Signaling from the Small GTP-binding Proteins Rac1 and Cdc42 to the c-Jun N-terminal Kinase/Stress-activated Protein Kinase Pathway

A ROLE FOR MIXED LINEAGE KINASE 3/PROTEIN-TYROSINE KINASE 1, A NOVEL MEMBER OF THE MIXED LINEAGE KINASE FAMILY*

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Certain small GTP-binding proteins control the enzymatic activity of a family of closely related serine-threonine kinases known as mitogen-activated protein kinases (MAPKs). In turn, these MAPKs, such as p44mapk and p42mapk, referred to herein as MAPKs, and stress-activated protein kinases, also termed c-Jun N-terminal kinases (JNKs), phosphorylate and regulate the activity of key molecules that ultimately control the expression of genes essential for many cellular processes. Whereas Ras controls the activation of MAPK, we and others have recently observed that two members of the Ras family of small GTP-binding proteins, Rac1 and Cdc42, regulate the activity of JNKs. The identity of molecules communicating Rac1 and Cdc42 to JNK is still poorly understood. It has been suggested that Pak1 is the most upstream kinase connecting these GTPases to JNK; however, we have observed that coexpression of Pak1 with activated forms of Cdc42 or Rac1 diminishes rather than enhances JNK activation. This prompted us to explore the possibility that kinases other than Pak might participate in signaling from GTP-binding proteins to JNK. In this regard, a computer-assisted search for proteins containing Pak-relevant motifs in vivo led to the identification of the mixed lineage kinase 3 (MLK3)/ protein-tyrosine kinase 1 (PTK1) family, a potential candidate for this function. In this study, we found that MLK3 overexpression is sufficient to activate JNK potently without affecting the phosphorylating activity of MAPK or p38. Furthermore, we present evidence that MLK3 binds the GTP-binding proteins Cdc42 and Rac1 in vivo and that MLK3 mediates activation of MEKK-SEK-JNK kinase cascade by Rac1 and Cdc42. Taken together, these findings strongly suggest that members of the novel MLK family of highly related kinases link small GTP-binding proteins to the JNK signaling pathway.

Work from a number of laboratories has established a role for certain small GTP-binding proteins controlling the enzymatic activity of a family of closely related serine-threonine kinases, known as mitogen-activated protein kinases (MAPKs)1 (1). In turn, MAPKs phosphorylate and regulate the activity of key molecules that ultimately control the expression of genes essential for many cellular processes, including cell growth, differentiation, programmed cell death, and neoplastic transformation. To date, MAPKs have been classified into five subfamilies: p44mapk and p42mapk, also known as extracellular signal-regulated kinases (ERKs) 1 and 2, respectively (1), referred to herein as MAPKs; stress-activated protein kinases (SAPKs), also termed c-jun N-terminal kinases (JNKs) (2); p38 and its alternatively spliced forms, CSBP1 and Mxi2 (3, 4); ERK5 (5); and ERK6 (6). Whereas Ras controls the activation of MAPK, we and others have recently observed that two members of the Ras family of small GTP-binding proteins, Rac1 and Cdc42, regulate the activity of JNKs (7, 8).

The identity of molecules communicating Rac1 and Cdc42 to JNK/SAPK is poorly understood. In this regard, the observations that a kinase termed Pak1 binds in vitro Rac1 and Cdc42 in a GTP-dependent manner and that both Rac1 and Cdc42 activate JNK in several cell lines provided strong evidence supporting a role for Pak1 as the most upstream kinase connecting these GTPases to JNK. However, expression of certain mutated, constitutively activated forms of Pak can induce JNK to a limited extent (9–11), coexpression of Pak1 with activated forms of Cdc42 or Rac1 fails to enhance JNK activation, and in some cases, it diminishes the elevation of JNK activity caused by these GTPases (Ref. 11 and see below). These observations prompted us to explore the possibility that kinases in addition to Pak might participate in signaling from GTP-binding proteins to JNK.

A computer-assisted search for proteins containing Pak-relevant Cdc42/Rac interactive binding (CRIIB) domains (12) led to the identification of the mixed lineage kinase 3 (MLK3)/protein-tyrosine kinase 1 (PTK1) family (13) of highly related serine-threonine kinases as a potential candidate for this function. In this study, we found that MLK3 overexpression is sufficient to potently activate JNK. Furthermore, we present evidence that MLK3 binds the GTP-binding proteins Cdc42 and Rac1 in vivo and that MLK3 mediates activation of MEKK-SEK-JNK kinase cascade by Rac1 and Cdc42. Taken together, these findings strongly suggest that

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1 The abbreviations used are: MAPK, mitogen-activated protein kinases; ERK, extracellular signal-regulated kinase; SAPK, stress-activated protein kinase; JNK, c-jun N-terminal kinase; CRIIB, Cdc42/Rac interactive binding; MLK3, mixed lineage kinase 3; PTK1, protein-tyrosine kinase 1; GST, glutathione S-transferase.

2 H. Teramoto, O. A. Coso, H. Miyata, T. Igishi, and J. S. Gutkind, unpublished observations.
Eco cDNAs as pCEF AU5 ras V12, pCEF AU5 rhoA QL, pCEF AU5 rac1 QL, and subcloned into the pcDNA3-HA vector as a Pak1 was kindly provided by Dr. Toru Miki, and Pak1 cDNA was cleotide will be made available upon request. pEBG SEK and pEBG erase chain reaction-directed mutagenesis. Sequence of each oligonu-

replacing lysine 144 for an arginine residue (KR mutant), using polym-

EcoBam subcloned into the pcDNA3 vector and pcDNA3-HA vector as a gen) encoding the HA nonapeptide (7).

sites of pcDNA3-HA, a modified pcDNA3 expression plasmid (Invitro-
mouse spleen cDNA as a template and cloned into the (3) was obtained by the polymerase chain reaction technique using

of Ras, RhoA, Rac1, and Cdc42 were described previously (7). p38 cDNA as expression plasmids for wildtype and constitutively activated forms

kinase (Fig. 1). Thus, based upon these data, we hypothesized that a
data in the presence of wild type MLK3, kindly provided by Dr. Richard Spritz (13), was

subcloned into the pcDNA3 vector and pcDNA3-HA vector as a a BamHI-EcoRI fragment. A kinase-deficient mutant of MLK3 was obtained by replacing lysine 144 for an arginine residue (KR mutant), using polymerase chain reaction-directed mutagenesis. Sequence of each oligonucleotide will be made available upon request. pEBG SEK and pEBG SEK KR were kindly provided by Dr. Leonard Zon (14). pcDNA3 rat Pak1 was kindly provided by Dr. Toru Miki, and Pak1 cDNA was subcloned into the pcDNA3-HA vector as a BamHI-EcoRI fragment.

pCEP AU5 ras V12, pCEP AU5 rhoA QL, pCEP AU5 rac1 QL, and pCEP AU5 cdc42 QL were obtained by subcloning the corresponding cDNAs as BglII-XhoI sites of pcDNA3-HA, a modified pcDNA3 expression plasmid (Invitro-
gen) encoding the HA nonapeptide (7).

PLasmids expressing epitope-tagged MAPK and

JNK, pcDNA3 HA-MAPK, and pcDNA3 HA-JNK, respectively, as well as expression plasmids for wild type and constitutively activated forms of Ras, RhoA, Rac1, and Cdc42 were described previously (7). p38 cDNA (3) was obtained by the polymerase chain reaction technique using mouse spleen cDNA as a template and cloned into the BglII and XhoI sites of pcDNA3-HA, a modified pcDNA3 expression plasmid (Invitro-
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Kinase Assays—MAPK activity in cells transfected with an epitope-
tagged MAPK (HA-ERK2, referred to herein as HA-MAPK) was deter-
mined as described previously (16), using myelin basic protein (Sigma) as a substrate. JNK assays in cells transfected with an epitope-tagged JNK (HA-JNK) were carried out as described previously (7), using purified, bacterially expressed GST-ATF2(96) fusion protein as a substrate. p38 assays in cells transfected with an epitope-tagged p38 (HA-
p38) were carried out following a protocol identical to that for HA-JNK assays, using purified, bacterially expressed GST-ATF2(96) fusion protein as a substrate. Pak kinase and MLK3 kinase assays in cells

transfected with an epitope-tagged Pak1 (HA-Pak1) and an epitope-
tagged MLK3 (HA-MLK3) were determined upon immunoprecipitation with the anti-HA specific monoclonal antibody 12CA5 (Babco). Immunoprecipitates were washed twice in lysis buffer and once in kinase reaction buffer (100 mM NaCl, 50 mM Tris, pH 7.5, 10 mM MgCl2, and 1 mM MnCl2). Kinase assays were performed using myelin basic protein as a substrate, similar to that for HA-MAPK. Immunocomplex-kinase reactions were fractionated by SDS-gel electrophoresis on 12% acryl-

amide gels, and autoradiography was performed with the aid of an intensifying screen.

Western Blots—Lyssates containing approximately 50 μg of total cellular
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MATERIALS AND METHODS

Cell Lines and Transfection—COS-7 cells were maintained in Dul-
becco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Cells were transfected by the DEAE-dextran method, adjusting the total amount of DNA to 5–10 μg/plate with vector alone (7).

DNA Constructs—Plasmids expressing epitope-tagged MAPK and

JNK, pcDNA3 HA-MAPK, and pcDNA3 HA-JNK, respectively, as well as expression plasmids for wild type and constitutively activated forms of Ras, RhoA, Rac1, and Cdc42 were described previously (7). p38 cDNA (3) was obtained by the polymerase chain reaction technique using mouse spleen cDNA as a template and cloned into the BglII and XhoI sites of pcDNA3-HA, a modified pcDNA3 expression plasmid (Invitro-
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amide gels, and autoradiography was performed with the aid of an intensifying screen.

Western Blots—Lysates containing approximately 50 μg of total cellular
cytoskeletal proteins or anti-HA (12CA5, Babco) or anti-AU5 (Babco) immuno-

RESULTS AND DISCUSSION

Pak-related kinases have been proposed to mediate the activ-

ation of JNK by two small GTP-binding proteins of the Rho

family, Rac1 and Cdc42 (7, 8). However, although expression of activated forms of Pak1 results in a limited increase in JNK activity (9–11), when wild type Pak1 was coexpressed with activated, GTPTase-deficient forms of Rac1 and Cdc42, it diminished rather than enhanced JNK activation (Fig. 1). Similar results were recently reported by others (11). For our experiments, we used epitope-tagged forms of Rac1 and Cdc42, thereby allowing us to confirm that Pak1 overexpression does not affect the expression of these small GTP-binding proteins (Fig. 1). Thus, based upon these data, we hypothesized that a kinase in addition to or other than Pak might participate in JNK activation by small GTPases.

We searched the GenBank® for proteins containing areas of homology to that in Pak1 but is involved in binding to Rac1 and Cdc42 (17). Similar analysis was recently reported by Burbelo et al. (12), leading to the identification of a conserved protein domain that was termed Pak-related CRIB domain

3 L. Vitale and J. S. Gutkind, manuscript in preparation.
Among these CRIB-containing proteins, only two kinases unrelated to Pak were identified, MLK3 (18), also known as PTK1 (13), or src-homology 3 domain-containing proline-rich kinase (19); and MLK2 (20), also known as MST (20). MLK3 is expressed in most tissues and cell lines (13, 18) and is related to the previously described MLK1 and MLK2 (20). MLK3 of human origin is a protein kinase containing a N-terminal SH3 domain followed by a CRIB domain, a kinase catalytic domain, a leucine zipper-like domain, and a carboxyl proline-rich region (12, 13). The function of the MLK family of related kinases is still unknown, but because kinases other than Pak might mediate JNK activation by small GTPases and the finding of a CRIB domain in MLK3 and MLK2, we set out to explore a role for this family of kinases in signaling to the JNK pathway.

Initially, we asked whether co-expression of MLK3 could enhance the activity of an epitope-tagged JNK. We observed that MLK3 was highly expressed upon transfection in COS-7 cells, and a very prominent band was readily observed in MLK3 transfected COS-7 cells (see below). Surprisingly, expression of MLK3 in COS-7 cells led to a nearly 20-fold increase in the in vitro phosphorylating activity of JNK (Fig. 1). Furthermore, MLK3 overexpression enhanced JNK stimulation when inactivated Ras, RhoA, Rac1, and Cdc42. Each AU5-tagged small GTP-binding protein was detectably expressed to nearly identical levels (Fig. 1 and data not shown). Western blot analysis revealed that Pak1 and MLK3 coimmunoprecipitated only with activated Rac1 and Cdc42 (Fig. 3). Furthermore, we did not detect Pak1 or MLK3 in parallel experiments using AU5-tagged wild type forms of Rac1 and Cdc42 (data not shown), thus suggesting that both Pak1 and MLK3 can physically associate in vivo with Rac1 and Cdc42. As an approach, we coexpressed Pak1 or MLK3 cDNAs with AU5 epitope-tagged forms of activated Ras, RhoA, Rac1, and Cdc42. Each AU5-tagged small GTP-binding protein was detectably expressed to nearly identical levels (Fig. 1 and data not shown). Western blot analysis revealed that Pak1 and MLK3 coimmunoprecipitated only activated Rac1 and Cdc42 (Fig. 3). Furthermore, we did not detect Pak1 or MLK3 in parallel experiments using AU5-tagged wild type forms of Rac1 and Cdc42 (data not shown), thus suggesting that both Pak1 and MLK3 can physically associate in vivo with Rac1 and Cdc42 only in their active state. We next asked whether activated Rac or Rho fails to elevate the enzymatic activity of either HA-tagged Pak1 or HA-tagged MLK3 (data not shown). In contrast, the MBP phosphorylating activity of both Pak1 and MLK3 was enhanced when coexpressed with activated forms of JNK/SAPKActivation by MLK3/PTK1 27227
Fig. 5. The pathway linking Rac1-Cdc42 to JNK. Schematic representation of protein kinase cascades leading to the activation of JNK. In the mammalian JNK signaling route, JNK, SEK, and MEKK represent the first three hierarchical levels, and we postulate that MLK3 and Pak represent the most upstream kinase in this cascade. The broken line corresponds to an unidentified signaling pathway that may be involved in JNK activation.

Rac1 and Cdc42 (Fig. 3b). These observations and the potent activating effect of MLK3 on the JNK pathway prompted us to investigate whether MLK3 participates in signaling from Rac1 and Cdc42 to JNK. For that, a mutant of MLK3 was generated by replacing a lysine in position 144 for arginine. This lysine represents a highly conserved residue among catalytic domains of most protein kinases and is known to be critical for binding of ATP (21). Analogous mutations prevent the enzymatic activity of other kinases and in some cases confer dominant negative activity (22). Thus, this mutation, designated MLK3 KR, would be expected to act as a dominant negative, kinase-deficient MLK3. As shown in Fig. 3b, both MLK3 and MLK3 KR were detectably expressed, as judged by Western blotting using an anti-MLK3 serum. The slower mobility of wild type MLK3 is likely to result from post-translational modification, such as phosphorylation, because when expressed in bacteria both MLK3 and MLK3 KR have identical mobilities in SDS-polyacrylamide gels (data not shown). Faster mobility of kinase inactive forms of MLK3 has been recently reported by others (19). Under these experimental conditions, we observed that whereas MLK3 overexpression enhanced JNK stimulation induced by Cdc42 and Rac1, the kinase-deficient mutant of MLK3, MLK3 KR, drastically reduced JNK stimulation by these GTPases. In contrast, MLK3 KR had no effect on JNK activation by MEKK, a putative downstream kinase (23). Taken together, these results suggest that MLK3 is downstream of Rac1 and Cdc42 and probably upstream of MEKK in a biochemical route linking these GTPases to JNK.

As discussed above, it has been proposed that Rac1 and Cdc42 communicate with JNK utilizing a kinase cascade sequentially including MEKK and SEK (7, 8, 14), the latter also referred to as MKK4 or JNKK1 (1). Because our findings strongly suggest that MLK3 can potently induce JNK activation, we asked whether the pathway linking MLK3 to JNK involves an independent kinase cascade or whether MLK3 acts upstream of SEK to enhance JNK activity. As an approach, we cotransfected COS-7 cells with plasmids expressing the N-terminal domain of GST fused to a wild type SEK or a kinase-deficient mutant of SEK, SEK KR (14). As shown in Fig. 4, Western blotting with anti-SEK antibodies revealed that both GST-SEK and GST-SEK KR were detectably expressed. Expression of SEK WT in COS-7 cells led to a 1–2-fold increase in the in vitro phosphorylating activity of JNK and enhanced JNK stimulation induced by MLK3 and MEKK. In contrast, the dominant negative mutant of SEK, SEK KR, decreased effectively JNK stimulation by MLK3 and MEKK. These observations strongly suggest that the signaling pathways activated by MLK3 and MEKK in COS-7 cells converge at the level of SEK. These observations together with the previous results led us to propose that the pathway linking the small GTP-binding proteins Rac1 and Cdc42 to JNK involves, sequentially, MLK3, MEKK, and SEK (Fig. 5). Whether Pak1 plays a major role in signaling to JNK or whether its primary role is to connect small GTP-binding proteins to cytoskeletal components, or both warrants further investigation.

The biological function of the MLK family of kinases is not known, but recent available evidence suggests a role for MLK3 in melanocyte proliferation (13). Whereas MLK3/PTK1 and MLK2, also known as MST (20), contain a CRIB domain (12), other members of this newly discovered family of related kinases, such as DLK (24), also known as ZPK (25), lack an obvious Cdc42 or Rac1 binding domain. Thus, although it is still possible that these kinases can associate directly or indirectly with small GTPases, these observations suggest that the MLK family of related kinases may mediate JNK activation by different stimuli, acting on Rac1- and Cdc42-dependent as well as -independent pathways. This question, as well as the potential role for the different MLK-related kinases in signaling from distinct classes of cell surface receptors to JNK, are under current investigation.

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REFERENCES

1. Seger, R. & Krobs, E. (1995) FASEB J. 9, 726–735
2. Kyriakis, J., Banerjee, P., Nikolakaki, E., Dai, T., Ruhie, E., Ahmad, M., Avruch, J. & Woodgett, J. (1994) Nature 369, 156–160
3. Han, J., Lee, J.-D., Bibbs, L. & Ulevitch, R. J. (1994) Science 265, 808–811
4. Zavos, A. S., Faccio, L., Gatto, J., Kyriakis, J. & Bret, R. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 10531–10534
5. Zhou, G., Bao, Z. & Dixon, J. E. (1996) J. Biol. Chem. 271, 12665–12669
6. Lechner, C., Zahalka, M., Gi, J., Meller, N. & Ullrich, A. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 4355–4359
7. Coso, O. A., Chiariello, M., Yu, J.-C., Teramoto, H., Crespo, P., Xu, N., Miki, T. & Gutkind, J. S. (1995) Cell 81, 1157–1166
8. Minden, A., Lin, A., Claret, F. X., Abo, A. & Karin, M. (1995) Cell 81, 1147–1157
9. Brown, J. L., Stowers, L., Baer, M., Trejo, J., Coughlin, S. & Chant, J. (1996) Curr. Biol. 6, 598–605
10. Bagrodia, S., Derijard, B., Davis, R. J. & Cerione, R. A. (1995) J. Biol. Chem. 270, 27995–27998
11. Frost, J. Xu, S., Hutchinson, M., Marcus, S. & Cobb, M. (1996) Mol. Cell. Biol. 16, 3707–3713
12. Burbelo, P. D., Drechsel, D. & Hall, A. (1995) J. Biol. Chem. 270, 29071–29074
13. Eze, K., Lee, S.-T., Strunk, K. M. & Spritz, R. A. (1994) Oncogene 9, 935–938
14. Sanchez, I., Hughes, R. T., Mayer, B. J., Yee, K., Woolfett, J. R., Avruch, J., Kyriakis, J. M. & Zon, L. I. (1994) Nature 372, 794–798
15. Lim, P., Jensen, A., Cowsert, L., Nakai, Y., Lim, L., Jin, X. & Sundberg, J. (1999) J. Infect. Dis. 182, 1263–1269
16. Crespo, P., Xu, N., Simonds, W. & Gutkind, J. S. (1994) Nature 369, 418–420
17. Manser, D., Leung, T., Sahiuddin, H., Zhou, Z. S. & Lim, L. (1994) Nature 367, 40–46
18. Ing, Y. L., Leung, I. W. L., Heng, H. H. Q., Tsui, L.-C. & Lassam, N. J. (1994) Oncogene 9, 1745–1750
19. Gallo, K. A., Mark, M. R., Scadden, D. T., Wang, Z., Gu, Q. & Godowski, P. J. (1994) J. Biol. Chem. 269, 15992–15998
20. Katoh, M., Hirai, M., Sugimura, T. & Teramda, M. (1995) Oncogene 10, 1447–1451
21. Kampe, M., Taylor, S. & Selfit, B. (1984) Nature 310, 589–592
22. Heidecker, G., Huleihel, M., Cleveland, J., Kolch, W., Beck, T., Lloyd, P., Pawson, T. & Rapp, U. (1990) Mol. Cell. Biol. 10, 2503–2512
23. Lange-Carter, C., Pleiman, C., Gardner, A., Blumer, K. & Johnson, G. (1994) Science 260, 315–319
24. Holzmann, L., Merritt, S. & Fan, G. (1994) J. Biol. Chem. 269, 30808–30817
25. Reddy, U. & Pleasure, D. (1994) Biochem. Biophys. Res. Commun. 202, 613–620