lating acid resistance in vivo, was corroborated by in vitro experiments. When *E. coli* strains isolated from the cattle were grown in the laboratory with a high concentration of glucose, acetic acid accumulated in the medium, pH declined, and the cell survival after acid shock was high (Fig. 3B). If the glucose concentration of the medium was low, little acid was produced, and cell survival was extremely low. Strains isolated from cattle fed forage or grain, and *E. coli* O157:H7 (ATCC 43895, CDC EDL 933) behaved similarly, and this result indicated that grain feeding was inducing acid resistance rather than selecting a different population of *E. coli*.

About 5% of our *E. coli* isolates ($n = 155$) were sorbitol negative, a diagnostic trait of O157:H7 (*14*), but none of these strains tested positive for O157:H7 antigens (*18*). The absence of *E. coli* O157:H7 in our cattle is not surprising. Previous workers have noted that nonpathogenic *E. coli* can often outgrow pathogenic strains, and this point is illustrated by at least three observations: (i) The percentage of O157:H7-positive animals in herds directly linked to outbreaks was less than 2% (*19*); (ii) even cattle experimentally inoculated with *E. coli* O157:H7 did not shed the bacterium for long periods of time (*20*); and (iii) *E. coli* O157:H7 numbers can be reduced by giving animals doses of nonpathogenic *E. coli* (*21*).

The finding that grain feeding increased both the number and acid resistance of *E. coli* in cattle could have significant implications for food safety. Although not all *E. coli* are pathogenic, there is always the risk that at least some cattle will harbor pathogenic strains. Acid resistance appears to be a factor in the dissemination (transmission) of *E. coli* from cattle to humans. Therefore, it is reasonable to suggest that the induction of acid resistance could increase the risk of foodborne illness. Our studies indicated that the time needed to decrease *E. coli* numbers was relatively short (Fig. 4A). Cattle adapted to a 90% grain diet had an acid-resistant *E. coli* count greater than 10^6 viable cells per gram. After change to a hay diet, the viable cell number immediately declined, and after 5 days the *E. coli* population was nearly 10^6-fold lower (Fig. 4B).

Grain feeding is a practice that promotes the production and efficiency of cattle production, and it is unlikely that American cattle will ever be fed diets consisting only of hay. However, our studies indicate that cattle could be given hay for a brief period immediately before slaughter to significantly reduce the risk of food-borne *E. coli* infection.

References

1. *Foodborne Pathogens: Risks and Consequences* (Center for Agricultural Science and Technology, Task Force Report number 122, CAST, Ames, IA, 1994).
2. R. V. Tauxe, *Emerging Infect. Dis.* **3**, 425 (1997).
3. J. E. Collins, *ibid*, p. 471.
4. R. L. Buchanan and M. P. Doyle, *Food Technol.* **51**, 69 (1997).
5. J. Lederberg, *Emerging Infect. Dis.* **3**, 417 (1997).
6. C. Su and L. J. Brandt, *Ann. Intern. Med.* **123**, 698 (1995).
7. S. C. Whipp, M. A. Rasmussen, W. C. Cray, *J. Am. Vet. Med. Assoc.* **204**, 1168 (1994).
8. L. W. Riley *et al.*, *N. Engl. J. Med.* **308**, 681 (1983).
9. J. Gorden and P. L. C. Small, *Infect. Immun.* **61**, 364 (1993).
10. M. M. Benjamin and A. R. Datta, *Appl. Environ. Microbiol.* **61**, 1669 (1995).
11. D. R. Waldo, *J. Anim. Sci.* **37**, 1062 (1973).
12. J. R. Russell, A. W. Youngand, N. A. Jorgensen, *ibid.* **52**, 1177 (1981).
13. E. C. C. Lin, in Escherichia coli *and* Salmonella *Cellular and Molecular Biology*, F. C. Neidhardt *et al.*, Eds. (American Society for Microbiology, Washington, DC, ed. 3, 1996), vol. 1, chap. 20.
14. A. D. Hitchins, P. Feng, W. D. Watkins, S. R. Rippey, L. A. Chandler, in *Food Drug Administration Bacteriological Analytical Manual* (Association of Official Analytical Chemists International, Gaithersburg, MD, ed. 8, 1995), chap. 4.
15. D. R. Caldwell and M. P. Bryant, *Appl. Microbiol.* **14**, 794 (1966).
16. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, New York, 1969).
17. R. L. Ott, *An Introduction to Statistical Methods and Data Analysis* (Wadsworth, Belmont, CA, 1993).
18. RIM *E. coli* O157:H7 Latex Test (Remel, Lenexa, KS).
19. G. L. Armstrong, J. Hollingsworth, J. G. Morris Jr., *Epidemiol. Rev.* **18**, 29 (1996).
20. W. C. Cray and H. W. Moon, *Appl. Environ. Microbiol.* **61**, 1586 (1995).
21. T. Zhao *et al.*, *J. Clin. Microbiol.* **36**, 641 (1998).

27 March 1998; accepted 3 August 1998

MP1: A MEK Binding Partner That Enhances Enzymatic Activation of the MAP Kinase Cascade

Hans J. Schaeffer,* Andrew D. Catling,* Scott T. Eblen, Lara S. Collier,† Anke Krauss,‡ Michael J. Weber§

Signal transduction is controlled both by regulation of enzyme activation and by organization of enzymatic complexes with nonenzymatic adapters, scaffolds, and anchor proteins. The extracellular signal–regulated kinase (ERK) cascade is one of several evolutionarily conserved mitogen-activated protein (MAP) kinase cascades important in the regulation of growth, apoptosis, and differentiation. A two-hybrid screen was conducted to identify nonenzymatic components of this signaling cascade that might be important in regulating its activity. A protein called MP1 (MEK Partner 1) was identified that bound specifically to MEK1 and ERK1 and facilitated their activation. When overexpressed in cultured cells, MP1 enhanced activation of ERK1 and activation of a reporter driven by the transcription factor Elk-1. Expression of MP1 in cells increased binding of ERK1 to MEK1. MP1 apparently functions as an adapter to enhance the efficiency of the MAP kinase cascade.

The MAP kinases ERK1 and ERK2 are components of a protein kinase cascade displaying evolutionary conservation of protein sequence and a three-kinase architecture (*1*). ERKs are activated by the MAP kinase kinases MEK1 or MEK2 . MEKs, in turn, are activated by members of the Raf family. In a related MAP kinase pathway in *Saccharomyces cerevisiae*, the pheromone response pathway, the specificity and efficiency of the enzymatic components are facilitated by a nonenzymatic "scaffolding protein," STE5, that directly interacts with the signaling enzymes of this pathway (*2*).

Although a scaffold-like component has not previously been reported for MAP kinase pathways in higher eukaryotic systems, our earlier work indicated that efficient signaling through the Raf-MEK-ERK pathway also appears to require an additional, unknown component (*3*). MEK1 has a proline-rich sequence (PRS) that spans residues 270 to 307, between kinase subdomains IX and X (*4*). A PRS is present in MEK1 and MEK2, but not in other members of the MAP kinase kinase family. Downstream signaling by MEK requires the PRS; MEK can be activated by mutation to display high enzymatic activity and to transform Rat1 cells, whereas activated MEK mutants that lack the PRS retain

Department of Microbiology and Cancer Center, University of Virginia Health Sciences Center, Charlottesville, VA 22908, USA.

*These authors contributed equally to this report.
†Present address: Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305, USA.
‡Present address: European Molecular Biology Laboratory, Developmental Biology, 69012 Heidelberg, Germany.
§To whom correspondence should be addressed. E-mail: MJW@Virginia.edu

comparable enzymatic activity but are unable to transform cells (*3*) or to activate ERKs in vivo (*5*). Thus, the ability of mutationally activated MEK to transform cells depends not only on the enzymatic activity of MEK, but also on the presence of the PRS. These findings indicated that a MEK partner that would interact with the PRS might be important for efficient MAP kinase signaling in mammalian cells.

To identify such MEK partners, we used a yeast two-hybrid screen (*6*) with MEK1 as bait, and isolated a cDNA encoding a 13.5-kD protein that we term MP1 (MEK Partner 1; GenBank accession number AF082526). Analysis of the predicted protein sequence revealed no obvious similarity to proteins in the GenBank, PDB, SwissProt, or PIR databases. However, we identified several clones identical to the MP1 cDNA sequence in mouse expressed sequence tag (EST) databases and highly conserved sequences (97.6% identity at the amino acid level) in human EST databases. RNA blot analysis of various mouse tissues showed similar amounts of a 1.4- to 1.5-kb MP1 transcript in heart, brain, lung, liver, muscle, and kidney, and smaller amounts in spleen and testis (*7*). MP1 protein was detected by immunoblotting in various cell lines (NIH 3T3, Rat1, and CCL39 fibroblasts and PC12 pheochromocytoma) (*7*).

To define the region of MEK1 that is necessary for MP1 binding, we fused various MEK1 deletion mutants to the Gal4 DNA binding domain and tested them for their ability to interact with MP1 in the two-hybrid system. Only full-length MEK1 and a fragment spanning the COOH-terminal half of MEK1 that includes the PRS (MEK1 220-393) interacted with MP1. Moreover, a MEK1 deletion mutant lacking the PRS (MEK1 ΔPRS) failed to give a detectable signal in the two-hybrid assay (Fig. 1A). This observation indicated that the PRS is necessary for association of MEK1 with MP1. MEK2 failed to bind detectably to MP1 in the two-hybrid system (*7*).

To determine whether MEK1 and MP1 interacted in mammalian cells, we tested whether the proteins would coimmunoprecipitate. CCL39 fibroblasts were transiently transfected with various HA-tagged MEK constructs and FLAG-tagged MP1. MEK1 was coimmunoprecipitated with antibody to FLAG (Fig. 1B). Mutationally activated MEK1 (S218, 222D) also coimmunoprecipitated with MP1 (*7*). However, neither MEK1 ΔPRS nor MEK2 were detected in MP1 immunoprecipitations, in agreement with the two-hybrid results.

To determine whether MP1 plays a role in the enzymatic activation of the Raf-MEK-ERK cascade, we developed an in vitro assay with purified recombinant B-Raf, MEK1, and MP1 (*8*). MEK1 was incubated with B-Raf alone or with B-Raf and MP1 in the presence of Mg^{2+} and [γ-^{32}P]adenosine triphosphate (ATP). Addition of MP1, but not lysozyme or boiled MP1 (*7*), enhanced MEK phosphorylation by B-Raf (Fig. 2A). Determination of MEK1 activation with a coupled MAP kinase–myelin basic protein (MBP) phosphorylation assay demonstrated that enhanced phosphorylation of MEK1 due to MP1 was quantitatively reflected in enhanced MEK1 activation (Fig. 2B). MP1 also enhanced activation of ERK by mutationally activated MEK1 in the absence of B-Raf (Fig. 2C). Thus, MP1 increases the ability of MEK1 to be activated by B-Raf and also enhances the ability of mutationally activated MEK to activate ERK in vitro.

Although these data suggest that MP1 fa-

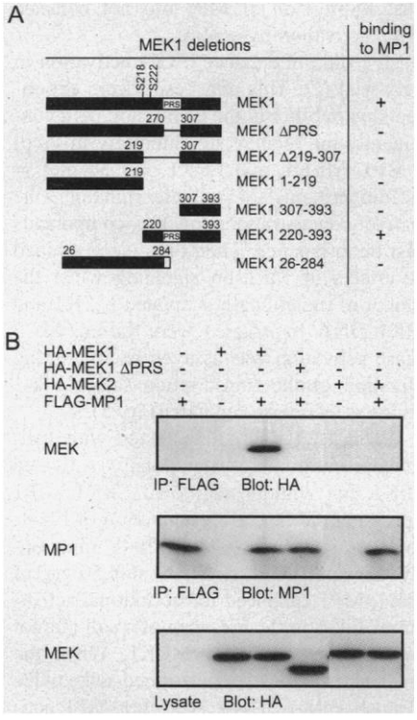

Fig. 1. Binding of MP1 to MEK. (**A**) Requirement of the MEK1 PRS for MP1 binding. MEK1 deletion mutants were constructed and tested for interaction with MP1 in the two-hybrid system. (**B**) Specific binding of MP1 to MEK1, but not to MEK1 ΔPRS or MEK2. CCL39 cells were cotransfected with FLAG-tagged MP1 and HA-tagged MEK constructs. FLAG-MP1 immunoprecipitates (*9*) were immunoblotted for MEK with antibody to HA (top panel) or for MP1 with antibody to FLAG (middle panel). Identical amounts of cell lysate were blotted with antibody to HA to verify expression of comparable amounts of MEK (lower panel). Epitope-tagged MEK expression constructs have been described (*3*).

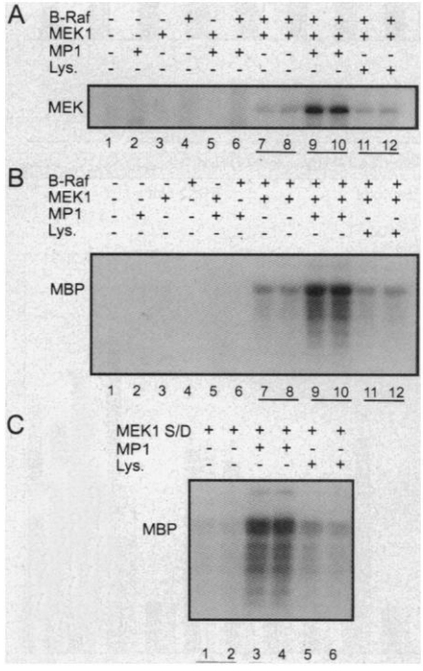

Fig. 2. Effect of MP1 on MEK in vitro. (**A**) Enhanced phosphorylation of MEK1 by B-Raf in vitro in the presence of MP1. Recombinant MEK1 was incubated with B-Raf alone, with B-Raf and MP1, or with B-Raf and lysozyme control in the presence of Mg^{2+}/[γ-^{32}P]ATP. Proteins were resolved by polyacrylamide gel electrophoresis and detected by autoradiography. (**B**) Enhanced activation of MEK1 by B-Raf in the presence of MP1. Reactions were performed as described in (A) except that MEK1 activity was measured in terms of its ability to activate recombinant ERK. ERK activity was measured using MBP as substrate in the presence of Mg^{2+}/[γ-^{32}P]ATP. Proteins were resolved by polyacrylamide gel electrophoresis and detected by autoradiography. (**C**) MP1 enhances the activity of mutationally activated MEK1 (MEK1 S/D). MEK1 S/D was incubated with recombinant ERK alone, with ERK and MP1, or ERK and lysozyme. ERK activity was later measured using MBP as substrate in the presence of Mg^{2+}/[γ-^{32}P]ATP. Proteins were resolved by polyacrylamide gel electrophoresis and detected by autoradiography.

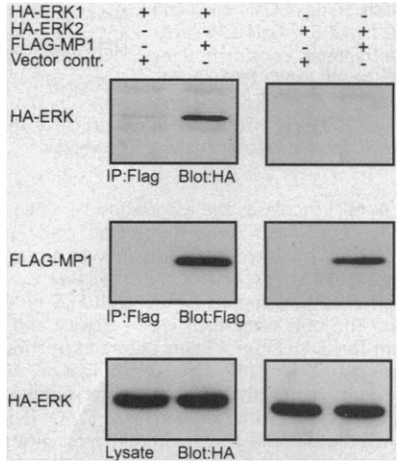

Fig. 3. Association of MP1 with ERK1 but not ERK2. CCl39 cells were transiently cotransfected with FLAG-tagged MP1 and HA-tagged ERK1 or ERK2 constructs. Immunoprecipitations and blots were done as in Fig. 1.

cilitates MEK1 function by binding specifically to the PRS, several lines of evidence indicate that its mechanism of action may be more complex. For example, MP1 was without effect on MEK1 previously activated by phosphorylation in vitro by B-Raf (*7*), suggesting that MP1 interacts preferentially with inactive or partially active conformers of MEK1. Moreover, even though MP1 specifically binds to MEK1 in vivo, it binds in vitro to MEK2 as well. Consistent with this, MP1 facilitates activation of both MEK1 and MEK2 in vitro. The differences in MP1 function in vivo and in vitro could reflect differences in posttranslational modification (for example, phosphorylation of MEK) or the requirement for additional factors.

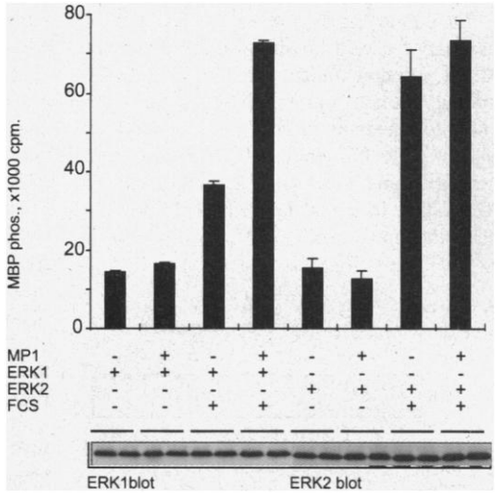

Fig. 4. Specific activation of ERK1, but not ERK2, after cotransfection with MP1. Duplicate dishes of NIH 3T3 cells were transiently cotransfected with HA-tagged ERK1 or ERK2 and either MP1 or control vector. After 24 hours, cells were deprived of serum for 4 hours and then either left untreated or stimulated with fetal calf serum (FCS, 10%) for 10 min. HA-tagged ERKs were immunoprecipitated and assayed with MBP as substrate, essentially as described (*10*).

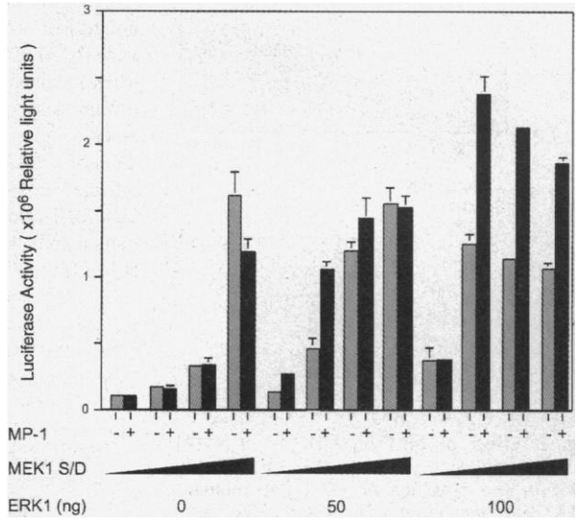

Fig. 5. Enhanced ERK1-stimulated gene transcription in the presence of MP1. COS cells were transfected with various amounts of MEK1, ERK1, and MP1 expression constructs, together with reporter plasmids, at a fixed total DNA concentration. After 5 hours, cells were washed with phosphate-buffered saline and then incubated for 19 hours in Dulbecco's modified Eagle's medium (DMEM) supplemented with FCS (1%). Cells were then harvested and luciferase activity was measured in duplicate dishes. Constructs were transfected at the following concentrations, per dish: 50 ng of Gal4-Elk-1 and 1000 ng of 5× Gal4-E1b–luciferase reporter construct; 0 or 2000 ng of pLNC MP1; 0, 25, 100, or 500 ng of MEK1 S/D; and 0, 50, or 100 ng of pCGN HA-ERK1. In all cases, the total amount of transfected DNA was kept constant by addition of the appropriate empty vector.

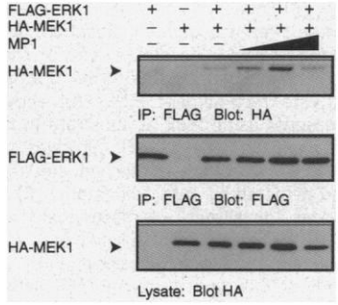

Fig. 6. MP1 increases the association between ERK1 and MEK1. COS-1 cells were transfected with FLAG-ERK1, HA-MEK1, and increasing amounts of untagged MP1 expression plasmids. After 5 hours, cells were washed and placed in DMEM supplemented with FCS (10%) for 15 hours. The cells were then washed twice and placed in serum-free DMEM for 4 hours before harvesting in hypotonic lysis buffer (*11*). After centrifugation, FLAG-ERK1 was immunoprecipitated from equal amounts of the soluble lysates with antibody to FLAG (Sigma) and washed as described (*11*). Immunoprecipitated FLAG-ERK1 and associated HA-MEK1 were analyzed by immunoblotting. Equal amounts of soluble lysate were immunoblotted for expression of HA-MEK1. The following amounts of each construct were used, as indicated, per 10-cm dish: 3 μg of HA-MEK1; 2 μg of FLAG-ERK1; and 0.1, 0.5, or 1 μg of MP1. The appropriate empty vector was used to keep total DNA amounts constant in each transfection.

When MP1 was immunoprecipitated from CCL39 cells transfected with HA-tagged ERKs and FLAG-tagged MP1, ERK1 but not ERK2 was associated with MP1 (Fig. 3). This raised the possibility that MP1 preferentially targets MEK1 to ERK1.

To test whether overexpression of MP1 affects activation of ERK1 in preference to ERK2, we transiently cotransfected NIH 3T3 cells with HA-tagged ERK1 or ERK2 and MP1 or control vector. After incubation in the absence of serum, cells were either left untreated or stimulated with serum, and MAP kinase activity was assessed in immune complex kinase assays. Enhanced ERK1 activity (by a factor of 1.5 to 2.5) was observed when coexpressed with MP1 (Fig. 4). Enhancement of ERK activation by MP1 in vivo was less than seen in vitro (Fig. 2), possibly because of the presence of endogenous MP1 in the cells. Expression of MP1 did not enhance ERK2 activation by serum.

MP1 did not enhance ERK1 activation in every trial (*7*). This may result from experimental variability in the expression (and consequently the relative stoichiometry in vivo) of MP1, MEK1, and ERK1. For adapter or scaffold proteins to facilitate signaling, the relative concentrations of the components must be in balance. Therefore, we examined the effects of MP1 on signaling when the amount of mutationally activated MEK1 and ERK1 DNA transfected were varied. MAP kinase activation was assayed by measuring activation of the transcription factor Elk-1 driving a luciferase reporter (Fig. 5).

When MP1 was cotransfected with various concentrations of mutationally activated MEK1, but without additional ERK1, MP1 expression did not affect activation of Elk-1. However, in cells transfected with mutationally activated MEK1, MP1, and 50 ng of ERK1, MP1 enhanced transcriptional activation of Elk-1 in the presence of small but not large amounts of input MEK1. When the same experiment was performed with twice as much ERK1 cDNA (100 ng), MP1 activated transcription at both high and low concentrations of input MEK1. Immunoblots confirmed that the expression of MEK and ERK was dose-dependent with respect to the amount of plasmid transfected, and that coexpression of MP1 did not influence expression of the other components (*7*). MP1 apparently stimulates activation of Elk-1 until ERK1 becomes limiting; increasing the ERK1 concentration allows the MP1 effect to occur at higher concentrations of MEK1. These data do not address the issue of isoform specificity because cells transfected by

mutationally activated MEK become transformed, activating both isoforms of MEK and ERK. However, this experiment clearly demonstrates the ability of MP1 to enhance signaling through the MAP kinase pathway, dependent on the relative stoichiometries of the components.

MP1 may participate in functionally linking a subset of components of the MAP kinase pathway by facilitating specific protein-protein interactions. To test this directly, we assessed the effect of varying amounts of MP1 expression on the binding of MEK1 with ERK1 (Fig. 6). Cells were transfected with DNAs encoding MEK1, ERK1, and increasing amounts of MP1. Expression of MP1 enhanced the coimmunoprecipitation of MEK1 with ERK1, except at the highest amounts of MP1 DNA, where a decrease in MEK1-ERK1 binding was observed. These results are consistent with a model where MP1 can form a ternary complex with MEK1 and ERK1, facilitating the interaction of the two enzymes; however, at high concentrations of MP1, binary MP1-ERK1 and MP1-MEK1 complexes would be favored.

In yeast, scaffolding proteins such as STE5 can enhance signaling by locally sequestering a subset of components. In the mammalian MAP kinase pathway, analogous functions may be distributed among several proteins, acting at different steps in the pathway, with MP1 facilitating the functional interactions involving MEK. The existence of multiple small proteins that serve adapter functions would provide enhanced flexibility in regulating the efficiency and specificity of the MAP kinase cascade.

References and Notes

1. M. H. Cobb and E. J. Goldsmith, *J. Biol. Chem.* **270**, 14843 (1995).
2. K. Y. Choi, B. Satterberg, D. M. Lyons, E. A. Elion, *Cell* **78**, 499 (1994); I. Herskowitz, *ibid.* **80**, 187 (1995); H. D. Madhani and G. R. Fink, *Trends Genet.* **14**, 151 (1998).
3. A. D. Catling, H. J. Schaeffer, C. W. Reuter, G. R. Reddy, M. J. Weber, *Mol. Cell. Biol.* **15**, 5214 (1995).
4. S. K. Hanks, A. M. Quinn, T. Hunter, *Science* **241**, 42 (1988).
5. A. D. Catling, unpublished data; A. Dang, J. A. Frost, M. H. Cobb, *J. Biol. Chem.* **273**, 19909 (1998).
6. C. T. Chien, P. L. Bartel, R. Sternglanz, S. Fields, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9578 (1991); J. W. Harper, G. R. Adami, N. Wei, K. Keyomarsi, S. J. Elledge, *Cell* **75**, 805 (1993). The two-hybrid screen was done using yeast strain CG1945 and a pACT mouse lymphoma cDNA library (Clontech MATCHMAKER GAL4 two-hybrid user manual). Full-length MEK1 was subcloned into pAS1 and expressed as a Gal4 DNA binding domain fusion protein. Approximately 2×10^6 clones of a mouse lymphoma library were screened, and seven independent clones coding for MP1 were isolated. MP1 clones with additional 5′ sequence were isolated from the same library by plaque hybridization. MP1 failed to interact with various control proteins including Lamin, p53, SNF1, Ras, c-Raf1, and the Gal4 DNA-binding domain alone. The cDNA of the longest clone isolated had an open reading frame of 372 nucleotides and coded for a protein with a predicted molecular size of 13.5 kD. The putative initiation codon is flanked by a consensus Kozak sequence. An in-frame stop codon 5′ of the predicted start site excludes the possibility of a longer open reading frame.
7. H. J. Schaeffer, A. D. Catling, S. T. Eblen, L. S. Collier, A. Krauss, M. J. Weber, data not shown.
8. MEK proteins tagged at the NH_2-terminus with GST and at the COOH-terminus with His_6 were purified from *Escherichia coli* by batch elution from glutathione-Sepharose (Pharmacia), followed by gradient elution from Ni^{2+}-NTA agarose (Qiagen). His_6-tagged MP1 was purified from baculovirus-infected Sf21 cells. GST–B-Raf was purified from baculovirus-infected Sf9 cells by elution from glutathione-Sepharose. The preparation contained a mixture of GST–B-Raf and 14-3-3 proteins and was essentially free of other contaminating proteins, as judged by Coomassie blue staining. Biochemical assays to assess the role of MP1 in MEK1 phosphorylation and activation in vitro used reaction conditions essentially as described (*3*).
9. For coimmunoprecipitation experiments, cells were lysed in FLAG lysis buffer [50 mM tris-HCl, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 0.5 mM EDTA, and 0.5 mM EGTA (pH 7.3) supplemented with 50 mM NaF, 5 mM $Na_4P_2O_7$, 0.2 mM sodium orthovanadate, and protease inhibitors] 24 hours after transfection. Clarified extracts were incubated for 2 hours with 20 μg of anti-FLAG affinity resin (M2, Kodak) at 4°C. MEK-MP1 coimmunoprecipitates were washed four times with FLAG lysis buffer. ERK-MP1 coimmunoprecipitates were washed twice with FLAG lysis buffer and twice with a phosphate-buffered saline solution containing 0.5 M NaCl.
10. T. Jelinek *et al.*, *Mol. Cell. Biol.* **14**, 8212 (1994).
11. M. Fukuda, Y. Gotoh, E. Nishida, *EMBO J.* **16**, 1901 (1997).

12. We thank W. Kolch for B-Raf baculovirus; C. Der for HA-ERK constructs; R. Maurer for reporter constructs; numerous members of the Parsons-Weber-Parsons groups for valuable discussions; S. Parsons, T. Parsons, D. Brautigan, and T. Sturgill for their comments on the manuscript; and S. Elledge for two-hybrid plasmids, a cDNA library, and help in establishing the two-hybrid screen. Supported by NIH grants CA39076 and GM47332 (M.J.W.) and a predoctoral fellowship of the Studienstiftung des Deutschen Volkes (H.J.S.).

10 March 1998; accepted 5 August 1998

A Mammalian Scaffold Complex That Selectively Mediates MAP Kinase Activation

Alan J. Whitmarsh, Julie Cavanagh, Cathy Tournier, Jun Yasuda, Roger J. Davis*

The c-Jun NH_2-terminal kinase (JNK) group of mitogen-activated protein (MAP) kinases is activated by the exposure of cells to multiple forms of stress. A putative scaffold protein was identified that interacts with multiple components of the JNK signaling pathway, including the mixed-lineage group of MAP kinase kinase kinases (MLK), the MAP kinase kinase MKK7, and the MAP kinase JNK. This scaffold protein selectively enhanced JNK activation by the MLK signaling pathway. These data establish that a mammalian scaffold protein can mediate activation of a MAP kinase signaling pathway.

The c-Jun NH_2-terminal kinase (JNK) group of MAP kinases represents one of three groups of MAP kinases that have been identified in mammalian cells (*1*). JNK is activated in cells exposed to environmental stress or in cells treated with proinflammatory cytokines. Targets of the JNK signaling pathway include the transcription factors ATF-2, Elk-1, c-Jun, and NFAT4. JNK is required for a number of cellular processes in both *Drosophila* and mammalian cells. These include early embryonic development, apoptosis, oncogenic transformation, and the immune response (*1*).

Similar to other MAP kinases, JNK is activated by dual phosphorylation on Thr and Tyr within protein kinase subdomain VIII by a MAP kinase kinase (MAPKK). Each MAPKK is phosphorylated and activated by a MAP kinase kinase kinase (MAPKKK). Distinct signaling modules activate the different MAP kinase groups. Components of the JNK signaling module include the MAPKKs MKK4 and MKK7, together with members of the MEK kinase (MEKK) and mixed-lineage kinase (MLK) groups of MAPKKKs. JNK activity can therefore be regulated by many protein kinases, some of which also regulate other MAP kinase signaling pathways. Because JNK also displays some overlap in substrate specificity with other MAP kinases, mechanisms must exist to achieve signaling specificity and to ensure the correct biological response to extracellular stimulation.

An emerging property of signal transduction pathways that might account for specificity is the formation of signaling complexes (*2*). These complexes may result from the physical interaction between components of particular signaling pathways or by the assembly of signaling molecules on anchor or scaffold proteins that localize their binding partners to specific subcellular compartments or to specific substrates. Multienzyme com-

Program in Molecular Medicine, Department of Biochemistry and Molecular Biology, University of Massachusetts Medical School and Howard Hughes Medical Institute, Worcester, MA 01605, USA.

*To whom correspondence should be addressed.