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Commentary

Mitogen-activated protein kinases: new signaling pathways functioning in cellular responses to environmental stress

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Summary

The mitogen-activated protein kinase (MAPK) superfamily consists of three main protein kinase families: the extracellular signal-regulated protein kinases (ERKs), the c-Jun N-terminal kinases (JNKs) and the p38 family of kinases. Each is proving to have major roles in the regulation of intracellular metabolism and gene expression and integral actions in many areas including growth and development, disease, apoptosis and cellular responses to external stresses. To date, this cellular signal transduction network has received relatively little attention from comparative biochemists, despite the high probability that MAPKs have critical roles in the adaptive responses to thermal, osmotic and oxygen stresses. The present article reviews the current understanding of the roles and regulation of ERKs, JNKs and p38, summarizes what is known to date about MAPK roles in animal models of anoxia tolerance, freeze tolerance and osmoregulation, and highlights the potential that studies of MAPK pathways have for advancing our understanding of the mechanisms of biochemical adaptation.

Key words: extracellular signal-regulated protein kinase, c-Jun-N-terminal kinase, p38 kinase, signal transduction, freeze tolerance, anoxia tolerance, osmoregulation.

Introduction

Less than 15 years ago, researchers studying the metabolic responses to mating phermones by yeast discovered novel protein kinases that interfered with mating phermone-induced growth arrest and mating-induced cell fusion (Courchesne et al., 1989; Elion et al., 1990). From these humble beginnings, studies of the mitogen-activated protein kinase (MAPK) superfamily have grown explosively and have fueled major advances in our understanding of the regulation of growth and development and the mechanisms by which cells respond to external stresses. MAPK signal transduction pathways have proved to be ubiquitous in eukaryotes and, to date, thousands of publications have documented their role in a myriad of cell functions. Indeed, the size of this field can be readily illustrated with a simple literature search. An October 2002 search of PubMed for the phrase ‘MAPK review’ produced 733 hits and included review articles on the roles of MAPKs in ischemic injury, liver regeneration, cardiac hypertrophy, rheumatoid arthritis, immune response, memory, cancer, osmotic responses, host–parasite interactions and apoptosis, to name just a few.

The MAPK superfamily

It is now recognized that the MAPK superfamily is made up of three main and distinct signaling pathways: the extracellular signal-regulated protein kinases (ERKs), the c-Jun N-terminal kinases or stress-activated protein kinases (JNK/SAPK), and the p38 family of kinases (Fig. 1). Each of the MAPK modules operates as a three-tier system. The MAPK, a serine/threonine kinase, is activated by a MAPK kinase (MAPKK), which is a ‘dual-specific’ kinase that phosphorylates at both Ser/Thr and Tyr sites, targeting a Thr-X-Tyr motif on the MAPK (where X is glutamate, proline or glycine for the ERK, JNK and p38 modules, respectively) (Hoeflich and Woodgett, 2001). Phosphorylation of the MAPK results in a conformational change and a >1000-fold increase in specific activity so that, in effect, MAPKs are inactive unless phosphorylated by their respective upstream kinases (Hoeflich and Woodgett, 2001). In turn, the MAPKK is activated by a MAPKK kinase (MAPKKK) that receives signals from stimulus-activated receptors on the cell surface or through interactions with GTP-binding proteins and/or other kinases. The MAPKs at the end of these signaling cascades phosphorylate their target proteins, many of which are nuclear proteins such as transcription factors. Hence, MAPKs have a key role in the regulation of many genes. The three MAPK modules are briefly summarized below. Another, lesser-known type of MAPK that has been

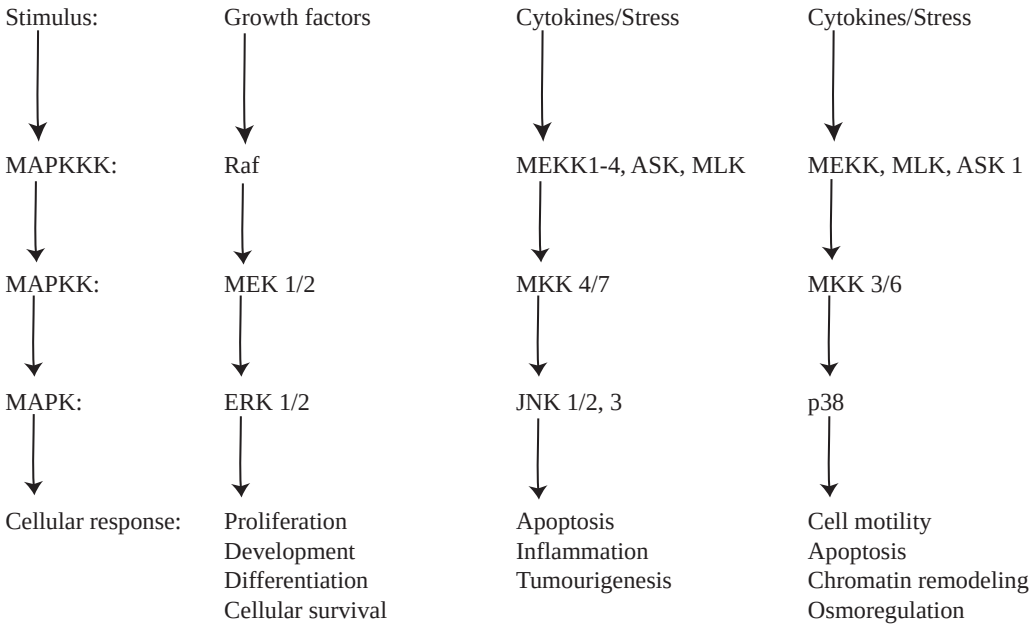


Fig. 1. Flow chart of the three MAPK modules (ERKs, JNKs, p38), showing stimuli, the three-tier regulatory cascade within each module (MAPK, MAPKK and MAPKKK levels) and the various cellular responses elicited by MAPK control.

named Big MAPK or ERK5 will not be discussed here but the reader is referred to Kato et al. (2000) for a recent review.

ERK module

The ERK module responds primarily to growth factors and mitogens and stimulates transcriptional responses in the nucleus. ERK1 and ERK2, the best-studied of the group, are activated by MAPK/ERK kinase (MEK) 1 and MEK2, which phosphorylate at the Thr-Glu-Tyr motif (Cobb and Goldsmith, 1995). The MEKs, in turn, are activated by c-Raf, the MAPKKK of this signaling pathway, that is in turn regulated by growth factor receptors and tyrosine kinases activating through Ras (Moodie and Wolfman, 1994). Upon translocation to the nucleus, ERKs are responsible for the phosphorylation of multiple substrates, depending on the initial stimulus. These include activators of transcription including p90 RSK S6 kinase (Frodin and Gammeltoft, 1999), MAPK-activated protein kinase-1, MAPKAP-K1, phospholipase A_2 and MSK, as well as transcription factors (Elk-1, Ets 1, Sap1a, m-Myc), STAT (signal transducers and activators of transcription) proteins such as Stat3, adapter proteins such as Sos, growth factor receptors such as epidermal growth factor (EGF), and the estrogen receptors (Denhardt, 1996). Generally, activation of an ERK signaling pathway has a role in mediating cell division, migration and survival. ERK1/2 and MEK1/2 are also strongly activated during muscle exercise and may provide the link between exercise and adaptive changes in skeletal muscle composition (Widegren et al., 2000).

JNK module

There are three types of JNKs. JNK1 and JNK2, gene products of alternative splicing, are widely expressed in many tissues, whereas JNK3 is brain-specific (Davis, 2000). JNKs respond to a variety of stress signals including heat shock, osmotic stress, pro-inflammatory cytokines, ischemia and UV exposure (Pombo et al., 1994; Hoeflich and Woodgett, 2001; Irving and Bamford, 2002). The JNKs are activated by dual phosphorylation at the Thr-Pro-Tyr motif by JNKK1 and JNKK2, also known as MAPK kinase 4 (MKK4) and MKK7 (Tournier et al., 1999). Upstream of the MKKs are their MAPKKKs, which include MEKKs 1-4, ASK, and a member of the mixed-lineage kinases (MLKs; Hirai et al., 1996). In turn, these are activated by GTP-binding proteins of the Rho family (Racs, Rhos, the Cdc42s) (Teramoto et al., 1996). The MLKs can also be activated by a germinal center kinase (GCK) family member, and thus activation of JNK can occur independently of the GTPases (Yuasa et al., 1998). JNKs are active as dimers to translocate across the nuclear membrane. JNKs were originally identified as the major kinases responsible for the phosphorylation of c-Jun, leading to increased activity of the AP-1 (activator protein-1) transcription factor (summarized in Shaulian and Karin, 2002); other nuclear transcription factors are also now known to be targets including ATF-2, Elk-1, Myc, Smad3, tumor suppressor p53, NFAT4, DPC4 and MADD, a cell death domain protein (Atfi et al., 1997; Zhang et al., 1998; Hoeflich and Woodgett, 2001). This selective focus on transcription factors contrasts with the actions of the ERK and p38 MAPKs, which phosphorylate targets both inside and outside the nucleus (Hoeflich and Woodgett, 2001). JNK-regulated transcription factors help to regulate gene expression in response to a variety of cellular stimuli, including stress events, growth factors and cytokines (Whitmarsh et al., 1995). Activation of the JNK signaling cascade generally results in apoptosis, although it has also been shown to promote cell survival under certain conditions (e.g. in cardiac myocytes after oxidative stress; Dougherty et al., 2002) and has important roles in determining cell fate during metazoan development (Lisovsky et al., 2002; Moreno et al., 2002) as well as involvement in tumorigenesis and inflammation.

p38 module

Enzymes in the p38 MAPK module are subject to dual phosphorylation at the Thr-Gly-Tyr motif and are generally activated by environmental stresses, including heat, osmotic and oxidative stresses, ionizing radiation and ischemia-induced vasoactive stresses, as well as inflammatory cytokines and tumor necrosis factor (TNF) receptor signaling (New and Han, 1998). The upstream kinases acting on p38 include MKKs 3 and 6. These upstream kinases have preferential effects on different p38 isoforms (Chan-Hui and Weaver, 1998), which are in turn activated by MEKKs, MLKs and ASK1 (Fig. 1). GTPases are responsible for the transmission of stress stimuli to the MAPKKKs of this pathway, including the Racs, the Rhos and the Cdc42s. The five p38 isoforms defined to date (p38α, p38β, p38γ, p38δ and p38-2) vary, based on their substrate specificity. The α and β isoforms of p38 are responsible for the activation of heat shock proteins (hsps) 25, 27 and the MAPK-activated protein (MAPKAP)-2. The γ and δ isoforms of p38 activate ATF2, and p38-2 phosphorylates ATF2 and Sap-1a (Stein et al., 1997). Other transcription factors affected by the p38 family include Stat1, Max/Myc complexes, MEF-2A/C, Elk-1 and CREB through the activation of MSK1. Therefore, the p38 subfamily is also involved in affecting cell motility, transcription and chromatin remodeling (Kyriakis and Avruch, 2001). Other substrates of the p38 signaling pathway include ATF2 and CHOP for regulation of gene expression, as well as MAPKAPK3, MAPKAPK5 and Mnk1 (Beyaert et al., 1996; Huot et al., 1997; Zhu and Lobie, 2000).

MAPKS and comparative biochemistry

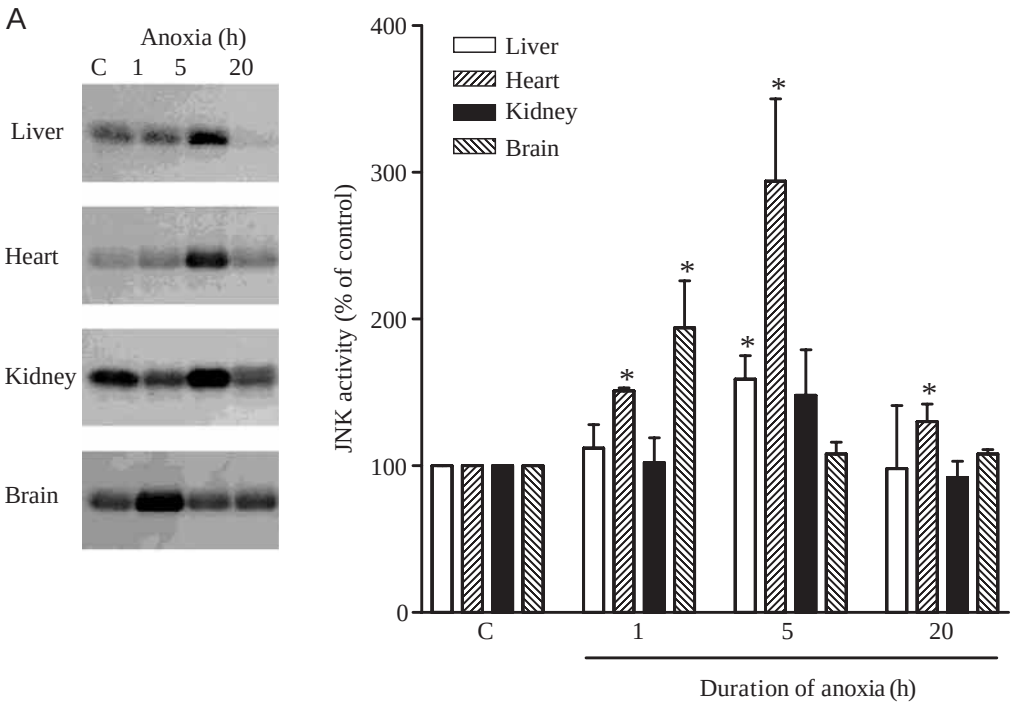
In the last decade thousands of studies have traced the involvement of MAPKs in many aspects of development, disease and cellular responses to stress. MAPKs have been shown to mediate a vast number of cellular responses including gene transcription, cytoskeletal organization, metabolite homeostasis, cell growth and apoptosis in response to many different extracellular signals (Kyriakis, 1999). However, in the field of comparative animal biochemistry, there has been relatively little exploration of MAPKs and their functions to date except for studies in embryology and development (e.g. Pozios et al., 2001; Moreno et al., 2002; Lisovsky et al., 2002). This is despite the obvious potential importance of these kinases in mediating organismal responses to multiple environmental stresses. Indeed, it is rather interesting that studies of MAPK action and regulation are very advanced with respect to their role in mediating environmental stress responses by plants but little progress has been made to assess similar stress responses in comparative animal models (both vertebrate and invertebrate). For example, a recent review by Wrzaczek and Hirt (2001) on MAPKs in plants states that there are 24 MAPK pathways known to date in *Arabidopsis thaliana*, with known roles in signaling multiple biotic and abiotic stresses such as wounding and pathogen infection, temperature stress or drought, as well as mediating cell cycle and developmental processes and the effects of some plant hormones (e.g. ethylene and auxin). MAPK pathways are particularly important in mediating plant responses to water-related stresses (drought, salinity) (Munnik and Meijer, 2001). Some recent studies from our laboratory and others that have begun to probe MAPK involvement in the biochemical responses by animals to anoxia, thermal, freezing and osmotic challenges are highlighted below.

Studies in our laboratory have analyzed multiple facets of biochemical adaptation by anoxia-tolerant and freeze-tolerant animals (for recent reviews, see Storey, 1996, 1999; Storey and Storey, 2001) and recently we began to look at the role that MAPKs play in these processes. To date, much of the research on MAPKs in vertebrates has centered on mammals, with frequent use of isolated cell systems where stressors and effectors can be applied *in vitro*. Our aim in initial studies was to find out how MAPKs responded to stresses imposed upon the whole animal *in vivo*. Data gathered from these initial studies have identified stress-specific, organ-specific and time-dependent responses by one, two or all three of the MAPK modules, and clearly show that MAPK signal transduction cascades have roles to play in metabolic adaptation by anoxia- or freezing-tolerant species. Initial work assessed three systems: (1) responses to whole animal freezing at –2.5°C by the freeze-tolerant wood frog *Rana sylvatica*, (2) responses to anoxic submergence at 7°C by anoxia-tolerant adult red-eared slider turtles *Trachemys scripta elegans*, and (3) responses to both anoxia at 5°C and freezing at –2.5°C by hatchling sliders that are tolerant of both stresses (Greenway and Storey, 1999, 2000a,b).

Our initial data showed one common result: ERKs have little or no involvement in the responses to freezing or anoxia in frogs and turtles. The only substantial response by ERKs was an increase in the content of active, phosphorylated ERK2 in frog brain as an early response to freezing (Greenway and Storey, 1999). Since the ERK pathway is believed to transduce signals primarily from growth factors and mitogens, this result is not surprising.

JNKs and p38 responded to freeze/thaw and JNKs responded to anoxia. JNK activities were not affected in wood frog organs over a 12 h freezing exposure but activities were reduced by 40–50% in turtle liver and heart over a 4 h freeze (Greenway and Storey, 1999, 2000a). By contrast, JNK appears to play a role in metabolic recovery after thawing in frog organs; JNK activity increased strongly after 90 min thawing in liver and kidney (rising approx. five- and fourfold, respectively) and after a longer time (4 h) in heart. JNK activity also rose during survivable anoxia exposure in tissues of both adult and hatchling turtles (Fig. 2A); in both cases, JNK rose to a peak after 5 h of anoxic submergence but fell with longer exposure (Greenway and Storey, 1999, 2000b). This suggests a role for JNK activation in the hypoxia transition period during the early hours of submergence with JNK suppressed again when metabolic arrest responses are fully developed to support long term anoxia survival.

The p38 MAPK was activated by freezing in wood frogs



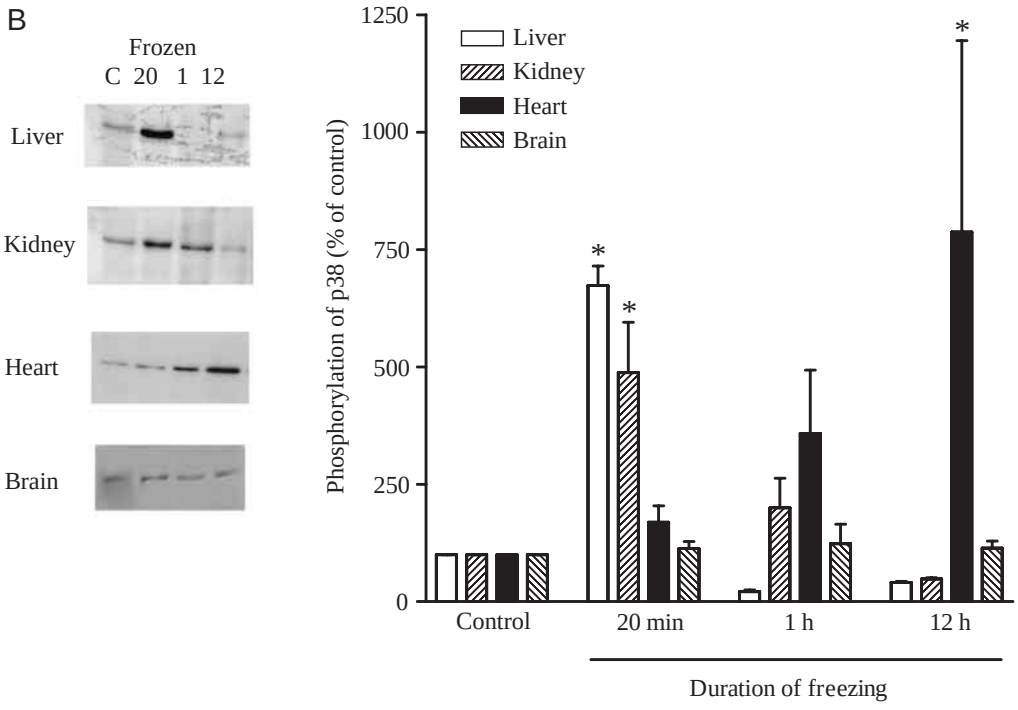


Fig. 2. MAPK responses in stress-tolerant animals. (A) JNK activity in organs from control (C) and anoxia-exposed (1, 5 and 20 h anoxic submergence at 5°C) hatchling turtles *Trachemys scripta elegans*. Left, representative autoradiograms of phosphorylated ^{32}P-c-Jun-GST; right densitometry results (means ± S.E.M.) for N=3 trials. (B) Amount of phosphorylated (active) p38 in organs from control (C) *versus* frozen (20 min, 1 and 12 h at –2.5°C) wood frogs *Rana sylvatica*. On the left are representative western blots using antibody to the phosphorylated (tyr 182) peptide; on the right are densitometry results, means ± S.E.M., N=3. *Significantly different from the corresponding control value, P<0.05. From Greenway and Storey (1999, 2000a).

(Greenway and Storey, 2000a). The amount of active, phosphorylated p38 increased strongly in liver and kidney within 20 min after freezing began but this was reversed by 60 min (Fig. 2B). This suggests that p38 may be involved in mediating one or more of the rapid, initial metabolic responses to freezing such as the upregulation of multiple genes (Storey, 1999; Storey and Storey, 2001). The activity of p38 in heart followed a different time course, with phospho-p38 (the active form) rising only after 1 h of freezing and continuing to 12 h. Organ-specific activation of p38 also occurred during thawing but the phospho-p38 content of wood frog liver and kidney did not respond to two component stresses of freezing (anoxia, dehydration) when each stress was applied individually (Greenway and Storey, 2000a). This suggests that p38 may stimulate metabolic responses that are unique to freeze/thaw. The amount of phospho-p38 was also unchanged in turtle

organs over the course of a 20 h anoxia exposure (Greenway and Storey, 2000b).

The lack of p38 responses to anoxia by anoxia- or freeze-tolerant animals contrasts with studies of anoxia/hypoxia-sensitive species. Ischemia–reperfusion damage in mammalian heart is linked with activation of p38, and attenuation of the p38 response to sustained ischemia by means of short preconditioning exposures (that transiently activate p38) resulted in improved recovery of function during reperfusion (Marais et al., 2001). In mammalian kidney, ischemia induced the activation of both JNK and p38 and phosphorylation of MKK 7, MKK4 and MKK 3/6; preconditioning attenuated these responses and correlated with improved survival (Park et al., 2001). Hence, although much research remains to be done, it is interesting to speculate that some of the significant differences in metabolic responses to anoxia between anoxia-tolerant and intolerant species are mediated *via* JNK and p38 signal transduction pathways.

MAPK pathways also responded to anoxia, hyperosmotic and thermal stresses as well as mechanical overload in the perfused heart of the frog *Rana ridibunda* (Aggeli et al., 2001a,b, 2002). Hyperosmotic stresses (*via* perfusion with high sorbitol, NaCl or KCl) stimulated a rapid phosphorylation of p38 that was readily reversible, whereas hypotonicity did not affect the enzyme; both high and low temperatures elevated phospho-p38 content (Aggeli et al., 2002). High perfusion pressure also stimulated a rapid (30 s) phosphorylation of p38 and a prolonged (up to 30 min) phosphorylation of JNK (Aggeli et al., 2001a). JNKs were also activated by anoxia/reoxygenation (Aggeli et al., 2001b). It is interesting that both of these MAPKs also respond to freeze/thaw in wood frog heart and, notably, freezing and thawing cause major changes in peripheral resistance and blood viscosity that affect heart work load.

The responses of the p38 MAPK (or its yeast/fungal homologue HOG1) to osmotic and volume stresses have been documented in many organisms including yeasts, fungi (Zhang et al., 2002), plants (Munnik and Meijer, 2001) and mammalian kidney (Chen and Gardner, 2002), liver (vom Dahl et al., 2001), and brain astrocytes (Xu et al., 2001). New studies by Kultz and Avila (2001) probed the role of MAPKs in osmosensory signaling pathways in gills of the fish *Fundulus heteroclitus*. Total ERK and JNK protein contents were not affected by either hyper- or hyposmotic stress, but p38 content rose significantly during hyperosmotic stress. However, the activity (amount of phosphoenzyme) of all three MAPKs increased significantly during hyposmotic stress and, oppositely, decreased under hyperosmotic stress. These data demonstrate a key role for MAPKs in salinity adaptation and will undoubtedly fuel a ‘renaissance’ of interest in the molecular mechanisms of osmoregulation in euryhaline organisms.

The above studies clearly indicate that MAPKs have key roles to play in animal responses to a wide variety of environmental stresses. Among the obvious next steps in these studies is to identify both the downstream targets of MAPK action and upstream signals that initiate MAPK activation in response to stress. MAPKs have major roles as regulators of gene expression and, hence, a major focus for MAPK studies in comparative systems will be on stress-induced gene expression. In this search, new cDNA array gene screening technology will prove to be critical. Indeed, its value is exemplified in a recent paper by Nahm et al. (2002), where cDNA array screening technology was used to identify 12 genes that were upregulated by hypertonicity in an inner medullary collecting duct cell line from mammalian kidney. Cell lines were then treated with inhibitors of various protein kinases and from this it was shown that MAPKs were commonly involved in the induction of hypertonicity responsive genes. This study emphasizes not only the key role of MAPKs in mediating responses to osmotic stresses in cells but also the tremendous potential of cDNA array screening for gaining a ‘global’ view of cellular responses to stress. Having identified the genes that are MAPK responsive, future studies can then go on trace the full regulatory cascade involved and characterize the adaptive function of the protein products of these upregulated genes.

A similar approach will undoubtedly prove effective in many comparative systems, particularly those that are amenable to *in vitro* study and treatment with externally added modifiers of MAPK activity (e.g. fish gill, frog or turtle hepatocytes, mollusc mantle). Indeed, we are finding cDNA array screening to be of great value in comparative systems. For example, a recent analysis of anoxia-induced gene expression in hepatopancreas of the marine snail, *Littorina littorea* using human 19 000 gene glass microarrays showed low cross-reactivity (only 18.35%), but nonetheless still allowed us to analyze the effects of anoxia on nearly 3500 genes, over 300 of which appeared to be upregulated in anoxia. These represented a wide selection of protein phosphatases and kinases, MAPK-interacting factors, translation factors, antioxidant enzymes and nuclear receptors (Larade and Storey, 2002). Few of these proteins have ever been implicated before in anoxia adaptation and this opens the door to expanded studies of the genes/proteins involved in anoxia tolerance and the signal transduction systems that regulate them.

Control of MAPKs

The MAPK signaling pathways are all cascades with at least 3 levels (MAPK, MAPKK and MAPKKK) (Fig. 1), and are susceptible to regulatory inputs at multiple levels within the cascade as well as *via* multiple mechanisms. These can include cell/tissue specific expression patterns for each MAPK module, specificity of the stimuli that can trigger each MAPK module, specificity of the substrates that are targets of each MAPK in each cell type, modification MAPK responses *via* activators, inhibitors, scaffolding proteins, sequential interactions with other proteins in a cascade, positive and negative feedback loops, and cross-talk among MAPK and other signaling pathways. Some examples of MAPK regulatory mechanisms are discussed below and these highlight some areas to be

considered in studying the roles of MAPKs in biochemical adaptation.

Regulation by MAPKKK specificity

One way to achieve specificity of a pathway is *via* the type of MAPKKK activated by a signal. For example, specificity in the ERK signaling cascade depends on which isoform of Raf (the MAPKKK of the pathway) is activated. Mouse knock-out studies have shown that each of the three isoforms (Raf-1, B-Raf, A-Raf) have distinct roles. Raf-1 has a general and crucial role in the development of all tissues, whereas A-Raf defects result in neurological and intestinal problems and B-Raf defects die because endothelial cells fail to mature, leading to vascular haemorrhaging (Hagemann and Rapp, 1999).

Regulation by scaffolding proteins

ERK activation is a cascade event that results in the formation of large, multimeric signaling complexes. For example, Raf interacts with Ras-GTP, MEKs then bind to Raf, and ERKs bind to the N terminus of MEK. Recently discovered is the MP1 protein (MEK partner 1), a scaffolding molecule that appears to bind to a subgroup of proteins within a MAPK module, and therefore it favors the specific activation of certain components within the module (Schaeffer et al., 1998). MP1 is believed to play a significant role in specificity within the MAPK module for it interacts only with ERK1 and MEK1, and not with ERK2 and MEK2, and hence favors the activation of ERK1. MP1 also augments Raf activation of MEK. Other potential scaffolding proteins in the ERK module are also being investigated. Thus, it is believed that ERK signaling is regulated dynamically by the binding of scaffolding proteins such as MP1, which affect protein–protein interactions, and therefore sway the status of stability within a module and affect the outcome of the stimulus.

The JNK cascade also has a scaffolding protein. JIP1 binds specifically to JNK1, as well as to MKK7, and the MKK7 activators MLK3 and DLK (dual leucine zipper-bearing kinase) (Whitmarsh et al., 1998). JIP1 is highly specific to binding and activating the JNK cascade, and is probably involved in organizing this signaling module in order to permit upstream regulation. The JNK signaling pathway is also regulated by adapter proteins that couple to the TNF receptors, the family of receptors that are probably the most important activators of this pathway. The TNFR-associated factors (TRAFs) couple to upstream activators of the JNK signaling pathway as well as to the stress-activated kinase itself (Bradley and Pober, 2001). The six TRAFs known to date are each activated in response to various TNFR signaling ligands.

Regulation by cellular location

ERK activity is also regulated by subcellular location. For example, during mitosis, studies have shown that activated ERKs associate with CENP-E, a centromeric protein (Zecevic et al., 1998), at the kinetochores, and on the mitotic apparatus (Willard and Crouch, 2001), implicating their importance during M phase. JNK colocalizes in certain cells with its MAPKKK, MLK2, along microtubules (Nagata et al., 1998). The subcellular location of downstream substrates of JNK is also affected by JNK phosphorylation, such as the transcription factor NFAT4 (nuclear factor of activated T cells), which is involved in differentiation and cytokine gene expression (Chow et al., 1997). Phosphorylation by JNK prohibits this transcription factor from entering the nucleus and thereby inhibits NFAT4 signaling. The regulation of the p38 signaling pathway by scaffolding and adapter proteins is not well understood but activated p38 can regulate the distribution of some of its substrates. For example, the phosphorylation of both p38 and its substrate, MAPKAP kinase 2, causes both proteins to be excluded from the nucleus (Ben-Levy et al., 1998). In addition, NFATc4, a substrate of p38 but not of JNK, must be dephosphorylated in order to enter the nucleus to activate transcription (Yang et al., 2002).

Regulation via *stimulus intensity*

For specific biological responses, the timing and duration of the stimulus also has a direct impact on the type of response that cells make to a signal as well as the cell type affected. Thus, sustained or transient signals through ERK, for example, will determine whether a cell's response is growth or differentiation (Kao et al., 2001). In turn, the duration of the signal, whether it is transient or prolonged, could rely on feedback pathways through phosphorylation, although the relevance of these feedback mechanisms has yet to be deciphered.

Phosphatases

Since MAPKs are activated by phosphorylation, the protein phosphatases that dephosphorylate MAPKs are a key element in their control. Three families of protein phosphatases are involved: Ser/Thr phosphatases, Tyr phosphatases and dual specificity Ser/Thr/Tyr phosphatases (Tamura et al., 2002). Some have received considerable attention. MAPK phosphatase-1 (MKP-1) and MKP-2 are not specific and dephosphorylate the ERKs, JNKs and p38 (Chu et al., 1996), whereas MKP-3 appears to be specific to ERK1 and ERK2 only and JNKs and p38 are inactivated by the phosphatase M3/6 (Muda et al., 1996). The activities of MKPs are also defined by their subcellular location. MKP-3 is known to be cytoplasmic, whereas MKP-1 is only found in the nucleus. In addition serine/threonine protein phosphatases 1 and 2A have been implicated in MAPK signaling cascades, although whether the dephosphorylation of their substrates has any pertinent role *in vivo* has yet to be determined.

Signaling crosstalk

Although the three MAPK modules run in parallel (Fig. 1), there is a considerable degree of cross-talk between them, which creates multiple opportunities for modulating or fine-tuning responses to different signals. Specificity of the MAPK signaling pathways is greatest at the level of specific MKK activation of individual MAPKs, where there is the least amount of cross-talk. Although some substrates are activated

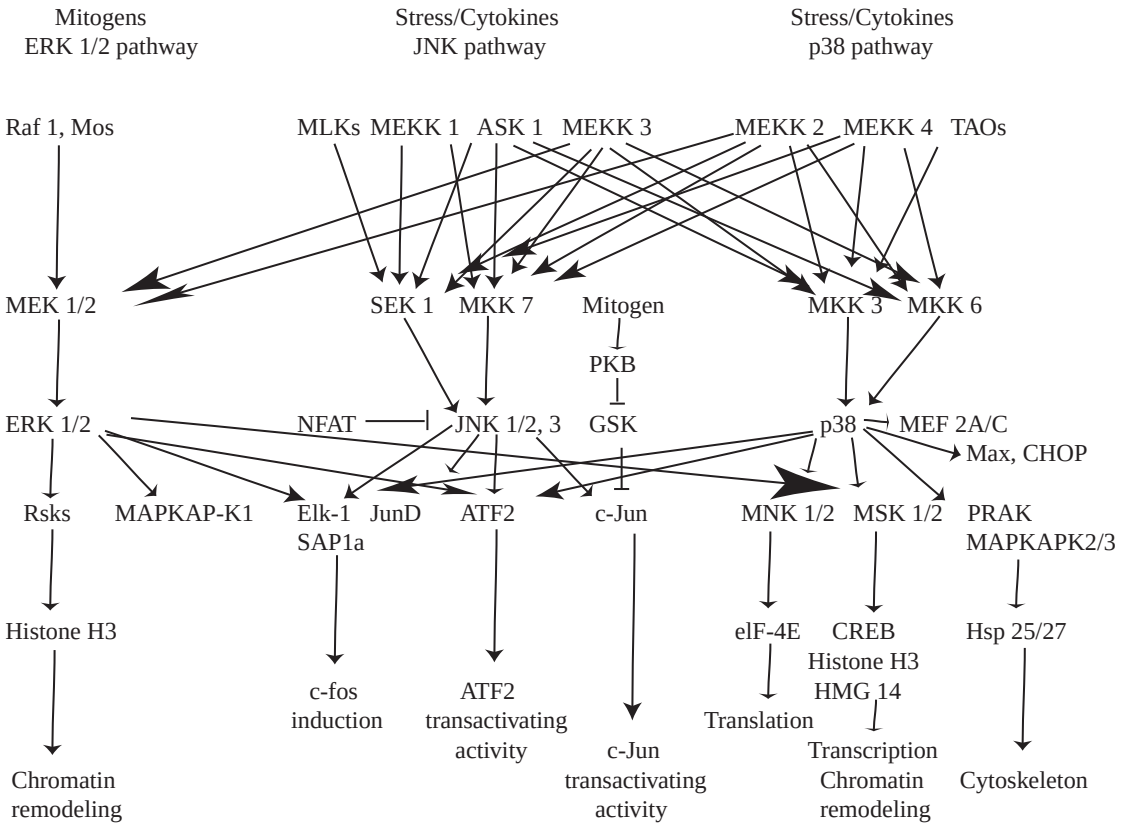


Fig. 3. Summary of MAPK signaling pathways, showing cross-talk between modules.

very specifically by only one of the three pathways, there is often considerable cross-talk at the MAPK substrate level (e.g. Elk-1 is phosphorylated by all three MAPK signaling pathways; Yordy and Muise-Helmericks, 2000). The MAPKKKs are also involved in cross-talk, although much remains to be elucidated at this level. Fig. 3 outlines various known cross-talk interactions among the MAPK pathways. Obviously, there is potential for a considerable level of communication between these MAPK modules, the type and amount of which can vary widely depending upon factors, including the length, intensity and timing of signal, type of cell, and cell-specific receptor distribution at the plasma membrane.

MAPK cascades can also influence other signaling pathways and *vice versa*; there are several known examples of MAPK influences on outside signaling pathways. For example, ERKs can activate the JAK-STAT pathways (Marshall, 1995). MAPKs can also converge on the same substrates. Thus, both ERK and p38 activate MNK1 (Fukunaga and Hunter, 1997), the MAPK signaling-integrating kinase 1, which is responsible for the activation of the eukaryotic translation initiation factor 4E, as well as MSK1. ERK, p38 and JNK all also phosphorylate the transcription activators MAPKAP kinase 3 and Elk-1, whereas p38 and JNK both phosphorylate ATF2, and ERK and JNK both activate c-Jun.

MAPK signaling cascades are influenced by other signaling pathways, including those linked with cAMP and calcium. The MEKs that activate ERKs can be activated by specific Raf isoforms based on the type of cell stimulated and the level of cAMP generated. Calcium signaling also affects ERK modules; it has been shown in cardiomyocytes and neurons that calcium influx results in MEF2 activation, a transcription factor that is a substrate for the calcium/calmodulin-dependent protein kinase IV (Mao et al., 1999; Lu et al., 2000). The activation of MEF2 correlates with the activation of p38, which is responsible for the phosphorylation of MEF2 on its activation site.

Conclusions and perspectives

In the field of comparative biochemistry, there is a great unexplored territory involving the roles of MAPKs in metabolic regulation and biochemical adaptation. Initial studies with comparative models have already linked MAPKs with the metabolic responses to anoxic, osmotic, thermal and freezing stresses, as well as to changes in mechanical workload, but these have no more than ‘scratched the surface’ of this new field. Effective assay methods are now available for assessing ERK, JNK and p38 responses to stimuli and these can be used as starting points for analyzing both the upstream triggers of MAPK activity (all the way to cell surface receptors) as well as the downstream consequences of MAPK action – transcription factors and gene activation. Particularly important will be identification of the nuclear targets of MAPK action and, in combination with new technology such as cDNA

array screening, these studies will undoubtedly bring many new insights into the biochemical mechanisms that underlie adaptation to multiple environmental stresses.

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References

Aggeli, I. K., Gaitanaki, C., Lazou, A. and Beis, I. (2001a). Stimulation of multiple MAPK pathways by mechanical overload in the perfused amphibian heart. *Am. J. Physiol.* **281**, R1689-R1698.

Aggeli, I. K., Gaitanaki, C., Lazou, A. and Beis, I. (2001b). Activation of multiple MAPK pathways (ERKs, JNKs, p38-MAPK) by diverse stimuli in the amphibian heart. *Mol. Cell. Biochem.* **221**, 63-69.

Aggeli, I. K., Gaitanaki, C., Lazou, A. and Beis, I. (2002). Hyperosmotic and thermal stresses activate p38-MAPK in the perfused amphibian heart. *J. Exp. Biol.* **205**, 443-454.

Atfi, A., Buisine, M., Mazars, A. and Gespach, C. (1997). Induction of apoptosis by DPC4, a transcriptional factor regulated by transforming growth factor-beta through stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) signaling pathway. *J. Biol. Chem.* **272**, 24731-24734.

Ben-Levy, R., Hooper, S., Wilson, R., Paterson, H. F. and Marshall, C. J. (1998). Nuclear export of the stress-activated protein kinase p38 mediated by its substrate MAPKAP kinase-2. *Curr. Biol.* **8**, 1049-1057.

Beyaert, R., Cuenda, A., Vanden Berghe, W., Plaisance, S., Lee, J. C., Haegeman, G., Cohen, P. and Fiers, W. (1996). The p38/RK mitogen-activated protein kinase pathway regulates interleukin-6 synthesis response to tumor necrosis factor. *EMBO J.* **15**, 1914-1923.

Bradley, J. R. and Pober, J. S. (2001). Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene* **20**, 6482-6491.

Chan-Hui, P. Y. and Weaver, R. (1998). Human mitogen-activated protein kinase kinase kinase mediates the stress-induced activation of mitogen-activated protein kinase cascades. *Biochem. J.* **336**, 599-609.

Chen, S. and Gardner, D. G. (2002). Osmoregulation of natriuretic peptide receptor signaling in inner medullary collecting duct. A requirement for p38 MAPK. *J. Biol. Chem.* **277**, 6037-6043.

Chow, C. W., Rincon, M., Cavanagh, J., Dickens, M. and Davis, R. J. (1997). Nuclear accumulation of NFAT4 opposed by the JNK signal transduction pathway. *Science* **278**, 1638-1641.

Chu, Y., Solski, P. A., Khosravi-Fa, R., Der, C. J. and Kelly, K. (1996). The mitogen-activated protein kinase phosphatases PAC1, MKP-1, and MKP-2 have unique substrate specificities and reduced activity in vivo toward the ERK2 sevenmaker mutation. *J. Biol. Chem.* **271**, 6497-6501.

Cobb, M. and Goldsmith, E. J. (1995). How MAP kinases are regulated. *J. Biol. Chem.* **270**, 14843-14846.

Courchesne, W. E., Kunisawa, R. and Thorner, J. (1989). A putative protein kinase overcomes pheromone-induced arrest of cell cycling in *S. cerevisiae*. *Cell* **58**, 1107-1119.

Davis, R. J. (2000). Signal transduction by the JNK group of MAP kinases. *Cell* **103**, 239-252.

Denhardt, D. T. (1996). Signal-transducing protein phosphorylation cascades mediated by Ras/Rho proteins in the mammalian cell: the potential for multiplex signalling. *Biochem. J.* **318**, 729-747.

Dougherty, C. J., Kubasiak, L. A., Prentice, H., Andreka, P., Bishopric, N. H. and Webster, K. A. (2002). Activation of c-Jun N-terminal kinase promotes survival of cardiac myocytes after oxidative stress. *Biochem. J.* **362**, 561-571.

Elion, E. A., Grisafi, P. L. and Fink, G. R. (1990). FUS3 encodes a cdc2+/CDC28-related kinase required for the transition from mitosis into conjugation. *Cell* **60**, 649-664.

Frodin, M. and Gammeltoft, S. (1999). Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. *Mol. Cell Endocrinol.* **151**, 65-77.

Fukunaga, R. and Hunter, T. (1997). MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. *EMBO J.* **16**, 1921-1933.

Greenway, S. C. and Storey, K. B. (1999). Discordant responses of mitogen-activated protein kinases to anoxia and freezing exposures in hatchling turtles. *J. Comp. Physiol.* **169**, 521-527.

Greenway, S. C. and Storey, K. B. (2000a). Activation of mitogen-activated protein kinases during natural freezing and thawing in the wood frog. *Mol. Cell Biochem.* **209**, 29-37.

Greenway, S. C. and Storey, K. B. (2000b). Mitogen-activated protein kinases and anoxia tolerance in turtles. *J. Exp. Zool.* **287**, 477-284.

Hagemann, C. and Rapp, U. R. (1999). Isotype-specific functions of Raf kinases. *Exp. Cell Res.* **253**, 34-46.

Hirai, S., Izawa, M., Osada, S., Spyrou, G. and Ohno, S. (1996). Activation of the JNK pathway by distantly related protein kinases, MEKK and MUK. *Oncogene* **12**, 641-650.

Hoeflich, K. P. and Woodgett, J. R. (2001). Signal transduction and gene expression in the regulation of natural freezing survival. In *Cell and Molecular Responses to Stress*, Vol. 2 (ed. K. B. Storey and J. M. Storey), pp. 175-193. Amsterdam: Elsevier Press.

Huot, J., Houle, F., Marceau, F. and Landry, J. (1997). Oxidative stress-induced actin reorganization mediated by the p38 mitogen-activated protein kinase/heat shock protein 27 pathway in vascular endothelial cells. *Circ. Res.* **80**, 383-392.

Irving, E. A. and Bamford, M. (2002). Role of mitogen- and stress-activated kinases in ischemic injury. *J. Cereb. Blood Flow Metab.* **22**, 631-647.

Kao, S., Jaiswal, R. K., Kolch, W. and Landreth, G. E. (2001). Identification of the mechanisms regulating the differential activation of the MAPK cascade by epidermal growth factor and nerve growth factor in PC12 cells. *J. Biol. Chem.* **276,** 18169-18177.

Kato, Y., Chao, T. H., Hayashi, M., Tapping, R. I. and Lee, J. D. (2000). Role of BMK1 in regulation of growth factor-induced cellular responses. *Immunol. Res.* **21**, 233-237.

Kultz, D. and Avila, K. (2001). Mitogen-activated protein kinases are in vivo transducers of osmosensory signals in fish gill cells. *Comp. Biochem. Physiol. B* **129**, 821-829.

Kyriakis, J. M. (1999). Making the connection: coupling of stress-activated ERK/MAPK signalling modules to extracellular stimuli and biological responses. *Biochem. Soc. Symp.* **64**, 29-48.

Kyriakis, J. M. and Avruch, J. (2001). Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol. Rev.* **81**, 807-869.

Larade, K. and Storey, K. B. (2002). A profile of the metabolic responses to anoxia in marine invertebrates. In *Cell and Molecular Responses to Stress*, Vol. 3 (ed. K. B. Storey and J. M. Storey), pp. 27-46. Amsterdam: Elsevier.

Lisovsky, M., Itoh, K. and Sokol, S. Y. (2002). Frizzled receptors activate a novel JNK-dependent pathway that may lead to apoptosis. *Curr. Biol.* **12**, 53-58.

Lu, J., McKinsey, T. A., Nicol, R. L. and Olson, E. N. (2000). Signal-dependent activation of the MEF2 transcription factor by dissociation from histone deacetylases. *Proc. Natl. Acad. Sci. USA* **97**, 4070-4075.

Mao, Z., Bonni, A., Xia, F., Nadal-Vicens, M. and Greenberg, M. E. (1999). Neuronal activity-dependent cell survival mediated by transcription factor MEF2. *Science* **286**, 785-790.

Marais, E., Genade, S., Huisamen, B., Strijdom, J. G., Moolman, J. A. and Lochner, A. (2001). Activation of p38 MAPK induced by a multi-cycle ischaemic preconditioning protocol is associated with attenuated p38 MAPK activity during sustained ischaemia and reperfusion. *J. Mol. Cell. Cardiol.* **33**, 769-778.

Marshall, C. J. (1995). Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* **80**, 179-185.

Moodie, S. A. and Wolfman, A. (1994). The 3Rs of life: Ras, Raf, and growth regulation. *Trends Genet.* **10**, 44-48.

Moreno, E., Basler, K. and Morata, G. (2002). Cells compete for decapentaplegic survival factor to prevent apoptosis in *Drosophila* wing development. *Nature* **416**, 755-759.

Muda, M., Theodosiou, A., Rodrigues, N., Boschert, U., Camps, M., Gillieron, C., Davies, K., Ashworth, A. and Arkinstall, S. (1996). The dual specificity phosphatases M3/6 and MKP-3 are highly selective for inactivation of distinct mitogen-activated protein kinases. *J. Biol. Chem.* **271**, 4319-4326.

Munnik, T. and Meijer, H. J. (2001). Osmotic stress activates distinct lipid and MAPK signalling pathways in plants. *FEBS Lett.* **498**, 172-178.

Nagata, K., Puls, A., Futter, C., Aspenstrom, P., Schaefer, E., Nakata, T., Hirokawa, N. and Hall, A. (1998). The MAP kinase kinase kinase MLK2 co-localizes with activated JNK along microtubules and associates with kinesin superfamily motor KIF3. *EMBO J.* **17**, 149-158.

Nahm, O., Woo, S. K., Handler, J. S. and Kwon, H. M. (2002). Involvement of multiple kinase pathways in stimulation of gene transcription by hypertonicity. *Am. J. Physiol.* **282**, C49-C58.

New, L. and Han, J. (1998). The p38 MAP kinase pathway and its biological function. *Trends Cardiovasc. Med.* **8**, 220-229.

Park, K. M., Chen, A. and Bonventre, J. V. (2001). Prevention of kidney ischemia/reperfusion-induced functional injury and JNK, p38 and MAPK kinase activation by remote ischemic pretreatment. *J. Biol. Chem.* **276**, 11870-11876.

Pombo, C. M., Bonventre, J. V., Avruch, J., Woodgett, J. R., Kyriakis, J. M. and Force, T. (1994). The stress-activated protein kinases are major c-Jun amino-terminal kinases activated by ischemia and reperfusion. *J. Biol. Chem.* **269**, 26546-26551.

Pozios, K. C., Ding, J., Degger, B., Upton, Z. and Duan, C. (2001). IGFs stimulate zebrafish cell proliferation by activating MAP kinase and PI3-kinase-signaling pathways. *Am. J. Physiol.* **280**, R1230-R1239.

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.

Shaulian, E. and Karin, M. (2002). AP-1 as a regulator of cell life and death. *Nat. Cell Biol.* **4**, E131-E136.

Stein, B., Yang, M. X., Young, D. B., Janknecht, R., Hunter, T., Murray, B. W. and Barbosa, M. S. (1997). p38-2, a novel mitogen-activated protein kinase with distinct properties. *J. Biol. Chem.* **272**, 19509-19517.

Storey, K. B. (1996). Metabolic adaptations supporting anoxia tolerance in reptiles: recent advances. *Comp. Biochem. Physiol. B* **113**, 23-35.

Storey, K. B. (1999). Stress-induced gene expression in freeze tolerant and anoxia tolerant vertebrates. In *Environmental Stress and Gene Regulation* (ed. K. B. Storey), pp. 1-23. Oxford: BIOS Scientific Publishers.

Storey, K. B. and Storey, J. M. (2001). Signal transduction and gene expression in the regulation of natural freezing survival. In *Cell and Molecular Responses to Stress*, Vol. 2 (ed. K. B. Storey and J. M. Storey), pp. 1-19. Amsterdam: Elsevier Press.

Tamura, S., Hanada, M., Ohnishi, M., Katsura, K., Sasaki, M. and Kobayashi, T. (2002). Regulation of stress-activated protein kinase signaling pathways by protein phosphatases. *Eur. J. Biochem.* **269**, 1060-1066.

Teramoto, H., Coso, O. A., Miyata, H., Igishi, T., Miki, T. and Gutkind, J. S. (1996). Signaling from the small GTP-binding proteins Rac1 and Cdc42 to the c-Jun N-terminal kinase/stress-activated protein kinase pathway. *J. Biol. Chem.* **271**, 27225-27228.

Tournier, C., Whitmarsh, A. J., Cavanagh, J., Barrett, T. and Davis, R. J. (1999). The MKK7 gene encodes a group of c-Jun NH2-terminal kinase kinases. *Mol. Cell* **19**, 1569-1581.

vom Dahl, S., Schliess, F., Graf, D. and Haussinger, D. (2001). Role of p38(MAPK) in cell volume regulation in perfused rat liver. *Cell Physiol. Biochem.* **11**, 285-294.

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.

Whitmarsh, A. J., Shore, P., Sharrocks, A. D. and Davis, R. J. (1995). Integration of MAP kinase signal transduction pathways at the serum response element. *Science* **269**, 403-407.

Widegren, U., Wretman, C., Lionikas, A., Hedin, G. and Henriksson, J. (2000). Influence of exercise intensity on ERK/MAP kinase signalling in human skeletal muscle. *Pflugers Arch.* **441**, 317-322.

Willard, F. S. and Crouch, M. F. (2001). MEK, ERK, and p90RSK are present on mitotic tubulin in Swiss 3T3 cells: a role for the MAP kinase pathway in regulating mitotic exit. *Cell Signal.* **13**, 653-664.

Wrzaczek, M. and Hirt, H. (2001). Plant MAP kinase pathways: how many and what for? *Biol. Cell.* **93**, 81-87.

Xu, D., Wang, L., Olson, J. E. and Lu, L. (2001). Asymmetrical response of p38 kinase activation to volume changes in primary rat astrocytes. *Exp. Biol. Med.* **226**, 927-933.

Yang, T. T., Xiong, Q., Enslen, H., Davis, R. J. and Chow, C. W. (2002). Phosphorylation of NFATc4 by p38 mitogen-activated protein kinases. *Mol. Cell. Biol.* **22**, 3892-3904.

Yordy, J. S. and Muise-Helmericks, R. C. (2000). Signal transduction and the Ets family of transcription factors. *Oncogene* **19**, 6503-6513.

Yuasa, T., Ohno, S., Kehrl, J. H. and Kyriakis, J. M. (1998). Tumor necrosis factor signaling to stress-activated protein kinase (SAPK)/Jun NH2-terminal kinase (JNK) and p38. *J. Biol. Chem.* **273**, 22681-22692.

Zecevic, M., Catling, A. D., Eblen, S. T., Renzi, L., Hittle, J. C., Yen, T. J., Gorbsky, G. J. and Weber, M. J. (1998). Active MAP kinase in mitosis: localization at kinetochores and association with the motor protein CENP-E. *J. Cell Biol.* **142**, 1547-1558.

Zhang, Y., Lamm, R., Pillonel, C., Lam, S. and Xu, J. R. (2002). Osmoregulation and fungicide resistance: the *Neurospora crassa* os-2 gene encodes a HOG1 mitogen-activated protein kinase homologue. *Appl. Environ. Microbiol.* **68**, 532-538.

Zhang, Y., Zhou, L. and Miller, C. A. (1998). A splicing variant of a death domain protein that is regulated by a mitogen-activated kinase is a substrate for c-Jun N-terminal kinase in the human central nervous system. *Proc. Natl. Acad. Sci. USA* **95,** 2586-2591.

Zhu, T. and Lobie, P. E. (2000). Janus kinase 2-dependent activation of p38 mitogen-activated protein kinase by growth hormone. *J. Biol. Chem.* **275**, 2103-2114.