Journal of Cellular Biochemistry 102:848–858 (2007)

# Integrated Activation of MAP3Ks Balances Cell Fate in Response to Stress

**Ann M. Winter-Vann and Gary L. Johnson***

Department of Pharmacology and the Lineberger Comprehensive Cancer Center,
1108 Mary Ellen Jones Bldg, Campus Box 7365, University of North Carolina School of Medicine,
Chapel Hill, North Carolina 27599-7365

**Abstract** In vivo, tissues and organs are exposed to numerous stressors that require cells to respond appropriately for viability and homeostasis. Cells respond to these stressors, which range from UV irradiation, heat shock, chemicals, and changes in osmolality, to oxidative stress and inflammatory cytokines, by activating pathways that protect cells from damage. If the stress is too great, cells commit to undergo apoptosis. Such cell fate decisions involve the stress-mediated activation of mitogen-activated protein kinase (MAPK) networks, ultimately under the control of MAPK kinase kinases, or MAP3Ks. It is the MAP3Ks that coordinate the localization, duration and magnitude of MAPK activation in response to cell stress. A single stressor may activate several MAP3Ks, each of which impacts the balance between survival and apoptotic signaling. In this prospect article, we review the specific MAP3Ks that integrate the physiological response to cell stressors. The interrelationships among different stressors are discussed, with an emphasis on how the balance of signaling through MAP3Ks controls the MAPK response to determine cell fate. J. Cell. Biochem. 102: 848–858, 2007. 

**Key words:** stress response; MAPK networks; MAP3Ks; integrated spatio-temporal signaling

Mitogen-activated protein kinases (MAPKs) are expressed in all cells yet regulate very specific biological responses to different stimuli. An understanding of how MAPKs are regulated provides insight into how the cell uses rather common enzymes (i.e., MAPKs) to control complex and highly specific physiological responses. MAPKs are part of a three kinase signaling module composed of the MAPK, a MAPK kinase (MAP2K), and a MAPK kinase kinase (MAP3K). MAP3Ks phosphorylate and activate MAP2Ks, which in turn phosphorylate and activate MAPKs.

Three general mechanisms are used in the control of the MAP3K-MAP2K-MAPK signaling module. First, different MAP3Ks and scaffolding proteins bind and organize signaling complexes that contain upstream regulators and sometimes contain downstream substrates of the MAPKs [Johnson et al., 2005]. It is these complexes that largely control specificity in the activation of different MAPK pathways. Second, the subcellular location of different MAP3K-MAP2K-MAPK modules is critical for specificity in controlling biological responses. For example, the scaffold protein KSR recruits the Raf-MEK1/2-ERK1/2 complex to growth factor receptor tyrosine kinases, and JIP1 scaffolds an MLK3-MKK4-JNK1/2 complex that binds to kinesin motors and associates with the microtubule cytoskeleton [Johnson et al., 2005].

Abbreviations used: MAPK, mitogen-activated protein kinase; MAPKKK or MAP3K, mitogen-activated protein kinase kinase kinase; MEKK1, MEK kinase 1; JNK, c-jun amino-terminal kinase; ERK, extracellular signal-regulated protein kinase; MEKK3, MEK kinase 3; MKK, mitogen-activated protein kinase kinase; OSM, osmosensing scaffold for MEKK3; MEKK4, MEK kinase 4; TAK1, transforming growth factor β activated kinase; UV, ultraviolet; JIP3, JNK-interacting protein 3; GADD45, growth arrest and DNA damage inducible 45; TAO, thousand and one amino acid kinase; ATM, ataxia telangiectasia mutated; Hsp, heat shock protein; ASK, apoptosis signal-regulating kinase; GSTM1-1, glutathione-*S*-transferase Mu 1-1; ROS, reactive oxygen species; Trx, thioredoxin; CHIP, C-terminus of Hsp70-interacting protein; TGFβ, transforming growth factor β; TNFα, tumor necrosis factor α.

Grant sponsor: American Cancer Society; Grant number: PF-06-023-01-CSM; Grant sponsor: NIH; Grant numbers: GM30324, GM62338, DK37871.

*Correspondence to: Gary L. Johnson, Department of Pharmacology, 1108 Mary Ellen Jones Bldg, Campus Box 7365, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7365. E-mail: glj@med.unc.edu

Received 3 July 2007; Accepted 9 July 2007

DOI 10.1002/jcb.21522

The third mechanism that controls MAPK signaling is the concentration of a specific MAPK module along with its duration of activation, which can profoundly affect the biological response. A transient activation of JNK or p38, for example, might have a protective effect in a cellular response to a stress stimulus, whereas a prolonged activation of the same MAPK might lead to a commitment to apoptosis. The concentration of the MAPK also influences the magnitude of the signal and thus influences the response. The magnitude and the duration of the activation of a specific MAPK to a given stimulus are related, in large part by the expression level of the MAPK, the MAP2K and the specific MAP3K within a cell.

In addition to phosphorylating cytoplasmic substrates, MAPKs including ERK1/2, JNK, p38, and ERK5 each selectively regulate transcription factors and hence the promoter activity of different genes for control of their expression. The duration and magnitude of MAPK activation can influence the induction of specific gene expression, thus dramatically altering cell fate. A good example of this effect is seen in the regulation of specific cytokines such as IL-6 and IL-8 [Johnson and Nakamura, 2007]. Cumulatively, these mechanisms are used to maintain cellular homeostasis through the fine control of differential activation and inactivation of MAPKs with both spatial and temporal accuracy. Disruption of this spatial and temporal control, as seen with Ras or BRaf mutations that constitutively activate ERK1/2, can result in human disease such as cancer [Dhillon et al., 2007].

Of the defined members of MAPK pathways, there are 11 MAPK genes, 7 MAP2K genes, and 21 MAP3K genes [Johnson et al., 2005]. The different motifs and domains encoded in the MAP3Ks control protein–protein interactions, protein–lipid interactions, and covalent modifications such as ubiquitination, sumoylation, and phosphorylation, and provide specificity for the stimulus-dependent activation of MAP2K-MAPK pathways. Many MAP3Ks are able to regulate more than one MAP2K-MAPK pathway and therefore coordinate the activation of two MAPKs to a single stimulus. For example, MEKK1 regulates both MKK1/2-ERK1/2 and MKK4-JNK1/2, coordinating the activation of the ERK1/2 and JNK pathways in response to cell shape changes. In contrast, MEKK4 regulates the MKK7-JNK and MKK3/6-p38 pathways, coordinating the JNK and p38 pathways in response to the binding of GADD45.

Based on the described functions, MAP3Ks should be thought of as "signaling hubs" for the integration of MAPK activation with physiological responses to different stimuli. In this prospective, we describe the role of different MAP3Ks in the control of MAPK networks, primarily JNK and p38, in the response to different stress stimuli. An interesting outcome from our analysis is the description of how multiple MAP3Ks are involved in the response to individual stress stimuli. The activation of multiple MAP3Ks provides the spatio-temporal control of MAPK pathways needed for the decision process that must be balanced in determining cell fate in response to a cellular stress, which often involves recovery and survival or a commitment to cell death.

## OSMOTIC STRESS

By altering sodium levels or adding sorbitol to culture media, osmotic stress is quite easy to study in cell culture. Although osmotic stress is unquestionably important for single celled organisms, how relevant are these conditions to multicellular organisms that are capable of maintaining a homeostatically controlled environment? Certainly the cells of the kidney are subject to dramatic changes in osmolality, but other tissues are not isolated from osmotic stress. Elevated osmolality is found in lymphatic tissue, liver and spleen, and quite possibly a number of other tissues as well [Go et al., 2004]. Further, hyperglycemic conditions such as poorly controlled diabetes are known to stimulate the production of sorbitol and increase osmolality [Obrosova, 2005]. Mammalian cells are exposed to osmotic stress on a frequent basis, and several MAPK pathways are poised to respond (Fig. 1).

MEK kinase 1 (MEKK1) is among those MAP3Ks that are stimulated by osmotic stress. Yujiri et al. [1998] demonstrated that within 30 min, MEKK1 responds to sorbitol-induced osmotic stress by activating both c-Jun amino-terminal kinase (JNK) and extracellular signal-regulated protein kinase (ERK1/2). In addition to its kinase domain, MEKK1 contains an E3 ubiquitin ligase activity. In an interesting twist, when osmotic stress is continued over 4–6 h, MEKK1 downregulates signaling through the JNK pathway by ubiquitinating the JNK

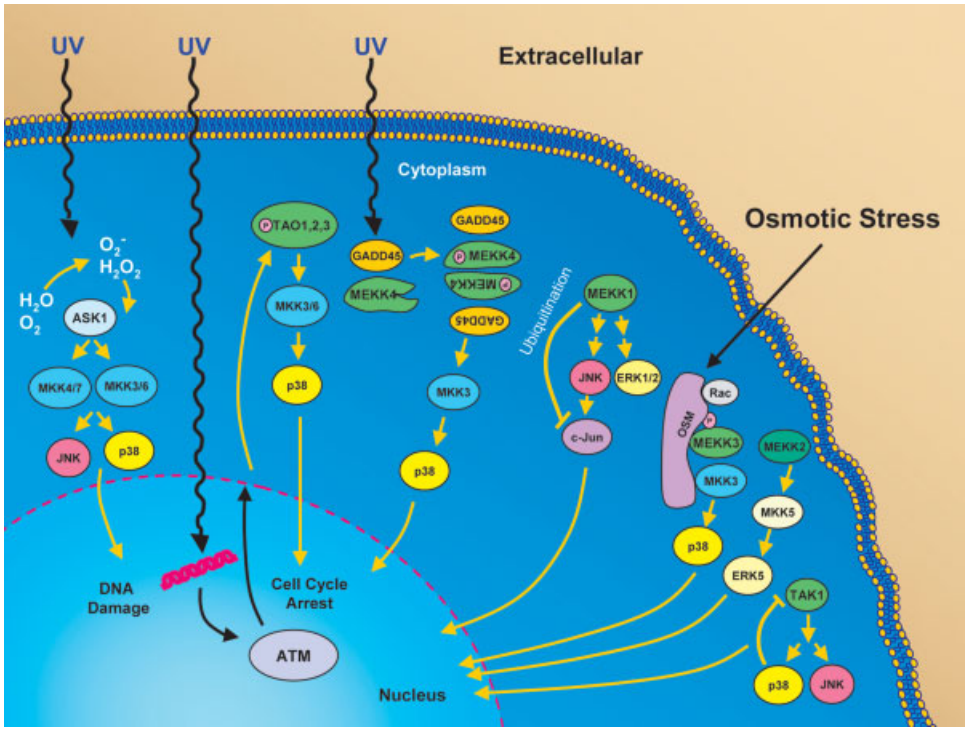


**Fig. 1.** In addition to generating reactive oxygen species (ROS) and DNA damage, ultraviolet light can activate the TAO, MEKK4, and MEKK1 pathways. These pathways can activate p38 and in some cases cause cell cycle arrest. Osmotic stress can cause cell shrinkage and cytoskeletal stress, and activates the MEKK1, MEKK2, MEKK3, and TAK1 MAP3K pathways. TAK and MEKK3 signal to p38. MEKK1 signals to JNK, but long-term stimulation of this pathway leads to MEKK1-stimulated ubiquitination of the JNK substrate, c-Jun, as described in the text.

substrate c-Jun, targeting it for degradation. Under these conditions, signaling through MEKK1 to JNK has a protective effect; subsequent degradation of c-Jun promotes apoptosis [Xia et al., 2007].

MEK kinases 2 and 3 (MEKK2 and MEKK3) also respond to osmotic stress. For MEKK2, activation by osmotic stress stimulates ERK5, a MAPK that regulates PPARγ, MEF2c, and specific gene expression in different cell types [Nakamura et al., 2006]. Following stimulation, MEKK3 is activated by autophosphorylation [Fritz et al., 2006] and signals downstream to MKK3/6 and p38. Activation of p38 then stimulates the transcription of genes containing the tonicity enhancer element (also known as the osmotic response element) [Padda et al., 2006]. The activation of MEKK3 is mediated by the binding of MEKK3, MKK3, and the small G-protein Rac1 to a scaffolding protein known as OSM (osmosensing scaffold for MEKK3, also called CCM2) [Uhlik et al., 2003].

Research in *Drosophila* and in yeast suggests that MEKK4 (*Drosophila* D-MEKK1 and Ssk2p in yeast) is also responsive to osmotic stress, activating p38. In yeast, mammalian MEKK4 is capable of replacing mutated Ssk2p, and responds to osmotic stress by signaling to the yeast homolog of p38 [Bettinger and Amberg, 2007]. In *Drosophila*, *D-MEKK1* mutant flies are sensitive to osmotic stress, where D-MEKK1 signals to p38 [Inoue et al., 2001].

One component of the cellular response to osmotic stress is an increase in the formation of reactive oxygen species (ROS; Fig. 1). ASK1 is highly responsive to ROS and potentially will be activated in response to any stimulus that elevates ROS in cells [Matsukawa et al., 2004]. As discussed below, prolonged activation of ASK1 can lead to cell death. The MAP3K transforming growth factor-β activated kinase-1 (TAK1) has also been shown to respond to osmotic stress, but TAK1 mediates only a modest, very transient rise in the phosphorylation of p38. TAK1 is feedback inhibited by p38 activation; in the presence of a p38 inhibitor, the activation of TAK1 in response to sorbitol is quite robust, and is maintained over a much

longer period of time [Cheung et al., 2003]. Since several other MAP3K pathways feed into p38, these data suggest that TAK1 activity can be suppressed not only by TAK1 signaling, but by the activation of other MAP3K pathways as well.

## UV IRRADIATION

UV irradiation strongly activates JNK and p38 [Ding et al., 2002; Gallagher et al., 2002; Song and Lee, 2007], probably through several different pathways (Fig. 1). MEKK1 is involved in the UV stimulated activation of JNK. In DU-145 cells, UVC stimulation increased the association of MEKK1, MKK4, and JIP3, a JNK scaffolding protein. Moreover, siRNA knockdown of MEKK1 significantly decreased the UV-stimulated phosphorylation of JNK [Song and Lee, 2007]. Gallagher et al. [2002] reported that following UV treatment, serine 67 of MEKK1 is dephosphorylated. This dephosphorylation of MEKK1 leads to an increased affinity for JNK, and correlates with increased JNK activation. MEKK1 signaling to JNK also stimulates the expression of the protease urokinase-type plasminogen activator [Witowsky et al., 2003]. Consistent with a UV-MEKK1-MKK4-JNK signaling pathway, urokinase-type plasminogen activator expression and protease activity is increased by UV irradiation [Miralles et al., 1998].

In contrast to these reports, Yujiri et al. [1998] found that the UV stimulation of JNK is normal in mouse embryonic stem cells lacking MEKK1, suggesting that MEKK1 is not the only MAP3K to respond to UV irradiation. Indeed, UV stimulation of *Drosophila* cells leads to the activation of D-p38 downstream of D-MEKK1 (the equivalent of mammalian MEKK4) [Zhuang et al., 2006]. Mammalian MEKK4, which activates p38 and JNK, is activated by a family of stress-inducible proteins known as growth arrest and DNA damage inducible 45 (GADD45) $\alpha/\beta/\gamma$. These proteins bind directly to MEKK4 and induce MEKK4 to form head-to-tail dimers, which then transautophosphorylate and become active [Miyake et al., 2007]. GADD45 proteins are induced by UV-stimulation, suggesting that MEKK4 may be activated by GADD45 proteins in response to UV irradiation, signaling to both p38 and JNK [Takekawa and Saito, 1998].

The TAO family of MAP3K (TAO1/2/3) is also activated by UV irradiation, increasing TAO autophosphorylation by two to threefold. siRNA knockdown of any of the TAO proteins led to a 50% decrease in the UV-stimulated activation of p38, and correlated with a decrease in p38 activation and a decrease in the UV-induced G2/M cell cycle arrest [Raman et al., 2007]. The DNA damage kinase ataxia telangiectasia mutated (ATM) phosphorylates TAO1 and 3 in vitro, suggesting that ATM lies upstream of UV-induced TAO activation and that perhaps it is UV-induced DNA damage, and not UV irradiation itself, that stimulates TAO activity.

In addition to inducing DNA damage, UV irradiation also produces ROS. This leads to the question of whether the activation of p38 and JNK in response to UV stimulation is due to the ultraviolet irradiation itself, or to the production of ROS (or in fact due to UV-induced DNA damage, as discussed above). Through the use of general and specific oxyradical scavengers, Ding et al. [2002] demonstrated that some, but not all, of the UV-stimulated activation of p38 and JNK is due to the production of oxygen radicals. In vivo, it is likely that all of these factors feed into overlapping pathways leading to the activation of p38 and JNK.

## HEAT SHOCK

Cells respond to elevated temperature by activating the p38 and JNK pathways (Fig. 2). The activation of JNK is normal in cells that lack MEKK1, suggesting that this MAP3K is not involved [Yujiri et al., 1998]. However, cellular responses to heat stress are decreased in cells expressing a kinase-inactive mutant of MEKK4 (K1361 $\rightarrow$ R). In control cells, heat shock stimulated the phosphorylation of p38 and of a downstream target, Hsp27. In $MEKK4^{K1361R}$ mouse embryo fibroblasts, these responses were greatly diminished. Hsp27 is involved in protecting the actin cytoskeleton during heat shock. As one functional consequence of the $MEKK4^{K1361R}$ mutation, heat shocked cells were unable to maintain their actin cytoskeletal structure [Abell et al., 2005].

In *Drosophila*, heat shock stimulates the phosphorylation and activation of p38 through both D-MEKK1 (MEKK4) and D-ASK1 [Zhuang et al., 2006]. Mammalian ASK1 is bound to and inhibited by glutathione-*S*-transferase Mu1-1 (GSTM1-1). Following stimulation by heat shock, GSTM1-1 dissociates from ASK1, allowing ASK1 to activate the p38 pathway [Dorion et al., 2002]. ASK1 signaling in response to heat

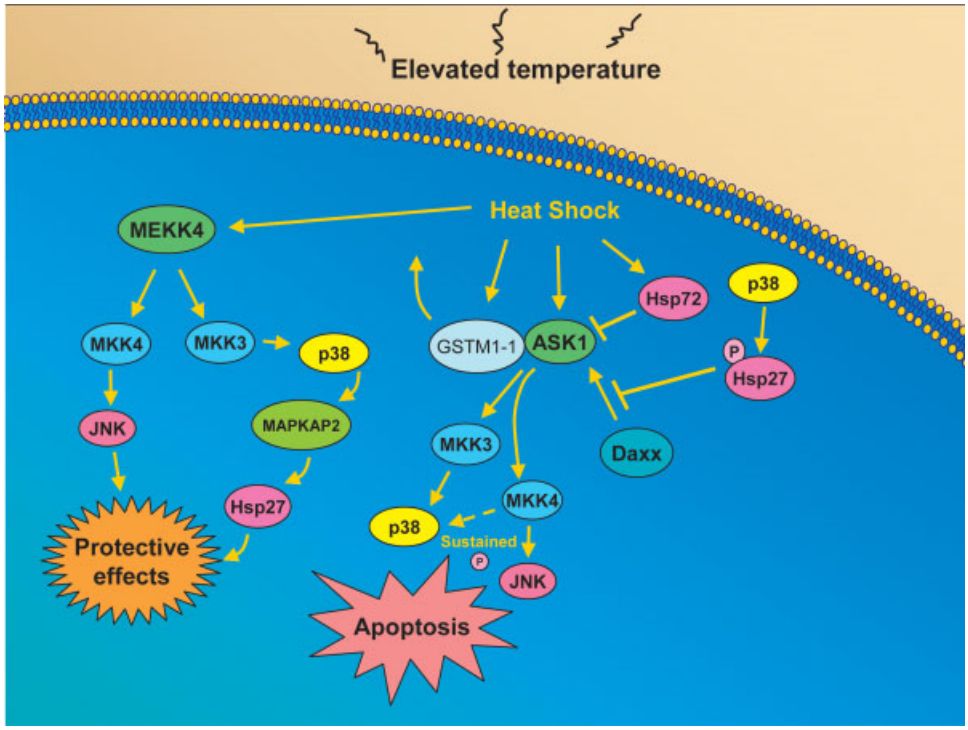


**Fig. 2.** MEKK4 is activated by heat shock, leading to stimulation of MKK3 and MKK4 followed by JNK and p38 activation. ASK1 signaling is stimulated by a decreased interaction with GSTM1-1. ASK1 is inhibited by Hsp72, and the activation of Hsp27 decreases the association of ASK1 with the pro-apoptotic protein Daxx.

shock is complicated, however, because ASK1 is also inhibited by heat shock. As Park et al. [2002] demonstrated, the heat shock protein Hsp72 binds to ASK1 and prevents its dimerization and activation. This interaction, stimulated by a mild heat shock, had the effect of protecting $H_2O_2$ treated cells from apoptosis.

ASK1 is further regulated by other heat shock proteins. Hsp90 forms a ternary complex with ASK1 and Akt. From within this complex, ASK1 is phosphorylated at a consensus Akt site and inactivated [Zhang et al., 2005]. ASK1 interacts with the pro-apoptotic protein Daxx to promote apoptosis. Upon activation, the small heat shock protein Hsp27 competes with this interaction and protects cells from apoptosis [Charette et al., 2000]. As mentioned above, MEKK4 activity promotes the phosphorylation and activation of Hsp27, suggesting that MEKK4 activity may inhibit ASK1-mediated apoptosis.

## OXIDATIVE STRESS

Reactive oxygen species consist of superoxide anion ($O_2^{\cdot -}$), hydroxyl radicals ($OH^{\cdot}$), and hydrogen peroxide ($H_2O_2$), as well as nitric oxide ($NO^{\cdot}$) and the highly reactive molecule peroxynitrite ($ONOO^-$). Oxidative stress is present when the production of ROS in a cell exceeds the cell's capacity to detoxify them. ROS can be produced by a number of cellular events, with a major source being aerobic respiration. Aerobic respiration produces a large amount of $O_2^{\cdot -}$ which is typically contained and reduced within the mitochondria. ROS can also be produced by inflammatory processes, ionizing radiation, and many chemotherapeutic drugs. UV irradiation also produces several distinct species of ROS [Ding et al., 2002]. It is common for one ROS to react with molecules within the cell and produce other ROS. These molecules vary dramatically in their half-lives, ranging from fractions of a second to hours, and also vary in their ability to cross cell membranes [Karihtala and Soini, 2007; Valko et al., 2007].

Several families of proteins have evolved to scavenge oxygen radicals. Within the mitochondria, the superoxide dismutases convert $O_2^{\cdot -}$ to oxygen and $H_2O_2$; these reactions are the major source of hydrogen peroxide within a cell. Catalase reduces hydrogen peroxide to water and oxygen, and peroxidases reduce peroxides. Thioredoxin along with glutathione and glutathione peroxidases reduce hydrogen peroxide

[reviewed in Karihtala and Soini, 2007]. The contribution of antioxidants from food is still poorly understood, but dietary antioxidants include vitamins C and E, glutathione, carotenoids, and flavonoids [Martindale and Holbrook, 2002].

These ROS are physiologically important on many levels. First of all, they directly damage DNA, proteins, and phospholipids. ROS can also physically modify many proteins, potentially inhibiting or increasing their enzymatic activity. Several protein tyrosine phosphatases can be inhibited by oxidation of the catalytic cysteine, whereas several cysteines in protein kinase C can be oxidized, leading to increased activity [Valko et al., 2007]. $NO^{\cdot}$ has been well characterized as a secondary messenger, capable of signaling to distant sites [Karihtala and Soini, 2007]. Hydrogen peroxide, a ROS with a relatively long half-life and good membrane permeability, can also function as a second messenger. $H_2O_2$ is involved in growth factor and oncogene signaling, stimulating cell proliferation and contributing to a transformed phenotype [Sundaresan et al., 1995; Bae et al., 1997; Joneson and Bar-Sagi, 1998; Suh et al., 1999]. Many tumors have elevated $H_2O_2$, which is thought to participate in increased cell growth [Fruehauf and Meyskens, 2007].

Oxidative stress activates both JNK and p38 through the activation of several MAP3Ks, the best characterized of which is ASK1. Stimulation of ASK1 by oxidative stress leads to the activation of p38 and JNK, followed by apoptosis. Although $H_2O_2$ can transiently activate p38 and JNK in $ASK1^{-/-}$ cells, ASK1 is necessary for sustained p38 and JNK activation as well as for apoptosis following sustained ROS elevation [Tobiume et al., 2001]. Hydrogen peroxide promotes the dimerization of ASK1, along with autophosphorylation at Thr 838. This autophosphorylation seems to be essential for ASK1 kinase activity, and forced dimerization of ASK1 is sufficient to stimulate autophosphorylation [Gotoh and Cooper, 1998; Matsukawa et al., 2004]. In addition to forming homodimers, the ASK1 and 2 proteins can form ASK1/ASK2 heterodimers. In the absence of ASK2, $H_2O_2$-stimulated JNK activation is greatly diminished, suggesting that ASK2 is necessary to activate that arm of the signaling pathway [Takeda et al., 2007].

The activation of ASK1 by oxidative stress is highly controlled because of the potential consequence of inducing apoptosis with pronounced and prolonged ASK1 activation. The redox-regulated protein thioredoxin binds to and inhibits ASK1 activation; under conditions of oxidative stress, thioredoxin is oxidized and releases ASK1, allowing for activation of ASK1 signaling [Saitoh et al., 1998]. Thioredoxin is also capable of stimulating the ubiquitination and degradation of ASK1, resulting in decreased signaling through this pathway [Liu and Min, 2002]. Oxidative stress induces the expression of many heat shock proteins. As mentioned above, cells are protected from $H_2O_2$-stimulated apoptosis through Hsp90/Akt and Hsp72-mediated inhibition of ASK1 [Park et al., 2002; Zhang et al., 2005]. In addition, the Hsp70 co-chaperone CHIP binds to and ubiquitinates ASK1, leading to ASK1 degradation and a decrease in $H_2O_2$-stimulated JNK activity [Hwang et al., 2005]. All together, these data suggest that ASK1 and ASK2 are activated by oxidative stress, leading to p38 and JNK activation and apoptosis, and that many mechanisms exist to modulate this activation and protect cells from ASK-mediated apoptosis. It is interesting to note that many tumors have modestly elevated levels of ROS, presumably related to their mitogenic effect [Suh et al., 1999; Fruehauf and Meyskens, 2007]. It has also been reported that many tumors and tumor-derived cell lines have elevated thioredoxin or glutathione-*S*-transferase Mu [Dolado et al., 2007; Karihtala and Soini, 2007], both of which inhibit ASK1; it is likely that overexpression of these proteins suppresses ASK1 activity, allowing tumor cells to evade apoptosis under conditions of chronic oxidative stress.

Other MAP3Ks are also activated by oxidative stress. Studies in *Drosophila* suggest that MEKK4 may afford protection from oxidative stress-mediated apoptosis [Brun et al., 2006]. In addition to MEKK4, MEKK1 also protects cells from apoptosis during oxidative stress. Within minutes of treatment with $H_2O_2$, cardiac myocytes activate JNK and p38. In cardiac myocytes that lack MEKK1, p38 activation is normal but activation of JNK is lost; these cells subsequently undergo apoptosis at a much higher rate [Minamino et al., 1999]. Interestingly, MEKK1 is also inhibited by oxidative stress. A cysteine within the ATP-binding site of MEKK1 is modified by glutathione under conditions of oxidative stress, and this specific modification inhibits MEKK1 activity [Cross

and Templeton, 2004]. Under the same high oxidative conditions, ASK1 activity was stimulated, suggesting that oxidative stress affects the balance between cell survival (MEKK1) and cell death (ASK1).

## ISCHEMIA/REPERFUSION INJURY

Ischemia/reperfusion injury is a special case of oxidative damage. Cells in an organ are deprived of oxygen for a period of time, after which oxygen is re-introduced. This is a fairly common pathological stress, occurring during myocardial infarction and stroke, as well as surgical procedures such as cardiopulmonary bypass or organ transplant. Some damage results from hypoxia, and can lead to necrosis. However, a great deal of damage occurs from a dramatic increase in ROS following re-oxygenation [Werns and Lucchesi, 1990; Valko et al., 2007]. This burst of oxidative stress damages cellular molecules as described above, and stimulates a number of signaling pathways leading to MAPK activation. Although ischemia stimulates p38 activity, subsequent reperfusion stimulates both the p38 and JNK pathways [Bogoyevitch et al., 1996] (Fig. 3).

MEKK1 is involved in JNK stimulation in response to reperfusion. As mentioned above, MEKK1 stimulates JNK activity in response to oxidative stress, protecting cardiac myocytes from apoptosis. In this case, signaling through MEKK1 to JNK can protect neighboring cells, because although p38 stimulated the production of tumor necrosis factor-α (TNF-α), JNK activity inhibited synthesis of TNFα, providing a survival signal to the cells [Minamino et al., 1999]. MEKK1 signaling in cardiac myocytes may have a negative long-term effect, however, because MEKK1-JNK signaling has been implicated in the process of cardiac hypertrophy, which can lead to mechanical dysfunction and heart failure [Bogoyevitch et al., 1996; Minamino et al., 1999]. These findings demonstrate the importance of the duration of a MAPK response, where a short JNK response can be protective but a prolonged response induces apoptosis.

In addition to activating MEKK1, reperfusion may also stimulate TAK1. Although TAK1

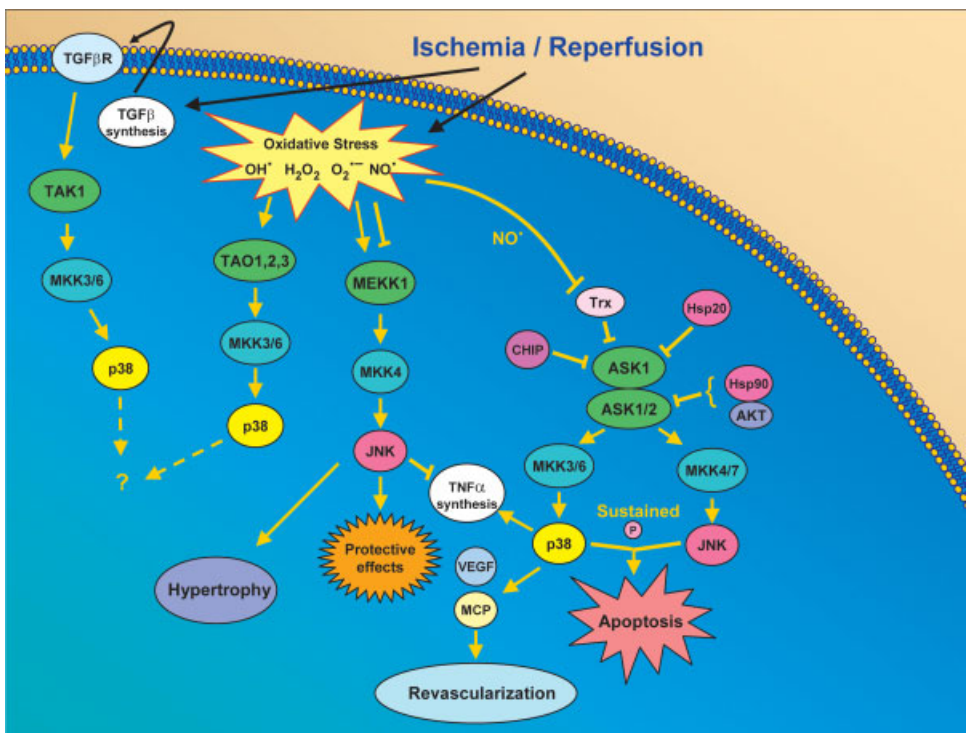


**Fig. 3.** Reperfusion following ischemia leads to a burst in the production of oxidative radicals. This can stimulate the TAK1, TAO, and ASK pathways, and can both stimulate and inhibit MEKK1 activation. The regulation of ASK is again rather complex, with ASK activity regulated by thioredoxin and the heat shock proteins Hsp20, Hsp90, and CHIP. Sustained activation of the ASK pathway leads to apoptosis, while activation of MEKK1 leads to protective effects.

activity was not affected by hypoxia, Frazier et al. [2007] reported an increase in TAK1 activity within 30 min of initiating reperfusion. This increase in TAK1 activity correlated with an increase in the phosphorylation of JNK and a physical association between TAK1 and JNK. Expression of a dominant-negative TAK1 led to increased apoptosis, suggesting that TAK1 signaling plays a protective role. In a rat model of cardiac infarction, both TAK1 levels as well as TAK1 activity were increased in neighboring, non-infarcted cardiac tissue, and this correlated with an increase in the phosphorylation of MKK3/6 and p38. The same tissues showed a parallel increase in transforming growth factor-β (TGF-β), suggesting that this cytokine may be driving TAK1 activation [Matsumoto-Ida et al., 2006].

During ischemia and reperfusion, levels of nitric oxide are greatly increased, leading both to increased nitration of proteins and to NO·-induced apoptosis [Andreka et al., 2001; Tao et al., 2006; Zweier and Talukder, 2006]. Cells are protected from NO·-induced apoptosis by JNK activation, in a signaling pathway that may go through MEKK1 [Andreka et al., 2001]. Another consequence of increased NO· is that thioredoxin is nitrated. As described by Tao et al. [2006], nitrated thioredoxin is unable to bind to ASK1 and protect cells from apoptosis. These findings again suggest that there is a complex, highly regulated integration and balance of multiple MAP3Ks that are activated in response to a stress like ischemia/reperfusion. It is the balance of timing, the magnitude of response and the multiplicity of MAPK responses that contribute to the determination of cell fate.

## DISCUSSION

The activation of MAPK signaling pathways in response to cell stress is a complex process. As shown in Figure 4, stressors themselves are overlapping in their biochemical consequence and cellular response. Pharmacologically, many chemotherapy drugs induce DNA damage, and also increase oxidative stress; other chemotherapeutics such as taxol induce cytoskeletal stress. Elevated sorbitol, a source of osmotic stress in diabetics, induces cytoskeletal stress by virtue of cell shrinkage but also leads to increased oxidative stress (Fig. 1). Ultraviolet light induces DNA damage, and also stimulates the production of several types of ROS (Fig. 1). Ischemia and reperfusion, along with oxidative stress in general, lead to increased cytokine production; cytokine release can activate a number of MAPK pathways, and can in turn lead to increased oxidative stress. Heat shock induces the expression of proteins that can alter MAP3K activation in response to other stressors (Fig. 2). Experimentally, it is interesting to isolate and study each cell stressor individually and discover which stimuli trigger which specific MAP3K and downstream MAPKs. From a physiological standpoint, what is essential to understand are the mechanisms by which cells

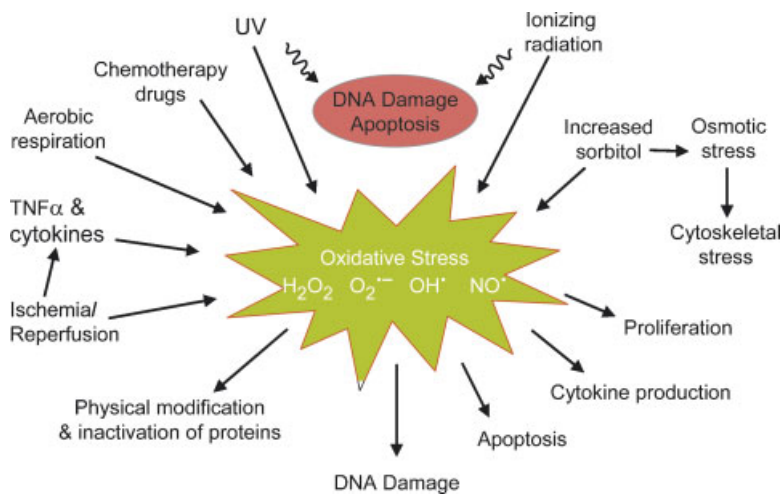


**Fig. 4.** A number of cellular stressors lead to an increase in intracellular levels of ROS, as shown here. Elevated ROS produce many effects, ranging from increased proliferation to increased cytokine production and apoptosis. ROS also damage or modify proteins, lipids, and DNA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

recognize different stressors and induce an integrated spatio-temporal activation of different MAP3Ks for the control of the MAPK signaling network for cellular homeostasis.

From the descriptions of cellular stress responses given above, clearly cell stress can activate both protective and pro-apoptotic MAP3K pathways at the same time. In the examples we have provided, the pro-apoptotic pathway was often controlled by ASK1, with protective responses involving MEKK1, MEKK4, TAO or TAK1. It is exactly this integration of multiple MAP3Ks we describe that is responsible for how the MAPK network is integrated into the physiological response to stressors. Several MAPK responses activated by specific MAP3Ks also have a feedback regulation to control the duration and magnitude of the response. For example, ubiquitination of c-jun is induced by persistent MEKK1 activation after prolonged osmotic stress [Xia et al., 2007]. In other cell conditions, one MAPK pathway can inhibit another within the activated MAPK signaling network; p38 is activated by several pathways, and p38 can phosphorylate and inhibit TAK1 [Cheung et al., 2003]. In addition, MEKK4 activity leads to the phosphorylation and activation of Hsp27 [Abell et al., 2005]; Hsp27 inhibits the interaction between ASK1 and Daxx to decrease apoptosis [Charette et al., 2000]. One stimulus may also have opposing effects on two pathways, such as the effect of ROS on MEKK1 and ASK1 signaling. MEKK1 is glutathionylated and inhibited during oxidative stress, but when thioredoxin is oxidized, it releases its inhibition of ASK1 [Cross and Templeton, 2004]. Finally, the expression of scaffolding proteins such as KSR, OSM, and JIP3 facilitates differential spatio-temporal signaling through specific MAP3K-MAP2K-MAPK modules by the scaffold's ability to organize specific protein complexes that include upstream regulators of specific MAP3Ks (i.e., GTPases, kinases, E3 ligases) [Uhlik et al., 2003; Song and Lee, 2007].

Osmotic changes, heat shock, ultraviolet irradiation, and oxidative stress are all fairly common cellular insults, and cells have evolved mechanisms to deal with them. Often, a low level or short-term stress will stimulate a protective or adaptive response, and larger or sustained stress will stimulate apoptosis. The switch between these two states is of tremendous importance in medicine, because controlling that balance point pharmacologically could enable cells to survive an ischemia/reperfusion insult, or perhaps stimulate apoptosis in tumor cells that use elevated ROS to increase cell proliferation. What is required is a detailed understanding of the integrated response of the MAPK signaling network that allows effective therapeutic intervention to protect cells and tissues from the stressors relevant in physiology and pathophysiology. The future will bring targeting of specific MAP3Ks or families of MAP3Ks to selectively inhibit stimulus-specific activation of MAPK networks and gene expression. Inhibition of specific MAP3Ks will leave intact the MAPK responses from other MAP3K pathways. Scientifically, what is needed are continued genetic, proteomic, and pharmacological approaches to define MAP3Ks as targets for control of the MAPK network in different diseases.

## ACKNOWLEDGMENTS

We would like to thank Betsy Clarke for the graphic illustrations. We are grateful to the American Cancer Society (AMW) and the NIH (GLJ) for financial support.

## REFERENCES

Abell AN, Rivera-Perez JA, Cuevas BD, Uhlik MT, Sather S, Johnson NL, Minton SK, Lauder JM, Winter-Vann AM, Nakamura K, Magnuson T, Vaillancourt RR, Heasley LE, Johnson GL. 2005. Ablation of MEKK4 kinase activity causes neurulation and skeletal patterning defects in the mouse embryo. Mol Cell Biol 25:8948–8959.

Andreka P, Zang J, Dougherty C, Slepak TI, Webster KA, Bishopric NH. 2001. Cytoprotection by Jun kinase during nitric oxide-induced cardiac myocyte apoptosis. Circ Res 88:305–312.

Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, Rhee SG. 1997. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. J Biol Chem 272: 217–221.

Bettinger BT, Amberg DC. 2007. The MEK kinases MEKK4/Ssk2p facilitate complexity in the stress signaling responses of diverse systems. J Cell Biochem 101: 34–43.

Bogoyevitch MA, Gillespie-Brown J, Ketterman AJ, Fuller SJ, Ben-Levy R, Ashworth A, Marshall CJ, Sugden PH. 1996. Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart. p38/RK mitogen-activated protein kinases and c-Jun N-terminal kinases are activated by ischemia/reperfusion. Circ Res 79:162–173.

Brun S, Vidal S, Spellman P, Takahashi K, Tricoire H, Lemaitre B. 2006. The MAPKKK Mekk1 regulates the

expression of Turandot stress genes in response to septic injury in Drosophila. Genes Cells 11:397–407.
Charette SJ, Lavoie JN, Lambert H, Landry J. 2000. Inhibition of Daxx-mediated apoptosis by heat shock protein 27. Mol Cell Biol 20:7602–7612.
Cheung PC, Campbell DG, Nebreda AR, Cohen P. 2003. Feedback control of the protein kinase TAK1 by SAPK2a/p38alpha. EMBO J 22:5793–5805.
Cross JV, Templeton DJ. 2004. Oxidative stress inhibits MEKK1 by site-specific glutathionylation in the ATP-binding domain. Biochem J 381:675–683.
Dhillon AS, Hagan S, Rath O, Kolch W. 2007. MAP kinase signalling pathways in cancer. Oncogene 26:3279–3290.
Ding M, Li J, Leonard SS, Shi X, Costa M, Castranova V, Vallyathan V, Huang C. 2002. Differential role of hydrogen peroxide in UV-induced signal transduction. Mol Cell Biochem 234-235:81–90.
Dolado I, Swat A, Ajenjo N, De Vita G, Cuadrado A, Nebreda AR. 2007. p38alpha MAP kinase as a sensor of reactive oxygen species in tumorigenesis. Cancer Cell 11: 191–205.
Dorion S, Lambert H, Landry J. 2002. Activation of the p38 signaling pathway by heat shock involves the dissociation of glutathione S-transferase Mu from Ask1. J Biol Chem 277:30792–30797.
Frazier DP, Wilson A, Dougherty CJ, Li H, Bishopric NH, Webster KA. 2007. PKC-alpha and TAK-1 are intermediates in the activation of c-Jun NH2-terminal kinase by hypoxia-reoxygenation. Am J Physiol Heart Circ Physiol 292:H1675–H1684.
Fritz A, Brayer KJ, McCormick N, Adams DG, Wadzinski BE, Vaillancourt RR. 2006. Phosphorylation of serine 526 is required for MEKK3 activity, and association with 14-3-3 blocks dephosphorylation. J Biol Chem 281:6236–6245.
Fruehauf JP, Meyskens FL, Jr. 2007. Reactive oxygen species: A breath of life or death? Clin Cancer Res 13: 789–794.
Gallagher ED, Xu S, Moomaw C, Slaughter CA, Cobb MH. 2002. Binding of JNK/SAPK to MEKK1 is regulated by phosphorylation. J Biol Chem 277:45785–45792.
Go WY, Liu X, Roti MA, Liu F, Ho SN. 2004. NFAT5/TonEBP mutant mice define osmotic stress as a critical feature of the lymphoid microenvironment. Proc Natl Acad Sci USA 101:10673–10678.
Gotoh Y, Cooper JA. 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.
Hwang JR, Zhang C, Patterson C. 2005. C-terminus of heat shock protein 70-interacting protein facilitates degradation of apoptosis signal-regulating kinase 1 and inhibits apoptosis signal-regulating kinase 1-dependent apoptosis. Cell Stress Chaperones 10:147–156.
Inoue H, Tateno M, Fujimura-Kamada K, Takaesu G, Adachi-Yamada T, Ninomiya-Tsuji J, Irie K, Nishida Y, Matsumoto K. 2001. A Drosophila MAPKKK, D-MEKK1, mediates stress responses through activation of p38 MAPK. EMBO J 20:5421–5430.
Johnson GL, Nakamura K. 2007. The c-jun kinase/stress-activated pathway: Regulation, function and role in human disease. Biochim Biophys Acta, in press.
Johnson GL, Dohlman HG, Graves LM. 2005. MAPK kinase kinases (MKKKs) as a target class for small-molecule inhibition to modulate signaling networks and gene expression. Curr Opin Chem Biol 9:325–331.
Joneson T, Bar-Sagi D. 1998. A Rac1 effector site controlling mitogenesis through superoxide production. J Biol Chem 273:17991–17994.
Karihtala P, Soini Y. 2007. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. Apmis 115:81–103.
Liu Y, Min W. 2002. Thioredoxin promotes ASK1 ubiquitination and degradation to inhibit ASK1-mediated apoptosis in a redox activity-independent manner. Circ Res 90:1259–1266.
Martindale JL, Holbrook NJ. 2002. Cellular response to oxidative stress: Signaling for suicide and survival. J Cell Physiol 192:1–15.
Matsukawa J, Matsuzawa A, Takeda K, Ichijo H. 2004. The ASK1-MAP kinase cascades in mammalian stress response. J Biochem (Tokyo) 136:261–265.
Matsumoto-Ida M, Takimoto Y, Aoyama T, Akao M, Takeda T, Kita T. 2006. Activation of TGF-beta1-TAK1-p38 MAPK pathway in spared cardiomyocytes is involved in left ventricular remodeling after myocardial infarction in rats. Am J Physiol Heart Circ Physiol 290:H709–H715.
Minamino T, Yujiri T, Papst PJ, Chan ED, Johnson GL, Terada N. 1999. MEKK1 suppresses oxidative stress-induced apoptosis of embryonic stem cell-derived cardiac myocytes. Proc Natl Acad Sci USA 96:15127–15132.
Miralles F, Parra M, Caelles C, Nagamine Y, Felez J, Munoz-Canoves P. 1998. UV irradiation induces the murine urokinase-type plasminogen activator gene via the c-Jun N-terminal kinase signaling pathway: Requirement of an AP1 enhancer element. Mol Cell Biol 18: 4537–4547.
Miyake Z, Takekawa M, Ge Q, Saito H. 2007. Activation of MTK1/MEKK4 by GADD45 through induced N-C dissociation and dimerization-mediated trans autophosphorylation of the MTK1 kinase domain. Mol Cell Biol 27:2765–2776.
Nakamura K, Uhlik MT, Johnson NL, Hahn KM, Johnson GL. 2006. PB1 domain-dependent signaling complex is required for extracellular signal-regulated kinase 5 activation. Mol Cell Biol 26:2065–2079.
Obrosova IG. 2005. Increased sorbitol pathway activity generates oxidative stress in tissue sites for diabetic complications. Antioxid Redox Signal 7:1543–1552.
Padda R, Wamsley-Davis A, Gustin MC, Ross R, Yu C, Sheikh-Hamad D. 2006. MEKK3-mediated signaling to p38 kinase and TonE in hypertonically stressed kidney cells. Am J Physiol Renal Physiol 291:F874–F881.
Park HS, Cho SG, Kim CK, Hwang HS, Noh KT, Kim MS, Huh SH, Kim MJ, Ryoo K, Kim EK, Kang WJ, Lee JS, Seo JS, Ko YG, Kim S, Choi EJ. 2002. Heat shock protein hsp72 is a negative regulator of apoptosis signal-regulating kinase 1. Mol Cell Biol 22:7721–7730.
Raman M, Earnest S, Zhang K, Zhao Y, Cobb MH. 2007. TAO kinases mediate activation of p38 in response to DNA damage. EMBO J 26:2005–2014.
Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, Ichijo H. 1998. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J 17:2596–2606.
Song JJ, Lee YJ. 2007. Differential activation of the JNK signal pathway by UV irradiation and glucose deprivation. Cell Signal 19:563–572.

Suh YA, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK, Lambeth JD. 1999. Cell transformation by the superoxide-generating oxidase Mox1. Nature 401:79–82.

Sundaresan M, Yu ZX, Ferrans VJ, Irani K, Finkel T. 1995. Requirement for generation of $H_2O_2$ for platelet-derived growth factor signal transduction. Science 270:296–299.

Takeda K, Shimozono R, Noguchi T, Umeda T, Morimoto Y, Naguro I, Tobiume K, Saitoh M, Matsuzawa A, Ichijo H. 2007. Apoptosis signal-regulating kinase (ASK) 2 functions as a mitogen-activated protein kinase kinase kinase in a heteromeric complex with A SK1. J Biol Chem 282: 7522–7531.

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

Tao L, Jiao X, Gao E, Lau WB, Yuan Y, Lopez B, Christopher T, RamachandraRao SP, Williams W, Southan G, Sharma K, Koch W, Ma XL. 2006. Nitrative inactivation of thioredoxin-1 and its role in postischemic myocardial apoptosis. Circulation 114:1395–1402.

Tobiume K, Matsuzawa A, Takahashi T, Nishitoh H, Morita K, Takeda K, Minowa O, Miyazono K, Noda T, Ichijo H. 2001. ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. EMBO Rep 2: 222–228.

Uhlik MT, Abell AN, Johnson NL, Sun W, Cuevas BD, Lobel-Rice KE, Horne EA, Dell'Acqua ML, Johnson GL. 2003. Rac-MEKK3-MKK3 scaffolding for p38 MAPK activation during hyperosmotic shock. Nat Cell Biol 5: 1104–1110.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84.

Werns SW, Lucchesi BR. 1990. Free radicals and ischemic tissue injury. Trends Pharmacol Sci 11:161–166.

Witowsky J, Abell A, Johnson NL, Johnson GL, Cuevas BD. 2003. MEKK1 is required for inducible urokinase-type plasminogen activator expression. J Biol Chem 278:5941–5946.

Xia Y, Wang J, Xu S, Johnson GL, Hunter T, Lu Z. 2007. MEKK1 mediates the ubiquitination and degradation of c-Jun in response to osmotic stress. Mol Cell Biol 27:510–517.

Yujiri T, Sather S, Fanger GR, Johnson GL. 1998. Role of MEKK1 in cell survival and activation of JNK and ERK pathways defined by targeted gene disruption. Science 282:1911–1914.

Zhang R, Luo D, Miao R, Bai L, Ge Q, Sessa WC, Min W. 2005. Hsp90-Akt phosphorylates ASK1 and inhibits ASK1-mediated apoptosis. Oncogene 24:3954–3963.

Zhuang ZH, Zhou Y, Yu MC, Silverman N, Ge BX. 2006. Regulation of Drosophila p38 activation by specific MAP2 kinase and MAP3 kinase in response to different stimuli. Cell Signal 18:441–448.

Zweier JL, Talukder MA. 2006. The role of oxidants and free radicals in reperfusion injury. Cardiovasc Res 70: 181–190.