Hematopoietic Progenitor Kinase 1 Is a Component of Transforming Growth Factor β-induced c-Jun N-terminal Kinase Signaling Cascade*

(Received for publication, November 16, 1998, and in revised form, February 24, 1999)

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The c-Jun N-terminal kinase (JNK) signaling pathway is involved in transforming growth factor β (TGF-β) signaling in a variety of cell systems. We report here that hematopoietic progenitor kinase 1 (HPK1), a novel Ste20-like protein serine/threonine kinase, serves as an upstream mediator for the TGF-β-activated JNK1 cascade in 293T cells. TGF-β treatment resulted in a time-dependent activation of HPK1, which was accompanied by similar kinetics of JNK1 activation. The activation of JNK1 by TGF-β treatment required a kinase-defective HPK1 mutant but not by a kinase-defective mutant of kinase homologous to Ste20/Sp61. This result indicates that HPK1 is specifically required for TGF-β-induced activation of JNK1. We also found that TGF-β-induced JNK1 activation was blocked by a kinase-defective mutant of TGF-β-activated kinase 1 (TAK1). In addition, interaction between HPK1 and TAK1 was observed in transient transfection assays, and this interaction was enhanced by TGF-β treatment. Both stress-activated protein kinase/extracellular signal-regulated kinase 7 (SEK) and mitogen-activated protein kinase kinase 7 (MKK7) are immediate upstream activators of JNK1. Although SEK and MKK7 acted downstream of TAK1, only a kinase-defective SEK mutant blocked TGF-β-induced activation of JNK1, indicating that the TGF-β signal is relayed solely through SEK, but not MKK7, in vivo. Furthermore, TGF-β-induced activating protein 1 activation was blocked by a HPK1 mutant, as well as by TAK1 and SEK mutants. Taken together, these studies establish a potential cascade of TGF-β-activated interacting kinases beginning with HPK1, a Ste20 homolog, and ending in JNK1 activation: HPK1 → TAK1 → SEK → JNK1.

Transforming growth factor β (TGF-β)† belongs to a family of multifunctional cytokines that regulate cell proliferation, cel-

* This work was supported by the National Institutes of Health Grants R01-AI38649 and R01-1A42532 (to T.-H. T.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ The abbreviations used are: TGF-β, transforming growth factor β; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MEKK, MAPK kinase 1; HPK1, hematopoietic progenitor kinase 1; KHS, kinase homologous to Ste20; GCK, germinal center kinase; HGK, HPK1/GCK-like kinase; SEK, stress-activated protein kinase/ERK kinase; MKK7, MAPK kinase 7; TAK1, TGF-β-activated kinase 1; MLK-3, mixed lineage kinase 3; HA, hemagglutinin; GST, glutathione S-transferase; AP-1, activating protein 1; MUK, MAPK upstream kinase; lular differentiation, apoptosis, cell adhesion and motility, and production of the extracellular matrix (reviewed in Refs. 1 and 2). TGF-β initiates its pleiotropic effects by binding a hetero-

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unknown function and by the lack of a Rac1/Cdc42-binding domain found in PAKs.

Although HPK1 is widely expressed in embryonic tissues, its expression is restricted to hematopoietic tissues in adults (19, 20). In transient transfection assays, HPK1 has been shown to interact with MEKK1 (19) and MLK3 (20), which, in turn, can activate SEK, thereby leading to the activation of JNK pathway. Because its physiological activator(s) has not been identified, the biological function of HPK1 and the mechanisms of HPK1 signaling are currently unknown. However, a potential association between HPK1 and several protein-tyrosine kinase receptors mediated by SH2/SH3 adapter proteins has recently been reported (26–28).

We have previously shown in transfected 293T cells that TAK1, a TGF-β-activated MAPKKK level kinase, serves downstream of HPK1 and upstream of SEK in the JNK pathway (10). Because TAK1 is a potential mediator of TGF-β signal transduction (29), we were prompted to investigate whether HPK1 is an in vivo component of the TGF-β-activated JNK signaling pathway. The studies presented here show that TGF-β treatment of 293T cells resulted in a marked and persistent increase in HPK1 activity and that HPK1 was required for the activation of JNK and activating protein 1 (AP-1) by TGF-β. These studies establish that HPK1 acts as an upstream activator for the TAK1-SEK-JNK1 module in relaying the TGF-β signal into the nuclei in 293T cells.

**EXPERIMENTAL PROCEDURES**

Reagents—[γ-32P]ATP was purchased from ICN Biomedicals (Irvine, CA). An enhanced chemiluminescence system was purchased from Amersham Pharmacia Biotech. TGF-β was purchased from R & D Systems (Minneapolis, MN). Anti-HA antibody (12C5) and anti-FLAG antibody (M2) were purchased from Roche Molecular Biochemicals and Eastman Kodak Co., respectively. Monoclonal anti-GST and polyclonal anti-MEK1 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal anti-JNK1 antibody (Ab101; Ref. 33), polyclonal anti-HPK1 antibody (Ab2025; Ref. 19), and polyclonal anti-HKG antibody (Ab2225; Ref. 24) were described previously. All other reagents were purchased from Sigma unless otherwise indicated. CAT Assays and Immunocomplex Kinase Assays—CAT assays (31) and immunocomplex kinase assays (32–35) were performed as described previously (33). For the activation of JNK and activating protein 1 (AP-1) by TGF-β, activator for the TAK1-SEK-JNK1 module in relaying the TGF-β expression is restricted to hematopoietic tissues in adults (19, 20). We have previously demonstrated that TAK1, a TGF-β-activated kinase, is a potent activator for JNK and serves downstream of HPK1, a MAPKKKK level kinase, in the JNK pathway (10). We were thus prompted to address whether HPK1 is involved in TGF-β signaling in vivo. Using an HPK1-specific antibody Ab2025 (19), we detected a high level of HPK1 expression in human embryonic kidney 293 and 293T cells (Fig. 1A). Cell fractionation analysis indicates that HPK1 is localized in the cytosol but not in the nucleus of 293 cells (Fig. 1A). To study the role of HPK1 in TGF-β signaling, 293T cells were transfected with FLAG-tagged HPK1, and following TGF-β treatment, the FLAG-HPK1 was immunoprecipitated with an anti-FLAG antibody (M2), and immunocomplex kinase assays were performed using myelin basic protein (MBP) as a substrate. Equivalent levels of FLAG-HPK1 expression were verified by immunoblotting with an anti-FLAG antibody (M2). C, TGF-β resulted in HPK1 activation with kinetics similar to HPK1 activation. Endogenous HPK1 was immunoprecipitated with an anti-JNK1 antibody (Ab101) from the cells transfected with FLAG-HPK1 after TGF-β treatment, and immunocomplex kinase assays were performed using GST-c-Jun (1–79) as a substrate.

**RESULTS**

**Concomitant Activation of HPK1 with JNK1 Following TGF-β Treatment**—The JNK signaling pathway is activated by TGF-β in a variety of cellular systems, including 293T cells (7–10). We have previously demonstrated that TAK1, a TGF-β-activated kinase, is a potent activator for JNK and serves downstream of HPK1, a MAPKKKK level kinase, in the JNK pathway (10). We were thus prompted to address whether HPK1 is involved in TGF-β signaling in vivo. Using an HPK1-specific antibody Ab2025 (19), we detected a high level of HPK1 expression in human embryonic kidney 293 and 293T cells (Fig. 1A). Cell fractionation analysis indicates that HPK1 is localized in the cytosol but not in the nucleus of 293 cells (Fig. 1A). To study the role of HPK1 in TGF-β signaling, 293T cells were transfected with FLAG-tagged HPK1, and following TGF-β treatment, the FLAG-HPK1 was immunoprecipitated with an anti-FLAG antibody (M2). HPK1 activity was determined by an immunocomplex kinase assay using myelin basic protein (MBP) as a substrate. TGF-β treatment resulted in a time-dependent activation of HPK1 (Fig. 1B). The increased kinase activity was not due to various levels of HPK1 because an equivalent expression of FLAG-HPK1 was detected by immunoblotting analysis with anti-FLAG antibody (M2) (Fig. 1B, bottom panel). Endogenous HPK1 immunoprecipitated with an anti-HPK1 antibody (Ab2025) was also activated by TGF-β with similar kinetics (data not shown). To address a possible link between HPK1 and
JNK in response to TFG-β, endogenous JNK1 was immunoprecipitated with an anti-JNK1 antibody (Ab101) from the FLAG-HPK1-transfected cell lysates, and its activity was determined by an immunocomplex kinase assay using GST-c-Jun (1–79) as substrate. Fig. 1C shows that endogenous JNK1 was activated by TFG-β with kinetics similar to that of HPK1. Thus, HPK1 was activated concomitantly with JNK1 in response to TGF-β in 293T cells.

TGF-β-induced JNK1 Activation Is Specifically Blocked by the HPK1 Mutant but Not by the KHS Mutant—To investigate the involvement of HPK1 in TGF-β-induced JNK1 activation, we attempted to block the signaling cascade with HPK1-M46, an HPK1 kinase-defective mutant in which methionine is substituted for lysine 46 (19). 293T cells were transfected with HA-JNK1 alone or with HA-JNK1 plus various amounts of HPK1-M46. Following TGF-β treatment, HA-JNK1 was immunoprecipitated with an anti-HA antibody (12CA5), and the JNK1 activity was measured. Fig. 2A shows that TGF-β-induced JNK1 activation was blocked by cotransfected HPK1 mutant (HPK1-M46) in a dose-dependent manner. In contrast, cotransfection of HA-JNK1 with KHS-KR, a kinase-defective mutant of KHS (22), another MAPKKKK level kinase, had no inhibitory effect on JNK1 activation by TGF-β. These data indicate that HPK1 is specifically required for the transduction of TGF-β signal to JNK1 activation. To further confirm the specific involvement of HPK1 in TGF-β-induced JNK1 activation, we tested the effect of the HPK1 mutant on JNK1 activation by UV-C, a potent stimulus for the JNK pathway (33, 34). UV-C-induced JNK1 activation was blocked by HGK-KR, a kinase-defective mutant of HGK, a MAPKKKK level kinase (24). However, cotransfected HPK1 mutant HPK1-M46 had no effect on UV-C-induced JNK1 activation (Fig. 2B). Thus, HPK1 acts as a specific mediator for TGF-β-induced JNK1 signaling pathway.

TAK1 Mutant Blocks TGF-β-induced JNK1 Activation—TAK1, a member of the MAPKKK family, has been shown as a potential mediator of TGF-β signal transduction (29). Our previous studies have shown that TAK1 is a potent JNK activator and a mediator of HPK1-induced JNK activation (10). Given that HPK1 is required for the activation of JNK1 by TGF-β (shown above), we wondered whether TAK1 is an in vitro component of the JNK1 signaling cascade triggered by TGF-β in 293T cells. To address this question, we investigated whether JNK1 activation by TGF-β is blocked by a kinase-defective mutant of TAK1, TAK1-K63W. Cotransfection of 293T cells with HA-JNK1 and TAK1-K63W abolished TGF-β-induced JNK1 activation (Fig. 3, lanes 3 and 4). We did not observe interference with TGF-β-induced activation of JNK1 by a dominant-negative mutant of MEKK1 (lanes 5 and 6). Further evidence for the specific involvement of TAK1 in TGF-β-induced JNK1 activation is provided by the inability of the kinase-defective mutant of MUK, another MAPK KK level kinase (36), to block the JNK1 activation by TGF-β (data not shown). Therefore, TAK1 is likely an in vitro signaling component of TGF-β-activated JNK1 pathway in 293T cells.

The HPK1-TAK1 Interaction Is Enhanced by TGF-β—Complex formation exists widely in signaling processes and is an important mechanism to facilitate signal transduction and maintain signaling specificity. This led us to investigate whether HPK1 interacts with TAK1 in vivo by a communoprecipitation assay. HA-tagged TAK1 and FLAG-tagged HPK1 were overexpressed by transient transfection in 293T cells. When an anti-HA antibody (12CA5) was used to precipitate HA-TAK1, FLAG-HPK1 was co-precipitated (Fig. 4A, left panel). Conversely, HA-TAK1 was co-precipitated with FLAG-HPK1 when an anti-FLAG antibody (M2) was used to precipitate FLAG-HPK1 (Fig. 4A, right panel). These data indicate that a complex was formed between HPK1 and TAK1. Moreover, TGF-β treatment significantly enhanced the complex formation between HPK1 and TAK1 (Fig. 4B). Thus, HPK1 interacted with TAK1 in 293T cells, and this interaction was enhanced in response to TGF-β.

TGF-β-induced Activation of JNK1 Is Only Blocked by a SEK Mutant but Not by an M KK7 Mutant—Both SEK (37) and M KK7 (13, 14) are kinases that lie immediately upstream of JNK. We were interested in determining which MAPK (either SEK, MKK7, or both) relays the TGF-β signal to JNK1 activation in 293T cells. We examined the ability of kinase-defective SEK and MKK7 mutants to block JNK1 activation by TAK1 and TGF-β. TAK-induced JNK1 activation was blocked by cotransfected SEK-KR (K63W) and HGK-KR, a kinase-defective mutant of HGK, a MAPKKKK level kinase (24). However, cotransfected HPK1 mutant HPK1-M46 had no effect on UV-C-induced JNK1 activation (Fig. 2B). Thus, HPK1 acts as a specific mediator for TGF-β-induced JNK1 signaling pathway.

FIG. 2. JNK activation by TGF-β is specifically blocked by a kinase-defective HPK1 mutant (HPK1-M46). A, 293T cells (1.5 × 10⁵ cells in 35-mm wells) were transfected with either HA-JNK (0.1 µg) alone or HA-JNK plus various amounts of HPK1-M46, as indicated by the italic numbers. As a control, cotransfections of HA-JNK with various amounts of a kinase-defective mutant of KHS (KHS-KR) were included. Empty vector was used to normalize the amount of transfected DNA. 36 h post-transfection, the cells were treated with TGF-β (10 ng/ml) for 12 h. Cell lysates were prepared, HA-JNK1 was immunoprecipitated with an anti-HA antibody (12CA5), and immunocomplex kinase assays were performed using GST-c-Jun (1–79) as a substrate. Equivalent levels of HA-JNK, HPK1-M46, and KHS-KR expression were verified by immunoblotting using anti-HA (12CA5), anti-FLAG (M2), and anti-GST antibodies, respectively (three bottom panels). B, UV-C-induced JNK activation is not sensitive to HPK1-M46. 293T cells (1.5 × 10⁵ cells in 35-mm wells) were transfected with HA-JNK alone (0.1 µg), HA-JNK plus 2 µg of HPK1-M46, or HA-JNK plus 2 µg of HGK-KR. Empty vector was used to normalize the amount of transfected DNA. 44 h post-transfection, the cells were treated with UV-C (300 J/m²) for 30 min. Cell lysates were prepared, HA-JNK1 was immunoprecipitated with an anti-HA antibody (12CA5), and immunocomplex kinase assays were performed using GST-c-Jun (1–79) as a substrate. Equivalent levels of HA-JNK1, HPK1-M46, and HGK-KR expression were verified by immunoblotting using anti-HA (12CA5), anti-FLAG (M2), and anti-HGK (Ab2228) antibodies, respectively (three bottom panels).
**HPK1 Activation by TGF-β**

**FIG. 3. TGF-β-induced JNK activation is blocked by the dominant-negative TAK1 mutant, TAK-K63W.** 293T cells (1.5 × 10^5 cells in 35-mm wells) were transfected with either HA-JNK1 (0.1 μg) alone (lanes 1 and 2) or HA-JNK1 plus various amounts of TAK-K63W (lanes 3 and 4) and MEKK1-KR (lanes 5 and 6), as indicated by the italic numbers. Empty vector was used to normalize the amount of transfected DNA. 36 h post-transfection, the cells were treated with TGF-β (10 ng/ml) for 12 h. Cell lysates were prepared, HA-JNK1 was immunoprecipitated with an anti-HA antibody (12CA5), and immuno-complex kinase assays were performed using GST-c-Jun (1–79) as a substrate. To ensure equivalent levels of HA-JNK, HA-TAK-K63W, and MEKK1-KR expression, an equal amount of each cell lysate was resolved by 10% SDS-polyacrylamide gel electrophoresis, and immunoblotting was performed with anti-HA (12CA5) and polyclonal anti-MEKK1 antibodies (three bottom panels).

abrogated by both SEK and M KK7 mutants in transient transfection assays (Fig. 5A). However, only the SEK mutant blocked TGF-β-induced JNK1 activation, whereas the M KK7 mutant exerted no effect (Fig. 5B). These data indicate that it is SEK but not M KK7 that acts downstream of TAK1 and serves as an endogenous regulator that relays the TGF-β signal to activate JNK1 in 293T cells.

**HPK1 Is Required for TGF-β-induced, AP-1-dependent Gene Expression**—The JNK signaling pathway has been implicated in AP-1-dependent gene expression because c-Jun, one component of the AP-1 complex, is a substrate for JNK in vivo (38). TGF-β also exerts many of its effects through AP-1 gene expression (39, 40). To further confirm the functional involvement of HPK1 in TGF-β-induced JNK1 activation, we investigated the effect of the HPK1 mutant on TGF-β-induced AP-1 activity. AP-1 activity was increased by TGF-β in a time-dependent manner, peaking at 24 h (data not shown). We cotransfected a chloramphenicol acetyltransferase (CAT) reporter construct containing multiple c-Jun-binding sites (5xTRE-CAT) with or without HPK1-M46 into 293T cells. TGF-β-stimulated AP-1 activity was blocked by HPK1-M46 (Fig. 6). As expected, cotransfection of the TAK1 mutant or the SEK mutant also abrogated the TGF-β-induced AP-1 activity (Fig. 6). The CAT activity of the various transfectants correlated closely with the activation of JNK1 (data not shown), indicating that JNK1 activation contributed to the TGF-β-induced, AP-1-dependent CAT activity. Taken together, these data indicate that HPK1 is required for TGF-β-induced, JNK1-mediated AP-1 activation.

**DISCUSSION**

There is a growing interest in the identification and characterization of Ste20-like kinases, because they might represent the first step in a number of linear cascades of sequentially activating serine/threonine kinases leading to the stimulation of members of the MAPK superfamily. Here we present the evidence that HPK1, a novel Ste20/PAK-like protein, serine/threonine kinase, and a potent activator of JNK, acts as an upstream regulator for the JNK1 signaling cascade triggered by TGF-β. The evidence includes: (i) HPK1 activation by TGF-β was accompanied by similar kinetics of JNK1 activation; (ii) TGF-β-induced activation of JNK1 was specifically blocked by an HPK1 kinase-defective mutant; (iii) TGF-β enhanced the interaction between HPK1 and TAK1, a TGF-β-activated MAPK-KKK level kinase, which is also required for TGF-β-induced JNK1 activation; and (iv) TGF-β induced, JNK1-mediated AP-1 activation was abrogated by an HPK1 kinase-defective mutant. The functional significance of the activation of HPK1 by TGF-β has not been completely understood. It has been recently shown that HPK1 specifically interacts with adaptor proteins, such as Crk, CrkL, and Grb2, and through these adaptor proteins connects to the membrane tyrosine kinase receptors (26–28). It is thus conceivable that the JNK cascade is activated through a specific interaction between HPK1 and an adaptor protein that connects it to the TGF-β receptor complex. This hypothesis is supported by the recent finding that GCK, another member of the HPK1 subfamily of mammalian Ste20 homologs, interacts in vivo with the tumor necrosis factor-α receptor 1-associated factor-2 and with MEKK1,
HPK1 Activation by TGF-β

A

SEK-AL
MKK7-K76E
TAK1
HA-JNK1

GST-cJun(1-79)

29 43
1 2 3 4 5 6

HA-JNK1
HA-TAK1
Flag-MKK7-K76E

GST-SEK-AL

B

TGF-β

SEK-AL
MKK7-K76E
HA-JNK1

GST-cJun(1-79)

29 43
1 2 3 4 5 6

HA-JNK1
Flag-MKK7-K76E

GST-SEK-AL

Fig. 5. JNK activation by TGF-β is blocked by the SEK mutant but not by the MKK7 mutant. A, both SEK and MKK7 mutants blocked TAK1-induced JNK activation. 293T cells (1.5 x 10⁶ cells in 35-mm wells) were transfected with HA-JNK (0.1 μg) alone (lane 1), HA-JNK plus TAK1 (2 μg, lane 2), or HA-JNK plus TAK1 and various amounts of either SEK (lanes 3 and 4) or MKK7 (lanes 5 and 6) mutants, as indicated by the italic numbers. Empty vector was used to normalize the amount of transfected DNA. Cell lysates were prepared at 48 h post-transfection, HA-JNK1 was precipitated with an anti-HA antibody (12CA5), and immunocomplex kinase assays were done using GST-c-Jun (1-79) as a substrate. Equivalent levels of HA-JNK, HA-TAK1, MKK7-K76E, and SEK-AL expression were verified by immunoblotting using anti-HA (12CA5), anti-HA (12CA5), anti-FLAG (M2), and monoclonal anti-GST antibodies, respectively (four bottom panels). B, only the SEK mutant blocked JNK1 activation by TGF-β. 293T cells (1.5 x 10⁶ cells in 35-mm wells) were transfected with HA-JNK1 (0.1 μg) alone (lanes 1 and 2), HA-JNK1 plus various amounts of either SEK (lanes 3 and 4) or MKK7 (lanes 5 and 6) mutants, as indicated by the italic numbers. Empty vector was used to normalize the amount of transfected DNA. 36 h post-transfection, the cells were treated with TGF-β (10 ng/ml) for 12 h. Cell lysates were prepared, HA-JNK1 was precipitated with an anti-HA antibody (12CA5), and immunocomplex kinase assays were performed using GST-c-Jun (1-79) as a substrate. Equivalent levels of HA-JNK, MKK7-K76E, and SEK-AL expression were verified by immunoblotting using anti-HA (12CA5), anti-FLAG (M2), and monoclonal anti-GST antibodies, respectively (three bottom panels).

Fig. 6. TGF-β-induced AP-1 activation is abrogated by the HPK1, TAK1, and SEK mutants. 293T cells (1.5 x 10⁶ cells in 35-mm wells) were transfected with 5xTRE-CAT (0.1 μg) alone, 5xTRE-CAT plus 2 μg of HPK1-M46, TAK1-K63W, and SEK-AL, and CAT activity was measured and compared with that induced by transfection of empty vector. The pCMV-β-gal reporter plasmid (0.15 μg) was used in all transfection assays to normalize variations in transfection efficiency. The results are expressed as fold induction.

TAK1 was originally identified as a TGF-β-responsive MAPKK level kinase (29). It has been shown that TAK1 can activate SEK, an immediate upstream activator kinase of JNK, in vitro (29), suggesting that TAK1 might be involved in mediating TGF-β-induced JNK activation. However, no direct evidence for this has been given so far, although TAK1 has been shown to mediate JNK activation by other stimuli, such as ceramide (42). Here we presented data showing that TGF-β-stimulated JNK1 and AP-1 activation in 293T cells was completely abrogated by a kinase-defective TAK1 mutant. Moreover, by using an immunoprecipitation approach, we found that HPK1 interacted with TAK1 and that this interaction was greatly enhanced by TGF-β. Thus, these results indicate that TAK1 mediates TGF-β signaling to JNK1 activation and subsequent AP-1 activation, although we have not yet examined the activation of TAK1 by TGF-β in our system. This conclusion is also in line with our previous observation that TAK1 acts downstream of HPK1 and mediates HPK1-induced JNK activation (10).

The MAPK cascades can be activated by multiple extracellular stimuli with little apparent commonality in the cellular effects, such as cytokines, hormones, growth factors, and stresses (11, 12). The mechanism that determines MAPK signaling specificity in response to diverse extracellular stimuli has not been fully characterized. We report here that TGF-β-induced JNK1 activation was only blocked by the SEK1 mutant but not the MKK7 mutant at the MAPKK level, and by the TAK1 mutant but not the MEKK1 and MUK mutants at the MAPKKK level. These results imply that a mechanism might exist to ensure the correct recruitment of TGF-β signaling components in vivo. This idea is supported by the recent discovery that the mammalian scaffold protein JIP1 preferentially binds MKK7 but not SEK, thus assembling the selective MAPK module MLK3-MKK7-JNK (43). The differential responses of SEK and MKK7 to extracellular stimuli have also been reported. For example, UV and anisomycin strongly activate SEK; however, MKK7 is only weakly activated (44). In addition, the differential activation of SEK and MKK7 was observed in MST/MLK2-dependent activation of JNK in which MST/MLK2 activates recombinant MKK7 more efficiently than recombinant SEK, whereas MEKK1 activates both to a similar extent (45). Therefore, the signaling leading to JNK activation may diverge upstream of JNK at the various levels of the kinase module.

An emerging property of signal transduction pathways that might account for signaling specificity is the formation of sig-
naling complexes. These complexes may result from the preferred physical interaction between kinases within a module (12, 46). The inducible interaction between HPK1 and TAK1 by TGF-β, combined with the requirement of HPK1 for TGF-β-induced JNK activation and the specific blockage of TGF-β-induced JNK activation by the TAK1 mutant, strongly indicates that HPK1 might have a recruiting function, coupling a TGF-β signal to TAK1.

Acknowledgments—We thank Drs. J. Blenis, J. Bruder, M. C.-T. Hu, M. Karin, K. Matsumoto, S. Ohno, D. Templeton, J. Woodgett, and L. I. Zon for providing valuable reagents; members of the Tan laboratory for the helpful discussions and critical reading of the manuscript; Roshi Afshar for technical assistance; and Mary Lowe for secretarial assistance.

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