Contribution of the ERK5/MEK5 Pathway to Ras/Raf Signaling and Growth Control*

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The activity of the catalytic domain of the orphan MAP kinase ERK5 is increased by Ras but not Raf-1 in cells, which suggests that ERK5 might mediate Raf-independent signaling by Ras. We found that Raf-1 does contribute to Ras activation of ERK5 but in a manner that does not correlate with Raf-1 catalytic activity. A clue to the mechanism of action of Raf-1 on ERK5 comes from the observation that endogenous Raf-1 binds to endogenous ERK5, suggesting the involvement of regulatory protein-protein interactions. This interaction is specific because Raf-1 binds only to ERK5 and not ERK2 or SAPK. Finally, we demonstrate the ERKS5/MEK5 pathway is required for Raf-dependent cellular transformation and that a constitutively active form of MEK5, MEK5DD, synergizes with Raf to transform NIH 3T3 cells. These observations suggest that ERK5 plays a large role in Raf-1-mediated signal transduction.

We have recently demonstrated that Ras contributes to activation of the newly discovered MAP kinase family member, ERK5 (1). However, unlike ERK1 and ERK2, ERK5 is not detectably stimulated by Raf-1 or its activated mutants. These findings initially suggested that Ras activates ERK5 by a Raf-independent mechanism. Many studies imply that Ras function is mediated by a convergence of Raf-dependent and Raf-independent signaling events (2, 3). The observation that the activity of the ERK5 catalytic domain is increased by Ras but not Raf-1 raises the possibility that ERK5 might contribute to Raf-independent signaling by Ras.

To address the possibility that ERK5 is regulated by a Raf-independent Ras effector pathway, we tested the capacities of a panel of Ras effector domain mutants to activate the catalytic domain of ERK5. These mutations uncouple the association of Ras with its multiple downstream partners (3). We found that all Ras effector domain mutants were defective in activating ERK5. We were surprised to find that Raf-1 complemented all of the mutants, including those that do not bind or activate Raf-1. The ability of Raf-1 to restore activation of the ERK5 catalytic domain by the Ras effector domain mutants did not correlate with the ability of Raf-1 to activate the ERK1,2 MAP kinase cascade. This finding suggests that Raf-1 and Ras coordinate to regulate ERK5 by a mechanism distinct from that of Raf-1 in ERK1,2 activation. To test for a possible role of complex formation between ERK5 and Raf-1, we examined their interactions in vitro and in intact cells. Not only do recombinant ERK5 and Raf-1 bind in vitro, endogenous ERK5 and Raf-1 associate as detected by co-immunoprecipitation. An important role for coordinated ERK5 regulation is suggested by our observation that dominant negative mutants of both ERK5 and its upstream regulator MEK5 inhibit Raf-dependent cellular transformation.

EXPERIMENTAL PROCEDURES

Mammalian Cell Culture and Transfection—293 cells were cultured, transfected, and harvested as described previously (1). Focus formation assays in NIH 3T3 cells were performed as described previously (8). Expression of HA-ERK5kin, Raf-1, and Ras and its corresponding mutants was monitored by immunoblotting with antibodies against the HA epitope or the appropriate proteins (HA, 12CA5, BABCO, Raf-1, SC-133, and GST, SC-138, Santa Cruz Biotechnology, Ras, OP40, and ERK5, CalBiochem).

Immunoprecipitation of ERK5—Immunoprecipitations were performed as described (1). Lysates containing equal amounts of ERK5kin, as assessed by immunoblotting with the anti-HA antibody, were used for immunoprecipitation. Kinase assays were performed with 20 μl of beads in a 50-μl reaction with final concentrations of 10 mM Hepes, pH 8.0, 10 mM MgCl2, 1 mM benzamidine, 50 μM ATP and [γ-32P]ATP (10–30 cpm/nmol) for 30 min at 30 °C, and 0.1 mg/ml GST-Myc (amino acids 1–103). To detect co-immunoprecipitating proteins, immunoprecipitates of transfected or endogenous ERK5 were resolved on gels and then blotted for transfected or endogenous Raf-1 as described above. Endogenous ERK5 was immunoprecipitated using a rabbit polyclonal antibody raised against the first 14 amino acids of ERK5 (CalBiochem 44266E) from lysates containing equal amounts of ERK5. Endogenous Raf-1 was detected in immunoprecipitates using a mouse monoclonal antibody (Transduction Laboratories, R19120).

DNA Constructs—cDNAs encoding ERK5 were obtained from J. D. Lee (4) and Jack Dixon (5) and used in initial studies. Subsequent studies utilized a human ERK5 cDNA generated using clone HIBBR16 (ATCC). HIBBR16 is missing the first 136 amino acids of ERK5. Kidney first-strand cDNA was used as a template in PCR to generate a 640-base pair clone corresponding to the amino terminus of ERK5. This PCR clone was cut with Nhel (site added by PCR) and AflIII (internal site), ligated to the EST clone, and sequenced to confirm that no mutations were introduced in the generation of this full-length ERK5 clone. Constructs were ERK5kin and ERK5kin(κ106M) in pCMV5HAB (6) and Myc-ERK5KKM, Myc-MEK5DD (S222D, T226D), and Myc-MEK5KKM (K106M) in pCMV5. Construction of MEK5DD, MEK5KM, and ERK5KM. Lys106 of MEK5 and Lys83 of ERK5 were mutated to Met by PCR with Vent polymerase and oligomers that spanned these amino acids.

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§ The abbreviations used are: MAP, mitogen-activated protein; ERK, extracellular signal-related kinase; MEK, mitogen-activated protein kinase/extracellular signal-related kinase; GST, glutathione S-transferase; PCR, polymerase chain reaction; PI 3-kinase, phosphatidylinositol 3-kinase.
Acids. The constructs were sequenced to ensure no additional mutations were incorporated. MEK5DD was subcloned from pGEX-KG using sites added in the construction of the original pGEX-KG vector (7). Wild-type Raf-1, Raf-1 S259A, and Raf-1 BXB in pCMV5 were kindly provided by Jeff Frost (University of Texas Southwestern). G12VRas effector domain mutants were transiently expressed in 293 cells along with HA-ERK5kin. The expression of Raf-1 BXB (amino acids 329–648) in pCDNAIII were transcribed/translated for 45 min in vitro with T7 DNA polymerase and rabbit reticulocyte lysates according to the manufacturer's protocols (Promega). Equal amounts of recombinant kinase proteins were used in the assays based on Comassie staining. Recombinant GST-tagged MAP kinase or GST alone was incubated with 25 μl of GST-agarose for 2 h at 4 °C with rotation in 0.5 ml of buffer C (20 mM Hepes, pH 7.6, 0.1 mM KCl, 2 mM EDTA, 20% glycerol, 1% Triton X-100, 1 mM dithiothreitol, and TM protease inhibitor mixture (Roche Molecular Biochemicals)). After incubation the beads were washed three times with buffer C. After the final wash, the residual buffer was removed, and 10 μl of 10 mM Hepes and 20 μl of 2× sample buffer were added prior to electrophoresis. Raf-1 was detected by immunoblotting. GST was detected by immunoblotting of the stripped blots to determine the amounts of GST-MAP kinases isolated by immunoblotting. GST was detected by immunoblotting of the sample buffer were added prior to electrophoresis. Raf-1 was detected on the beads.

**RESULTS**

Raf-1 Participates in but Is Not Sufficient for Activation of ERK5 Downstream of Ras—Ras has multiple effectors that are required for the full expression of its activity (3, 9–14). Previously we demonstrated that a constitutively active form of Raf activates the catalytic domain of ERK5 (ERK5kin) (1). However, unlike the ERK1,2 MAP kinase module, the constitutively active mutants of Raf-1, Raf BXB and Raf-1 S259A, do not activate ERK5. The capacity of distinct Ras effector pathways to activate ERK5kin was assessed with the activated Ras effector domain mutants G12V/T35S, G12V/Y40C, and G12V/E37G. These Ras effector domain mutants have been used previously to distinguish among effector pathways activated by Ras (3). The Ras effector domain mutants were transiently transfected into 293 cells along with HA-ERK5kin. The expression levels of ERK5kin, Ras, and Raf-1 were measured by immunoblotting with antisera specific to each protein (Fig. 1B). Expression of Ras mutants was approximately equal with the exception of G12V/T35S, which was expressed consistently less well (Fig. 1B and data not shown). The activity of HA-ERK5kin was measured in immune complexes using GST-Myc as the substrate. As shown in Fig. 1, A and C, for G12V/Y40C, none of the effector domain mutants activated ERK5kin as well as G12VRas did (Fig. 1, A and C, and data not shown). These data also confirm previous results showing that neither wild type nor constitutively active mutants of Raf-1 activate ERK5kin (Fig. 1C, and data not shown) (1).

Co-expression of Ras effector mutants with downstream effectors can enhance the signaling output of the effector mutants if they provide the necessary complementing function (11). We thus tested whether co-expression of known downstream effectors would restore ERK5kin activation by the Ras effector domain mutants. To our surprise, expression of either wild type or a constitutively active form of Raf-1, Raf-1 S259A, restored the capacity of all three of the Ras effector domain mutants to activate ERK5kin to the same extent as G12VRas did (Fig. 1, A and C, and data not shown). In marked contrast, Raf-1 BXB, which lacks the amino-terminal regulatory domain, was unable to restore activation of ERK5kin (Fig. 1, A and C, and data not shown). Despite significant activation of ERK1,2 (not shown) (1). Thus, the capacity of Raf-1 to restore ERK5kin activation by Ras is distinct from its ability to activate ERK1,2. This is consistent with our previously reported finding that Raf-1 is neither a MEK5 kinase (7) nor does it activate ERK5kin (Fig. 1C) (1).

We also tested whether other candidate Ras effectors, Ral guanine nucleotide dissociation stimulator, PI 3-kinase, and protein kinase Cζ, activate ERK5kin alone or when co-expressed with the Ras effector domain mutants. None of them restored activation of ERK5kin by the Ras effector domain mutants (not shown). ERK5kin was also not activated by co-expression of Raf-1 with protein kinase Cζ, Ral guanine nucleotide dissociation stimulator, or PI 3-kinase in the absence of an activated isoform of Ras (data not shown). These results suggest that none of the candidate Ras effectors tested other than Raf-1 plays a major role in activation of ERK5kin.

**ERK5 Binds to Raf-1**—Because it appears that full-length Raf-1 is required, but its catalytic activity is not sufficient, to restore activation of ERK5kin, we tested the ability of Raf-1 to interact with ERK5. HA-ERK5 or HA-ERK5kin were immunoprecipitated from cells co-transfected with Raf-1. The immunoprecipitates were immunoblotted with antibodies to Raf-1 (Fig. 2). All forms of ERK5 co-immunoprecipitated with Raf-1, Raf-1 S259A, and Raf-1 BXB (Fig. 2). More Raf was consistently detected in ERK5 immunoprecipitates from cells expressing constitutively active Raf-1 (Fig. 2).

The interaction of Raf-1 and ERK5 was further examined by determining if recombinant GST-ERK5kin bound to in vitro translated Raf-1 BXB. Immunoblotting of proteins on glutathione-agarose beads following extensive washing demonstrated that Raf-1 BXB was detected only when GST-ERK5kin was present (Fig. 3A). To examine the specificity of the interaction of ERK5kin with Raf-1 BXB, we compared the capacity of other MAP kinase family members, GST-ERK2 and GST-SAPK, to interact with Raf-1. Strikingly, neither of these kinases interacted with Raf-1 BXB (Fig. 3A). The specificity of the Raf-1-ERK5 interaction is further demonstrated upon examining im-
munoblots of proteins with anti-GST, which showed that significantly less GST-ERK5kin was bound to the beads than the other MAP kinases (Fig. 3A). Kinase-dead ERK5 also bound Raf-1 but less well than ERK5kin (Fig. 3B). In addition, kinase-dead Raf-1 BXB bound less well than Raf-1 BXB (Fig. 3B). These data support the idea that the activity states of these kinase influence their association.

Finally, we examined the interaction of endogenous ERK5 and Raf-1 by co-immunoprecipitation from cells. No Raf-1 was detected in immunoprecipitates of ERK5 from unstimulated cells (Fig. 4, Vector). We have previously characterized mutations in the ERK5 upstream activator MEK5 that result in constitutively active or kinase dead proteins (7). Expression of constitutively active MEK5, MEK5DD, activates ERK5 (data not shown). In the presence of MEK5DD, Raf-1 was easily detected bound to ERK5 (Fig. 4, MEK5K-M (HA)). On the other hand, kinase-dead MEK5 (KM) does not promote their association (Fig. 4, MEK5K-M (HA)). These data are consistent with the observation that ERK5 KM bound less well to Raf than did wild type ERK5, suggesting that the interaction may be activity dependent. Thus, under certain conditions endogenous ERK5 and Raf-1 are associated in cells.

The ERK5 Pathway Modulates Raf-induced Cellular Transformation—Our findings suggest that Raf-1 participates in the activation of ERK5 by a novel mechanism and, by implication, that ERK5 plays a role in Raf-1 signaling. To test the importance of the MEK5/ERK5 pathway in Raf-1 signaling, we examined the consequences of expression of MEK5DD or MEK5KM on the induction of cellular transformation by oncogenic Raf-1 (Raf-1 BXB). We first chose to examine effects of MEK5 mutants, because constitutively active mutants of MAP kinase family members have not been generated. Co-expression of MEK5DD with Raf-1 BXB caused a 3-fold increase in focus formation when compared with cells expressing Raf-1 BXB alone (Fig. 5A). MEK5DD did not induce foci of transformed cells when expressed alone. Thus, MEK5DD synergizes with Raf-1 BXB to transform NIH 3T3 cells. In contrast, co-expression of MEK5KM with Raf-1 BXB resulted in a significant reduction in the number of foci formed compared with fibroblasts expressing Raf-1 BXB alone (Fig. 5A). Consistent with these findings, ERK5KM also inhibited transformation by

FIG. 2. ERK5 binds to Raf-1. A and B, cells were co-transfected with either Raf-1, S259A Raf-1 (Raf-259), or Raf-1 BXB (Raf BXB) with empty vector or ERK5kin. Immunoprecipitations (IP) were performed using the anti-HA antibody. Immunoprecipitations were then immunoblotted using an anti-Raf-1 antibody. Lysates expressing equal amounts of ERK5kin were used for immunoprecipitation as assessed by immunoblotting with the anti-HA antibody.

FIG. 3. In vitro binding of ERK5 to Raf-1. A and B, Raf-1 BXB (BXB) or Raf-1 BXB(KM) (BXB (K-M)), translated using rabbit reticulocyte lysates, were incubated with glutathione-beads prebound to GST-ERK5kin, GST-ERK5kinKM, GST-ERK2, or GST-SAPK. The washed beads were subjected to immunoblot analysis using anti-Raf-1 antibody (top) or the same blots stripped and reprobed with anti-GST antibody (bottom).

FIG. 4. Co-immunoprecipitation of endogenous Raf-1 and ERK5. Top, cells were transfected with empty vector, MEK5DD, or MEK5KM. Immunoprecipitations (IP) were performed on lysates containing equivalent amounts of Raf-1 and ERK5 or lysis buffer control using anti-ERK5 antibody. Immunoprecipitations were then immunoblotted using anti-Raf-1 antibody. Bottom, expression of MEK5DD and MEK5KM in lysates as assessed by immunoblotting with anti-HA antibody.

MEK5DD on the induction of cellular transformation by oncogenic Raf-1 (Raf-1 BXB). We first chose to examine effects of MEK5 mutants, because constitutively active mutants of MAP kinase family members have not been generated. Co-expression of MEK5DD with Raf-1 BXB caused a 3-fold increase in focus formation when compared with cells expressing Raf-1 BXB alone (Fig. 5A). MEK5DD did not induce foci of transformed cells when expressed alone. Thus, MEK5DD synergizes with Raf-1 BXB to transform NIH 3T3 cells. In contrast, co-expression of MEK5KM with Raf-1 BXB resulted in a significant reduction in the number of foci formed compared with fibroblasts expressing Raf-1 BXB alone (Fig. 5A). Consistent with these findings, ERK5KM also inhibited transformation by
DISCUSSION

We previously demonstrated that oncogenic Ras activates the catalytic domain of ERK5 (ERK5kin) (1). In an attempt to delineate the molecular players downstream of Ras we uncovered a role of Raf-1 in the activation of ERK5. Ras mutants with a partial loss of function were incapable of activating ERK5kin. However, the addition of Raf-1 to these mutants restored the activation by Ras. The ability of Raf-1 to restore activation did not correlate with Raf-1 catalytic activity, suggesting a novel mechanism of action of Raf-1 in activation of ERK5kin. In addition, Raf-1 is apparently not a MEK5 kinase (7). Together these findings imply that the ERK5 pathway is distinct from the ERK1/2 pathways in its regulation downstream of Ras and Raf-1.

Further evidence for a role for Raf-1 in the regulation of ERK5 was the finding that Raf-1 and ERK5 interact. Raf-1 co-immunoprecipitated with HA-ERK5 from 293 cells and bound to GST-ERK5 in vitro. More significantly, in the presence of MEK5DD, Raf-1 and ERK5 endogenous to 293 cells co-immunoprecipitated. The specificity of the interaction is supported by the lack of detectable binding of Raf-1 to ERK2 or SAPK. This specificity is consistent with the work of Wang et al. (15) who saw no Raf-1 binding to three other MAP kinase family members. Thus, ERK5 is a novel Raf-1 partner that may participate in mediating Raf-1 responses in cells.

We have been unable to show that Raf-1 is sufficient to activate ERK5 or MEK5 under conditions in which MEK1 and ERK2 are activated (1, 7). Thus, our previous work in combination with the experiments described above suggests that although Raf-1 is not likely to be a MEK5 kinase, it plays a role in the activation of this MAP kinase module. Perhaps the function of Raf-1 is to cause the formation of functional ERK5 complexes. The proper formation of multi-protein complexes is an essential part of the regulation of numerous signaling pathways (14, 16–19). The importance of protein associations in the actions of serine/threonine kinases has been amply demonstrated by the necessity of proteins such as Ste5p, a scaffold for the MAP kinase cascade in the yeast pheromone response pathway, for the functioning of the cascades that they support.

The effector pathway(s) mediating Ras regulation of ERK5 remains to be determined. Our results suggest that Raf-1 participates in ERK5 activation together with a novel Ras-dependent signaling event. The Ras effector mutants used in this study discriminate among PI 3-kinase, Ral guanine nucleotide dissociation stimulator, and Raf-1 family members. However, there are likely to be other uncharacterized Ras partners that will associate with one or more of these mutants. The observation that all three mutants cooperated with Raf-1 to activate ERK5 suggests that an uncharacterized Ras partner(s) may be involved. Consistent with this perception, we found that overexpression of Ral guanine nucleotide dissociation stimulator or expression of activated variants of PI 3-kinase failed to cooperate with Raf-1 to activate ERK5 (not shown). A similar genetic argument for the presence of a novel Ras effector pathway was made using a myoblast differentiation model system (11).

The ability of Raf-1 to enhance activation of ERK5 by Ras effector domain mutants suggests that the MEK5/ERK5 cascade is involved in Raf-1 signaling. Using active and inhibitory forms of MEK5 and kinase-dead ERK5, we observed a functional interaction of the MEK5/ERK5 pathway in Raf-dependent transformation of fibroblasts. To our knowledge, this is the first demonstration of a role for the MEK5/ERK5 pathway in cellular transformation. Previously, we and others demonstrated that MEK5 does not activate ERK2 (5, 7). Furthermore, MEK5DD significantly activates ERK5kin in cells but does not

Raf-1 BXB (Fig. 5C). Thus, blockade of the MEK5/ERK5 pathway interferes with the ability of Raf-1 BXB to transform fibroblasts.

FIG. 5. Modulation of Raf transformation by the MEK5/ERK5 pathway. A, NIH 3T3 cells were transfected with the indicated constructs and plated in medium plus 5% calf serum. 14 days post-transfection, plates were scored for the appearance of foci of morphologically and growth-transformed cells. Quantitations shown are from three independent experiments. Error bars represent the standard deviation from the mean. B, cells from the above transfections were also plated in medium containing G418 to select for transfected populations. Lysates from the stably transfected cells were analyzed for Raf-1 BXB (rafBXB) expression. A representative immunoblot is shown: lane 1, Raf-1 BXB alone; lane 2, Raf-1 BXB plus MEK5KM; lane 3, Raf-1 BXB plus MEK5DD; lane 4, MEK5DD alone. C, experiments were performed as described in A. Results are representative of one of two independent experiments.
activate ERK2. Thus, the synergy of MEK5DD and Raf-1 in cell transformation is not because of enhanced ERK2 activation by MEK5DD either directly or through autocrine pathways. Therefore, the MEK5/ERK5 pathway is required for Raf-1 transformation of fibroblasts via a mechanism independent of the best characterized Raf-1 effector pathway.

In conclusion, we demonstrate that although Raf-1 does not appear to be a direct activator of the MEK5/ERK5 pathway, there is an intimate relationship between Raf-1 and the MEK5/ERK5 MAP kinase module. Our future work will be directed toward determining the mechanism of regulation of this pathway and investigating the importance of Raf-1-ERK5 binding for their functions.

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REFERENCES
1. English, J. M., Pearson, G., Baer, R., and Cobb, M. H. (1998) J. Biol. Chem. 273, 3854–3860
2. Joneson, T., White, M. A., Wigler, M. H., and Barsagi, D. (1996) Science 271, 810–812
3. White, M. A., Nicolette, C., Minden, A., Polverino, A., Van Aelst, L., Karin, M., and Wigler, M. H. (1995) Cell 80, 533–541
4. Kato, Y., Kravchenko, V. V., Tapping, R. I., Han, J., Ulevitch, R. J., and Lee, J. D. (1997) EMBO J. 16, 7054–7066
5. Zhou, G., Bao, Z. Q., and Dixon, J. E. (1995) J. Biol. Chem. 270, 12665–12669
6. Xu, S., Bobbins, D. J., Christerson, L. B., English, J. M., Vanderbilt, C. A., and Cobb, M. H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5291–5295
7. English, J. M., Vanderbilt, C. A., Xu, S., Marcus, S., and Cobb, M. H. (1995) J. Biol. Chem. 270, 28897–28902
8. Mineo, C., Anderson, R. W., and White, M. A. (1997) J. Biol. Chem. 272, 10345–10348
9. Khorrami-Far, R., Selski, P. A., Clark, G. J., Kinch, M. S., and Der, C. J. (1995) Mol. Cell. Biol. 15, 643–6453
10. Marshall, C. J. (1996) Curr. Opin. Cell Biol. 8, 197–204
11. Ramocki, M. B., Johnson, S. E., White, M. A., Ashendel, C. L., Konieczny, S. F., and Taparowsky, E. J. (1997) Mol. Cell. Biol. 17, 3547–3555
12. Qiu, R.-G., Chen, J., Kirn, D., McCormick, F., and Symons, M. (1995) Nature 374, 457–459
13. Zwartkruis, F., Woltius, R. M., Nabben, N. M., Franke, B., and Bos, J. L. (1998) EMBO J. 17, 5905–5912
14. Therrien, M., Michaud, N. R., Rubin, G. M., and Morrison, D. K. (1996) Genes & Dev. 10, 2684–2695
15. Wang, S., Ghosh, R. N., and Chellappan, S. P. (1998) Mol. Cell. Biol. 18, 7487–7498
16. Choi, K.-Y., Satterberg, B., Lyons, D. M., and Elion, E. A. (1994) Cell 78, 499–512
17. Marcus, S., Polverino, A., Barr, M., and Wigler, M. (1991) Proc. Natl. Acad. Sci. U. S. A. 91, 7762–7766
18. Whitmarsh, A. J., Cavanagh, J., Tourneur, C., Yasuda, J., and Davis, R. J. (1998) Science 281, 1671–1674
19. Pawson, T., and Scott, J. D. (1997) Science 278, 2075–2080

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