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 A. D. Catling, unpublished data; A. Dang, J. A. Frost, M. H. Cobb, *J. Biol.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

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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-

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plexes can be created through these interactions. Examples include the MAP kinase complexes coordinated by the scaffold proteins Ste5p and Pbs2p in the yeast *Saccharomyces cerevisiae* (*3*). Ste5p binds components of the pheromone mating response pathway and Pbs2p can coordinate components of the osmoregulatory pathway. These scaffold proteins appear to play a dual regulatory role. First, they ensure precise regulation of the MAP kinase pathway by colocalizing and facilitating phosphorylation of successive members of the cascade. Second, they insulate the MAP kinase pathway to prevent cross talk with functionally unrelated members of other MAP kinase signaling modules.

The cytoplasmic protein JIP-1 (JNK interacting protein-1) (*4*) binds selectively to the MAP kinase JNK but not to other related MAP kinases, including p38 and ERK (Fig. 1A). Overexpression of JIP-1 causes cytoplasmic retention of JNK and thereby inhibits gene expression mediated by the JNK signaling pathway (*4*). In addition, JIP-1 suppresses the effects of JNK on apoptosis and malignant transformation (4). Overexpressed JIP-1 can therefore function to inhibit nuclear signaling by the JNK pathway. To determine whether JIP-1 could act as a scaffold protein for the JNK signaling module, we tested whether JIP-1 interacted with additional components of the JNK pathway. We expressed epitope-tagged JIP-1 in cells together with vectors encoding epitope-tagged MAPKKs. Coprecipitation analysis demonstrated that the JNK-specific activator MKK7 was detected in JIP-1 precipitates (Fig. 1B). The association of MKK7 with JIP-1 was confirmed by coimmunoprecipitation of epitope-tagged JIP-1 with MKK7 (Fig. 1C). The JNK activator MKK4 (which can also activate p38 MAP kinase) did not interact with JIP-1 (Fig. 1, B and C). MKK3 and MKK6 (which activate the p38 group of MAP kinases) and MEK1 (which activates the ERK group of MAP kinases) did not coprecipitate with JIP-1 (Fig. 1B). Thus, JIP-1 preferentially binds the MAPKK MKK7.

JNK is activated by many MAPKKKs that display varying promiscuity toward the various MAP kinase groups (*5*). These include MEKK1 (*5*) and the mixed-lineage kinases MLK3 and DLK (*6*), which preferentially activate JNK; MEKK3 (*7*), which activates both ERK and JNK MAP kinases; and MEKK4 (*8*), which preferentially activates p38 MAP kinase. These MAPKKKs were tested for interaction with JIP-1 by coprecipitation assays. MLK3 and DLK coprecipitated with JIP-1 (Fig. 2A) and JIP-1 coprecipitated with MLK3 and DLK (Fig. 2B). Weak binding of JIP-1 to MEKK3, but not to MEKK1 or MEKK4, was also detected (Fig. 2A). cRAF-1, a MAPKKK specific for the ERK signaling pathway, also did not interact with JIP-1 (Fig. 2A). Thus, JIP-1 preferentially binds to the MLK group of MAPKKKs.

Signaling molecules that function upstream of MAPKKKs in the JNK signal transduction pathway include Rho proteins and kinases related to *Saccharomyces cerevisiae* Ste20p (*1*). We detected no binding of the Rho family proteins Cdc42 or Rac1 to JIP-1 (*9*). We also tested the interaction of JIP-1 with three mammalian Ste20p-related protein kinases: p21-activated kinase-3 (PAK3) (*10*), hematopoietic progenitor kinase-1 (HPK1) (*11*), and kinase homologous to Ste20 (KHS) (*12*). PAK-3 did not coprecipitate with JIP-1, and KHS interacted weakly with JIP-1 (*9*). In contrast, HPK1 coprecipitated with JIP-1 (Fig. 3A) and JIP-1 was detected in HPK1 immunoprecipitates (*9*), indicating that JIP-1 interacts with specific mammalian Ste20p-related protein kinases.

JNK, MLK3, and DLK were detected in MKK7 immunoprecipitates (*9*). These data suggest that MKK7, DLK, MLK3, and HPK1 could be tethered to JIP-1 through an interaction with JNK. A mutated JIP-1 molecule with an in-frame deletion of the JNK binding domain did not bind JNK but did bind MKK7, DLK, MLK3, and HPK1 (Fig. 3A). Thus, binding of JIP-1 to upstream components of the JNK signaling module does not require JNK.

The MKK7, DLK, MLK3, and HPK1 protein kinases did not bind to the NH_2-terminal region of JIP-1 (residues 1 to 282), which encompasses the JNK binding domain (Fig. 3B). However, MKK7, DLK, MLK3, and HPK1 did bind to a COOH-terminal fragment of JIP-1 (residues 283 to 660) (Fig. 3B). A smaller COOH-terminal fragment (amino acids 471 to 660) bound DLK, MLK3, and HPK1 but not MKK7 (Fig. 3B). An alternatively spliced variant of JIP-1 (JIP-1b), which has an insertion of 47 amino acids in the COOH-terminal region (*13*), also bound to MKK7, DLK, and MLK3 (*9*). Therefore, the JIP-1 sequence required for binding MKK7 is distinct from that required for

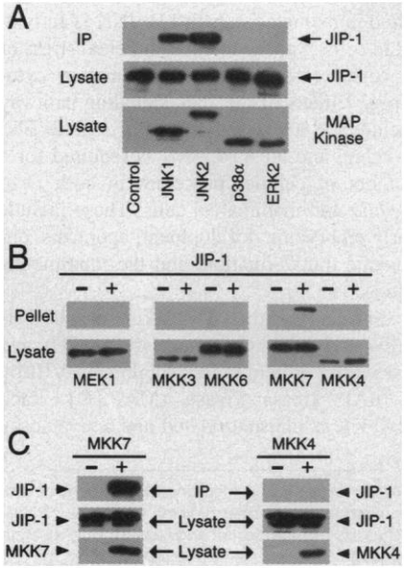

Fig. 1. Selective binding of JIP-1 to the MAP kinase JNK and the MAP kinase kinase MKK7. (**A**) Epitope-tagged JIP-1 (T7-Tag) was expressed in cells with the HA-tagged MAP kinases ERK2, p38α, JNK1, and JNK2 (*15, 16*). The MAP kinases were immunoprecipitated with an antibody to HA. The presence of JIP-1 in the immunoprecipitates (IP) was detected on immunoblots probed with an antibody to T7-Tag. The amount of JIP-1 and MAP kinases in the cell lysates was examined by protein immunoblot analysis. (**B**) JIP-1 was expressed in cells as a GST fusion protein together with epitope-tagged MEK1, MKK3, MKK4, MKK6, or MKK7 (*15, 16*). JIP-1 was precipitated from cell lysates with glutathione-agarose, and the MAPKKs present in the pellet were detected by protein immunoblot analysis. The amount of the MAPKKs in the cell lysates was examined by protein immunoblot analysis. (**C**) Epitope-tagged JIP-1 (T7-Tag) was expressed in cells with Flag-tagged MKK4 or MKK7 (*15, 16*). The presence of JIP-1 in Flag IP was detected by protein immunoblot analysis with an antibody to T7-Tag. The amount of the MAPKKs in the cell lysates was examined by protein immunoblot analysis.

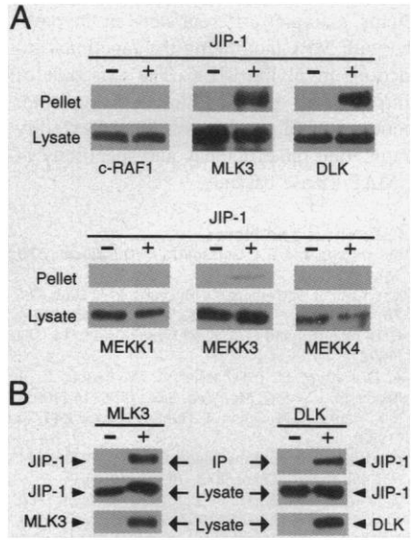

Fig. 2. Selective binding of JIP-1 to the mixed-lineage group of MAPKKKs. (**A**) JIP-1 was expressed in cells as a GST fusion protein together with the epitope-tagged MAPKKKs (*15, 16*). The presence of MAPKKKs in glutathione-agarose precipitates (pellet) was assayed by protein immunoblot analysis. The amount of the MAPKKKs in the cell lysates was examined by protein immunoblot analysis. (**B**) Epitope-tagged JIP-1 was coexpressed in cells with epitope-tagged MLK3 or DLK (*15, 16*). The presence of JIP-1 in the MLK3 and DLK immunoprecipitates (IP) was examined by protein immunoblot analysis. The amount of the MAPKKKs in the cell lysates was examined by protein immunoblot analysis.

binding DLK, MLK3, or HPK1. Because HPK1 binds to MLK3, but not DLK (*11*), it is possible that MLK3 mediates the interaction of HPK1 with JIP-1 (*9*).

To test whether upstream components of the JNK signaling pathway interact directly with JIP-1, we examined binding of bacterially expressed proteins (Fig. 3D). Glutathione *S*-transferase (GST) and GST-JIP-1 fusion proteins were expressed in bacteria, purified, and immobilized on glutathione-agarose beads. Bacterially expressed JNK1, MKK7, or MLK3 was incubated with immobilized GST-JIP-1 fusion proteins. JNK bound to an NH_2-terminal region of JIP-1 (residues 127 to 282). Both MKK7 and MLK3 bound to a COOH-terminal fragment of JIP-1 (residues 283 to 660). Thus, JNK, MKK7, and MLK3 appear to bind directly to JIP-1.

The expression of JIP-1 in cultured cells causes cytoplasmic retention of JNK (*4*). MLK3 and DLK are predominantly localized to the cytoplasm (*9*), whereas MKK7 was detected primarily in the nucleus (Fig. 3C). Expression of the cytoplasmic protein JIP-1 reduced the amount of nuclear MKK7 and increased the amount of MKK7 in the cytoplasm (Fig. 3C). JIP-1 did not alter the subcellular distribution of MKK4. Similar results were obtained in experiments using JIP-1b, an alternatively spliced JIP-1 variant (*9, 13*). These data indicate that JIP-1 interacts with JNK and MKK7 in vivo.

The binding of JIP-1 to multiple JNK signaling pathway components indicates that JIP-1 may contribute to the regulation of JNK function. Previous studies demonstrated that the overexpression of JIP-1 causes marked inhibition of nuclear signaling mediated by the sequestration of JNK in the cytoplasm (*4*). We examined the effect of JIP-1 overexpression on the response of cells after exposure to the cytokine interleukin-1 (IL-1) and to environmental stress, including ultraviolet (UV) radiation

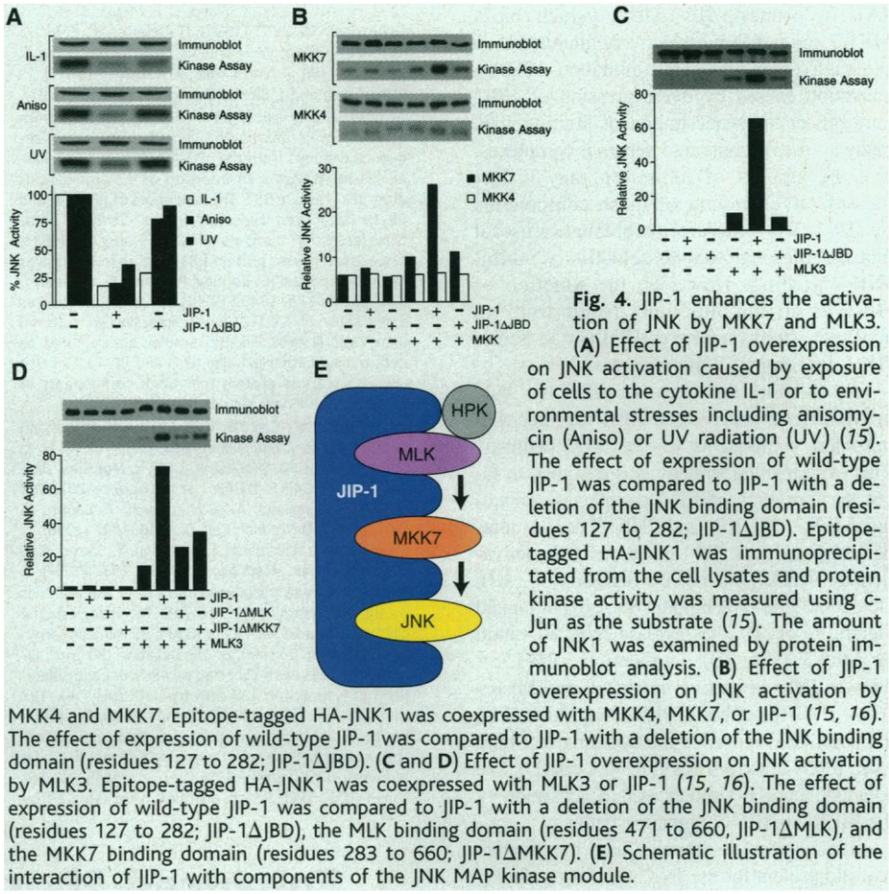

Fig. 4. JIP-1 enhances the activation of JNK by MKK7 and MLK3. (**A**) Effect of JIP-1 overexpression on JNK activation caused by exposure of cells to the cytokine IL-1 or to environmental stresses including anisomycin (Aniso) or UV radiation (UV) (*15*). The effect of expression of wild-type JIP-1 was compared to JIP-1 with a deletion of the JNK binding domain (residues 127 to 282; JIP-1ΔJBD). Epitope-tagged HA-JNK1 was immunoprecipitated from the cell lysates and protein kinase activity was measured using c-Jun as the substrate (*15*). The amount of JNK1 was examined by protein immunoblot analysis. (**B**) Effect of JIP-1 overexpression on JNK activation by MKK4 and MKK7. Epitope-tagged HA-JNK1 was coexpressed with MKK4, MKK7, or JIP-1 (*15, 16*). The effect of expression of wild-type JIP-1 was compared to JIP-1 with a deletion of the JNK binding domain (residues 127 to 282; JIP-1ΔJBD). (**C** and **D**) Effect of JIP-1 overexpression on JNK activation by MLK3. Epitope-tagged HA-JNK1 was coexpressed with MLK3 or JIP-1 (*15, 16*). The effect of expression of wild-type JIP-1 was compared to JIP-1 with a deletion of the JNK binding domain (residues 127 to 282; JIP-1ΔJBD), the MLK binding domain (residues 471 to 660, JIP-1ΔMLK), and the MKK7 binding domain (residues 283 to 660; JIP-1ΔMKK7). (**E**) Schematic illustration of the interaction of JIP-1 with components of the JNK MAP kinase module.

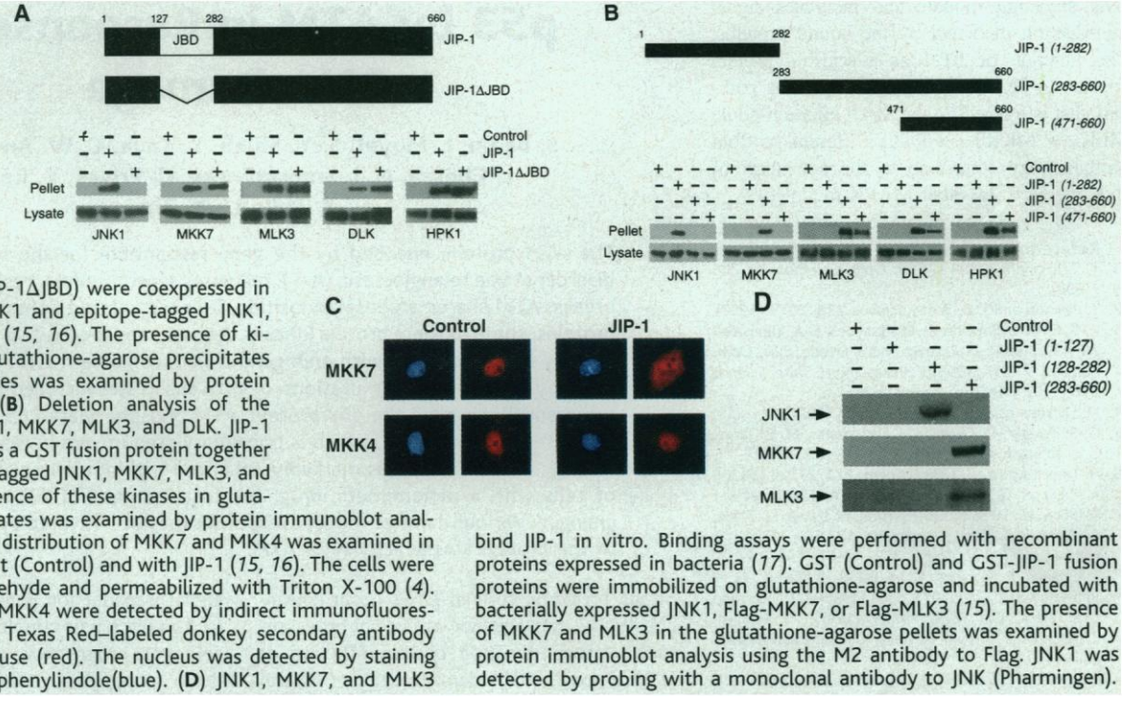

Fig. 3. Binding of JIP-1 to MKK7, MLK, and HPK1 independently of JNK. (**A**) The binding of JIP-1 to HPK1, MLK3, DLK, and MKK7 is independent of JNK. GST (Control), GST-tagged JIP-1, and a GST-tagged JIP-1 mutant with an in-frame deletion of the JNK binding domain (JIP-1ΔJBD) were coexpressed in cells together with HPK1 and epitope-tagged JNK1, MKK7, MLK3, and DLK (*15, 16*). The presence of kinases in the reduced glutathione-agarose precipitates (pellets) and cell lysates was examined by protein immunoblot analysis. (**B**) Deletion analysis of the binding of JIP-1 to JNK1, MKK7, MLK3, and DLK. JIP-1 was expressed in cells as a GST fusion protein together with HPK1 or epitope-tagged JNK1, MKK7, MLK3, and DLK (*15, 16*). The presence of these kinases in glutathione-agarose precipitates was examined by protein immunoblot analysis. (**C**) The subcellular distribution of MKK7 and MKK4 was examined in cells transfected without (Control) and with JIP-1 (*15, 16*). The cells were fixed with paraformaldehyde and permeabilized with Triton X-100 (*4*). Transfected MKK7 and MKK4 were detected by indirect immunofluorescence analysis using a Texas Red–labeled donkey secondary antibody immunoglobulin to mouse (red). The nucleus was detected by staining with 4′,6-diamidino-2-phenylindole(blue). (**D**) JNK1, MKK7, and MLK3 bind JIP-1 in vitro. Binding assays were performed with recombinant proteins expressed in bacteria (*17*). GST (Control) and GST-JIP-1 fusion proteins were immobilized on glutathione-agarose and incubated with bacterially expressed JNK1, Flag-MKK7, or Flag-MLK3 (*15*). The presence of MKK7 and MLK3 in the glutathione-agarose pellets was examined by protein immunoblot analysis using the M2 antibody to Flag. JNK1 was detected by probing with a monoclonal antibody to JNK (Pharmingen).

and anisomycin. IL-1 preferentially activates MKK7 and UV radiation and anisomycin preferentially activate MKK4. The overexpressed JIP-1 (which binds JNK and MKK7) decreased JNK activation caused by each stimulus (Fig. 4A). In contrast, JIP-1ΔJBD (which binds MKK7 and not JNK) selectively inhibited IL-1 signaling (Fig. 4A). This inhibition of JNK activation caused by overexpression of JIP-1 may reflect the sequestration of limiting JNK pathway components into separate complexes, and the amount of inhibition may reflect the selective binding of these components by JIP-1. JIP-1 therefore represents a useful pharmacological tool to selectively inhibit JNK signaling. However, the function of JIP-1 as an inhibitor may result from an artificial situation where JIP-1 (or an active JIP-1 fragment) is overexpressed.

To examine the effect of JIP-1 on JNK activation, we coexpressed JIP-1 together with upstream components of the JNK pathway. This experimental strategy was designed to favor the formation of JIP-1 complexes containing MLK3, MKK7, and JNK. These studies demonstrated that JIP-1 enhanced JNK activation by MKK7 but not by MKK4 (Fig. 4B). Deletion of the JNK binding domain eliminated the ability of JIP-1 to increase JNK activation by MKK7 (Fig. 4B). Furthermore, JIP-1 enhanced JNK activation mediated by MLK3 (Fig. 4C). This effect of JIP-1 was reduced by the separate deletion of the domains that bind JNK, MKK7, and MLK3 (Fig. 4, C and D). We conclude that JIP-1 enhances JNK activation by the MLK → MKK7 signaling pathway.

JIP-1 appears to function as a mammalian scaffold protein for the JNK signaling pathway (Fig. 4E). JIP-1 binds multiple components of a JNK signaling module and facilitates signal transduction mediated by the bound proteins. The function of JIP-1 as a scaffold protein appears to be selective for the signaling pathway that is formed by the MAP kinase module MLK → MKK7 → JNK. Different scaffold proteins may contribute to the activation of JNK mediated by other MAPK modules.

References and Notes

1. Y. T. Ip and R. J. Davis, *Curr. Opin. Cell Biol.* **10**, 205 (1998).
2. T. Pawson and J. D. Scott, *Science* **278**, 2075 (1997).
3. K.-Y. Choi, B. Satterberg, D. M. Lyons, E. A. Elion, *Cell* **78**, 499 (1994); D. E. Levin and B. Errede, *Curr. Opin. Cell Biol.* **7**, 197 (1995); F. Posas and H. Saito, *Science* **276**, 1702 (1997).
4. M. Dickens *et al.*, *Science* **277**, 693 (1997).
5. G. R. Fanger, P. Gerwins, C. Widmann, M. B. Jarpe, G. L. Johnson, *Curr. Opin. Genet. Dev.* **7**, 67 (1997).
6. H. Teramoto *et al.*, *J. Biol. Chem.* **271**, 27225 (1996); A. Rana *et al.*, *ibid.*, p. 19025; L. A. Tibbles *et al.*, *EMBO J.* **15**, 7026 (1996); S. Hirai, M. Izawa, S. Osada, G. Spyrou, S. Ohno, *Oncogene* **12**, 641 (1996); S. Hirai *et al.*, *J. Biol. Chem.* **272**, 15167 (1997).
7. J. L. Blank, P. Gerwins, E. M. Elliott, S. Sather, G. L. Johnson, *J. Biol. Chem.* **271**, 5361 (1996); K. Deacon and J. L. Blank, *ibid.* **272**, 14489 (1997); H. Ellinger-Ziegelbauer, K. Brown, K. Kelly, U. Siebenlist, *ibid.*, p. 2668.
8. P. Gerwins, J. L. Blank, G. L. Johnson, *ibid.* **272**, 8288 (1997); M. Takekawa, F. Posas, H. Saito, *EMBO J.* **16**, 4973 (1997).
9. A. J. Whitmarsh, C. Tournier, J. Cavanagh, unpublished observation.
10. S. Bagrodia, B. Derijard, R. J. Davis, R. A. Cerione, *J. Biol. Chem.* **270**, 27995 (1995).
11. M. C. Hu, W. R. Qiu, X. Wang, C. F. Meyer, T. H. Tan, *Genes Dev.* **10**, 2251 (1996); F. Kiefer *et al.*, *EMBO J.* **15**, 7013 (1996); W. Wang, G. Zhou, M. C. T. Hu, Z. Yao, T. H. Tan, *J. Biol. Chem.* **272**, 22771 (1997).
12. R. M. Tung, and J. Blenis, *Oncogene* **14**, 653 (1997).
13. An alternatively spliced JIP-1 cDNA was isolated from a murine brain library. The variant JIP-1 protein sequence deduced from the cDNA sequence (designated JIP-1b) contains an insertion of 47 amino acids after JIP-1 residue 557. The sequences of JIP-1 (*4*) and JIP-1b have been deposited in the GenBank database (accession numbers AF003115 and AF054611, respectively). A rat protein (IB1) similar to JIP-1b was recently reported [C. Bonney, P. Nicod, G. Waeber, *J. Biol. Chem.* **273**, 1843 (1998)]. IB1 was identified as a regulator of GLUT2 gene expression in cultured pancreatic β cells. Further studies are required to determine whether IB1, like JIP-1 and JIP-1b, can also act as a scaffold protein that binds components of the JNK MAP kinase signaling module.
14. B. Dérijard *et al.*, *Cell* **76**, 1025 (1994); M. Wartmann and R. J. Davis, *J. Biol. Chem.* **269**, 6695 (1994); H. K. Sluss, T. Barrett, B. Dérijard, R. J. Davis, *Mol. Cell. Biol.* **14**, 8376 (1994); B. Dérijard *et al.*, *Science* **267**, 682 (1995); J. Raingeaud, A. J. Whitmarsh, T. Barrett, B. Derijard, R. J. Davis, *Mol. Cell. Biol.* **16**, 1247 (1996); C. Tournier, A. J. Whitmarsh, J. Cavanagh, T. Barrett, R. J. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7337 (1997).
15. Plasmid DNA was transfected into COS-7 and 293 cells by the lipofectamine method (Life Technologies). The cells were treated with IL-1 (10 nM; 20 min), anisomycin (10 μg/ml; 30 min), or UV radiation (80 J/m^2; 60 min). The cells were collected at 48 hours and solubilized in lysis buffer [20 mM tris-HCl (pH 7.4), 137 mM NaCl, 2 mM EDTA, 25 mM β-glycerophosphate, 2 mM pyrophosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, leupeptin (10 μg/ml), 10% (v/v) glycerol, 1% Triton X-100]. GST fusion proteins were isolated by incubation with glutathione-agarose (Pharmacia-LKB) beads (20 μl) for 3 hours at 4°C. Epitope-tagged proteins were immunoprecipitated by incubation for 3 hours at 4°C with the monoclonal antibodies M2-Flag (IBI-Kodak), hemagglutinin (HA) (Boehringer-Mannheim), or T7-Tag (Novagen Inc.) bound to protein G–Sepharose (Pharmacia-LKB). HPK1 was immunoprecipitated with a rabbit polyclonal antibody to HPK1. Immunoprecipitated proteins were examined by SDS–polyacrylamide gel electrophoresis and detected by immunoblot analysis. Protein kinase activity was measured by the in-gel method with substrate polymerized in the gel (0.25 mg/ml) [B. Dérijard *et al.*, *Cell* **76**, 1025 (1994)].
16. Mammalian expression vectors for JNK1, JNK2, JIP-1, p38α, MEKK1, MKK3, MKK4, MKK6, MKK7, and c-RAF1 have been described (*4*, *14*). The mammalian expression vectors pCDNA3-HA-MLK3, pCMV5-HA-MEK1, pCMV-HA-ERK2, pSRα-T7-DLK, pCEVHA-MEKK3-F, pCDNAI-MTK1/MEKK4, pSRα-HPK1, pEBG-KHS, and pJ3H-PAK3 were provided by S. Gutkind, N. Ahn, M. Weber, A. Rana, U. Siebenlist, H. Saito, T. Tan, J. Blenis, and R. Cerione, respectively. The expression plasmid pCDNA3-Flag-MLK3 was constructed by replacing the HA tag sequence in pCDNA3-HA-MLK3 with the Flag sequence. Full-length JIP-1 and JIP-1 deletion mutants were expressed as GST fusion proteins using the vector pEBG or with a T7-Tag fused to the NH_2-terminus using the vector pCDNA3.
17. Bacterial expression of GST-cJun (1 to 79), GST-JNK1, and GST-JIP-1 (127 to 282) have been described (*4*). Expression plasmids for GST-JIP-1 (1 to 127) and GST-JIP-1 (283 to 660) were constructed by subcloning polymerase chain reaction fragments of JIP-1 into pGEX-4T-1 (Pharmacia-LKB). Bacterial expression plasmids for epitope-tagged MKK7 and MLK3 were constructed by inserting Flag-MKK7 and Flag-MLK3 (encoding amino acids 1 to 204) into pRSETA (Invitrogen).

18. We thank our colleagues for providing essential reagents, T. Barrett for DNA sequence analysis, and K. Gemme for secretarial assistance. Supported by a grant from the National Cancer Institute. R.J.D. is an Investigator of the Howard Hughes Medical Institute.

23 March 1998; accepted 4 August 1998

Enhanced Phosphorylation of p53 by ATM in Response to DNA Damage

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The ATM protein, encoded by the gene responsible for the human genetic disorder ataxia telangiectasia (A-T), regulates several cellular responses to DNA breaks. ATM shares a phosphoinositide 3-kinase–related domain with several proteins, some of them protein kinases. A wortmannin-sensitive protein kinase activity was associated with endogenous or recombinant ATM and was abolished by structural ATM mutations. In vitro substrates included the translation repressor PHAS-I and the p53 protein. ATM phosphorylated p53 in vitro on a single residue, serine-15, which is phosphorylated in vivo in response to DNA damage. This activity was markedly enhanced within minutes after treatment of cells with a radiomimetic drug; the total amount of ATM remained unchanged. Various damage-induced responses may be activated by enhancement of the protein kinase activity of ATM.

Strand breaks in cellular DNA occur continuously as a consequence of normal processes such as recombination or the infliction of DNA damage. DNA damage triggers several signal transduction pathways that lead either to damage repair coupled with attenuation of cell cycle progression, or to programmed cell death (apoptosis). A junction of such path-