nucleus.

The importance of nuclear uptake of MKK for cell regulation has been substantiated by studies showing that constitutively active mutants of MKK are most potent as inducers of NIH 3T3 cell transformation when they lack the NES (25). This suggests that transformation is enhanced by stable nuclear localization of MKK. This effect was correlated with increased levels of activated ERK in the nucleus and was blocked by MAP kinase phosphatase 1 (25). Thus, the striking nuclear redistri-

characteristics of nuclear translocation of various MKK mutants in mitotic cells. Cells were transfected with MKK1 WT (A–C), K97M (D–F), S218A/S222A (G–I), and S218E/S222D (J–M) and grown in 10% serum and DMEM. Cells were fixed, permeabilized, and costained with anti-HA and anti-phospho-MEK1/2 primary antibodies, followed by anti-mouse-Texas Red and anti-goat-fluorescein isothiocyanate secondary antibodies. Panels show anti-HA (D), anti-lamin B (E), and DAPI (F) staining of the same cells. Mitotic cells were identified by the appearance of condensed chromatin in DAPI staining (arrows). Experiments were repeated two times with similar results.

One of the ambiguities confounding the issue of MKK translocation is that nuclear localization of wild type MKK is never observed in response to acute stimulation under normal cellular conditions (5, 10, 11). Therefore, the striking nuclear redistribution we observe during prophase suggests a mitotic role for MKK in the regulation of normal growing cells. Our results indicate that mitotic nuclear localization of NES-intact MKK also requires phosphorylation at the activation lip, similar to the characteristics for serum-dependent uptake of the NES deletion mutants. This most likely involves activation of known upstream components Src and Raf-1, which have been shown to also be active early in mitosis (27–29). We hypothesize that these events also regulate translocation at the level of import, in analogy to serum-PMA stimulation. However, the fact that nuclear localization is observed with MKK containing an intact NES indicates that a mechanism for retarding export of MKK must occur during mitosis, thus stabilizing nuclear pools of active MKK and ERK.

An important function of MKK activation during mitosis may be to promote M phase entry in somatic cells, similar to its role during M phase progression in meiosis. This is suggested by recent experiments showing that the MKK inhibitor PD98059 or dominant negative mutants of MKK both delay M phase entry in synchronized NIH 3T3 cells.2 Later in mitosis,

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2 J. H. Wright, E. Munar, P. Andreassen, R. Margolis, R. Seger, and E. G. Krebs, submitted for publication.
ERK activation appears to inhibit anaphase entry. This is suggested by studies in cell free extracts of *Xenopus* embryos demonstrating that active ERK promotes metaphase arrest and blockscdc2-cyclin B inactivation (30–33). We have also found that active ERK appears to regulate a phosphoepitope recognized by the 3F3/2 antibody (34), which is believed to regulate kinetochore attachment in metaphase cells (22). Thus, available evidence supports both positive and negative roles for the MKK-ERK pathway during mitotic progression. Presumably, nuclear uptake of MKK during mitosis is involved in controlling the activity state of nuclear pools of ERK to regulate the timing of events in mitosis.

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