Cell, Vol. 103, 239–252, October 13, 2000, Copyright ©2000 by Cell Press

Signal Transduction by the JNK Group of MAP Kinases

Review

Roger J. Davis*
Howard Hughes Medical Institute
Program in Molecular Medicine
Department of Biochemistry and Molecular Biology
University of Massachussetts Medical School
Worcester, Massachusetts 01605

Cells respond to changes in the physical and chemical properties of the environment. These changes include alterations in the amount of nutrients, growth factors, cytokines, and adhesion to the cell matrix. In addition, cells respond to physical stimulation mediated by osmolarity, heat, pH, redox, radiation, and mechanical stress. These physical and chemical cues control many aspects of cell function including migration, proliferation, differentiation, and death. The decision making process that cells employ to mount an appropriate response to a specific stimulus is critical for normal life. Many signal transduction pathways cooperate and participate in this process. Recent studies have established that mitogen-activated protein kinases (MAPK) play an important regulatory role.

Genetic studies have identified five MAPK pathways in the budding yeast *Saccharomyces cerevisiae* (Schaeffer and Weber, 1999). These MAPK are essential for mating (Fus3p), osmoregulation (Hog1p), sporulation (Smk1p), cell wall biosynthesis (Smk1p), and filamentation (Kss1p), and these enzymes form a group with related structures and biochemical properties. Each MAPK is activated by dual phosphorylation of a tripeptide motif (Thr-Xaa-Tyr) located in the activation loop (T-loop). This phosphorylation is mediated by a MAPK kinase (MAPKK) that is activated by phosphorylation by a MAPKK kinase (MAPKKK). These MAPK are therefore activated by a kinase signaling cascade.

MAPK signaling pathways have also been identified in higher organisms. In mammals, three major groups of MAPK have been identified (Schaeffer and Weber, 1999). Each of these groups of MAPK is activated by a protein kinase cascade (Figure 1). The ERK and p38 groups of MAPK are related to enzymes found in budding yeast and contain the dual phosphorylation motifs Thr-Glu-Tyr and Thr-Gly-Tyr, respectively. The c-Jun NH_2-terminal kinases (JNK), also known as stress-activated MAP kinases (SAPK), represent a third group of MAPK that has been identified in mammals. JNK contains the dual phosphorylation motif Thr-Pro-Tyr.

Biochemical studies led to the identification and purification of JNK as a "p54 microtubule-associated protein kinase" that was activated by cycloheximide (Kyriakis and Avruch, 1990). JNK was found to bind the NH_2-terminal activation domain of c-Jun (Adler et al., 1992; Hibi et al., 1993) and to phosphorylate c-Jun on Ser-63 and Ser-73 (Pulverer et al., 1991). Subsequently, JNK was molecularly cloned (Derijard et al., 1994; Kyriakis et al., 1994). JNK is activated by treatment of cells with cytokines (e.g., TNF and IL-1) and by exposure of cells to many forms of environmental stress (e.g., osmotic stress, redox stress, and radiation) (reviewed by Ip and Davis, 1998).

The purpose of this review is to summarize recent advances that have been made toward understanding the JNK signaling pathway. It is now known that JNK is required for embryonic morphogenesis and that this signaling pathway contributes to the regulation of cell proliferation and apoptosis. JNK also contributes to the function of some differentiated cells. Thus, the JNK signal transduction pathway is implicated in multiple physiological processes.

JNK Regulates AP-1 Transcription Activity

Phosphorylation of c-Jun on the sites that are phosphorylated by JNK (Ser-63 and Ser-73) causes increased transcription activity (Pulverer et al., 1991; Smeal et al., 1991). Interestingly, JNK also phosphorylates other AP-1 proteins, including JunB, JunD, and ATF2 (Ip and Davis, 1998). In each case, the sites of phosphorylation correspond to Ser/Thr-Pro motifs located in the activation domain of the transcription factor. Substrate recognition by JNK requires a docking site to tether the kinase to the substrate (Hibi et al., 1993). The mechanism that accounts for JNK-dependent regulation of AP-1 transcription activity is unclear, but a role for the coactivator CBP/p300 has been proposed (Arias et al., 1994). Additional JNK-dependent processes may also contribute to the regulation of AP-1 activity. Thus, JNK may regulate the intrinsic histone acetylase activity of ATF2 (Kawasaki et al., 2000) and may regulate the ubiquitin-mediated degradation of AP-1 proteins (Fuchs et al., 1998c).

A critical role for JNK appears to be the regulation of AP-1 transcription activity. This conclusion is supported by genetic analysis of Jun and JNK in *Drosophila* and by the analysis of AP-1 transcription activity in murine cells with targeted disruptions of genes that encode components of the JNK pathway (reviewed by Ip and Davis, 1998). JNK appears to be essential for AP-1 activation caused by stress and some cytokines, but is not required for AP-1 activation in response to other stimuli (Yang et al., 1997a). The precise role of AP-1 in the response to JNK activation is likely to be modified by the activity of other transcription factors that interact with AP-1 on the promoters of target genes.

The JNK Group of MAPK

The JNK protein kinases are encoded by three genes (Table 1). The *Jnk1* and *Jnk2* genes are expressed ubiquitously. In contrast, the *Jnk3* gene has a more limited pattern of expression and is largely restricted to brain, heart, and testis. These genes are alternatively spliced to create ten JNK isoforms (Gupta et al., 1996). Transcripts derived from all three genes encode proteins with and without a COOH-terminal extension to create both 46 kDa nd 55 kDa isoforms. The functional significance of these splice variants is unclear. A second form of

*To whom correspondence should be addressed (e-mail: roger.davis@umassmed.edu).

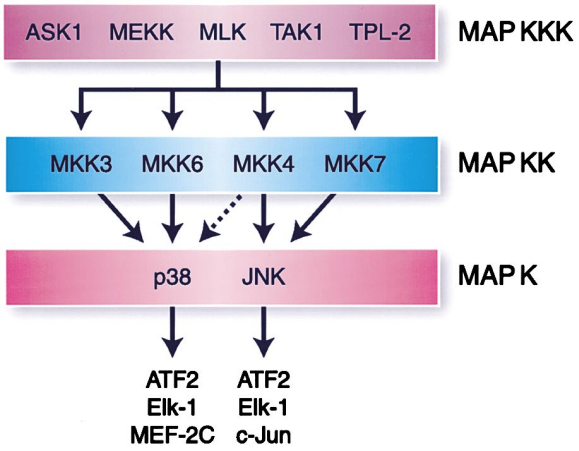

Figure 1. Stress-Activated MAPK Signaling Modules

The JNK and p38 MAPK are activated by dual phosphorylation on Thr and Tyr caused by members of the MAPKK group of protein kinases. The MAPKK are activated, in turn, by phosphorylation mediated by a group of MAPKKK. Stress-activated MAPK signaling modules can be created through the sequential actions of a MAPKKK, a MAPKK, and a MAPK.

alternative splicing is restricted to the *Jnk1* and *Jnk2* genes and involves the selection of one of two alternative exons that encodes part of the kinase domain. This alternative splicing influences the substrate specificity of the JNK isoforms by altering the ability of JNK to interact with docking sites on substrates (Gupta et al., 1996). These docking sites are present in MAPKK, MAPK phosphatases, and MAPK substrates and appear to mediate interactions with a common surface on JNK (Tanoue et al., 2000). Initial studies indicated that the docking and substrate specificities of JNK1 and JNK2 were different. For example, c-Jun was preferentially bound and phosphorylated by JNK1, while ATF2 was preferentially bound and phosphorylated by JNK2 (Kallunki et al., 1994; Sluss et al., 1994). However, it is now clear that these differences reflect the particular spliced isoforms that were examined. Different tissues express distinct repertoires of spliced JNK isoforms and the particular spliced isoform that preferentially targets a specific substrate can be encoded by either the *Jnk1* or the *Jnk2* genes (Gupta et al., 1996). The analysis of *Jnk* gene disruptions in mice confirms that there is extensive complementation between the *Jnk* genes and that there are also tissue-specific defects in signal transduction that may reflect the JNK isoform profile of individual tissues (Table 1). This complicates the analysis of *Jnk* knockout mice and indicates the need for studies of compound mutants that lack expression of all JNK isoforms (Tournier et al., 2000).

Mice deficient of JNK1 or JNK2 appear to be morphologically normal. However, these mice are immunodeficient due to severe defects in T cell function (Constant et al., 2000). No evidence for a defect in T cell activation (proliferation and IL-2 secretion) was obtained. Instead, the deficiency was identified as a requirement of JNK for the appropriate differentiation of CD4 T helper cells into effector cells (Dong et al., 1998, 2000; Yang et al., 1998). The mechanism was reported to be mediated, in part, by alterations in the function of the NFAT1 transcription factor (Dong et al., 1998; Chow et al., 2000) and by defects in IFNγ secretion (Yang et al., 1998). In contrast, one group has reported that JNK2 may be required for T cell activation at low levels of stimulation (Sabapathy et al., 1999a) and that the mechanism is mediated, in part, by JNK-dependent regulation of IL-2 mRNA stability (Chen et al., 2000). Nevertheless, there is agreement amongst all of these reports that at moderate and high levels of immune challenge, JNK is not required for T cell activation. However, JNK is required for effector T cell function (Dong et al., 2000). An important question concerns why T cells are sensitive to defects in JNK1 or JNK2 expression. This may result from the pattern of JNK expression in murine T cells (Weiss et al., 2000). Immature T cells (thymocytes) express high levels of JNK1 and JNK2. However, JNK expression is down-regulated in peripheral T cells. JNK is therefore expressed at low levels in naïve T cells, but the expression of JNK is up-regulated following immune challenge. The low level of JNK expression in naïve T cells may account for the sensitivity of these cells to targeted disruption of the *Jnk* genes.

Insight into the function of JNK protein kinases has recently been achieved through the determination of the atomic structure of JNK3 (Xie et al., 1998). Figure 2 illustrates the structure of the inactive complex of JNK3 with an ATP analog. The overall fold is typical of protein kinases and is similar to other MAPK and consists of two domains with an active site cleft. One significant difference between JNK3 and other MAPK is that the ATP binding site is well-ordered in the inactive structure. The low activity appears to result from misalignment of active site residues and the location of the T-loop, which blocks access of substrates to the active site. MAPKK activate JNK by phosphorylation of the T-loop on Thr and Tyr (Figure 2). Inactivation of JNK is mediated by a group of phosphatases, including Ser phosphatases, Tyr phosphatases, and dual specificity phosphatases (Keyse, 2000). The mechanism of JNK activation by dual phosphorylation is unclear, but it is likely that this phosphorylation may alter the structure of the T-loop and cause realignment of the NH_2- and COOH-terminal domains to create a functional active site. A crystal structure of activated JNK3 will be required to identify the structural changes that occur during activation.

JNK Is Activated by Two Dual-Specificity Protein Kinases

The JNK protein kinases are activated by phosphorylation on Thr and Tyr by MKK4 (also known as SEK1) and MKK7. These protein kinases are expressed as a group of alternatively spliced isoforms. Three MKK4 protein kinases with distinct NH_2-terminal regions have been identified and six MKK7 protein kinase isoforms with different NH_2 termini and COOH termini have been described. The mechanism that creates the different MKK4 isoforms has not been defined. In contrast, detailed studies of the *Mkk7* gene demonstrate that the isoforms result from both alternative splicing and the utilization of different promoters (Tournier et al., 1999). These different forms of MKK4 and MKK7 are biochemically distinct (basal activity and inducibility) and are differentially

Table 1. Components of the Mammalian JNK Signal Transduction Pathway

	Alternative Name	JNK Pathway Characterization[a]	Gene Disruption[a]
MAPKKK			
ASK1	MAPKKK5	1, 2	
ASK2	MAPKKK6	3	
DLK	MUK, ZPK	4, 5	
LZK		6	
MEKK1		7–9	10–14
MEKK2		15	10
MEKK3		15	16
MEKK4	MTK1	17, 18	
MLK1		19	
MLK2	MST	20	
MLK3	SPRK, PTK1	21–23	
TAK1		24	
Tpl-2	Cot	25	
MAPKK			
MKK4	SEK1, SERK1, SKK1, JNKK1	8, 26, 27	28–32
MKK7	SEK2, SKK4, JNKK2	33–38	39
MAPK			
JNK1	SAPKγ, SAPK1c	40–42	39, 43–47
JNK2	SAPKα, SAPK1a	41, 42, 48, 49	39, 44–46, 50–52
JNK3	SAPKβ, SAPK1b, p49F12	41, 42, 53	54
Scaffolds			
JIP1	IB1	55–57	
JIP2	IB2	58, 59	
JIP3	JSAP	60, 61	

[a] References: [1]Ichijo, H., et al. Science *275*, 90–94 (1997); [2]Wang, X.S., et al. J. Biol. Chem. *271*, 31607–31611 (1996); [3]Wang, X.S., et al. Biochem. Biophys. Res. Commun. *253*, 33–37 (1998); [4]Hirai, S., et al. Oncogene *12*, 641–650 (1996); [5]Fan, G., et al. J. Biol. Chem. *271*, 24788–24793 (1996); [6]Sakuma, H., et al. J. Biol. Chem. *272*, 28622–28629 (1997); [7]Minden, A., et al. Science *266*, 1719–1723 (1994); [8]Yan, M., et al. Nature *372*, 798–800 (1994); [9]Lange-Carter, C.A., et al. Science *260*, 315–319 (1993); [10]Yujiri, T., et al. J. Biol. Chem. *274*, 12605–12610 (1999); [11]Minamino, T., et al. Proc. Natl. Acad. Sci. USA *96*, 15127–15132 (1999); [12]Yujiri, T., et al. Proc. Natl. Acad. Sci. USA *97*, 7272–7277 (2000); [13]Yujiri, T., et al. Science *282*, 1911–1914 (1998); [14]Xia, Y., et al. Proc. Natl. Acad. Sci. USA *97*, 5243–5248 (2000); [15]Blank, J.L., et al. J. Biol. Chem. *271*, 5361–5368 (1996); [16]Yang, J., et al. Nat. Genet. *24*, 309–313 (2000); [17]Gerwins, P., et al. J. Biol. Chem. *272*, 8288–8295 (1997); [18]Takekawa, M., et al. EMBO J. *16*, 4973–4982 (1997); [19]Dorow, D.S., et al. Eur. J. Biochem. *213*, 701–710 (1993); [20]Hirai, S., et al. J. Biol. Chem. *272*, 15167–15173 (1997); [21]Rana, A., et al. J. Biol. Chem. *271*, 19025–19028 (1996); [22]Teramoto, H., et al. J. Biol. Chem. *271*, 27225–27228 (1996); [23]Tibbles, L.A., et al. EMBO J. *15*, 7026–7035 (1996); [24]Yamaguchi, K., et al. Science *270*, 2008–2011 (1995); [25]Salmeron, A., et al. EMBO J. *15*, 817–826 (1996); [26]Derijard, B., et al. Science *267*, 682–685 (1995); [27]Lin, A., et al. Science *268*, 286–290 (1995); [28]Swat, W., et al. Immunity *8*, 625–634 (1998); [29]Yang, D., et al. Proc. Natl. Acad. Sci. USA *94*, 3004–3009 (1997); [30]Nishina, H., et al. J. Exp. Med. *186*, 941–953 (1997); [31]Nishina, H., et al. Development *126*, 505–516 (1999); [32]Ganiatsas, S., et al. Proc. Natl. Acad. Sci. USA *95*, 6881–6886 (1998); [33]Tournier, C., et al. Proc. Natl. Acad. Sci. USA *94*, 7337–7342 (1997); [34]Moriguchi, T., et al. EMBO J. *16*, 7045–7053 (1997); [35]Yao, Z., et al. J. Biol. Chem. *272*, 32378–32383 (1997); [36]Wu, Z., et al. Mol. Cell Biol. *17*, 7407–7416 (1997); [37]Lu, X., et al. J. Biol. Chem. *272*, 24751–24754 (1997); [38]Lawler, S., et al. FEBS Lett. *414*, 153–158 (1997); [39]Dong, C., et al. Nature *405*, 91–94 (2000); [40]Derijard, B., et al. Cell *76*, 1025–1037 (1994); [41]Sanchez, I., et al. Nature *372*, 794–798 (1994); [42]Gupta, S., et al. EMBO J. *15*, 2760–2770 (1996); [43]Dong, C., et al. Science *282*, 2092–2095 (1998); [44]Tournier, C., et al. Science *288*, 870–874 (2000); [45]Kuan, C.Y., et al. Neuron *22*, 667–676 (1999); [46]Sabapathy, K., et al. Mech. Dev. *89*, 115–124 (1999); [47]Constant, S.L., et al. J. Immunol *165*, 2671–2676 (2000); [48]Kallunki, T., et al. Genes Dev *8*, 2996–3007 (1994); [49]Sluss, H.K., et al. Mol. Cell. Biol. *14*, 8376–8384 (1994); [50]Yang, D.D., et al. Immunity *9*, 575–585 (1998); [51]Sabapathy, K., et al. Curr. Biol. *9*, 116–125 (1999); [52]Chu, W.M., et al. Immunity *11*, 721–731 (1999); [53]Mohit, A.A., et al. Neuron *14*, 67–78 (1995); [54]Yang, D.D., et al. Nature *389*, 865–870 (1997); [55]Dickens, M., et al. Science *277*, 693–696 (1997); [56]Whitmarsh, A.J., et al. Science *281*, 1671–1674 (1998); [57]Bonny, C., et al. J. Biol. Chem. *273*, 1843–1846 (1998); [58]Negri, S., et al. Genomics *64*, 324–330 (2000); [59]Yasuda, J., et al. Mol. Cell. Biol. *19*, 7245–7254 (1999); [60]Ito, M., et al. Mol. Cell. Biol. *19*, 7539–7548 (1999); [61]Kelkar, N., et al. Mol. Cell. Biol. *20*, 1030–1043 (2000).

activated by upstream MAPKKK (Tournier et al., 1999). The MKK7 protein kinase is primarily activated by cytokines (e.g., TNF and IL-1) and MKK4 is primarily activated by environmental stress. Comparison of the biochemical properties of MKK4 and MKK7 demonstrates that while both protein kinases can activate JNK by dual phosphorylation on Thr and Tyr, there are significant differences in substrate specificity. First, MKK4, but not MKK7, can also activate p38 MAPK. Whether MKK4 is a physiologically relevant activator of p38 MAPK is unclear because p38 MAPK is also activated by MKK3 and MKK6 (Figure 1). Studies of compound mutant *Mkk3*$^{-/-}$ *Mkk6*$^{-/-}$ cells will be required to resolve this question. Second, although MKK4 and MKK7 are dual specificity protein kinases and do phosphorylate JNK on both Thr and Tyr, MKK4 and MKK7 appear to preferentially phosphorylate JNK on Tyr and Thr, respectively (Lawler et al., 1998). This difference in specificity suggests that MKK4 and MKK7 may cooperate to activate JNK under some circumstances.

The MKK4 and MKK7 protein kinases are activated by dual phosphorylation at two sites in the T-loop by MAPKKK. Recent studies indicate an important role for the protein phosphatase PP2Cα in the inactivation process (Takekawa et al., 1998). The MAPKK may also be inactivated during infection of cells with pathogens; for example, by the *Yersinia pestis* virulence factor, YpoJ, and anthrax lethal factor (Duesbery et al., 1998; Orth et al., 1999). Immunofluorescence analysis demonstrates that both MKK4 and MKK7 are present in the cytoplasm and the nucleus (Tournier et al., 1999). Indeed, MKK7 may accumulate in the nucleus following exposure of

Figure 2. The Atomic Structure of JNK

A ribbon diagram illustrates the three-dimensional structure of the inactive (nonphosphorylated) form of JNK3. The active site is occupied by the ATP analog adenylyl imidodiphosphate (yellow) and two Mg^{2+} ions (orange). The T-loop is colored red and the two sites of activating phosphorylation (Thr and Tyr) are indicated as red balls. Disordered regions are indicated with dashes.

cells to stress (Merritt et al., 1999). The nuclear localization of MKK4 and MKK7 contrasts with the cytoplasmic location (caused by nuclear export) of the ERK activators MEK1 and MEK2. JNK may therefore be activated in the nucleus and the cytoplasm.

Targeted gene disruption experiments in mice demonstrate that both MKK4 and MKK7 are required for embryonic development (Table 1). The cause of *Mkk7*$^{-/-}$ embryonic death is unclear (Dong et al., 2000). However, the death of *Mkk4*$^{-/-}$ embryos appears to be caused by liver apoptosis (Ganiatsas et al., 1998; Nishina et al., 1999). This phenotype is similar to that observed for *c-Jun*$^{-/-}$ embryos (Hilberg et al., 1993). However, it is unlikely that defects in c-Jun phosphorylation contribute to the *Mkk4*$^{-/-}$ phenotype because mutational removal of the JNK phosphorylation sites in c-Jun causes no obvious embryonic abnormality (Behrens et al., 1999). The mechanism that accounts for the defect in liver development observed in *Mkk4*$^{-/-}$ embryos therefore remains to be established.

Since *Mkk4*$^{-/-}$ and *Mkk7*$^{-/-}$ mice are not viable, the function of these protein kinases has been studied in *Rag*$^{-/-}$ blastocyst complementation assays. Initial studies indicated that *Mkk4*$^{-/-}$ thymocytes and peripheral T cells exhibited increased apoptotic responses and that *Mkk4*$^{-/-}$ T cells were also defective in IL-2 secretion and proliferation (Nishina et al., 1997). However, these conclusions have not been supported by another study which demonstrates that *Mkk4*$^{-/-}$ B and T cells develop normally, that *Mkk4*$^{-/-}$ deficiency does not prevent JNK activation in T cells, and that the mice develop lymphadenopathy with polyclonal expansion of both the B and T cell compartments (Swat et al., 1998). Further studies are required to resolve the differences between these reports. However, the observation that *Mkk4* gene disruption does not block JNK activation in T cells indicates that *Mkk7* may complement the *Mkk4* deficiency. A critical role for MKK7 is supported by the results of the analysis of *Mkk7*$^{-/-}$ T cells which are defective in JNK activation (Dong et al., 2000). However, Mkk7$^{-/-}$ CD4+ T cells do not exhibit defects in activation, including IL-2 secretion. Studies of compound mutant cells deficient of both the *Mkk4* and *Mkk7* genes will be critical for future studies.

The JNK Pathway Is Activated by a Large Group of MAPKKK

Several MAPKKK have been reported to activate the JNK signaling pathway. These include members of the MEKK group (MEKK1 though 4), the mixed-lineage protein kinase group (MLK1, MLK2, MLK3, DLK, and LZK), the ASK group (ASK1 and ASK2), TAK1, and TPL2 (Table 1). In most cases, the evidence is based upon transfection assays (overexpression and dominant-negative experiments) and in vitro protein kinase assays that demonstrate phosphorylation and activation of MKK4 and/or MKK7. However, these data do not establish whether these MAPKKK are physiological regulators of the JNK pathway. It is also unclear which MAPKKK are relevant to specific physiological stimuli. This uncertainty is caused by possible functional redundancy and by the promiscuity of function observed in overexpression and in vitro assays. Thus, many of these MAPKKK can activate more than one MAPK pathway and also the NF-κB pathway in transfection assays. Whether this reflects the in vivo function or whether it is an artifact of overexpression is unclear. These same considerations apply to the numerous Ste20-related protein kinases that activate the JNK pathway in transfection assays, although in this case the problem is further confounded because the mechanism by which these Ste20-related protein kinases activate the JNK cascade is unknown. The biochemical properties of Ste20-related protein kinases and MAPKKK have recently been reviewed in detail (Fanger et al., 2000).

Gene disruption studies represent an important step toward understanding the function of MAPKKK in the JNK pathway. Such studies have not been reported for most of the candidate MAPKKK. However, significant progress has been made toward understanding the function of the MEKK group of MAPKKK using gene targeting methods in mice (Table 1). Mice deficient of MEKK1 do not have gross morphological defects except for an eyelid closure disability (Yujiri et al., 2000). In contrast, MEKK3 deficiency causes embryonic lethality due to failure of embryonic angiogenesis and cardiovascular development (Yang et al., 2000). Whether these defects are due to alterations in JNK signaling or changes in another pathway is unclear. Mice deficient of MEKK2 or MEKK4 have not yet been reported. Studies

of *Mekk1*$^{-/-}$ cells indicate defects in JNK activation in response to a limited number of stimuli, including microtubule destabilizing drugs, cold shock, reovirus infection, serum, and lysophosphatidic acid (Yujiri et al., 1998, 2000). In contrast, an independently created clone of *Mekk1*-disrupted ES cells was found to be defective in JNK activation in response to all stimuli tested (Xia et al., 2000). This stronger phenotype may be a consequence of the strategy used for gene targeting, which resulted in the expression of a large fragment of MEKK1 that may act as a dominant-negative. Nevertheless, these data do establish that MEKK1 can function as a component of the JNK signaling pathway. Studies of the functional consequence of *Mekk1* gene disruption indicate defects in cell migration and increased apoptosis in response to microtubule destabilizing drugs. Whether these defects are due to changes in JNK activity or to changes in other signaling pathways that may be regulated by MEKK1 is unclear.

Mechanism of MAPKKK Activation

Several lines of evidence indicate that Rho family GTPases mediate the activation JNK by some stimuli (Coso et al., 1995; Minden et al., 1995). The function of Rho proteins is reviewed in this issue of *Cell* (Bar-Sagi and Hall, 2000 [this issue of *Cell*]). Potential targets of Rho family GTPases include several members of the mixed-lineage protein kinase group and MEKK group of MAPKKK (Fanger et al., 2000). Signal transduction pathways that activate Rho proteins include tyrosine kinases (Schlessinger, 2000 [this issue of *Cell*]). Thus, Rho proteins may mediate the activation of JNK caused by receptor tyrosine kinases. However, alternative mechanisms of JNK activation may also contribute to tyrosine kinase signaling. Thus, the adaptor protein Nck and the Ste20-like protein kinase NIK may mediate JNK activation by Eph receptors (Becker et al., 2000).

The activation of JNK by cytokine receptors appears to be mediated by the TRAF group of adaptor proteins (Liu et al., 1996). Activation of the TNF receptor leads to recruitment of TRAF2, which is required for JNK activation (Yeh et al., 1997). This adaptor protein has been reported to bind MEKK1 (Baud et al., 1999) and ASK1 (Nishitoh et al., 1998). The mechanism of TRAF2 action on MEKK1 is unclear. However, it has been demonstrated that the effect of TRAF2 on ASK1 requires the prior dissociation of ASK1 from the inhibitor thioredoxin (Liu et al., 2000) and is likely to involve regulated ASK1 dimerization mediated by reactive oxygen species (Gotoh and Cooper, 1998). Whether MEKK1 and ASK1 serve nonredundant functions in the TRAF2 pathway and whether there are roles for additional MAPKKK in this signaling pathway is unclear.

A role for TRAF proteins in the activation of JNK by the IL-1 receptor has also been established. The IL-1 receptor recruits TRAF6, which is required for JNK activation (Lomaga et al., 1999). Two possible mediators of TRAF6 have been reported. First, TRAF6 has been reported to bind MEKK1 (Baud et al., 1999) although this interaction may be mediated by the adaptor protein ECSIT (Kopp et al., 1999). The ECSIT adaptor protein appears to increase MEKK1 activity via proteolytic processing. Second, TRAF6 binds the MAPKKK TAK1 via the adaptor protein TAB2 (Takaesu et al., 2000). A similar adaptor complex involving XIAP/TAB1 appears to link TAK1 with BMP receptors (Yamaguchi et al., 1999). The mechanism of TAK1 activation involves autophosphorylation of the T-loop (Kishimoto et al., 2000).

It is possible that TRAF adaptor proteins may be involved in the activation of the JNK pathway by many stimuli. For example, the effects of endoplasmic reticulum stress on JNK activation appears to be mediated by the recruitment of TRAF2 to the transmembrane protein kinase IRE1 (Urano et al., 2000). However, it is likely that other adaptor proteins contribute to the activation of the JNK pathway. Thus, a role for a group of GADD45-related molecules in the activation of MEKK4 has been reported (Takekawa and Saito, 1998), although this has been questioned by more recent studies (Sheikh et al., 2000). Nevertheless, studies to identify the role of other potential adaptor molecules that mediate activation of MAPKKK are warranted.

Molecular Scaffold Proteins Assemble JNK Signaling Modules

Protein–protein interactions are thought to be critical for the normal function of the JNK signaling pathway. Indeed, signaling specificity may be mediated through the formation of protein complexes. These complexes may involve the interaction between kinase components of the signaling module or interactions with another protein. Studies of budding yeast have established that both types of complexes participate in the generation of signaling specificity (reviewed by Whitmarsh and Davis, 1998). First, the osmosensing MAPK Hog1p binds to the MAPKK Pbs2p as part of a multiprotein complex that includes the MAPKKK Ste11p. Second, the mating pathway MAPK Fus3p binds to the scaffold protein Ste5p together with the MAPKK Ste7p and the MAPKKK Ste11p. Examples of these types of complexes have been identified in mammalian cells (Whitmarsh and Davis, 1998). Complexes similar to Pbs2p have been defined for MKK4 and MEKK protein kinases (Xia et al., 1998; Cheng et al., 2000). These complexes may mediate JNK activation by forming sequential binary complexes, but it is possible that these proteins may also form larger assemblies of signaling molecules (Figure 3). Recently, scaffold proteins have also been identified in mammalian cells. One example is MP1, which interacts with the MAPK ERK1 and the MAPKK MEK1 (Schaeffer et al., 1998). Scaffold proteins for the JNK group of MAPK have also been identified. These include the JNK interacting protein (JIP) group of putative scaffolds (Figure 3).

The JIP1 and JIP2 proteins are closely related proteins that bind to JNK, MKK7, and mixed-lineage protein kinases (Whitmarsh et al., 1998; Yasuda et al., 1999). Transfection assays demonstrate that these JIP proteins potentiate JNK activation by mixed-lineage protein kinases. The JIP1 and JIP2 proteins contain an SH3 domain and a PTB domain within the COOH-terminal region. The PTB domain has been reported to interact with p190 RhoGEF (Meyer et al., 1999), the reelin receptor ApoER2 (Stockinger et al., 2000), and with the LDL receptor–related protein and Megalin (Gotthardt et al., 2000). However, the role of the SH3 domain has not yet been determined. The JIP1 and JIP2 proteins are most

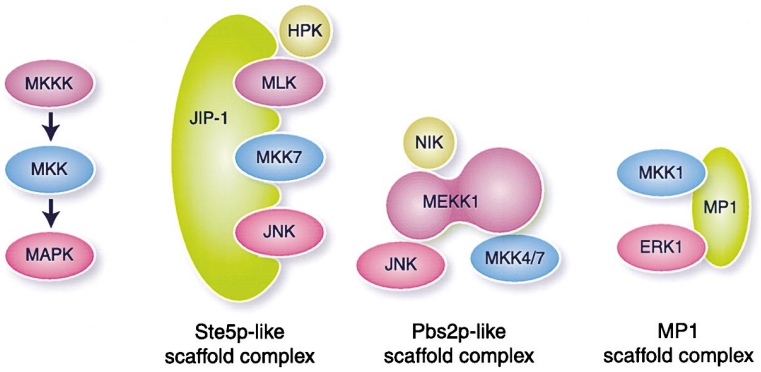

Figure 3. Mammalian Scaffold Proteins Organize MAPK Signaling Pathways

MAPK signaling modules can be structurally organized by interactions of MAPKKK, MAPKK, and MAPK with scaffold proteins. These complexes may also include Ste20-related protein kinases (e.g., HPK1 and NIK). The protein kinases that form the signaling module may interact individually with a scaffold protein like JIP-1. This type of scaffold assembly is similar to that described for the yeast scaffold protein Ste5p. A second type of scaffold assembly that has been described requires that one protein kinase interacts with the other protein kinases that form the signaling module. The MAPKKK MEKK1 is representative of this class of scaffold assembly and is similar to the Pbs2p scaffold in yeast. A third scaffold that functions within the ERK1 MAPK pathway has been described (MP1) which also interacts with MEK1. These scaffolds are thought to organize MAPK signaling modules to function in response to appropriate physiological stimulation.

highly expressed in the insulin-secreting β-cells of the pancreas and in neurons. Analysis of subcellular localization demonstrates that the JIP proteins accumulate in axonal and dendritic growth cones and appear to be present at synapses (Yasuda et al., 1999; Pellet et al., 2000). The JIP1 and JIP2 proteins were also identified independently as nuclear transcription factors (IB1 and IB2) that exhibit sequence-specific DNA binding activity and are proposed to regulate the expression of the insulin gene and the glucose transporter GLUT2 gene (Bonny et al., 1998; Negri et al., 2000). The potential role of the JIP proteins as transcription factors is intriguing. However, the DNA binding activity of JIP proteins has only been detected in Southwestern blots and several studies indicate that the JIP proteins appear to be excluded from the nucleus. In addition, the effects of JIP overexpression on gene expression may be mediated by changes in JNK activity. Further studies are required to determine whether the JIP proteins do function as transcription factors under certain circumstances.

Studies of the human *Jip1* gene have led to the identification of missense mutations that segregate with type II diabetes (Waeber et al., 2000). This analysis indicates that *Jip1* is a candidate maturity onset diabetes of the young (MODY) gene. Transfection assays and antisense oligonucleotide experiments suggest that JIP1 regulates pancreatic β-cell apoptosis (Bonny et al., 2000). These data suggest that the JNK signaling pathway may be relevant to β-cell pathology.

The JIP3 protein is structurally unrelated to the JIP1 and JIP2 proteins. However JIP3, like JIP1 and JIP2, binds to the MAPK JNK, the MAPKK MKK7, and members of the mixed-lineage group of MAPKKK (Kelkar et al., 2000). An alternatively spliced variant of JIP3 has been reported (JSAP) which also appears to function as a scaffold protein for the JNK signaling pathway (Ito et al., 1999). However, JSAP was reported to interact with the module JNK, MKK4, and MEKK1. Comparative studies of these proteins are warranted to confirm these differences in protein interactions. Nevertheless, both of these spliced forms of JIP3 are reported to potentiate JNK signaling.

The JIP proteins have been proposed to act as molecular scaffolds that organize the JNK signal transduction pathway in response to specific stimuli (Whitmarsh and Davis, 1998). Theoretical considerations indicate that a bound JNK module will not function to amplify signals. Instead, the assembly of the JNK module by a scaffold protein may lead to the efficient activation of JNK within a restricted region of the cell by a particular stimulus. Dynamic regulation of the subcellular localization of the scaffold would also be possible. An important question relates to the mechanism by which the scaffold potentiates signaling through the kinase cascade. The bound signaling molecules may interact more efficiently because of increased local concentration. Furthermore, since both MKK7 and JNK must be activated by dual phosphorylation, the tethering of molecules to the scaffold may increase the processivity of phosphorylation and thus increase activation. Since JIP1, JIP2, and JIP3 appear to act as dimers, it is likely that the kinase cascade may function within the dimer *in trans* rather than *in cis* (Yasuda et al., 1999; Kelkar et al., 2000). Structural analysis of the scaffold complexes will be critical for understanding the the molecular mechanism that accounts for the observed potentiation of JNK signaling. Furthermore, gene knockout studies will be required to establish the role of JIP proteins as scaffolds for the JNK signaling pathway.

It is likely that the JIP group of putative scaffold proteins represents only one of several groups of proteins that may assemble a functional JNK signaling module. For example, recent studies have established that the actin binding protein filamin may function as a putative scaffold for the assembly of a cytokine receptor module that activates JNK (Marti et al., 1997; Leonardi et al., 2000). Binding assays demonstrate that filamin interacts with MKK4 and TRAF2. Additional components of the JNK signaling module may also be assembled by filamin. Thus, studies of *Drosophila* indicate interactions of filamin with Tube and Toll (Edwards et al., 1997). Analysis of filamin-deficient melanoma cells demonstrates that filamin appears to be required for JNK activation caused by TNF. Together, these data establish that filamin is a putative scaffold protein that may function in a cytokine receptor signaling pathway that lead to JNK activation.

The JNK-Dependent Apoptotic Signaling Pathway

The JNK pathway is activated by the exposure of cells to stress. However, the role of JNK in the stress-

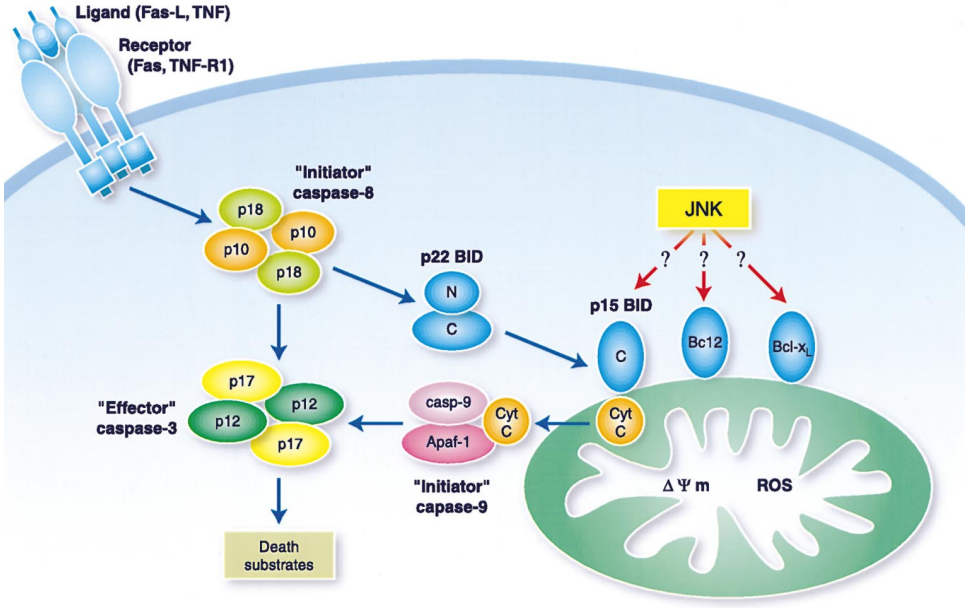

Figure 4. Role of the JNK Signaling Pathway in Stress-Induced Apoptosis

The caspase apoptotic machinery is illustrated in a simplified cartoon. Effector caspases, including caspase-3, are activated by initiator caspases that are activated by cell surface death receptors (caspase-8) and by the mitochondrial pathway (caspase-9). JNK is not required for death receptor signaling, but is required for caspase-9 activation by the mitochondrial pathway (Tournier et al., 2000). Potential targets of JNK include members of the Bcl2 group of apoptotic regulatory proteins.

response is unclear. It is possible that JNK may mediate some of the effects of stress on cells. Alternatively, JNK activation may represent a protective response that is initiated by the exposure to stress. The specific role of JNK may therefore depend upon the cellular context. Indeed, the JNK pathway has been implicated in both apoptosis and survival signaling (reviewed by Ip and Davis, 1998).

Initial studies of the role of JNK in apoptosis/survival signaling were performed by investigating neuronal cell death in response to neurotrophic factor withdrawal (Xia et al., 1995). Transfection assays using dominant-negative and gain-of-function components of the JNK pathway demonstrated that JNK contributed to the apoptotic response. Furthermore, it was found that JNK-dependent apoptosis was suppressed by activation of survival pathways (e.g., ERK MAPK and Akt/PKB). This role for JNK in stress-induced neuronal cell death has been confirmed in studies of mice with targeted disruption of the neuronal gene *Jnk3* (Yang et al., 1997b). The *Jnk3*$^{-/-}$ mice are developmentally normal, but are severely defective in the apoptotic response to excitotoxins. A similar defect was observed in mice with a germ-line mutation in the *c-Jun* gene that replaced the JNK phosphorylation sites with Ala (Behrens et al., 1999). Together, these data suggest that JNK and c-Jun may mediate a transcription-dependent apoptotic signaling pathway in neurons.

The transcriptional targets of the apoptotic JNK signaling pathway have not been established. A systematic analysis of gene expression in wild-type and *Jnk3*$^{-/-}$ mice will be required to identify the physiologically important JNK-responsive genes. However, progress in this area has been made by studies of candidate genes. One example is the observation that Fas-L is induced by JNK and may lead to autocrine or paracrine death signaling (Faris et al., 1998; Kasibhatla et al., 1998). However, it is not clear that defects in Fas-L or Fas block JNK-dependent apoptosis. Furthermore, targeted disruption of the gene that encodes the adaptor protein FADD, which is required for Fas-induced apoptosis, does not eliminate stress-induced apoptosis (Yeh et al., 1998). The expression of Fas-L may therefore contribute to JNK-induced apoptosis, but it does not appear to be essential for the function of JNK.

One potential target of pro-apoptotic signaling by JNK is the tumor suppressor p53. JNK phosphorylates murine p53 on Ser-34 in vitro (Milne et al., 1995). This phosphorylation site is not conserved in human p53. However, it has been reported that human p53 is also a JNK substrate (Fuchs et al., 1998b). Binding to JNK was reported to destabilize p53 by promoting ubiquitin-mediated degradation (Fuchs et al., 1998a). Conversely, when JNK is activated in cells exposed to stress, JNK was reported to phosphorylate p53, inhibit ubiquitin-mediated degradation, and thus stabilize the p53 protein (Fuchs et al., 1998b). These data suggest that JNK may be important for controlling the level of p53 expression by regulating the half-life of p53. However, more recent studies demonstrate that JNK is not required for ultraviolet (UV) radiation–induced accumulation of p53 (Tournier et al., 2000). Thus, JNK may contribute to the regulation of p53 stability, but JNK is not essential for this process. Furthermore, p53 does not appear to be required for JNK-induced apoptosis (Chen and Tan, 2000). The potential role of p53 as a target of JNK signaling is therefore unclear. However, it is possible that JNK may regulate other aspects of p53 function. For example, since c-Jun can repress the p53 promoter, it is possible that JNK may regulate transcription of the p53 gene (Schreiber et al., 1999).

Another potential target of pro-apoptotic signaling by JNK is the transcription factor c-Myc. Recent studies indicate that c-Myc interacts with JNK and is phosphorylated on Ser-62 and Thr-71 (Noguchi et al., 1999). Apoptosis induced by ectopic c-Myc expression in serum-starved cells is associated with increased JNK activity. In addition, dominant-negative experiments suggest that JNK signaling may participate in c-Myc stimulated apoptosis. However, since JNK-induced apoptosis does not require either ectopic c-Myc expression or serum starvation, the role of c-Myc phosphorylation by JNK is unclear.

The considerations outlined above indicate that a new approach that allows the direct biochemical analysis of apoptotic signaling in the absence of JNK is required. Targeted gene knockout experiments in mice provided an opportunity for this analysis. Disruption of the *Jnk1*, *Jnk2*, and *Jnk3* genes in mice (Table 1) causes no obvious phenotypic abnormalities. Mice with compound mutations in *Jnk1* plus *Jnk3* or *Jnk2* plus *Jnk3* are also viable. In contrast, compound mutations of the two ubiquitously expressed genes *Jnk1* and *Jnk2* causes early embryonic death associated with defects in neuronal apoptosis and exencephaly (Kuan et al., 1999; Sabapathy et al., 1999b). Primary murine embryo fibroblasts (MEF) prepared from *Jnk1*$^{-/-}$ *Jnk2*$^{-/-}$ embryos lack expression of both JNK protein and JNK activity (Tournier et al., 2000). The absence of JNK in these cells reflects the neuronal-specific expression pattern of the *Jnk3* gene. These *Jnk* null MEF represent a powerful model system for the analysis of JNK-induced apoptosis. The *Jnk* null MEF exhibit no defects in Fas-induced apoptosis, indicating that JNK is not required for death receptor signaling mediated by the initiator capase-8 (Figure 4). In contrast, the *Jnk* null MEF exhibited profound defects in stress-induced apoptosis. Defects were observed in the apoptotic response to UV radiation, to the translational inhibitor anisomycin and to the DNA alkylating agent methylmethanesulfonate. The defect in apoptosis was caused by the failure of activation of effector caspases, including caspase-3 (Tournier et al., 2000). These data establish that the JNK signaling pathway is required for the response to some, but not all, apoptotic stimuli. This conclusion has been confirmed by genetic analysis of JNK-dependent apoptosis in *Drosophila* (Adachi-Yamada et al., 1999) and also by the observation that JNK-deficiency causes defects in thymocyte apoptosis (Rincon et al., 1998; Sabapathy et al., 1999a).

Mechanism of JNK-Dependent Apoptosis

The biochemical defect in the stress-induced apoptosis of *Jnk* null MEF was localized to the mitochondria (Tournier et al., 2000). Decreased mitochondrial membrane potential is a late event in the apoptotic program that is dependent on caspase activation. The *Jnk* null MEF were found to be defective in the mitochondrial depolarization response to UV radiation. Furthermore, the *Jnk* null MEF were also defective in an early mitochondrial response to JNK activation, the release of cytochrome c. The failure to release cytochrome c is significant because cytochrome c acts together with Apaf-1 to activate initiator caspase-9. Furthermore, the defect in cytochrome c release probably reflects a defect in the release of other mitochondrial pro-apoptotic molecules, including AIF and Smac/DIABLO.

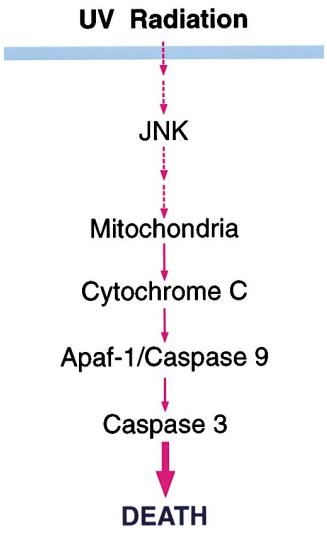

Figure 5. A Genetic Pathway that Mediates Stress-Induced Apoptosis

Targeted gene disruptions in mice have led to the identification of several genes that are required for stress-induced apoptosis. Thus, the apoptotic response to UV radiation is suppressed in the absence of JNK (Tournier et al., 2000), cytochrome c (Li et al., 2000), Apaf-1 (Yoshida et al., 1998), initiator caspase-9 (Hakem et al., 1998; Kuida et al., 1998), and the effector caspase-3 (Woo et al., 1998). The genes encoding these proteins describe a genetic pathway required for stress-induced cell death.

The recognition that JNK is a component of the stress-induced apoptotic signaling mechanism provides a genetic framework for further analysis of this signaling pathway. Targeted gene disruptions in mice have led to the identification of several genes that are required for stress-induced apoptosis (Figure 5). Thus, the apoptotic response to UV radiation is suppressed in the absence of JNK (Tournier et al., 2000), cytochrome c (Li et al., 2000), Apaf-1 (Yoshida et al., 1998), initiator caspase-9 (Hakem et al., 1998; Kuida et al., 1998), and the effector caspase-3 (Woo et al., 1998). The genes encoding these proteins describe a genetic pathway that is required for stress-induced cell death.

The observation that JNK is required for the stress-induced release of mitochondrial cytochrome c (Tournier et al., 2000) provides an explanation for findings reported in previous studies of the role of JNK in apoptotic signaling (reviewed by Chen and Tan, 2000). Thus, JNK is not required for death receptor signaling mediated by caspase-8, but is required for stress-induced apoptosis mediated by the mitochondrial/caspase-9 pathway. These two caspase signaling pathways, which converge on the activation of the effector caspase-3, are differentially utilized by specific apoptotic stimuli (Figure 4). However, although these pathways are distinct, functional interactions between these pathways have been described. For example, the mitochondrial pathway can provide an important amplification signal for death receptor signaling. The involvement of the mitochondrial pathway in death receptor signaling is most obvious in cells where death receptor signaling

causes a delayed apoptotic response. It may therefore be significant that JNK signaling has been implicated in slow killing by the Fas death receptor, but not in the rapid killing caused by Fas ligation. These considerations indicate that JNK may contribute to death receptor signaling under some circumstances. Furthermore, the transcription-dependent apoptotic signaling by JNK (leading to autocrine/paracrine secretion of death ligands) and the transcription-independent apoptotic signaling mechanism (leading to cytochrome c release) are not mutually exclusive. It is possible that these two mechanisms may function separately, but these mechanisms may also cooperate to induce death.

A significant question that remains concerns the molecular mechanism that accounts for the function of JNK in apoptotic signal transduction. JNK activation has been reported to induce cytochrome c release (Hatai et al., 2000), but the mechanism is unclear. One possibility is that JNK phosphorylates the transcription factor c-Jun. Indeed, mutation of the JNK phosphorylation sites on c-Jun leads to partial protection against UV-induced apoptosis (Behrens et al., 1999), suggesting that c-Jun might mediate the effects of JNK on cytochrome c release. However, the effect of UV to cause apoptosis does not require new gene expression (Tournier et al., 2000). Thus, it is likely that the relevant target of JNK required for cytochrome c release is present in normal cells and that JNK does not mediate apoptosis in this system by regulating gene expression. Potential targets of JNK that may regulate cytochrome c release include members of the Bcl2 group of apoptotic regulatory proteins. Several studies indicate that the anti-apoptotic proteins Bcl2 and Bcl-x_L are phosphorylated by JNK in vitro on sites that are phosphorylated in vivo (Maundrell et al., 1997; Yamamoto et al., 1999). Phosphorylation on these sites inhibits the anti-apoptotic function of Bcl2 and Bcl-x_L. This provides an elegant mechanism that could account for JNK signaling because the Bcl2 proteins are known to regulate mitochondrial cytochrome c release (Gross et al., 1999). However, there are two problems with this potential mechanism. First, there is disagreement concerning the function of phosphorylation. One study indicates that Bcl2 phosphorylation on the same sites may be anti-apoptotic rather than pro-apoptotic (Ito et al., 1997), while other studies indicate that the function of phosphorylation is to regulate ubiquitin-mediated degradation rather than the anti-apoptotic properties of Bcl2 (Breitschopf et al., 2000). The second problem concerns the identification of JNK as a kinase that phosphorylates Bcl2 in vivo. This is because many stimuli that activate JNK do not cause Bcl2 phosphorylation and, conversely, other stimuli cause marked Bcl2 phosphorylation (e.g., microtubule disrupting drugs) without activating JNK. These data demonstrate that the anti-apoptotic proteins Bcl2 and Bcl-x_L may not be physiological substrates of JNK. However, it is possible that JNK could be targeted to Bcl2 or Bcl-x_L under specialized circumstances in the presence of an adaptor molecule that mediates the interaction. The existance of an adaptor molecule that might have this property has not been established.

Further studies will be required to establish the mechanism of action of JNK in pro-apoptotic signaling to the mitochondria. Potential targets include the pro-apoptotic BH3-only members of the Bcl2 group (Tournier et al., 2000).

Role of JNK in Signaling Cell Survival

Although it is established that JNK contributes to some apoptotic responses, it is not clear that apoptosis represents the only functional consequence of JNK activation. This is most obvious when one considers that most stimuli that activate JNK do not cause apoptosis. For example, most forms of environmental stress do not cause apoptosis under conditions that are sufficient for JNK activation. This is partly because the JNK-dependent apoptotic signaling pathway can be blocked by activation of survival signaling pathways (Xia et al., 1995). Examples of these survival pathways include NF-κB, Akt/PKB, and ERK. Thus, the JNK pathway functions within the overall context of the state of activation of other signaling pathways. The absence of an apoptotic response to JNK activation also appears to correlate with the time course of JNK activation—sustained activation, but not transient activation, of JNK is associated with apoptosis (Chen and Tan, 2000). Many cytokines (e.g., TNF) cause only transient JNK activation, which does not contribute to the apoptotic response (Liu et al., 1996). What is the role of cytokine-induced JNK activation? One possibility is that JNK may contribute to survival signaling (Reinhard et al., 1997). Cells may interpret transient JNK activation as a survival signal because of the activation state of other signaling pathways within the cell. The strongest evidence in favor of this hypothesis derives from the analysis of compound mutant *Jnk1*$^{-/-}$ *Jnk2*$^{-/-}$ embryos that exhibit increased apoptosis within the developing forebrain (Kuan et al., 1999; Sabapathy et al., 1999b). In addition, it has been reported that integrin-mediated survival signaling can be mediated by the JNK pathway (Almeida et al., 2000). These data are consistent with the hypothesis that JNK may mediate survival signaling under specific circumstances. Further studies are required to determine the genetic and biochemical basis for the role of JNK in survival signaling.

Role of JNK in Tumor Development

Several lines of evidence suggest that JNK plays an important role in tumor cells. Thus, it is established that Ras-induced transformation requires c-Jun (Johnson et al., 1996) and that Ras induces c-Jun phosphorylation on sites that are phosphorylated by JNK (Pulverer et al., 1991; Smeal et al., 1991). Furthermore, Ras-induced tumorigenicity is suppressed by mutation of the JNK phosphorylation sites on c-Jun (Behrens et al., 2000). In addition, it has been reported that JNK is constitutively activated in several tumor cell lines and that the transforming actions of several oncogenes have been reported to be JNK dependent (based on dominant-negative approaches) (reviewed by Ip and Davis, 1998). These data strongly support the hypothesis that JNK is relevant to cancer.

Although a function for JNK is implicated in cancer, the mechanism of JNK action is unclear. During tumor development, it is likely that JNK-dependent stress-induced apoptosis must be suppressed. This would imply that components of the JNK pathway are potential

tumor suppressor genes. Since the *Jnk1* and *Jnk2* genes are largely functionally redundant, it is unlikely that either of these genes could represent a tumor suppressor. However, it is possible that proteins which inhibit JNK activity, like Evi-1 and perhaps GSTπ, could act as oncogenes by suppressing JNK activity (Adler et al., 1999; Kurokawa et al., 2000). In contrast, the *Mkk4* and *Mkk7* genes are potential tumor suppressors because these genes are not functionally redundant. Indeed, the *Mkk4* gene has been identified as a candidate tumor suppressor gene (Teng et al., 1997; Su et al., 1998) and as a candidate metastasis suppressor gene (Yoshida et al., 1999). It is intriguing that somatic mutations in *Mkk4* have been identified in cancer patients. However, do the homozygous *Mkk4* mutations precede tumor development? Could these mutations represent a consequence of chemotherapy or tumor-associated chromosomal instability? Further studies are required to more fully establish the significance of these somatic *Mkk4* mutations in disease. Studies to test whether the *Mkk7* gene is a candidate tumor suppressor are also warranted.

Established tumor cells must adopt mechanisms to inhibit JNK-dependent apoptosis. Tumor cells may activate survival pathways that function dominantly with respect to the JNK pro-apoptotic pathway (e.g., PTEN mutations may activate Akt/PKB signaling). Alternatively, the targets of pro-apoptotic JNK signaling may be altered (e.g., mutated, expressed at a different level, or post-translationally modified) to suppress JNK-dependent apoptosis. Finally, it is possible that, in the context of a tumor cell (e.g., loss of p53 or Rb pathways), JNK activation may be interpreted as an anti-apoptotic or growth signal. Recent investigations using anti-sense JNK oligonucleotides support the conclusion that JNK functions differently in normal and tumor cells. These studies demonstrate that JNK is required for stress-induced apoptosis of primary cells (Garay et al., 2000; Ho et al., 2000). In contrast, anti-sense JNK oligonucleotides inhibit the growth of tumor cells and can induce apoptosis (Bost et al., 1999; Potapova et al., 2000). These studies imply that JNK inhibition may be useful for tumor therapy. Furthermore, these studies indicate that genetic interactions with cancer-associated genes may be critical for determining the outcome of JNK signaling.

Role of the JNK Signaling Pathway in Embryonic Morphogenesis

The JNK protein kinases are not present in yeast, but JNK but has been identified in nematodes (Kawasaki et al., 1999) and insects (Riesgo-Escovar et al., 1996; Sluss et al., 1996). In the nematode *Caenorhabditis elegans*, JNK is required for the normal function of GABAergic motor neurons, but does not appear to be essential for embryonic morphogenesis. In contrast, genetic studies of *Drosophila melanogaster* demonstrate that JNK is required for embryonic epithelial cell sheet movements and epithelial planar polarity.

JNK is required for at least two morphogenetic processes that involve epithelial cell sheet movements: dorsal closure and thorax closure (reviewed by Ip and Davis, 1998; Leppa and Bohmann, 1999). Detailed studies of the process of dorsal closure demonstrate that JNK is required for Jun phosphorylation and expression of the TGF-β family protein decapentaplegic (Dpp) in the cells that form the leading edge of the lateral epithelial cell sheet. Fos is also required for Dpp expression, indicating that JNK may trigger Dpp expression by activation of an AP-1 complex composed of Fos-Jun heterodimers. Dpp expression is required for the elongation and spreading of the lateral epithelial cells to cover the amnioserosa on the dorsal surface of the developing embryo. A similar process may account for the role of JNK in thorax closure.

An important role for JNK in the establishment of epithelial planar polarity has been reported (Mlodzik, 1999). This pathway involves interactions between JNK and the Wnt signaling pathway. Genetic analysis demonstrates that Dishevelled may represent an important intermediate in this interaction. Dishevelled, which causes JNK activation, also functions in the Wnt pathway (Boutros et al., 1998). Biochemical studies confirm that Dishevelled activates JNK and further implicate the scaffold protein axin in this process (Moriguchi et al., 1999; Zhang et al., 2000). The mechanism of JNK function in the establishment of planar polarity is unclear.

Further studies of the role of JNK in embryonic morphogenesis in *Drosophila* are warranted because it is likely that new molecular insight into the function of JNK will be gained. These studies may also provide insight into the role of the JNK pathway in mammalian embryonic morphogenesis. Gene knockout studies demonstrate that the JNK signaling pathway is required for murine embryonic viability. JNK appears to be required for normal apoptosis in the developing embryo, but further studies are required to define the embryonic defects that are caused by JNK signaling deficiency in mammalian embryos.

Concluding Remarks

The JNK signal transduction pathway is implicated in many pathological conditions, including cancer. JNK may also mediate cardiac hypertrophic responses (Wang et al., 1998), ischemia/reperfusion injury to the heart and kidney (He et al., 1999; Garay et al., 2000), endothelial cell apoptosis caused by diabetes-associated hyperglycemia (Ho et al., 2000), and pancreatic β-cell apoptosis associated with diabetes (Bonny et al., 2000). JNK has also been implicated in several neurodegenerative diseases (Yang et al., 1997b). The JNK signaling pathway therefore represents a potential target for therapeutic intervention. A goal for future studies will be to establish whether JNK directly contributes to these disease processes and to define the molecular mechanism of JNK function.

A key fundamental question that remains unresolved concerns the ability of cells to interpret JNK activation in different ways depending upon context (e.g., survival signaling versus apoptosis). It is likely that this ability is mediated by interactions of JNK with other signaling pathways within the cell. The combinatorial actions of transcription factors on gene promoters suggests one possible mechanism by which JNK-activated AP-1 could lead to different outcomes in different contexts.

In conclusion, progress has been made toward under-

standing the function of the JNK pathway. Many of the components of this signaling pathway have now been identified. Research during the next few years will be exciting because we will begin to uncover basic molecular mechanisms and gain biological insight into the function of JNK.

Acknowledgments

I apologize to those whose work was not cited or discussed because of space limitations. I thank Richard Flavell, Mercedes Rincon, and members of the Davis laboratory for discussions. Keith Wilson and Michael S. Su are thanked for providing the crystal structure illustration (Figure 2). Support for the studies performed in this laboratory was received from the National Cancer Institute. R. J. D. is an investigator of the Howard Hughes Medical Institute.

References

Adachi-Yamada, T., Fujimura-Kamada, K., Nishida, Y., and Matsumoto, K. (1999). Distortion of proximodistal information causes JNK-dependent apoptosis in *Drosophila* wing. Nature *400*, 166–169.

Adler, V., Polotskaya, A., Wagner, F., and Kraft, A.S. (1992). Affinity-purified c-Jun amino-terminal protein kinase requires serine/threonine phosphorylation for activity. J. Biol. Chem. *267*, 17001–17005.

Adler, V., Yin, Z., Fuchs, S.Y., Benezra, M., Rosario, L., Tew, K.D., Pincus, M.R., Sardana, M., Henderson, C.J., Wolf, C.R., et al. (1999). Regulation of JNK signaling by GSTβ. EMBO J. *18*, 1321–1334.

Almeida, E.A., Ilic, D., Han, Q., Hauck, C.R., Jin, F., Kawakatsu, H., Schlaepfer, D.D., and Damsky, C.H. (2000). Matrix survival signaling: from fibronectin via focal adhesion kinase to c-Jun NH_2-terminal kinase. J. Cell Biol. *149*, 741–754.

Arias, J., Alberts, A.S., Brindle, P., Claret, F.X., Smeal, T., Karin, M., Feramisco, J., and Montminy, M. (1994). Activation of cAMP and mitogen responsive genes relies on a common nuclear factor. Nature *370*, 226–229.

Bar-Sagi, D., and Hall, A. (2000). Ras and Rho GTPases: a family reunion. Cell *103*, this issue, 227–238.

Baud, V., Liu, Z.G., Bennett, B., Suzuki, N., Xia, Y., and Karin, M. (1999). Signaling by proinflammatory cytokines: oligomerization of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain. Genes Dev. *13*, 1297–1308.

Becker, E., Huynh-Do, U., Holland, S., Pawson, T., Daniel, T.O., and Skolnik, E.Y. (2000). Nck-interacting Ste20 kinase couples Eph receptors to c-Jun N-terminal kinase and integrin activation. Mol. Cell. Biol. *20*, 1537–1545.

Behrens, A., Sibilia, M., and Wagner, E.F. (1999). Amino-terminal phosphorylation of c-Jun regulates stress-induced apoptosis and cellular proliferation. Nat. Genet. *21*, 326–329.

Behrens, A., Jochum, W., Sibilia, M., and Wagner, E.F. (2000). Oncogenic transformation by ras and fos is mediated by c-Jun N-terminal phosphorylation. Oncogene *19*, 2657–2663.

Bonny, C., Oberson, A., Steinmann, M., Schorderet, D.F., Nicod, P., and Waeber, G. (2000). IB1 reduces cytokine-induced apoptosis of insulin-secreting cells. J. Biol. Chem. *275*, 16466–16472.

Bonny, C., Nicod, P., and Waeber, G. (1998). IB1, a JIP-1-related nuclear protein present in insulin-secreting cells. J. Biol. Chem. *273*, 1843–1846.

Bost, F., McKay, R., Bost, M., Potapova, O., Dean, N.M., and Mercola, D. (1999). The Jun kinase 2 isoform is preferentially required for epidermal growth factor-induced transformation of human A549 lung carcinoma cells. Mol. Cell. Biol. *19*, 1938–1949.

Boutros, M., Paricio, N., Strutt, D.I., and Mlodzik, M. (1998). Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling. Cell *94*, 109–118.

Breitschopf, K., Haendeler, J., Malchow, P., Zeiher, A.M., and Dimmeler, S. (2000). Posttranslational modification of Bcl-2 facilitates its proteasome-dependent degradation: molecular characterization of the involved signaling pathway. Mol. Cell. Biol. *20*, 1886–1896.

Chen, Y.R., and Tan, T.H. (2000). The c-Jun N-terminal kinase pathway and apoptotic signaling. Int. J. Oncol. *16*, 651–662.

Chen, C.Y., Gherzi, R., Andersen, J.S., Gaietta, G., Jurchott, K., Royer, H.D., Mann, M., and Karin, M. (2000). Nucleolin and YB-1 are required for JNK-mediated interleukin-2 mRNA stabilization during T-cell activation. Genes Dev. *14*, 1236–1248.

Cheng, J., Yang, J., Xia, Y., Karin, M., and Su, B. (2000). Synergistic interaction of MEK kinase *2*, c-Jun N-terminal kinase (JNK) kinase *2*, and JNK1 results in efficient and specific JNK1 activation. Mol. Cell. Biol. *20*, 2334–2342.

Chow, C.W., Dong, C., Flavell, R.A., and Davis, R.J. (2000). c-Jun NH_2-terminal kinase inhibits targeting of the protein phosphatase calcineurin to NFATc1. Mol. Cell. Biol. *20*, 5227–5234.

Constant, S.L., Dong, C., Yang, D.D., Wysk, M., Davis, R.J., and Flavell, R.A. (2000). JNK1 is required for T cell-mediated immunity against *Leischmania major* infection. J. Immunol. *165*, 2671–2676.

Coso, O.A., Chiariello, M., Yu, J.C., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gutkind, J.S. (1995). The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. Cell *81*, 1137–1146.

Derijard, B., Hibi, M., Wu, I.H., Barrett, T., Su, B., Deng, T., Karin, M., and Davis, R.J. (1994). JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. Cell *76*, 1025–1037.

Dong, C., Yang, D.D., Wysk, M., Whitmarsh, A.J., Davis, R.J., and Flavell, R.A. (1998). Defective T cell differentiation in the absence of Jnk1. Science *282*, 2092–2095.

Dong, C., Yang, D.D., Tournier, C., Whitmarsh, A.J., Xu, J., Davis, R.J., and Flavell, R.A. (2000). JNK is required for effector T-cell function but not for T-cell activation. Nature *405*, 91–94.

Duesbery, N.S., Webb, C.P., Leppla, S.H., Gordon, V.M., Klimpel, K.R., Copeland, T.D., Ahn, N.G., Oskarsson, M.K., Fukasawa, K., Paull, K.D., and Vande Woude, G.F. (1998). Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor. Science *280*, 734–737.

Edwards, D.N., Towb, P., and Wasserman, S.A. (1997). An activity-dependent network of interactions links the Rel protein Dorsal with its cytoplasmic regulators. Development *124*, 3855–3864.

Fanger, G.R., Schlesinger, T.K., and Johnson, G.L. (2000). Control of MAPK signaling by Ste20- and Ste11-like kinases. In Signaling Networks and Cell Cycle Control, J.S. Gutkind, ed. (Totowa, New Jersey: Humana Press), pp. 183–211.

Faris, M., Latinis, K.M., Kempiak, S.J., Koretzky, G.A., and Nel, A. (1998). Stress-induced Fas ligand expression in T cells is mediated through a MEK kinase 1-regulated response element in the Fas ligand promoter. Mol. Cell. Biol. *18*, 5414–5424.

Fuchs, S.Y., Adler, V., Buschmann, T., Yin, Z., Wu, X., Jones, S.N., and Ronai, Z. (1998a). JNK targets p53 ubiquitination and degradation in nonstressed cells. Genes Dev. *12*, 2658–2663.

Fuchs, S.Y., Adler, V., Pincus, M.R., and Ronai, Z. (1998b). MEKK1/JNK signaling stabilizes and activates p53. Proc. Natl. Acad. Sci. USA *95*, 10541–10546.

Fuchs, S.Y., Fried, V.A., and Ronai, Z. (1998c). Stress-activated kinases regulate protein stability. Oncogene *17*, 1483–1490.

Ganiatsas, S., Kwee, L., Fujiwara, Y., Perkins, A., Ikeda, T., Labow, M.A., and Zon, L.I. (1998). SEK1 deficiency reveals mitogen-activated protein kinase cascade crossregulation and leads to abnormal hepatogenesis. Proc. Natl. Acad. Sci. USA *95*, 6881–6886.

Garay, M., Gaarde, W., Monia, B.P., Nero, P., and Cioffi, C.L. (2000). Inhibition of hypoxia/reoxygenation-induced apoptosis by an antisense oligonucleotide targeted to JNK1 in human kidney cells. Biochem. Pharmacol. *59*, 1033–1043.

Gotoh, Y., and Cooper, J.A. (1998). Reactive oxygen species- and dimerization-induced activation of apoptosis signal-regulating kinase 1 in tumor necrosis factor-alpha signal transduction. J. Biol. Chem. *273*, 17477–17482.

Gotthardt, M., Trommsdorff, M., Nevitt, M.F., Shelton, J., Richardson, J.A., Stockinger, W., Nimpf, J., and Herz, J. (2000). Interactions

of the low density lipoprotein receptor gene family with cytosolic adaptor and scaffold proteins suggest diverse biological functions in cellular communication and signal transduction. J. Biol. Chem. *275*, 25616–25624.

Gross, A., McDonnell, J.M., and Korsmeyer, S.J. (1999). BCL-2 family members and the mitochondria in apoptosis. Genes Dev. *13*, 1899–1911.

Gupta, S., Barrett, T., Whitmarsh, A.J., Cavanagh, J., Sluss, H.K., Derijard, B., and Davis, R.J. (1996). Selective interaction of JNK protein kinase isoforms with transcription factors. EMBO J. *15*, 2760–2770.

Hakem, R., Hakem, A., Duncan, G.S., Henderson, J.T., Woo, M., Soengas, M.S., Elia, A., de la Pompa, J.L., Kagi, D., Khoo, W., et al. (1998). Differential requirement for caspase 9 in apoptotic pathways in vivo. Cell *94*, 339–352.

Hatai, T., Matsuzawa, A., Inoshita, S., Mochida, Y., Kuroda, T., Sakamaki, K., Kuida, K., Yonehara, S., Ichijo, H., and Takeda, K. (2000). Execution of ASK1-induced apoptosis by the mitochondria-dependent caspase activation. J. Biol. Chem. *275*, 26576–26581.

He, H., Li, H.L., Lin, A., and Gottlieb, R.A. (1999). Activation of the JNK pathway is important for cardiomyocyte death in response to simulated ischemia. Cell Death Differ. *6*, 987–991.

Hibi, M., Lin, A., Smeal, T., Minden, A., and Karin, M. (1993). Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. Genes Dev. *7*, 2135–2148.

Hilberg, F., Aguzzi, A., Howells, N., and Wagner, E.F. (1993). c-Jun is essential for normal mouse development and hepatogenesis. Nature *365*, 179–181.

Ho, F.M., Liu, S.H., Liau, C.S., Huang, P.J., and Lin-Shiau, S.Y. (2000). High glucose-induced apoptosis in human endothelial cells is mediated by sequential activations of c-Jun NH_2-terminal kinase and caspase-3. Circulation *101*, 2618–2624.

Ip, Y.T., and Davis, R.J. (1998). Signal transduction by the c-Jun N-terminal kinase (JNK)—from inflammation to development. Curr. Opin. Cell Biol. *10*, 205–219.

Ito, T., Deng, X., Carr, B., and May, W.S. (1997). Bcl-2 phosphorylation required for anti-apoptosis function. J. Biol. Chem. *272*, 11671–11673.

Ito, M., Yoshioka, K., Akechi, M., Yamashita, S., Takamatsu, N., Sugiyama, K., Hibi, M., Nakabeppu, Y., Shiba, T., and Yamamoto, K.I. (1999). JSAP1, a novel jun N-terminal protein kinase (JNK)-binding protein that functions as a Scaffold factor in the JNK signaling pathway. Mol. Cell. Biol. *19*, 7539–7548.

Johnson, R., Spiegelman, B., Hanahan, D., and Wisdom, R. (1996). Cellular transformation and malignancy induced by ras require c-jun. Mol. Cell. Biol. *16*, 4504–4511.

Kallunki, T., Su, B., Tsigelny, I., Sluss, H.K., Derijard, B., Moore, G., Davis, R., and Karin, M. (1994). JNK2 contains a specificity-determining region responsible for efficient c-Jun binding and phosphorylation. Genes Dev. *8*, 2996–3007.

Kasibhatla, S., Brunner, T., Genestier, L., Echeverri, F., Mahboubi, A., and Green, D.R. (1998). DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kappa B and AP-1. Mol. Cell *1*, 543–551.

Kawasaki, M., Hisamoto, N., Iino, Y., Yamamoto, M., Ninomiya-Tsuji, J., and Matsumoto, K. (1999). A *Caenorhabditis elegans* JNK signal transduction pathway regulates coordinated movement via type-D GABAergic motor neurons. EMBO J. *18*, 3604–3615.

Kawasaki, H., Schiltz, L., Chiu, R., Itakura, K., Taira, K., Nakatani, Y., and Yokoyama, K.K. (2000). ATF-2 has intrinsic histone acetyltransferase activity which is modulated by phosphorylation. Nature *405*, 195–200.

Kelkar, N., Gupta, S., Dickens, M., and Davis, R.J. (2000). Interaction of a mitogen-activated protein kinase signaling module with the neuronal protein JIP3. Mol. Cell. Biol. *20*, 1030–1043.

Keyse, S.M. (2000). Protein phosphatases and the regulation of mitogen-activated protein kinase signalling. Curr. Opin. Cell Biol. *12*, 186–192.

Kishimoto, K., Matsumoto, K., and Ninomiya-Tsuji, J. (2000). TAK1 mitogen-activated protein kinase kinase kinase is activated by autophosphorylation within its activation loop. J. Biol. Chem. *275*, 7359–7364.

Kopp, E., Medzhitov, R., Carothers, J., Xiao, C., Douglas, I., Janeway, C.A., and Ghosh, S. (1999). ECSIT is an evolutionarily conserved intermediate in the Toll/IL-1 signal transduction pathway. Genes Dev. *13*, 2059–2071.

Kuan, C.Y., Yang, D.D., Samanta Roy, D.R., Davis, R.J., Rakic, P., and Flavell, R.A. (1999). The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. Neuron *22*, 667–676.

Kuida, K., Haydar, T.F., Kuan, C.Y., Gu, Y., Taya, C., Karasuyama, H., Su, M.S., Rakic, P., and Flavell, R.A. (1998). Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. Cell *94*, 325–337.

Kurokawa, M., Mitani, K., Yamagata, T., Takahashi, T., Izutsu, K., Ogawa, S., Moriguchi, T., Nishida, E., Yazaki, Y., and Hirai, H. (2000). The Evi-1 oncoprotein inhibits c-Jun N-terminal kinase and prevents stress-induced cell death. EMBO J. *19*, 2958–2968.

Kyriakis, J.M., and Avruch, J. (1990). pp54 microtubule-associated protein 2 kinase. A novel serine/threonine protein kinase regulated by phosphorylation and stimulated by poly-L-lysine. J. Biol. Chem. *265*, 17355–17363.

Kyriakis, J.M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E.A., Ahmad, M.F., Avruch, J., and Woodgett, J.R. (1994). The stress-activated protein kinase subfamily of c-Jun kinases. Nature *369*, 156–160.

Lawler, S., Fleming, Y., Goedert, M., and Cohen, P. (1998). Synergistic activation of SAPK1/JNK1 by two MAP kinase kinases *in vitro*. Curr. Biol. *8*, 1387–1390.

Leonardi, A., Ellinger-Ziegelbauer, H., Franzoso, G., Brown, K., and Siebenlist, U. (2000). Physical and functional interaction of filamin (actin-binding protein-280) and tumor necrosis factor receptor-associated factor 2. J. Biol. Chem. *275*, 271–278.

Leppa, S., and Bohmann, D. (1999). Diverse functions of JNK signaling and c-Jun in stress response and apoptosis. Oncogene *18*, 6158–6162.

Li, K., Li, Y., Shelton, J.M., Richardson, J.A., Spencer, E., Chen, Z.J., Wang, X., and Williams, R.S. (2000). Cytochrome c deficiency causes embryonic lethality and attenuates stress-induced apoptosis. Cell *101*, 389–399.

Liu, Z.G., Hsu, H., Goeddel, D.V., and Karin, M. (1996). Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kB activation prevents cell death. Cell *87*, 565–576.

Liu, H., Nishitoh, H., Ichijo, H., and Kyriakis, J.M. (2000). Activation of apoptosis signal-regulating kinase 1 (ASK1) by tumor necrosis factor receptor-associated factor 2 requires prior dissociation of the ASK1 inhibitor thioredoxin. Mol. Cell. Biol. *20*, 2198–2208.

Lomaga, M.A., Yeh, W.C., Sarosi, I., Duncan, G.S., Furlonger, C., Ho, A., Morony, S., Capparelli, C., Van, G., Kaufman, S., et al. (1999). TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Genes Dev. *13*, 1015–1024.

Marti, A., Luo, Z., Cunningham, C., Ohta, Y., Hartwig, J., Stossel, T.P., Kyriakis, J.M., and Avruch, J. (1997). Actin-binding protein-280 binds the stress-activated protein kinase (SAPK) activator SEK-1 and is required for tumor necrosis factor-alpha activation of SAPK in melanoma cells. J. Biol. Chem. *272*, 2620–2628.

Maundrell, K., Antonsson, B., Magnenat, E., Camps, M., Muda, M., Chabert, C., Gillieron, C., Boschert, U., Vial-Knecht, E., Martinou, J.C., and Arkinstall, S. (1997). Bcl-2 undergoes phosphorylation by c-Jun N-terminal kinase/stress-activated protein kinases in the presence of the constitutively active GTP-binding protein Rac1. J. Biol. Chem. *272*, 25238–25242.

Merritt, S.E., Mata, M., Nihalani, D., Zhu, C., Hu, X., and Holzman, L.B. (1999). The mixed lineage kinase DLK utilizes MKK7 and not MKK4 as substrate. J. Biol. Chem. *274*, 10195–10202.

Meyer, D., Liu, A., and Margolis, B. (1999). Interaction of c-Jun

amino-terminal kinase interacting protein-1 with p190 rhoGEF and its localization in differentiated neurons. J. Biol. Chem. *274*, 35113–35118.

Milne, D.M., Campbell, L.E., Campbell, D.G., and Meek, D.W. (1995). p53 is phosphorylated in vitro and in vivo by an ultraviolet radiation-induced protein kinase characteristic of the c-Jun kinase, JNK1. J. Biol. Chem. *270*, 5511–5518.

Minden, A., Lin, A., Claret, F.X., Abo, A., and Karin, M. (1995). Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell *81*, 1147–1157.

Mlodzik, M. (1999). Planar polarity in the *Drosophila* eye: a multifaceted view of signaling specificity and cross-talk. EMBO J. *18*, 6873–6879.

Moriguchi, T., Kawachi, K., Kamakura, S., Masuyama, N., Yamanaka, H., Matsumoto, K., Kikuchi, A., and Nishida, E. (1999). Distinct domains of mouse dishevelled are responsible for the c-Jun N-terminal kinase/stress-activated protein kinase activation and the axis formation in vertebrates. J. Biol. Chem. *274*, 30957–30962.

Negri, S., Oberson, A., Steinmann, M., Sauser, C., Nicod, P., Waeber, G., Schorderet, D.F., and Bonny, C. (2000). cDNA cloning and mapping of a novel islet-brain/JNK-interacting protein. Genomics *64*, 324–330.

Nishina, H., Fischer, K.D., Radvanyi, L., Shahinian, A., Hakem, R., Rubie, E.A., Bernstein, A., Mak, T.W., Woodgett, J.R., and Penninger, J.M. (1997). Stress-signalling kinase Sek1 protects thymocytes from apoptosis mediated by CD95 and CD3. Nature *385*, 350–353.

Nishina, H., Vaz, C., Billia, P., Nghiem, M., Sasaki, T., De la Pompa, J.L., Furlonger, K., Paige, C., Hui, C., Fischer, K.D., et al. (1999). Defective liver formation and liver cell apoptosis in mice lacking the stress signaling kinase SEK1/MKK4. Development *126*, 505–516.

Nishitoh, H., Saitoh, M., Mochida, Y., Takeda, K., Nakano, H., Rothe, M., Miyazono, K., and Ichijo, H. (1998). ASK1 is essential for JNK/SAPK activation by TRAF2. Mol. Cell *2*, 389–395.

Noguchi, K., Kitanaka, C., Yamana, H., Kokubu, A., Mochizuki, T., and Kuchino, Y. (1999). Regulation of c-Myc through phosphorylation at Ser-62 and Ser-71 by c-Jun N-terminal kinase. J. Biol. Chem. *274*, 32580–32587.

Orth, K., Palmer, L.E., Bao, Z.Q., Stewart, S., Rudolph, A.E., Bliska, J.B., and Dixon, J.E. (1999). Inhibition of the mitogen-activated protein kinase kinase superfamily by a Yersinia effector. Science *285*, 1920–1923.

Pellet, J.B., Haefliger, J.A., Staple, J.K., Widmann, C., Welker, E., Hirling, H., Bonny, C., Nicod, P., Catsicas, S., Waeber, G., and Riederer, B.M. (2000). Spatial, temporal and subcellular localization of islet-brain 1 (IB1), a homologue of JIP-1, in mouse brain. Eur. J. Neurosci. *12*, 621–632.

Potapova, O., Gorospe, M., Dougherty, R.H., Dean, N.M., Gaarde, W.A., and Holbrook, N.J. (2000). Inhibition of c-Jun N-terminal kinase 2 expression suppresses growth and induces apoptosis of human tumor cells in a p53-dependent manner. Mol. Cell. Biol. *20*, 1713–1722.

Pulverer, B.J., Kyriakis, J.M., Avruch, J., Nikolakaki, E., and Woodgett, J.R. (1991). Phosphorylation of c-jun mediated by MAP kinases. Nature *353*, 670–674.

Reinhard, C., Shamoon, B., Shyamala, V., and Williams, L.T. (1997). Tumor necrosis factor alpha-induced activation of c-jun N-terminal kinase is mediated by TRAF2. EMBO J. *16*, 1080–1092.

Riesgo-Escovar, J.R., Jenni, M., Fritz, A., and Hafen, E. (1996). The *Drosophila* Jun-N-terminal kinase is required for cell morphogenesis but not for DJun-dependent cell fate specification in the eye. Genes Dev. *10*, 2759–2768.

Rincon, M., Whitmarsh, A., Yang, D.D., Weiss, L., Derijard, B., Jayaraj, P., Davis, R.J., and Flavell, R.A. (1998). The JNK pathway regulates the in vivo deletion of immature CD4(+)CD8(+) thymocytes. J. Exp. Med. *188*, 1817–1830.

Sabapathy, K., Hu, Y., Kallunki, T., Schreiber, M., David, J.P., Jochum, W., Wagner, E.F., and Karin, M. (1999a). JNK2 is required for efficient T-cell activation and apoptosis but not for normal lymphocyte development. Curr. Biol. *9*, 116–125.

Sabapathy, K., Jochum, W., Hochedlinger, K., Chang, L., Karin, M., and Wagner, E.F. (1999b). Defective neural tube morphogenesis and altered apoptosis in the absence of both JNK1 and JNK2. Mech. Dev. *89*, 115–124.

Schaeffer, H.J., Catling, A.D., Eblen, S.T., Collier, L.S., Krauss, A., and Weber, M.J. (1998). MP1: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade. Science *281*, 1668–1671.

Schaeffer, H.J., and Weber, M.J. (1999). Mitogen-activated protein kinases: specific messages from ubiquitous messengers. Mol. Cell. Biol. *19*, 2435–2444.

Schlessinger, J. (2000). Cell signaling by receptor tyrosine kinases. Cell *103*, this issue, 211–225.

Schreiber, M., Kolbus, A., Piu, F., Szabowski, A., Mohle-Steinlein, U., Tian, J., Karin, M., Angel, P., and Wagner, E.F. (1999). Control of cell cycle progression by c-Jun is p53 dependent. Genes Dev. *13*, 607–619.

Sheikh, M.S., Hollander, M.C., and Fornace, A.J., Jr. (2000). Role of Gadd45 in apoptosis. Biochem. Pharmacol. *59*, 43–45.

Sluss, H.K., Barrett, T., Derijard, B., and Davis, R.J. (1994). Signal transduction by tumor necrosis factor mediated by JNK protein kinases. Mol. Cell. Biol. *14*, 8376–8384.

Sluss, H.K., Han, Z., Barrett, T., Davis, R.J., and Ip, Y.T. (1996). A JNK signal transduction pathway that mediates morphogenesis and an immune response in *Drosophila*. Genes Dev. *10*, 2745–2758.

Smeal, T., Binetruy, B., Mercola, D.A., Birrer, M., and Karin, M. (1991). Oncogenic and transcriptional cooperation with Ha-Ras requires phosphorylation of c-Jun on serines 63 and 73. Nature *354*, 494–496.

Stockinger, W., Brandes, C., Fasching, D., Hermann, M., Gotthardt, M., Herz, J., Schneider, W.J., and Nimpf, J. (2000). The reelin receptor ApoER2 recruits JNK-interacting proteins-1 and 2. J. Biol. Chem. *275*, 25625–25632.

Su, G.H., Hilgers, W., Shekher, M.C., Tang, D.J., Yeo, C.J., Hruban, R.H., and Kern, S.E. (1998). Alterations in pancreatic, biliary, and breast carcinomas support MKK4 as a genetically targeted tumor suppressor gene. Cancer Res. *58*, 2339–2342.

Swat, W., Fujikawa, K., Ganiatsas, S., Yang, D., Xavier, R.J., Harris, N.L., Davidson, L., Ferrini, R., Davis, R.J., Labow, M.A., et al. (1998). SEK1/MKK4 is required for maintenance of a normal peripheral lymphoid compartment but not for lymphocyte development. Immunity *8*, 625–634.

Takaesu, G., Kishida, S., Hiyama, A., Yamaguchi, K., Shibuya, H., Irie, K., Ninomiya-Tsuji, J., and Matsumoto, K. (2000). TAB2, a novel adaptor protein, mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway. Mol. Cell *5*, 649–658.

Takekawa, M., and Saito, H. (1998). A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. Cell *95*, 521–530.

Takekawa, M., Maeda, T., and Saito, H. (1998). Protein phosphatase 2Calpha inhibits the human stress-responsive p38 and JNK MAPK pathways. EMBO J. *17*, 4744–4752.

Tanoue, T., Adachi, M., Moriguchi, T., and Nishida, E. (2000). A conserved docking motif in MAP kinases common to substrates, activators and regulators. Nat. Cell Biol. *2*, 110–116.

Teng, D.H., Perry, W.L., 3rd, Hogan, J.K., Baumgard, M., Bell, R., Berry, S., Davis, T., Frank, D., Frye, C., Hattier, T., et al. (1997). Human mitogen-activated protein kinase kinase 4 as a candidate tumor suppressor. Cancer Res. *57*, 4177–4182.

Tournier, C., Whitmarsh, A.J., Cavanagh, J., Barrett, T., and Davis, R.J. (1999). The MKK7 gene encodes a group of c-Jun NH_2-terminal kinase kinases. Mol. Cell. Biol. *19*, 1569–1581.

Tournier, C., Hess, P., Yang, D.D., Xu, J., Turner, T.K., Nimnual, A., Bar-Sagi, D., Jones, S.N., Flavell, R.A., and Davis, R.J. (2000). Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. Science *288*, 870–874.

Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding,

H.P., and Ron, D. (2000). Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science *287*, 664–666.

Waeber, G., Delplanque, J., Bonny, C., Mooser, V., Steinmann, M., Widmann, C., Maillard, A., Miklossy, J., Dina, C., Hani, E.H., et al. (2000). The gene MAPK8IP1, encoding islet-brain-1, is a candidate for type 2 diabetes. Nat. Genet. *24*, 291–295.

Wang, Y., Su, B., Sah, V.P., Brown, J.H., Han, J., and Chien, K.R. (1998). Cardiac hypertrophy induced by mitogen-activated protein kinase kinase *7*, a specific activator for c-Jun NH_2-terminal kinase in ventricular muscle cells. J. Biol. Chem. *273*, 5423–5426.

Weiss, L., Whitmarsh, A.J., Yang, D.D., Rincon, M., Davis, R.J., and Flavell, R.A. (2000). Regulation of c-Jun NH(2)-terminal kinase (Jnk) gene expression during T cell activation. J. Exp. Med. *191*, 139–146.

Whitmarsh, A.J., and Davis, R.J. (1998). Structural organization of MAP-kinase signaling modules by scaffold proteins in yeast and mammals. Trends Biochem. Sci. *23*, 481–485.

Whitmarsh, A.J., Cavanagh, J., Tournier, C., Yasuda, J., and Davis, R.J. (1998). A mammalian scaffold complex that selectively mediates MAP kinase activation. Science *281*, 1671–1674.

Woo, M., Hakem, R., Soengas, M.S., Duncan, G.S., Shahinian, A., Kagi, D., Hakem, A., McCurrach, M., Khoo, W., Kaufman, S.A., et al. (1998). Essential contribution of caspase 3/CPP32 to apoptosis and its associated nuclear changes. Genes Dev. *12*, 806–819.

Xia, Z., Dickens, M., Raingeaud, J., Davis, R.J., and Greenberg, M.E. (1995). Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science *270*, 1326–1331.

Xia, Y., Wu, Z., Su, B., Murray, B., and Karin, M. (1998). JNKK1 organizes a MAP kinase module through specific and sequential interactions with upstream and downstream components mediated by its amino-terminal extension. Genes Dev. *12*, 3369–3381.

Xia, Y., Makris, C., Su, B., Li, E., Yang, J., Nemerow, G.R., and Karin, M. (2000). MEK kinase 1 is critically required for c-Jun N-terminal kinase activation by proinflammatory stimuli and growth factor-induced cell migration. Proc. Natl. Acad. Sci. USA *97*, 5243–5248.

Xie, X., Gu, Y., Fox, T., Coll, J.T., Fleming, M.A., Markland, W., Caron, P.R., Wilson, K.P., and Su, M.S. (1998). Crystal structure of JNK3: a kinase implicated in neuronal apoptosis. Structure *6*, 983–991.

Yamaguchi, K., Nagai, S., Ninomiya-Tsuji, J., Nishita, M., Tamai, K., Irie, K., Ueno, N., Nishida, E., Shibuya, H., and Matsumoto, K. (1999). XIAP, a cellular member of the inhibitor of apoptosis protein family, links the receptors to TAB1–TAK1 in the BMP signaling pathway. EMBO J. *18*, 179–187.

Yamamoto, K., Ichijo, H., and Korsmeyer, S.J. (1999). BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. Mol. Cell. Biol. *19*, 8469–8478.

Yang, D., Tournier, C., Wysk, M., Lu, H.T., Xu, J., Davis, R.J., and Flavell, R.A. (1997a). Targeted disruption of the MKK4 gene causes embryonic death, inhibition of c-Jun NH_2-terminal kinase activation, and defects in AP-1 transcriptional activity. Proc. Natl. Acad. Sci. USA *94*, 3004–3009.

Yang, D.D., Kuan, C.Y., Whitmarsh, A.J., Rincon, M., Zheng, T.S., Davis, R.J., Rakic, P., and Flavell, R.A. (1997b). Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the *Jnk3* gene. Nature *389*, 865–870.

Yang, D.D., Conze, D., Whitmarsh, A.J., Barrett, T., Davis, R.J., Rincon, M., and Flavell, R.A. (1998). Differentiation of CD4+ T cells to Th1 cells requires MAP kinase JNK2. Immunity *9*, 575–585.

Yang, J., Boerm, M., McCarty, M., Bucana, C., Fidler, I.J., Zhuang, Y., and Su, B. (2000). Mekk3 is essential for early embryonic cardiovascular development. Nat. Genet. *24*, 309–313.

Yasuda, J., Whitmarsh, A.J., Cavanagh, J., Sharma, M., and Davis, R.J. (1999). The JIP group of mitogen-activated protein kinase scaffold proteins. Mol. Cell. Biol. *19*, 7245–7254.

Yeh, W.C., Shahinian, A., Speiser, D., Kraunus, J., Billia, F., Wakeham, A., de la Pompa, J.L., Ferrick, D., Hum, B., Iscove, N., et al. (1997). Early lethality, functional NF-kappaB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. Immunity *7*, 715–725.

Yeh, W.C., Pompa, J.L., McCurrach, M.E., Shu, H.B., Elia, A.J., Shahinian, A., Ng, M., Wakeham, A., Khoo, W., Mitchell, K., et al. (1998). FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. Science *279*, 1954–1958.

Yoshida, H., Kong, Y.Y., Yoshida, R., Elia, A.J., Hakem, A., Hakem, R., Penninger, J.M., and Mak, T.W. (1998). Apaf1 is required for mitochondrial pathways of apoptosis and brain development. Cell *94*, 739–750.

Yoshida, B.A., Dubauskas, Z., Chekmareva, M.A., Christiano, T.R., Stadler, W.M., and Rinker-Schaeffer, C.W. (1999). Mitogen-activated protein kinase kinase 4/stress-activated protein/Erk kinase 1 (MKK4/SEK1), a prostate cancer metastasis suppressor gene encoded by human chromosome 17. Cancer Res. *59*, 5483–5487.

Yujiri, T., Sather, S., Fanger, G.R., and Johnson, G.L. (1998). Role of MEKK1 in cell survival and activation of JNK and ERK pathways defined by targeted gene disruption. Science *282*, 1911–1914.

Yujiri, T., Ware, M., Widmann, C., Oyer, R., Russell, D., Chan, E., Zaitsu, Y., Clarke, P., Tyler, K., Oka, Y., et al. (2000). MEK kinase 1 gene disruption alters cell migration and c-Jun NH_2-terminal kinase regulation but does not cause a measurable defect in NF-kB activation. Proc. Natl. Acad. Sci. USA *97*, 7272–7277.

Zhang, Y., Neo, S.Y., Han, J., and Lin, S.C. (2000). Dimerization choices control the ability of axin and dishevelled to activate c-Jun NH_2-terminal kinase/stress-activated protein kinase. J. Biol. Chem. *275*, 25008–25014.