Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4925–4929, May 1994
Cell Biology

Mammalian mitogen-activated protein kinase kinase kinase (MEKK) can function in a yeast mitogen-activated protein kinase pathway downstream of protein kinase C

KENDALL J. BLUMER*†, GARY L. JOHNSON‡, AND CAROL A. LANGE-CARTER‡

*Department of Cell Biology and Physiology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110; and ‡Division of Basic Sciences, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206

Communicated by Philip W. Majerus, February 7, 1994

ABSTRACT **Mitogen-activated protein kinase cascades are conserved in fungal, plant, and metazoan species. We expressed murine MAP kinase kinase kinase (MEKK) in the yeast *Saccharomyces cerevisiae* to determine whether this kinase functions as a general or specific activator of genetically and physiologically distinct MAP-kinase-dependent signaling pathways and to investigate how MEKK is regulated. Expression of MEKK failed to correct the mating deficiency of a *ste11*Δ mutant that lacks an MEKK homolog required for mating. MEKK expression also failed to induce expression of a reporter gene controlled by the *HOG1* gene product (Hog1p), a yeast MAP kinase homolog involved in response to osmotic stress. Expression of MEKK did correct the cell lysis defect of a *bck1*Δ mutant that lacks an MEKK homolog required for cell-wall assembly. MEKK required the downstream MAP kinase homolog in the *BCK1*-dependent pathway, demonstrating that it functionally replaces the *BCK1* gene product (Bck1p) rather than bypassing the pathway. MEKK therefore selectively activates one of three distinct MAP-kinase-dependent pathways. Possible explanations for this selectivity are discussed. Expression of the MEKK catalytic domain, but not the full-length molecule, corrected the cell-lysis defect of a *pkc1*Δ mutant that lacks a protein kinase C homolog that functions upstream of Bck1p. MEKK therefore functions downstream of the *PKC1* gene product (Pkc1p). The N-terminal noncatalytic domain of MEKK, which contains several consensus protein kinase C phosphorylation sites, may, therefore, function as a negative regulatory domain. Protein kinase C phosphorylation may provide one mechanism for activating MEKK.**

Mitogen-activated protein kinases (MAPKs), or extracellular signal-response kinases, are activated in response to various mitogens, growth factors, and oncogene products in eukaryotic cells (1–4). MAPKs are activated via an evolutionarily conserved network of protein kinases (Fig. 1). In these pathways, MAPK is phosphorylated and activated by MEK (mitogen-activated protein or extracellular signal-response kinase kinase) (5–9), and MEK can be phosphorylated and activated by Raf-1 kinase (10–12) or MEK kinase (MEKK) (13).

Raf and MEKK are positioned in these networks such that they potentially interact with different types of upstream signaling molecules, thereby linking various signaling pathways to the activation of MAPK. Raf can function downstream of protein kinase C (14–16), and downstream of Ras (17–19); indeed, Raf and Ras physically interact (20–23). However, full activation of Raf may involve the synergistic action of multiple factors because protein kinase C partially activates Raf (15), and there is evidence that Ras and protein kinase C can function in distinct as well as interdependent signaling pathways (3, 24, 25). Upstream regulatory molecules that control MEKK function in mammalian cells are currently undefined.

The yeast *Saccharomyces cerevisiae* possesses three signaling networks that employ MAPK homologs (Fig. 1), one for responding to mating pheromones (2, 6, 26–30), a second for sensing osmotic stress (31–33), and a third for assembling the cell wall (34–37). Protein kinases in these signaling networks function independently of one another because mutations that disrupt one pathway do not substantially affect the other pathways (2, 27, 31–37).

The existence of physiologically distinct MAPK pathways in yeast implies that the MEKK homolog in each network responds to specific upstream signals. Genetic analysis supports this hypothesis (Fig. 1). In the mating pheromone response pathway, an MEKK homolog (*STE11* gene product, Ste11p; the p here and elsewhere indicates a specific gene product) functions downstream of Ste5p (26, 29), a protein of unknown biochemical function (38, 39). In the cell wall assembly pathway, an MEKK homolog (*BCK1* gene product, Bck1p) functions downstream of a protein kinase C-like enzyme (35). In the mating pheromone response pathway of the yeast *Schizosaccharomyces pombe*, an MEKK homolog (*byr2* gene product, Byr2p) interacts with and functions downstream of *ras1* (21).

Here we have expressed MEKK in the yeast *S. cerevisiae* to assess whether it can function as a general or specific activator of genetically and physiologically distinct MAPK-dependent signaling networks and to address the relationship between MEKK and potential upstream regulatory molecules.

MATERIALS AND METHODS

Yeast Strains, Growth Conditions, Mating Tests, Gene Disruptions, and β-Galactosidase Assays. Strains of *S. cerevisiae* used were as follows: EG123 (*MATa leu2-3,112 ura3-52 trp1-1 his4 can*^{r}) (35); 1788 (*MATa*/*MATα* isogenic diploid of EG123) (35); DL251 (*bck1*Δ::*URA3*/*bck1*Δ::*URA3* derivative of 1788) (35); DL394 (*pkc1*Δ::*LEU2*/*pkc1*Δ::*LEU2* derivative of 1788) (35); DL456 (*mpk1*Δ::*TRP1*/*mpk1*Δ::*TRP1* derivative of 1788) (36); AG38-18B (*MATa leu2-3,112 ura3-52 trp1 his3 ade2*) (A. Grishin, personal communication); KBY207 (*ste11*Δ::*URA3* derivative of AG38-18B) (this study); GG18 (*MATa ura3 leu2 his3 trp1 ade8 URA3*::*CTT1-18*), *CTT1-18* is a *CTT1–lacZ* fusion that is induced by osmotic stress (0.4 M NaCl) (40) (M. Gustin, personal communication); and DC17 (*MATα his1*) (26). Rich (YPD and YPGal) and selective (SD and SGal) media were supplemented as necessary with osmotically supporting agents (10% or 1 M sorbitol). For

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; MEKK, mitogen-activated protein kinase kinase kinase.

†To whom reprint requests should be addressed.

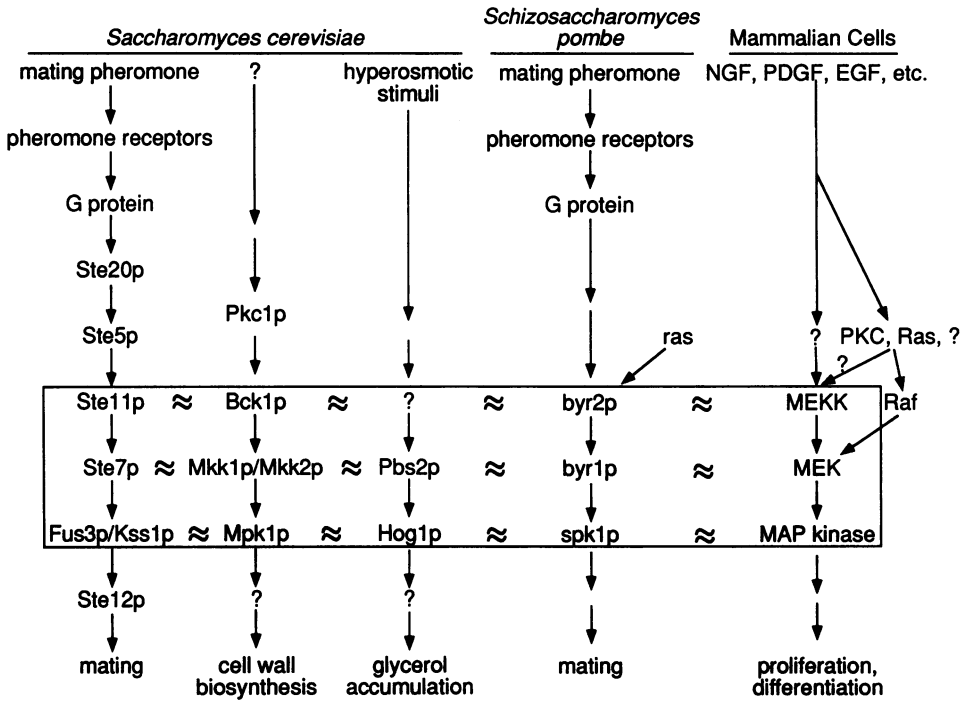

FIG. 1. Conserved MAPK signaling pathways in yeast and mammalian cells. Three pathways in *S. cerevisiae* and one in *Schizosaccharomyces pombe* involve protein kinases that are structurally related to mammalian MEKK, MEK, and MAPK (enclosed in the box). Homologous kinases in these pathways are indicated. An MEKK or Raf homolog has yet to be reported for the *HOG1*-dependent pathway. Connections among vertebrate MEKK, protein kinase C, and Ras are hypothetical. Factors that act upstream of MEKK and Raf in mammalian cells have been omitted for clarity. NGF, nerve growth factor; PDGF, platelet-derived growth factor; EGF, epidermal growth factor.

quantitative mating tests (41), MEKK expression was induced from the *GAL* promoter, and cells were mated with an excess of a mating-type tester strain (DC17) for 12 h at 30°C on YPGal medium. Quantitative mating efficiency was expressed relative to the number of diploids produced by mating of wild-type control cells that were treated identically. The *STE11* gene was disrupted by transforming AG38-18B with *Xba* I-cut pNC276; disruptions were confirmed by Southern blot analysis. Assays of β-galactosidase activity were performed as described (29).

Plasmids, MEKK, and MEK-1 Constructions. Sources of plasmids were as follows: pSL307 (41) (J. Thorner, University of California, Berkeley), pNC276 (B. Errede, University of North Carolina), pRS424GAL1,10 and pRS425GAL1,10 (C. Hug, personal communication), pPGK (42) (D. Tipper, University of Massachusetts Medical Center), and pBH21 (D. Jenness, personal communication). pRS314PGK was constructed by ligating a *PGK* promoter–terminator cassette (*Xho* I–*Pst* I fragment from pPGK) with *Xho* I/*Pst* I-cut pRS314. To express the MEKK cDNA in yeast, the entire MEKK coding region was amplified by PCR and inserted into pCRII (Invitrogen). An *Eco*RI fragment encompassing the MEKK coding sequence was excised and inserted into *Eco*RI-cut pRS424GAL1,10, pRS425GAL1,10, and pRS314PGK, to yield pRS424-GALMEKK, pRS425GALMEKK, and pRS314PGKMEKK, respectively. Plasmids constructed from independent PCRs functioned identically in yeast, indicating that functionally significant mutations did not occur. To express the catalytic domain of MEKK, sequences encoding the N-terminal domain of MEKK were deleted as follows. pRS424GALMEKK, pRS425GALMEKK, and pRS314PGKMEKK were digested partially with *Eco*RI and to completion with *Nco* I. After their ends were blunted, DNA fragments were circularized to yield plasmids pRS424GALMEKKCAT, pRS425GALMEKKCAT, and pRS314PGKMEKKCAT. Truncated MEKK is expressed from an in-frame methionine codon (corresponding to Met-353 in full-length MEKK), thereby omitting 88% of the N-terminal domain including all of the consensus protein kinase C phosphorylation sites. MEK-1 was cloned into a yeast expression plasmid by inserting a *Bam*HI–*Hin*dIII fragment from plasmid pRSETA-MEK1 (G.L.J., unpublished data) into *Bam*HI–*Hin*dIII-cut pBH21 (containing the *ADH1* promoter and the *LEU2* gene) to yield pADHMEK.

Immunological Methods. Standard procedures for yeast cell lysis, gel electrophoresis, and immunoblot analysis were employed (43). Immunoblots were developed by using the enhanced chemiluminescence procedure (Amersham). The anti-MEKK antibody has been described (13). Tyrosine phosphorylation of Hog1p was detected by immunoblot methods employing anti-phosphotyrosine monoclonal antibodies (4G10, Upstate Biotechnology, Lake Placid, NY), as described (33).

RESULTS

Expression of MEKK and the MEKK Catalytic Domain in Yeast. To determine whether MEKK can act as a general or specific activator of three MAPK cascades in yeast (Fig. 1), we constructed plasmids that direct the expression of full-length MEKK or a truncated form of the kinase lacking its N-terminal noncatalytic domain. Because the N-terminal regions of yeast MEKK homologs function genetically as negative regulatory domains (26, 29, 35), full-length MEKK may require upstream factors for its activation, and N-terminally truncated MEKK may be hyperactive, thereby bypassing its requirement for upstream signaling elements.

Immunoblot experiments were performed that employed extracts of yeast cells expressing full-length MEKK or the MEKK catalytic domain from the inducible *GAL1* promoter (Fig. 2). The major immunoreactive species migrated more slowly than expected, but sequence analysis indicated that these discrepancies were not due to cloning artifacts (data not shown). The major immunoreactive species detected in cells expressing full-length MEKK was a polypeptide of 110 kDa, larger than expected for the primary translation product (77 kDa). This species is phosphorylated *in vivo* (K.J.B., unpublished data), which may partly explain its slower mobility. Also detected were polypeptides (33–46 kDa) that probably are N-terminal truncations produced by proteolysis or initiation of translation at internal in-frame methionine codons because they were recognized by a C-terminal-specific antibody. Truncated species are also observed in mammalian cells that overexpress MEKK (13). In yeast cells expressing the MEKK catalytic domain, a major 46-kDa polypeptide

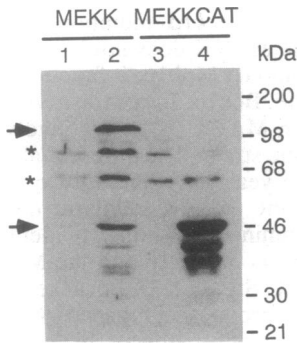

FIG. 2. Expression of MEKK and the MEKK catalytic (MEKKCAT) domain in yeast. Strain AG38-18B harboring pRS424-GALMEKK or pRS424GALMEKKCAT was grown in selective medium containing either glucose (lanes 1 and 3) or galactose (lanes 2 and 4) as the carbon source. Total cell extracts (300 μg of protein) resolved by SDS/PAGE were analyzed by immunoblot methods that employed antibodies raised against a C-terminal peptide sequence of mouse MEKK. Immunoreactive species were detected by enhanced chemiluminescence. The major forms of MEKK expressed under inducing conditions (galactose) are indicated by arrows. Cross-reacting species observed when MEKK expression was repressed (glucose) are indicated by stars; the levels of these species were variable. Positions of molecular mass markers are indicated.

was detected, somewhat larger than the expected size of 36 kDa. The 46-kDa species is also phosphorylated *in vivo* (K.J.B., unpublished data). Smaller products were also detected (33–41 kDa). Desitometric scanning indicated that full-length MEKK expression levels were 2- to 4-fold lower than those of the MEKK catalytic domain (data not shown).

MEKK Does Not Function in the Mating MAPK Pathway. We first examined whether MEKK can function in the mating pheromone-response pathway. Full-length and truncated MEKK were expressed from the *GAL* promoter in a *ste11*Δ mutant that lacks an MEKK homolog required for mating. Quantitative mating abilities of transformants grown under repressing (glucose) and inducing (galactose) conditions were determined. Mating was not detected ($<10^{-7}$ of the wild-type mating efficiency) whether or not full-length or truncated MEKK was expressed (data not shown). Furthermore, *ste11*Δ mutants failed to mate when full-length or truncated MEKK was overexpressed along with vertebrate MEK-1 (data not shown). Similarly, expression of full-length or truncated MEKK failed to induce expression of a pheromone-regulated *FUS1–lacZ* reporter gene (pSL307, data not shown). In addition, overexpression of full-length or truncated MEKK did not significantly reduce the mating efficiency of wild-type cells (data not shown). Hence there was no evidence that MEKK interacts in a positive or negative way with components of the mating pheromone-response pathway.

MEKK Does Not Activate the Osmotic Stress MAPK Pathway. Complementation tests could not be used to examine whether murine MEKK can function in the osmotic stress MAPK pathway because an MEKK homolog in this pathway has yet to be identified. Instead, we examined whether expression of full-length or truncated MEKK resulted in activation (as indicated by increased phosphotyrosine content) of Hog1p, the MAPK homolog in the pathway, or induced expression of a *CTT1–lacZ* fusion, which is induced by osmotic stress in a *HOG1*-dependent manner (ref. 40; M. Gustin, personal communication). Results of these experiments failed to reveal any evidence that MEKK can activate the *HOG1*-dependent pathway (data not shown).

MEKK Complements a *bck1*Δ Mutation. The *BCK1* gene of *S. cerevisiae* encodes an MEKK homolog of the MAPK network required for cell wall biosynthesis (Fig. 1) (34–37).

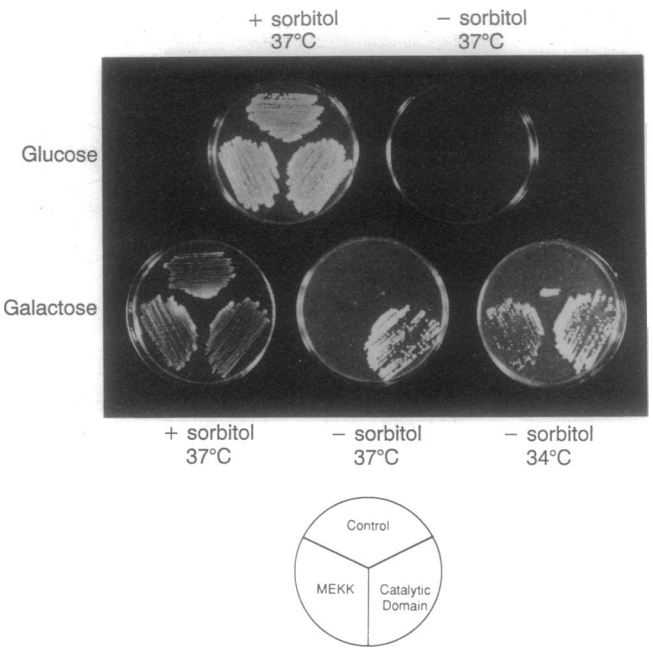

FIG. 3. Expression of MEKK corrects the osmotic sensitivity of *bck1*Δ mutants. A *bck1*Δ::*URA3* mutant (DL251) carrying pRS424-GAL (Control), pRS424GALMEKK (MEKK), or pRS424-GALMEKKCAT (Catalytic Domain) was grown in selective medium containing 10% sorbitol and either glucose or galactose as the carbon source. Cells in logarithmic phase were streaked on YPD or YPGal plates either lacking or containing 10% sorbitol, as indicated. Plates were incubated at the indicated temperatures for 3 days and photographed.

The temperature-sensitive lysis phenotype of *bck1*Δ mutants was used to determine whether MEKK can function in this pathway. Fig. 3 shows the growth phenotypes of a *bck1*Δ mutant carrying a control plasmid or plasmids that direct the galactose-inducible expression of full-length or truncated MEKK. As expected, under repressing conditions (glucose) *bck1*Δ mutants carrying control plasmids or MEKK constructs failed to grow at 37°C in the absence of an osmotic stabilizer (10% sorbitol). Under inducing conditions (galactose), *bck1*Δ mutants expressing full-length MEKK were able to grow in the absence of sorbitol and form colonies at 34°C but not at 37°C. When *bck1*Δ mutants expressed MEKK from a stronger constitutive promoter (*PGK*), they formed colonies at 37°C in the absence of sorbitol (data not shown). Expression of truncated MEKK from the *GAL* (Fig. 3) or *PGK* (data not shown) promoter allowed *bck1*Δ mutants to grow in the absence of sorbitol at 34°C and 37°C.

MEKK could suppress a *bck1*Δ mutation by bypassing the MAPK network or by faithfully substituting for the *BCK1* gene product (Bck1p). Bypass suppression was ruled out because expression of full-length or truncated MEKK failed to rescue the lysis defect of an *mpk1*Δ mutant (Fig. 4), which lacks the MAPK homolog in the pathway.

Truncated MEKK Bypasses the Requirement for a Protein Kinase C Homolog Encoded by *PKC1*. Yeast cells lacking Pkc1p lyse when grown in the absence of osmotic stabilizer (1 M sorbitol) (44, 45). The *pkc1*Δ mutant defect can be suppressed by certain dominant *BCK1* mutations (35); Bck1p, therefore, acts downstream of Pkc1p. To determine whether MEKK also functions downstream of Pkc1p, we tested whether expression of full-length or truncated MEKK suppresses a *pkc1* deletion mutation. The constitutive *PGK* promoter was used to express full-length MEKK or its catalytic domain. As shown in Fig. 5, a *pkc1*Δ mutant expressing truncated MEKK formed colonies in the absence of 1 M sorbitol, whereas the same cells expressing full-length

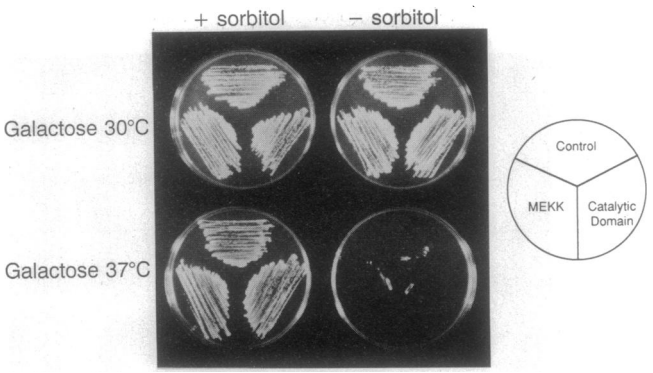

FIG. 4. Expression of MEKK does not correct the osmotic sensitivity of an *mpk1*Δ mutant. An *mpk1*Δ::*TRP1* mutant (DL456) carrying pRS425GAL (Control), pRS425GALMEKK (MEKK), or pRS425GALMEKKCAT (Catalytic Domain) was treated as described in Fig. 3. Cells were streaked on YPGal plates with or without 10% sorbitol, incubated at the indicated temperatures for 3 days, and photographed.

MEKK did not, even at reduced temperatures (22°C, data not shown).

Differences in expression levels might partially explain why expression of the MEKK catalytic domain suppressed a *pkc1*Δ mutation whereas full-length MEKK did not. However, this explanation seemed insufficient for two reasons. (*i*) Levels of full-length and truncated MEKK differed only 2- to 4-fold (Fig. 1). (*ii*) Full-length MEKK did not even partially suppress a *pkc1*Δ mutation (cells failed to grow even upon prolonged incubations at reduced temperatures). It was therefore more likely that full-length MEKK is significantly less active than the MEKK catalytic domain. Thus we suggest that the N-terminal region of MEKK functions genetically as a negative regulatory domain.

DISCUSSION

We have expressed full-length and N-terminally truncated forms of mammalian MEKK in the yeast *S. cerevisiae* and determined their ability to function in physiologically and genetically distinct MAPK cascades that are required for mating, response to osmotic stress, and cell-wall assembly. Our results address the signaling selectivity of MEKK and its relatives and suggest one way that mammalian MEKK may be regulated by upstream signaling elements.

Yeast cells defective in the MEKK-related kinase Bck1p or the MAPK homolog Mpk1p are defective in cell-wall assembly and lyse at nonpermissive temperatures (35, 37). We find that expression of full-length MEKK or the MEKK catalytic

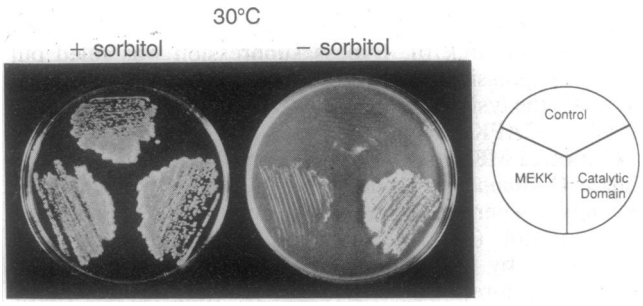

FIG. 5. Expression of the MEKK catalytic domain corrects the osmotic sensitivity of a *pkc1* mutant. A *pkc1*Δ::*LEU2* mutant (DL394) containing pRS314PGK (Control), pRS314PGKMEKK (MEKK), or pRS314PGKMEKKCAT (Catalytic Domain) was grown in selective medium containing 1 M sorbitol. Cells were then streaked on YPD plates with or without 1 M sorbitol, as indicated. Plates were incubated at 30°C for 3 days and photographed.

domain suppresses a *bck1*Δ mutation but not an *mpk1*Δ mutation. Because MEKK does not bypass the requirement for the MAPK homolog Mpk1p, it functions by replacing its homologous kinase, Bck1p. In contrast, we find that MEKK fails to function in MAPK pathways involved in mating or response to osmotic stress. The differential ability of MEKK to function in one yeast MAPK pathway but not others is striking because the MEKK catalytic domain displays a similar degree of amino acid sequence identity (≈36%) with the catalytic domains of Bck1p and the MEKK homolog of the mating pathway, Ste11p (13). Because an MEKK-related kinase (*NPK1* gene product) of tobacco (46) can also function in the *BCK1*-dependent pathway but not in the *STE11*-dependent pathway, members of the MEKK family in yeast and multicellular organisms can function as specialized rather than generalized activators of MAPK cascades in yeast. Whether Raf or Mos likewise displays selective signaling activity toward various yeast MAPK pathways remains to be examined. We predict that MEKK can function in a subset of the MAPK-dependent signaling pathways in mammalian cells, a speculation that can be tested once appropriate constitutively active or dominant-negative forms of MEKK are generated. The N-terminally truncated and apparently hyperactive form of MEKK described here should prove useful.

Selective signaling among components of metazoan, plant, and fungal MAPK networks could occur by various mechanisms. MEKK may function in the *BCK1*-dependent MAPK pathway because this network requires low level of activity. In contrast, the *STE11*-dependent and *HOG1*-dependent pathways may require a level of kinase activity exceeding that provided by vertebrate MEKK. Consistent with this possibility, a *bck1*Δ mutation can be suppressed by overexpressing the downstream MEK homolog (*MKK1* gene product) (34), whereas a *ste11* mutation cannot be suppressed by overexpressing Ste7p, its cognate MEK homolog (26).

Differences in intrinsic kinase activity might not fully explain selective signaling by MEKK in yeast. Even if MEKK has rather low activity, we still might have expected to observe detectable levels of mating or reporter gene induction in the sensitive assays we employed. Furthermore, expression of a hyperactive form of MEKK (catalytic domain) fails to complement a *ste11* mutation to a detectable degree even when its mammalian substrate, MEK-1, is coexpressed.

MEKKs might function in certain signaling pathways because they preferentially recognize specific isoforms of MEK-related kinases. Consistent with this hypothesis are heterologous expression studies employing components of other MAPK cascades (47, 48), and the observation that mutations disrupting one MEKK homolog in yeast do not substantially affect the other two MAPK signaling networks (27, 35). Accordingly, MEKK might function selectively because it is more closely related structurally to Bck1p. Indeed, MEKK and Bck1p are more alike because they lack a 36-amino acid insertion that is present in Ste11p between kinase subdomains II and III. This or other regions of MEKK-related protein kinases, may, therefore, influence their ability to discriminate among various MEK homologs.

Selective signaling by mammalian MEKK in yeast might also imply that various yeast MAPK pathways are activated by different biochemical mechanisms. For example, activation of the mating MAPK pathway may involve formation of ternary complexes that include the MEKK, MEK, and MAPK homologs, Ste11p, Ste7p, Fus3p/Kss1p, and possibly Ste5p, which functions genetically upstream of Ste11p (26, 29). Although the existence of such complexes has not been demonstrated directly, various combinations of Ste5p, Ste11p, Ste7p, and Fus3p/Kss1p do interact with one another in the yeast two-hybrid system (J. Printen and G. F. Sprague,

Jr., personal communication; B. Satterberg and E. Elion, personal communication), and Ste5p coimmunoprecipitates with Ste11p and Fus3p (J. Kranz and E. Elion, personal communication). Thus, according to the ternary complex model, MEKK does not function in the *STE11*-dependent pathway because it fails to assemble or it assembles improperly or inefficiently with other components of the complex. Whether signaling in the *BCK1*-dependent pathway also involves complex formation or sequential action of the protein kinases is currently unknown. Clearly, establishing the mechanisms responsible for selective signal transduction by components of MAPK cascades requires further genetic and biochemical investigation.

Our results address whether a functional link exists between MEKK and protein kinase C. Because expression of the MEKK catalytic domain suppresses a *pkc1*Δ mutation, MEKK functions downstream of Pkc1p. Furthermore, two lines of evidence address whether mammalian MEKK responds to signals transduced by yeast Pkc1p. (*i*) Our genetic data indicate that the N-terminal region of MEKK, which contains several consensus protein kinase C phosphorylation sites, functions genetically as a negative regulatory domain because removal of this region enables the MEKK catalytic domain to activate the *BCK1*-dependent MAPK pathway in the absence of Pkc1p. Indeed, MEKK, Ste11p, and the MEKK homolog of *Schizosaccharomyces pombe*, Byr2p, share some homology in their N-terminal domains (13), which function genetically as negative regulatory domains (26, 29, 35). (*ii*) Pkc1p can phosphorylate the N-terminal regulatory domain of Bck1p (C.-Y. Chen and D. Levin, personal communication). Although other models are possible, we speculate that protein kinase C in yeast, and perhaps mammalian cells as well, phosphorylates and participates in the activation of MEKK.

We thank B. Errede, M. Gustin, C. Hug, D. Jenness, D. Levin, and D. Tipper for gifts of strains and plasmids, and E. Elion, D. Levin, and G. F. Sprague, Jr., for communicating results prior to publication. This work was supported by the National Institutes of Health (Grants GM44592 to K.J.B.; DK37871, GM30324, and CA58187 to G.L.J.) and the Lucille P. Markey Charitable Trust (to K.J.B.).

1. Crews, C. M. & Erikson, R. L. (1993) *Cell* **74**, 215–217.
2. Errede, B. & Levin, D. E. (1993) *Curr. Opin. Cell Biol.* **5**, 254–260.
3. Leevers, S. J. & Marshall, C. J. (1992) *Trends Cell Biol.* **2**, 283–286.
4. Perrimon, N. (1993) *Cell* **74**, 219–222.
5. Crews, C. M., Alessandrini, A. & Erikson, R. L. (1992) *Science* **258**, 478–480.
6. Errede, B., Gartner, A., Zhou, Z., Nasmyth, K. & Ammerer, G. (1993) *Nature (London)* **362**, 261–264.
7. Kosako, H., Nishida, E. & Gotoh, Y. (1993) *EMBO J.* **12**, 787–794.
8. Wu, J., Harrison, J. K., Vincent, L. A., Haystead, C., Haystead, T. A., Michel, H., Hunt, D. F., Lynch, K. R. & Sturgill, T. W. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 173–177.
9. Zheng, C. F. & Guan, K. L. (1993) *J. Biol. Chem.* **268**, 11435–11439.
10. Dent, P., Haser, W., Haystead, T. A. J., Vincent, L. A., Roberts, T. M. & Sturgill, T. W. (1992) *Science* **257**, 1404–1407.
11. Howe, L. R., Leevers, S. J., Gómez, N., Nakielny, S., Cohen, P. & Marshall, C. J. (1992) *Cell* **71**, 335–342.
12. Kyriakis, J. M., App, H., Zhang, X. F., Banerjee, P., Brautigan, D. L., Rapp, U. R. & Avruch, J. (1992) *Nature (London)* **358**, 417–421.
13. Lange-Carter, C. A., Pleiman, C. M., Gardner, A. M., Blumer, K. J. & Johnson, G. L. (1993) *Science* **260**, 315–319.
14. Bruder, J. T., Heidecker, G. & Rapp, U. R. (1992) *Genes Dev.* **6**, 545–556.
15. Kolch, W., Heidecker, G., Kochs, G., Hummel, R., Vahidi, H., Mischak, H., Finkenzeller, G., Marmé, D. & Rapp, U. R. (1993) *Nature (London)* **364**, 249–252.
16. Sozeri, O., Vollmer, K., Liyanage, M., Frith, D., Kour, G., Mark, G. E. & Stable, S. (1992) *Oncogene* **7**, 2259–2262.
17. Kolch, W., Heidecker, G., Troppmair, J., Yanagihara, K., Bassin, R. H. & Rapp, U. R. (1993) *Oncogene* **8**, 361–370.
18. Reed, J. C., Yum, S., Cuddy, M. P., Turner, B. C. & Rapp, U. R. (1991) *Cell Growth Diff.* **2**, 235–243.
19. Williams, N. G., Roberts, T. M. & Li, P. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2922–2926.
20. Moodie, S. A., Willumsen, B. M., Weber, M. J. & Wolfman, A. (1993) *Science* **260**, 1658–1661.
21. Van Aelst, L., Barr, M., Marcus, S., Polverino, A. & Wigler, M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6213–6217.
22. Vojtek, A. B., Hollenberg, S. M. & Cooper, J. A. (1993) *Cell* **74**, 205–214.
23. Zhang, X., Settleman, J., Kyriakis, J. M., Takeuchi-Suzuki, E., Elledge, S. J., Marshall, M. S., Bruder, J. T., Rapp, U. R. & Avruch, J. (1993) *Nature (London)* **364**, 308–313.
24. Alexandropoulos, K., Qureshi, S. A. & Foster, D. A. (1993) *Oncogene* **8**, 803–807.
25. Krook, A., Rapoport, M. J., Anderson, S., Pross, H., Zhou, Y. C., Denhardt, D. T., Delovitch, T. L. & Haliotis, T. (1993) *Mol. Cell. Biol.* **13**, 1471–1479.
26. Cairns, B. R., Ramer, S. W. & Kornberg, R. D. (1992) *Genes Dev.* **6**, 1305–1318.
27. Rhodes, N., Connell, L. & Errede, B. (1990) *Genes Dev.* **4**, 1862–1874.
28. Sprague, G. F., Jr., & Thorner, J. W. (1992) in *The Molecular and Cellular Biology of the Yeast Saccharomyces*, eds. Jones, E. W., Pringle, J. R. & Broach, J. R. (Cold Spring Harbor Lab. Press, Plainview, NY), Vol. 2, pp. 657–744.
29. Stevenson, B. J., Rhodes, N., Errede, B. & Sprague, G. F., Jr. (1992) *Genes Dev.* **6**, 1293–1304.
30. Zhou, Z., Gartner, A., Cade, R., Ammerer, G. & Errede, B. (1993) *Mol. Cell. Biol.* **13**, 2069–2080.
31. Boguslawski, G. & Polazzi, J. O. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5848–5852.
32. Boguslawski, G. (1992) *J. Gen. Microbiol.* **138**, 2425–2432.
33. Brewster, J. L., de Valoir, T., Dwyer, N. D., Winter, E. & Gustin, M. C. (1993) *Science* **259**, 1760–1763.
34. Irie, K., Takase, M., Lee, K. Y., Levin, D. E., Araki, H., Matsumoto, K. & Oshima, Y. (1993) *Mol. Cell. Biol.* **13**, 3076–3083.
35. Lee, K. & Levin, D. E. (1992) *Mol. Cell. Biol.* **12**, 172–182.
36. Lee, K. S., Irie, K., Gotoh, Y., Watanabe, Y., Araki, H., Nishida, E., Matsumoto, K. & Levin, D. E. (1993) *Mol. Cell. Biol.* **13**, 3067–3075.
37. Torres, L., Martin, H., Garcia-Saez, M. I., Arroyo, J., Molina, M., Sanchez, M. & Nombela, C. (1991) *Mol. Microbiol.* **5**, 2845–2854.
38. Mukai, Y., Harashima, S. & Oshima, Y. (1993) *Mol. Cell. Biol.* **13**, 2050–2060.
39. Perlman, R., Yablonski, D., Simchen, G. & Levitzki, A. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 5474–5478.
40. Marchler, G., Schuller, C., Adam, G. & Ruis, H. (1993) *EMBO J.* **12**, 1997–2003.
41. Sprague, G. F., Jr. (1991) *Methods Enzymol.* **194**, 77–93.
42. Kang, Y.-S., Kane, J., Kurjan, J., Stadel, J. M. & Tipper, D. (1990) *Mol. Cell. Biol.* **10**, 2582–2590.
43. Blumer, K. J., Reneke, J. E. & Thorner, J. (1988) *J. Biol. Chem.* **263**, 10836–10842.
44. Levin, D. E. & Bartlett-Heubusch, E. (1992) *J. Cell Biol.* **116**, 1221–1229.
45. Paravacini, G., Cooper, M., Friedli, L., Smith, D. J., Carpenter, J.-L., King, L. S. & Payton, M. A. (1992) *Mol. Cell. Biol.* **12**, 4896–4905.
46. Banno, H., Hirano, K., Nakamura, T., Irie, K., Nomoto, S., Matsumoto, K. & Machida, Y. (1993) *Mol. Cell. Biol.* **13**, 4745–4752.
47. Hughes, D. A., Ashworth, A. & Marshall, C. J. (1993) *Nature (London)* **364**, 349–352.
48. Neiman, A. M., Stevenson, B. J., Xu, H. P., Sprague, G. F., Jr., Herskowitz, I., Wigler, M. & Marcus, S. (1993) *Mol. Biol. Cell.* **4**, 107–120.