Analysis of the Interaction between c-Jun and c-Jun N-terminal Kinase in Vivo*

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Regulation of c-Jun transcriptional activity is believed to depend on a physical interaction with c-Jun N-terminal kinase (JNK) that facilitates signal-regulated phosphorylation of multiple regulatory phosphoacceptor sites within the activation domain. Here we have investigated the structural requirements and consequences of regulatory phosphorylation for the interaction between c-Jun and JNK in vivo. We show that binding of JNK to c-Jun in vivo does not require JNK catalytic activity or the presence of the potential phosphoacceptor sites within c-Jun and that JNK retains the capacity to bind to a pseudo-phosphorylated mutant of c-Jun where these sites are replaced by phospho-mimetic aspartic acid residues. The c-Jun delta region docking site is essential for interaction with JNK in vivo but is not sufficient, because a c-Jun mutant that retains this region but that lacks the C-terminal DNA-binding domain fails to interact. Experiments using purified recombinant c-Jun and JNK proteins show that the c-Jun DNA-binding domain harbors an auxiliary interaction domain that has the potential to bind to JNK independently. Our results suggest that JNK can be tethered passively to c-Jun in situ through multiple interacting regions and, when activated, can stimulate c-Jun phosphorylation without necessarily dissociating from its substrate. Auxiliary interactions mediated by the DNA-binding domain could play a role in targeting JNK preferentially to c-Jun in specific homo- or heterodimeric complexes.

The c-Jun N-terminal kinase (JNK)1 or stress-activated protein kinase pathway is one of several mitogen-activated protein kinase (MAPK) pathways or modules that have been identified in vertebrate cells (1–3). Although the overall architecture of MAPK modules is conserved, they are functionally distinct and activated by signals that elicit different cellular responses, ranging from cell proliferation to apoptosis (4, 5). It is generally assumed that specific cellular responses are achieved by coupling individual MAPK pathways to distinct effector molecules, although how substrate targeting is determined is poorly understood.

The JNK pathway is stimulated by a wide range of cellular stresses such as UV and ionizing radiation, metabolic inhibitors, inflammatory cytokines, and chemotherapeutic drugs (2, 3, 5). The c-Jun proto-oncoprotein, the prototypic member of the AP-1 family of vertebrate transcription factors (6, 7), is an important effector of the JNK pathway. In particular, regulation of c-Jun by JNK modulates stress-induced apoptosis in certain cell types (8, 9) and may also play a role in triggering cell cycle arrest and inducing DNA repair processes in response to genotoxic damage (10, 11).

c-Jun contains a C-terminal basic region-leucine zipper (bZIP) DNA-binding domain and an N-terminal transactivation domain (7), and its activity can be regulated at a number of levels, notably via heterodimerization with Fos or other bZIP partner proteins and through phosphorylation of clusters of serine/threonine residues in the C or N terminus that either inhibit DNA binding or enhance transcriptional activity, respectively (reviewed in Refs. 12 and 13). Two major serine phosphoacceptor sites have been mapped within the c-Jun transactivation domain at positions 63 and 73 (Ser63–Ser73) (14–17). Additionally, two threonine residues at positions 91 and 93 (Thr91–Thr93) are thought to be modified by JNK under certain circumstances (17–20), but regulation of their phosphorylation is less well characterized.

Phosphorylation of N-terminal c-Jun phosphoacceptor sites by JNK probably stimulates c-Jun transcriptional activity by facilitating recruitment of the p300/CBP coactivator (13). JNK binds to c-Jun, and it seems likely that this is a primary determinant of signal transduction specificity, because regulatory phosphorylation is thought to depend on this interaction (21, 22). Binding of JNK depends on a small region in the N-terminal half of c-Jun (the delta region) (18, 21, 23). This region is thought to act as a docking site for JNK, which, although physically distinct from the phosphoacceptor sites, exerts a major influence on the efficiency with which these residues in c-Jun are phosphorylated in vitro, most probably by increasing the effective local concentration of kinase (21, 24).

Thus, a recent model proposes that association of JNK with the delta region facilitates subsequent translocation of the adjacent phosphoacceptor region into the kinase catalytic cleft, leading to catalysis and subsequent dissociation of the kinase from its substrate (24).

Despite its potential functional importance, the physical ba-
sis of the interaction between c-Jun and JNK is relatively poorly understood, because the kinase docking site is currently defined solely by a natural deletion found in the oncogenic derivative, v-Jun (6, 25), and the extent and amino acid sequence of the interaction domain per se has not been defined. Furthermore, it remains unclear whether binding of JNK to c-Jun is modulated by regulatory phosphorylation and whether it has functional significance over and above facilitating catalysis.

To gain further insight into these issues, we used a yeast two-hybrid system to analyze the structural requirements for and influence of JNK catalytic activity and substrate phosphorylation on the interaction between c-Jun and JNK in vivo. The results of this analysis suggest that JNK associates with c-Jun through multiple contacts in vivo and, after activation, can phosphorylate its substrate without necessarily dissociating from the complex.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions—**Routine manipulations of nucleic acids were performed as described (26). Vectors used to construct in-frame fusion proteins with the DNA-binding domain (BD) or the activation domain (AD) of yeast GAL4 were pGBT9 and pGAD424, respectively (CLONTECH) (27).

Plasmids encoding BD-JNK, BD-c-Jun, BD-c-Jun4ala, AD-c-Jun, and AD-v-Jun have been described (22). A catalytically inactive JNK derivative containing alanine in place of lysine at amino acid 55 in the ATP-binding site (JNK K→A) (28) was generated by PCR and cloned into pGBT9. Fragments encoding the basic region-leucine zipper domains of Fra-2 (amino acids 107–200) or c-Jun (amino acids 186–314) were generated by PCR and cloned into pGBT9.

c-Jun deletion variants were created by PCR and cloned into pGAD424 to generate AD-c-Jun 1–280, AD-c-Jun 1–256, AD-c-Jun 1–182, AD-c-Jun 31–59, AD-c-Jun 186–314, and AD-c-Jun Δ 183–256. A c-Jun mutant containing aspartic acid residues in place of Ser63–Ser73 and Thr91–Thr93 (c-Jun4asp) was created by site-directed mutagenesis using the QuickChange™ system (Stratagene). Subsequently the N-terminal halves of AD-c-Jun in pGAD424 and BD-c-Jun in pGBT9 were replaced with corresponding fragments from c-jun4ala or c-jun4asp to generate AD-c-Jun4ala, AD-c-Jun4asp, and BD-c-Jun4asp, respectively. The authenticity of all PCR-generated coding regions and, where appropriate, the presence of desired mutations were verified by sequencing. The sequences of the PCR primers used for these constructions are available on request. Throughout this manuscript the numbering of c-Jun amino acids is based on the sequence of avian c-Jun.

** Yeast Strain and Methods—**Yeast strain SFY526 has been described previously (27). Yeast cultures were grown and transformed using the lithium acetate method as described (29), except that 5 μl of plasmid DNA was used. β-galactosidase activity in liquid yeast cultures was quantified as described (29).

**Recombinant Protein Purification, Gel Retardation Analysis, and GST Pull-down Experiments—**c-Jun, Δ N c-Jun (amino acids 186–314), and bZIP Fos proteins were expressed and purified from Escherichia coli as described previously (30). A DNA fragment encoding rat stress-activated protein kinase c1 (corresponding to human JNK2) was generated by PCR and cloned into pGEX4T-1. GST, GST-JNK, GST-c-Jun 1–79 (a kind gift of I. Morgan), and GST-Erk (pGEX-WT-Erk2; a kind gift of C. Marshall) were expressed and purified from E. coli using conventional procedures (31). DNA binding reactions were performed by incubating purified Jun proteins (100 ng) with 1 ng of 3P-labeled collagenase TRE oligonucleotide probe (colTRE) (32) for 30 min at 4 °C as described previously (30). Where indicated the DNA binding reactions were supplemented with purified GST, GST-JNK, or GST-Erk (200 ng), or 3 μl of polyclonal anti-Jun (730) antiserum and incubated for 15 min at room temperature prior to addition of the colTRE probe. To generate Jun-bZIP Fos heterodimer complexes, binding reactions were supplemented with purified bZIP Fos (200 ng) prior to addition of probe and/or GST proteins.

For GST pull-down experiments, 4 μg of GST-c-Jun 1–79 protein was bound to 40 μl of glutathione beads and incubated with 1000 μg of cell extract prepared from avian fibroblasts treated with 10 mg/ml anisomycin (Sigma) for 15 min to activate JNK (33). After extensive washing, the reaction was split in half, and in vitro kinase assays in the presence or absence of 50 μM ATP were performed (21). Subsequently, each reaction mixture was separated into bead-bound and soluble fractions and analyzed by SDS gel electrophoresis. JNK, GST, and c-Jun proteins were visualized by Western blotting using antibodies specific for the phosphorylated, active form of JNK (New England Biolabs), a polyclonal serum raised against an unrelated GST fusion protein, or an antibody specific for phosphorylated c-Jun (KM1, Santa Cruz Biotechnology), respectively.

**RESULTS**

**Physical Interaction between JNK and c-Jun in Vivo Is Independent of Kinase Catalytic Activity or Phosphorylation of the Regulatory Phosphoacceptor Sites Ser63–Ser73 and Thr91–Thr93—**To determine whether phosphorylation of Ser63–Ser73 and Thr91–Thr93 in c-Jun modulates binding of JNK in vivo, we first evaluated the effect of JNK catalytic activity on the interaction between the proteins using a yeast two-hybrid approach (22). JNK can be active in yeast because it can both complement a null mutation of the yeast Hog1 gene, which encodes a MAPK homologue required for growth in high osmolarity medium (34), and stimulate c-Jun transcriptional activity via phosphorylation (22). Accordingly, we expressed hybrid proteins containing the DNA-binding domain of GAL4 and JNK3 (BD-JNK) or a mutant JNK lacking catalytic activity (BD-JNK K→A; see “Experimental Procedures” for details), together with GAL4 activation domain-tagged c-Jun (AD-c-Jun) (22). As shown in Fig. 1a, AD-c-Jun interacted equally well with both BD-JNK and BD-JNK K→A, demonstrating that JNK catalytic activity is dispensable for binding to c-Jun in vivo. Furthermore, a mutant derivative of c-Jun in which the Ser63–Ser73 and Thr91–Thr93 phosphoacceptor sites were replaced with nonphosphorylatable alanine residues (AD-c-Jun4ala) (22) interacted with BD-JNK at least as strongly as wild-type AD-c-Jun (Fig. 1b). Thus, neither JNK catalytic activity nor the presence of the JNK target sites in the c-Jun activation domain are required for binding of the kinase to its substrate in vivo.

**Amino Acid Substitutions That Mimic Activatory Phosphorylation of Ser63–Ser73 and Thr91–Thr93 Do Not Prevent the c-Jun-JNK Interaction in Vivo—**Active JNK that has been captured from cell extracts by an affinity matrix consisting of an N-terminal fragment of c-Jun has been reported to be released when Ser63–Ser73 (and to a lesser extent Thr91–Thr93) become phosphorylated (21), suggesting that catalysis leads to dissociation of the kinase from its substrate in vitro. To determine whether modification of Ser63–Ser73 and Thr91–Thr93 would prevent or weaken the interaction with JNK in vivo, we constructed a mutant in which all four of these residues were replaced with negatively charged aspartic acid residues to mimic phosphorylation (c-Jun4asp).

Mutants containing aspartic acid in place of Ser63–Ser73 and Thr91–Thr93 have been shown to acquire many of the physical and functional characteristics that result from authentic phosphorylation of these sites (19, 35). To verify that this was the case in yeast, we fused c-Jun4asp to the DNA-binding domain of GAL4 and compared transactivation by the resulting protein with BD-c-Jun and BD-c-Jun4ala (Fig. 1c). As expected, the aspartic acid substitutions resulted in a marked increase (approximately 6–7-fold) in transactivation activity, indicating that these mutations mimic at least some attributes of phosphorylated c-Jun in yeast.

We subsequently fused c-Jun4asp to the GAL4 activation domain and found that this mutant retained the capacity to interact with BD-JNK (Fig. 1b), indicating that phospho-mimetic mutations do not prevent binding of JNK in vivo. The aspartic acid mutations did not enhance transactivation in the two-hybrid assay, because in this case activation is primarily attributable to the heterologous GAL4 activation domain (22). To resolve the apparent discrepancy between the effects of regulatory phosphorylation or phospho-mimetic amino acid substitutions on binding of JNK to c-Jun in vitro (21) and in...
vivo (Fig. 1, b and c), we decided to analyze JNK-c-Jun interaction in vitro. Chicken embryo fibroblasts were treated with anisomycin to activate JNK (33), and the activated kinase was captured from cell extracts using an affinity matrix consisting of the N-terminal 79 amino acids of c-Jun fused to glutathione S-transferase and immobilized on glutathione beads (GST-Jun). The mixture was split in half, and kinase reactions were performed in the presence and absence of ATP as indicated. The samples were separated into bead-bound (lanes P) and free fractions (lanes S) and analyzed by Western blotting using antibodies specific for active, phosphorylated JNK (anti-P-JNK, upper panel), GST (anti-GST, middle panel), or phosphorylated c-Jun (KM-1, lower panel). Lane C contains GST-Jun that had not been incubated with cell extract. The positions of JNK1 and 2, GST-Jun and phosphorylated GST-Jun (GST-P-Jun) are marked.

JNK can remain bound to phosphorylated c-Jun.

Structural Requirements for c-Jun-JNK Interaction in Vivo—It is well established that binding of JNK to c-Jun in vitro depends on a short peptide termed the delta domain that is deleted from the v-Jun oncoprotein and that is thought to constitute a specific docking site for the kinase. This is also true in vivo because unlike AD-c-Jun, AD-v-Jun did not interact with BD-JNK (Fig. 3b) (22), even though both interacted equally with the leucine zipper domain of Fra-2 (Fig. 3c).

To determine whether the delta docking site is sufficient for interaction in vivo as it is in vitro, we tested interaction between BD-JNK and an N-terminal segment of c-Jun spanning amino acids 1–182 (AD-c-Jun 1–182). This mutant contains the delta domain and would therefore be expected to interact with JNK in vitro. Unexpectedly, however, AD-c-Jun 1–182 did not interact measurably with BD-JNK (Fig. 3b). Western blotting experiments indicated that this was not due to lack of expression of AD-c-Jun 1–182 (data not shown). Thus, although the delta region is necessary for interaction with JNK in yeast it is not sufficient, because sequences within the C-terminal half of c-Jun are also required for JNK binding in this context.

The segment of c-Jun deleted from AD-c-Jun 1–182 provides both DNA binding and dimerization functions. It has been proposed on the basis of in vitro experiments (24) that dimerization stabilizes the interaction between JNK and c-Jun in vitro. To determine whether defective dimerization explained the failure of AD-c-Jun 1–182 to interact with JNK, additional c-Jun mutants with shorter C-terminal deletions that removed sequences within the C-terminal half of c-Jun were constructed (see Fig. 3a). Both of the resulting hybrid proteins retained a proline- and glutamine-rich (P-Q) domain and were capable of interacting with BD-JNK, although their apparent affinity for JNK was markedly decreased (approximately 8–10-fold) in comparison with full-length AD-c-Jun (Fig. 3b).

To confirm that these C-terminally deleted c-Jun proteins exhibited the predicted dimerization properties, we examined their capacity to interact with the leucine zipper domain of Fra-2 fused to the GAL4 DNA-binding domain (BD-FraZip, Fig. 3c). Both AD- and v-Jun interacted equally well with BD-FraZip; however, none of the C-terminally truncated c-Jun mutants
interacted significantly, confirming that these proteins were defective for heterodimerization in vivo. Similar results were obtained when homodimerization was tested using the basic region-leucine zipper domain of c-Jun (amino acids 186–314) as a "bait" protein (BD-JunZip; data not shown).

Thus, these two-hybrid experiments indicated that a region within the c-Jun C terminus between amino acids 182 and 256 was absolutely required for the interaction of c-Jun monomers with JNK in vivo. To test whether this region also contributed to the affinity of JNK binding in the context of c-Jun dimers, a c-Jun mutant carrying an internal deletion that removed amino acids 183–256 formed heterodimers equally as or better than AD-c-Jun and AD-v-Jun with BD-FraZip and BD-JunZip (Fig. 4, b and c). However, its interaction with BD-JNK was severely compromised (Fig. 4a), confirming that the deleted region is required for optimal binding of JNK in vivo.

**JNK Interacts with the c-Jun C Terminus in Vitro**—The region between c-Jun amino acids 182 and 256 contains a P-Q domain and parts of the basic region DNA-binding domain. We considered the possibility that this region might make additional direct contacts with JNK in vivo; however, as with AD-c-Jun 1–182, c-Jun amino acids 186–314 did not interact detectably with BD-JNK (data not shown). As an alternative approach to this question, we therefore used purified recombinant proteins expressed in E. coli to determine whether the c-Jun C terminus alone could interact with JNK in vitro.

Purified c-Jun protein forms a specific homodimer complex with an oligonucleotide containing the consensus TRE from the collagenase gene promoter (colTRE) (32) in a gel retardation assay (Fig. 5a, arrow). When purified GST-JNK was added to this binding reaction a more slowly migrating complex formed (Fig. 5a, asterisk) that was not observed on addition of either GST alone or an analogous GST-ERK fusion protein. Control experiments demonstrated that GST-JNK did not bind to DNA alone in the absence of c-Jun.

Several observations indicated that this more slowly migrating species represented a ternary complex of c-Jun and GST-JNK bound to DNA. Firstly, anti-c-Jun antibodies disrupted both the homodimer and slower mobility complexes, demonstrating that c-Jun was a component of both (Fig. 5b). Secondly, glutathione beads adsorbed with GST-JNK could deplete c-Jun-DNA complexes from solution, whereas incubation with beads alone or GST-adsorbed beads were without effect (Fig. 5e).

To determine whether the c-Jun C terminus could interact directly with JNK in this context, we expressed a C-terminal fragment of c-Jun spanning amino acids 186–314 (ΔN c-Jun) in E. coli and analyzed its interaction with GST-JNK. ΔN c-Jun contains the complete c-Jun bZIP DNA-binding domain but lacks the N-terminal delta and transactivation domains. ΔN c-Jun bound the colTRE oligonucleotide as a distinct homodimer of higher electrophoretic mobility than full-length

**Fig. 4. Deletion of the proline- and glutamine-rich domain severely impairs c-Jun-JNK interaction.** The GAL4 activation domain was fused to full-length c-Jun, v-Jun (AD-c/v-Jun), or a deletion mutant of c-Jun lacking amino acids 183–256 (AD-c-JunΔ183–256) in pGAD424. The resulting plasmids were transformed into the yeast strain SFY526 in combination with BD-JNK (a) or fusion proteins between the DNA-binding domain of GAL4 and the leucine zipper domains of Fra-2 (BD-FraZip; b) or c-Jun (BD-JunZip; c). Methods are as described above for panel a.
removed by centrifugation, and the supernatant was analyzed by gel beads loaded with GST or GST-JNK. After incubation, the beads were on its own.

Therefore, although a single copy of the C-terminal auxiliary interaction domain in combination with one copy of the delta c-Jun and GST-JNK were preincubated with either pre-immune (PI) or polyclonal anti-Jun antiserum prior to the addition of the colTRE oligonucleotide probe. Complexes are denoted with either an arrow or an asterisk as in panel a, c, depletion of c-Jun-DNA complexes from solution by GST-JNK. Binding reactions containing purified c-Jun and colTRE probe were incubated with glutathione beads (GSH Beads) or beads loaded with GST or GST-JNK. After incubation, the beads were removed by centrifugation, and the supernatant was analyzed by gel retardation as in panel a.

c-Jun homodimers (Fig. 6a, arrow). Importantly, as seen with full-length c-Jun homodimers, addition of GST-JNK but not of GST alone led to the formation of a more slowly migrating species, demonstrating the formation of specific ternary complexes by direct contacts between JNK and DNA-bound Δ N c-Jun (Fig. 6a, asterisks). Thus, the c-Jun DNA-binding domain harbors an auxiliary JNK interaction domain and under certain circumstances can bind to JNK in isolation.

In vivo, c-Jun predominantly forms heterodimers with Fos-related proteins. We therefore wished to analyze the effect of heterodimerization on the c-Jun-JNK interaction in vitro. When full-length c-Jun was mixed with a purified fragment of c-Fos encompassing only the basic region-leucine zipper domain (bZIP Fos) c-Jun-bZIP Fos heterodimer complexes binding to DNA were readily detected (Fig. 6b, lane 3) by the appearance of a complex with higher electrophoretic mobility than c-Jun homodimers (Fig. 6b, lane 1). Addition of purified GST-JNK resulted in a marked retardation of this complex, corresponding sites in v-Jun effectively (17, 21, 36). Consequently, JNK neither binds to nor phosphorylates the corresponding sites in v-Jun effectively (17, 21–23, 36).

Although the role of JNK as a positive regulator of c-Jun function is well established, less is known about the structural basis of the c-Jun-JNK interaction or the mechanism through which phosphorylation stimulates c-Jun transcriptional activity. In particular, it remains unclear whether phosphorylation modulates JNK binding in vivo and whether the physical interaction between the kinase and its effector has functional significance over and above facilitating catalysis. To gain further insight into these issues, we have investigated the interaction between JNK and c-Jun using a yeast two-hybrid approach.

Using mutant derivatives of JNK and c-Jun we found that neither JNK catalytic activity nor phosphorylation of either the major (Ser63–Ser73) or less well characterized (Thr91–Thr93) c-Jun regulatory sites was required for binding of JNK in vivo. We also found that JNK could bind to a pseudo-phosphorylated mutant of c-Jun where Ser63–Ser73 and Thr91–Thr93 were replaced with negatively charged aspartic acid residues. This was unexpected, because it had previously been suggested that phosphorylation of Ser63–Ser73 (and possibly Thr91–Thr93) dissociated JNK from a GST-c-Jun affinity matrix (21). In similar capture experiments using GST-c-Jun 1–79 we found that the bulk of the active JNK remained bound after quantitative phosphorylation of the matrix, although a small amount of JNK was released independently of modification. This may account for the apparent discrepancy with previous work (21),
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because in these earlier experiments only JNK kinase activity was monitored, and the physical presence of JNK molecules could not be traced. At all events, both the yeast two-hybrid and GST pull-down experiments reported here are consistent with the notion that inactive JNK could exist in a preformed complex with c-Jun in vivo and that JNK could remain associated with phosphorylated c-Jun after kinase activation and catalysis.

A further unexpected finding was that the regions of c-Jun required for efficient interaction with JNK in yeast differ significantly from those defined previously in vitro. Although the delta region docking site is essential in both settings, in contrast to what has been observed in vitro (21, 36) it is not sufficient, because c-Jun sequences within or adjacent to the DNA-binding domain also exert a major influence on binding. Thus, deletions that remove part or all of the c-Jun bZIP domain greatly weaken interaction with JNK, whereas a mutant that additionally lacks an adjacent P-Q region (Fig. 3a) fails to interact at all.

Loss of dimerization potential likely accounts in part for the effect of the c-Jun bZIP deletions, because each of these mutants is defective for this function in vivo, and JNK has been reported to bind more avidly to c-Jun homodimers than monomers (24). This cannot be the sole explanation, however, because a mutant lacking the P-Q region but capable of dimerization might be part of the mechanism of JNK action. Thus, if JNK dimers exist in cells, then our model predicts that these will interact most avidly with c-Jun homodimers as shown in Fig. 7 and less avidly with heterodimers formed between c-Jun and other bZIP partner proteins, unless the partner in question also provides additional JNK interaction domains. Further work will be required to evaluate this possibility.

Finally, we have shown that JNK can form ternary complexes with DNA-bound c-Jun in vitro, leading to the intriguing question of whether such complexes exist in vivo. Stable association of JNK with DNA-bound c-Jun prior to kinase activation would clearly provide a powerful mechanism for ensuring rapid and selective activation of c-Jun transcriptional activity in response to signals that activate the JNK pathway. However, conflicting reports exist regarding the cellular localization of active and inactive JNK. In some cell types, JNK resides both in the cytoplasm and the nucleus under normal conditions but becomes concentrated in the nucleus after application of stresses which activate JNK (41, 42). In other systems, however, JNK becomes activated in the nucleus following stress (43, 44). SEK1, an upstream JNK kinase (45), resides both in the cytoplasm and the nucleus under normal conditions, whereas activation of SEK1 rapidly increased in the nucleus after stimulation (43). Our own preliminary results using confocal microscopy suggest co-localization of c-Jun and JNK within the nucleus of unstimulated chicken embryo fibroblasts. However, further investigation will be needed to determine whether JNK becomes activated while it resides in nuclear ternary complexes with c-Jun.

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REFERENCES

1. Cano, E., and Mahadevan, L. C. (1995) Trends Biochem. Sci. 20, 117–122
2. Kyriakis, J. M., and Avruch, J. (1996) Bioessays 18, 567–577
3. Kyriakis, J. M., and Avruch, J. (1996) J. Biol. Chem. 271, 24313–24316
4. Marshall, C. J. (1995) Cell 80, 179–185
5. Cosulich, S., and Clarke, P. (1996) Curr. Biol. 6, 1586–1588
6. Nishimura, T., and Vogt, P. K. (1988) Oncogene 3, 659–663
7. Abate, C., and Curran, T. (1998) Semin. Cancer Biol. 1, 19–26
8. Xia, Z., Dickens, M., Raineteau, J., Davis, E. J., and Greenberg, M. E. (1995) Science 270, 1326–1331
9. Verheij, M., Boor, R., Lin, X. H., Yao, B., Jarvis, W. D., Grant, S., Birrer, M. J., Stahr, E., Zou, L. J., Kyriakis, J. M., Haimovitz-Friedman, A., Fuku, Z., and Kolesnick, R. N. (1996) Nature 380, 75–79
10. Potapova, O., Haghighi, A., Bost, F., Liu, C., Birrer, M. J., Gjerset, R., and Mercola, D. (1997) J. Biol. Chem. 272, 14041–14044
11. Nehme, A., Baskaran, R., Aebi, S., Fink, D., Nebel, S., Cenni, B., Wang, J. Y., Howell, S. B., and Christen, R. D. (1997) Cancer Res. 57, 3253–3257
12. Karin, M. (1995) J. Biol. Chem. 270, 16483–16486
13. Karin, M., Liu, Z., and Zandi, E. (1997) Curr. Opin. Cell Biol. 9, 240–246
14. Binetruy, T., Smeal, T., and Karin, M. (1991) Nature 351, 122–127
15. Pulverer, B. J., Kyriakis, J. M., Avruch, J., Nikolakaki, E., and Woodgett, J. R. (1993) Nature 363, 870–877
16. Smeal, T., Binetruy, B., Mercola, D. A., Birrer, M., and Karin, M. (1991) Nature 354, 494–496
17. Black, E. J., Caudin, A. D., Woodgett, J. R., Kilbey, A., and Gillespie, D. A. (1994) Oncogene 9, 2363–2368
18. Derijard, B., Hibi, M., Wu, I. H., Barrett, T., Su, B., Deng, T., Karin, M., and E. J. Black and D. A. F. Gillespie, unpublished observation.
