



TOPICAL REVIEW

Stress-activated Protein Kinases: Activation, Regulation and Function

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ABSTRACT. The response of cells to extracellular stimuli is mediated in part by a number of intracellular kinase and phosphatase enzymes. Within this area of research the activation of the p42 and p44 isoforms of mitogen-activated protein (MAP) kinases have been extensively described and characterised as central components of the signal transduction pathways stimulated by both growth factors and G-protein-coupled receptor agonists. Signaling events mediated by these kinases are fundamental to cellular functions such as proliferation and differentiation. More recently, homologues of the p42 and p44 isoforms of MAP kinase have been described, namely the stress-activated protein kinases (SAPKs) or alternatively the c-jun N-terminal kinases (JNKs) and p38 MAP kinase (the mammalian homologue of yeast HOG1). These MAP kinase homologues are integral components of parallel MAP kinase cascades activated in response to a number of cellular stresses including inflammatory cytokines (e.g., Interleukin-1 (IL-1) and tumour necrosis factor- α (TNF- α), heat and chemical shock, bacterial endotoxin and ischaemia/cellular ATP depletion. Activation of these MAP kinase homologues mediates the transduction of extracellular signals to the nucleus and are pivotal events in the regulation of the transcription events that determine functional outcome in response to such stresses. In this review we highlight the identification and characterisation of the stress-activated MAP kinase homologues, their role as components of parallel MAP kinase pathways and the regulation of cellular responses following exposure to cellular stress. CELL SIGNAL 9;6:403–410, 1997. © 1997 Elsevier Science Inc.

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INTRODUCTION

Historical Perspectives

Stress-activated protein kinases were initially identified as the major protein kinase activity that phosphorylated the microtubule associated-protein-2 (MAP-2) recovered from rat liver tissue following treatment of the animals with the protein synthesis inhibitor cycloheximide [1]. This 54kDa activity was termed a MAP kinase isoform based on its 40–45% sequence homology with the p42/44 MAP kinases [2] and in common with the p42/44 MAP kinases was a proline-directed kinase, requiring dual specific phosphorylation of both tyrosine and threonine residues for maximal activation [3]. Molecular cloning of the p54 protein revealed a subfamily of MAP kinase proteins encompassing three distinguishable activities, termed p46, p54 α ; and p55 β [2]. The activation of these kinases contrasted with that of the p42/44 MAP kinases in that they were poorly activated in

response to both growth factors and phorbol ester. They were, in fact, highly responsive to inflammatory cytokines, heat shock and a number of cellular metabolic inhibitors [2]. Their activation was additionally described to be responsible for the N-terminal phosphorylation of the nuclear proto-oncogene c-jun, similar to previously described activities termed c-jun N-terminal kinases (JNKs) [2, 4–6]. Therefore, it became apparent that stress-activated MAP kinases and JNKs were indeed the same entity. At present the SAPK/JNK isozymes appear to be the product of three distinct genes though alternative splice variants of these genes may account for up to 10 identifiable forms [7] resulting in displayed molecular weights of 46 and 55 kDa, respectively.

In addition to the JNK proteins, a parallel stress-activated MAP kinase was also identified in mammalian cell types, analogous to a component of the high osmolarity glycerol responsive 1 (HOG1) pathway in yeast. Attention focused upon this MAP kinase homologue as it emerged from independent studies to be the intracellular kinase termed p38, phosphorylated and activated in response to hyperos-

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molarity and bacterial endotoxin [8, 9], the direct activator of the serine kinase MAPKAP kinase-2, responsible for phosphorylation of the small heat shock proteins hsp27; termed reactivating kinase (RK) or p40 [10, 11] and human homologues involved in the regulation of Il-1 and TNF α synthesis by endotoxin-stimulated monocytes, termed cytokine-suppressive anti-inflammatory drug (CSAID) binding proteins 1 and 2 (CSBPs 1 & 2) [12, 13].

While these initial studies clearly identified both cellular stress and cytokines as major activators of the SAPK cascade, evidence now supports a role for this pathway in the cellular effects of an increasingly large number of extracellular stimuli. This includes ceramides [14, 15], G-protein-coupled receptor agonists [16], vasoactive peptides [17], chronic hypoxia [Scott P. H., Paul A., Robinson C. J. M., Belham C. M., Gould G. W. and Plevin R., unpublished data], cell stretching [19] ischaemia/reperfusion [20–22] and integrin clustering [23] and suggests the potential of multiple signalling events contributing to the activation of the SAPKs and their involvement in a vast array of functional responses (Table 1). A major thrust of current research is in the identification and function of the major intermediates of the SAPK cascades, the intracellular targets of SAPK homologues and the physiological function of these pathways. This review will focus upon these aspects.

Activation of the Stress-activated Protein Kinases Requirement for Upstream Dual Specific Activating Kinases: MKK Homologues

As with the MAP kinase cascades evident in both yeast and mammalian cells, the SAPK pathways align themselves into a well conserved, three component, sequential kinase cascade consisting of MAP kinase homologue (or SAPK/JNK/p38 MAP kinase), MAP kinase kinase homologue (or MKK/MEK/SEK/JNKK) and MAP kinase kinase kinase (or MEKK) (see Fig. 1). Predictably, the activation of the SAPKs relies on their phosphorylation at specific dual phosphorylation motifs, namely the sequences Thr-Pro-Tyr (TPY) for JNK [2] and Thr-Glu-Tyr (TGY) for p38 MAP kinase, respectively [3, 24, 25] (see Fig. 2). These residues are specifically phosphorylated by MKK/MEK homologues distinct from MKK/MEKs 1 and 2, that are responsible for the activation of the classical p42/44 MAP kinase isoforms.

Initial studies examining the activation of SAPKs involved the identification of the existence of an upstream activator of RK/p38 MAP kinase distinct from MKK/MEKs 1/2, initially termed RK kinase (RKK) [10]. This was developed further following the independent isolation of a number of mammalian cDNA clones for distant but related MKK/MEK homologues termed SAPK/ERK kinase-1 or SEK1 [26] MKK/MEK isoforms 3 and 4 [27] and JNK kinase (JNKK) [28]. It became apparent that SEK1 [26] MKK/MEK4 [27] and JNKK [28] represented a common mammalian homologue capable of activating both JNK and p38 MAP kinase *in vitro* [26–28] without effect upon the p42/44 MAP kinase isoforms. However, these early studies also indicated that although MKK/MEK4 could serve as a dual function kinase,

activating both JNK and p38 MAP kinase, MKK/MEK3-mediated activation of p38 MAP kinase could occur independently of MKK/MEK4 activation [26–30].

Recent studies have now clarified these initial findings. Fractionation of sorbitol-stimulated PC-12 cells has illustrated the existence of two distinct resolvable SAPK-activating activities defined as SAPK kinases (SAPKK-1 and 2) [31] which have been identified as the previously isolated MKK/MEK4 and 3, respectively [26–28]. In these cells SAPKK-1 can act as a dual functional kinase, activating both JNK and p38 MAP kinase whilst SAPKK-2, although minimally activated, serves as a distinct and specific activator of p38 MAP kinase, thus supporting the notion of both concomitant regulation of JNK and p38 MAP kinase by MKK/MEK4 and selective activation of p38 MAP kinase by MKK/MEK3 in neuronal cells.

Further studies conducted in other cell types [31, 32], however, have provided evidence that this subfamily of MKK/MEK homologues is not restricted to two members alone and that other upstream activators of JNK and p38 MAP kinase may exist which are regulated in a cell specific manner. In particular, chromatographic fractionation of human KB cells following osmotic stress has identified up to five separable SAPKKs including a further predominant activator of p38 MAP kinase distinct from SAPKK-2, termed SAPKK-3 [31]. This enzyme constitutes the vast majority of p38 MAP kinase-activating activity in these particular cells and is estimated to be up to 10 times more active towards p38 MAP kinase than that of SAPKK-2 (or MKK/MEK3). Further experiments have defined this isoform of SAPK-activating activity to be similar to the recently described MKK/MEK homologue(s) defined as MKK/MEKs 6 and 6b, novel specific activators of p38 MAP kinase [31, 33–36]. This homologue is highly expressed in many peripheral tissues including both skeletal and cardiac muscle but is absent from brain. Thus, MKK/MEK6 is likely to be a major activator of p38 MAP kinase in a number of cells of non-neuronal origin. This is true even in cells where MKK/MEK3 is co-expressed with MKK/MEK6 presumably due to the marked differences in specific activity between the two enzymes [31].

The diversity of the SAPKK activities is not restricted to the regulation of p38 MAP kinase alone, but extends further to the regulation of JNKs. Other novel JNK-activating activities, immunologically distinct from MKK/MEK4, have also been identified; they are termed SAPKK-4 and SAPKK-5 [31] and are consistent with similar novel JNK-activating activities resolved following chromatography of extracts prepared from osmotically shocked fibroblasts [32]. Whilst these activities remain to be characterised and confirmed as unique MKK homologues, such findings further underscore the increasing complexity of the upstream regulation and activation of the SAPK isoforms (see Fig. 3) by diverse dual-specific activating kinases.

Activation of MKK Homologues: Phosphorylation by MEKKs

As predicted from the sequential nature of the SAPK pathways, the activation of the relevant MKK/MEK/SAPKK ac-

TABLE 1. Summary of physiological responses putatively initiated by SAPK activation

Cell type	Stimulus	JNK activation	p38 activation	Functional outcome	Reference
PC12	NGF-withdrawal	Yes	Yes	Apoptosis	[87]
Endothelial	Ceramide, environmental stress	Yes	ND	Apoptosis	[15]
HL-60	Ceramide	Yes	ND	Apoptosis	[14]
Jurkat T cells	Fas-ligation, γ -radiation	Yes	ND	Apoptosis	[84, 86]
NIH 3T3	Carbachol	Yes	ND	Proliferation	[16]
GN4	Angiotensin II	Yes	ND	Proliferation	[17]
Cardiac myocytes	Stretch	Yes	ND	Hypertrophy	[19]
Perfused heart	Ischaemia/reperfusion	Yes	Yes	Regrowth/repair	[21, 22]
Kidney	Ischaemia/reperfusion	Yes	No	Repair	[20]
Monocytes	LPS	ND	Yes	Il-1, TNF α synthesis	[12]
Neutrophils	TNF α , LPS	ND	Yes	Apoptosis	[91]
Platelets	Collagen	ND	Yes	Aggregation	[23]
	Thrombin	ND	Yes	Activation	[80]
Macrophages	LPS		Yes	COX-2/iNOS induction	[90, 91]
L292/HeLa	TNF α	ND	Yes	Il-6 synthesis	[89]
KB	Il-1	ND	Yes	Glucose transport	[79]

ND = not determined.

tivity requires its phosphorylation by an upstream kinase analogous to that of the Raf kinase isoforms that participate in the classical MAP kinase pathway as MKK/MEK/SAPKK activators (see Fig. 1). This has been partly elucidated by the finding that the novel serine/threonine kinase initially termed MEKK1 functions as an upstream activator of MKK/

MEK4/SEK-1/JNKK [29, 37]. This kinase was originally isolated as a cDNA that encoded a novel Raf/Ste11-like kinase able to phosphorylate and activate MKK/MEK1 and MKK/MEK2 *in vitro* [38, 39] and initially thought to function as kinase responsible for mediating p42/44 MAP kinase activation by heterotrimeric G-protein-coupled receptor agonists.

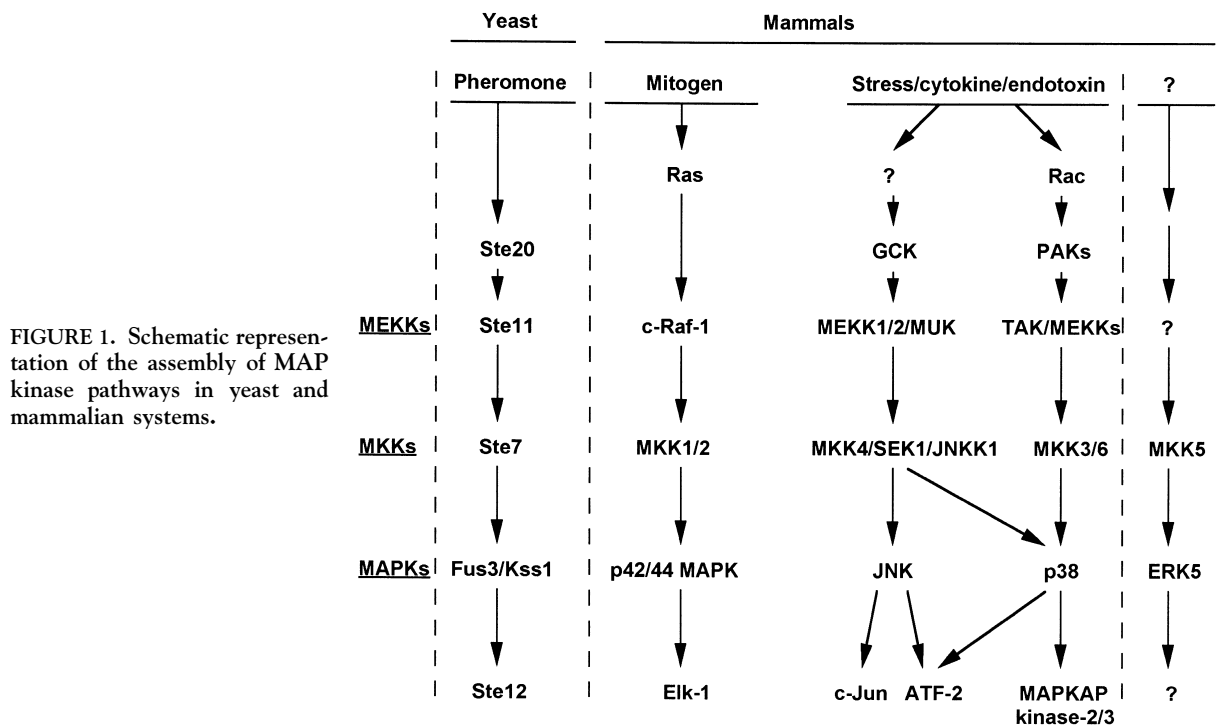


FIGURE 1. Schematic representation of the assembly of MAP kinase pathways in yeast and mammalian systems.

<u>Phosphorylation motif</u>	<u>MAPK homologue</u>
--TPY--	JNK
--TGY--	p38
--TEY--	p42/44 MAPK

FIGURE 2. Dual specific Thr/Tyr phosphorylation motifs within amino acid sequences of MAP kinase homologues.

Further cotransfection studies identified MEKK1 as a strong activator of MKK/MEK4/SEK-1/JNK as detailed above [29, 37]. In this role MEKK1 was also found to participate in both growth factor and TNF-stimulated activation of JNK [37]. Moreover, these studies further illustrated that Raf-1 contributes directly to activation of p42/44 MAP kinase but not to JNK activation, whereas MEKK contributed to JNK activation but only caused p42/44 MAP kinase activation following high cellular overexpression [37]. Therefore, MEKK1 is able to couple functionally the stress agents to c-jun phosphorylation defined by a MEKK1/MKK4 (MEK4)/JNK sequential cascade.

More recently the complexity in the regulation of the SAPK pathways, at the level of MEKK, has been extended following the isolation of further distinct isoforms of MEKK, namely MEKKs 2 and 3 [40]. Cellular expression of MEKK2 or MEKK3 results in the activation of both JNK and the classical p42/44 MAP kinase isoforms, but not that of p38 MAP kinase. Transfection assays further showed that MEKK2 preferentially activated JNK whereas MEKK3 preferentially activated p42/44 MAP kinase [40]. Other MEKK-like proteins such as MUK, related to the mixed-lineage kinases (MLKs), a family of kinases that remain undefined functionally, can also act as regulators of MKK/MEK4 and JNK activity [41]. This may further expand the number of proteins able to regulate the JNK pathway. However, it is apparent that there is no significant homology between the structures of MUK and MEKK, except the well conserved catalytic domain [41], and therefore MUK itself may be active in the capacity of MKK/MEK-activator only under conditions where MEKKs 1–3 are not activated.

The documented inability of MEKKs 1–3 to regulate p38 MAP kinase activation has raised the obvious question regarding the identity of the MEKK-like protein(s) that mediate activation of the parallel and distinct p38 MAP kinase pathway. One such candidate is the MEKK-like proteins termed TAK-1, isolated as a novel MEKK that was able to suppress the transcriptional deficiency of a Ste7 (MKK/MEK homologue) mutation in the yeast pheromone pathway [42]. This murine cDNA was found to encode a transforming growth factor β (TGF β)-activated kinase (hence TAK-1) responsible for mediating transcriptional regula-

tion. In subsequent characterisation of the assembly and activation of the p38 MAP kinase pathway by the novel MKK/MEK homologue MKK/MEK6, TAK-1 has been demonstrated to represent the third component of p38 MAP kinase pathway [36]. Thus, functional coupling of stress-activation of cells to p38 MAP kinase and transcription factor activation may be defined by a TAK-1/MKK3/6 (MEK3/6)/p38 MAP kinase sequential cascade (see Fig. 1). Further characterisation of this protein has led to identification of novel regulators/activators of this kinase, TAK1 binding proteins TAB1 and TAB2 respectively [43], therefore extending the number of components lying upstream of p38 MAP kinase.

At present the relative contribution of each MEKK or MEKK like protein(s) to activation of individual SAPK isoforms is unclear. A specific MEKK isoform may regulate either a single or multiple MKK/MEK homologue(s) depending upon the cellular distribution of the components of the SAPK pathway and the activating stimuli. Consequently, this may in turn generate significant differences in both the magnitude and kinetics of SAPK activation in response to a given agent.

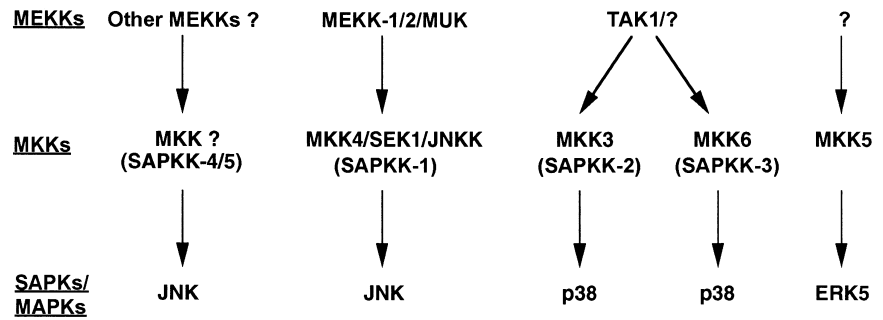
Upstream of MEKKs: Regulation of SAPK Cascades by Low Molecular Weight G-proteins and p21-activated Kinases (PAKs)

By virtue of the role that p21^{ras} plays in the upstream events initiating activation of the Raf/MEK/MAP kinase pathway by growth factors [reviewed in 44], much interest has centred on the role of small molecular weight guanosine 5-triphosphate (GTP)-binding proteins in the stress-related pathways. This includes not only p21^{ras} itself but also the members of the Rho subfamily; Rac and Cdc42 [45].

In particular, evidence now supports a role for Rac and Cdc42 lying upstream of JNK and p38 MAP kinase cascades. Constitutively active forms of both monomeric G-proteins have been shown to stimulate the activation of JNK1 [46–49] and p38 MAP kinase [49, 50], but not p42/44 MAP kinase, indicating a level of specificity in the activation of each pathway. These signalling molecules are likely to serve as critical intermediates as dominant negative Rac or Cdc42 have been reported to inhibit JNK and p38 MAP kinase activity in response to Il-1 [49], EGF [48, 51] muscarinic receptor activation and heterotrimeric G-protein $\beta\gamma$ subunit complexes [47].

Some evidence does support a role for p21^{ras} in the regulation of SAPK activation. It has been found that expression of constitutively active Ras, Ha-Ras or D12 Ras, results in increased activity of JNK [37, 46] while JNK activation in response to EGF is abrogated under conditions of expression of dominant negative Ras (N17) [37]. However, a number of these studies use substantial overexpression of Ras or cell types where Ras function is unusual, thus caution is required in the interpretation of these results. These findings are further complicated due to the potential of functional interrelationships between the small GTPases. Ras can activate

FIGURE 3. Complexity in the upstream regulation of MAP kinase homologues: phosphorylation by MAPKK homologues.



Rac in some cell types [52] and conversely Rac can play a role in Ras-mediated transformation [53]. Therefore, Ras activation may functionally precede Rac in the initiation of the JNK/SAPK cascade [48] and may well be a point at which the classical MAP kinase and SAPK pathways bifurcate.

Another perplexity, however, is that for some agents such as TNF α and anisomycin, activation of JNK appears to be both Ras and Rac-independent [47, 51], suggesting other mechanisms of transducing signals to downstream intermediates of the stress pathways which are independent of the activation of monomeric G-proteins. Thus, even at this level within the cascade, considerable divergence is observed and a substantial degree of agonist specificity still exists in the activation of the pathway.

Although a recent study has now positioned Rac upstream of MEKK1 in the JNK cascade in a manner analogous to Ras, a further level of complexity has been identified. Evidence has also accumulated in support of an involvement of members of a family of serine/threonine protein kinases called p21-activated kinases (PAKs), that are the mammalian homologues of Ste20 in yeast, as intermediates in SAP kinase pathway. Three related enzymes termed PAK1 (α PAK), PAK2 (γ PAK) and PAK3 (β PAK) [49, 54–56] have been shown to be substrates for, and become activated by, binding to Cdc42 and Rac *in vitro*. A role for PAK in the activation of JNK and p38 MAP kinase is indicated by the finding that constitutively active PAK or PAK1/2 overexpression activates JNK/SAPK α [49, 57, 58] and p38 MAP kinase [50] in a number of cell types. Furthermore, Rac-activated JNK1 is inhibited by the expression of the PAK1 N-terminal regulatory domain [48] and activation of JNK and p38 MAP kinase by Il-1, Rac and Cdc42 is inhibited by dominant-negative catalytically inactive PAK [49, 50]. Both Il-1 and the G-protein coupled receptor agonist, fMetLeuPhe (fMLP), can activate PAK *in vivo* [50, 59] and therefore it may emerge that PAK-dependent activation of both JNK and p38 MAP kinase can occur in response to a number of physiological activators of the SAPK pathway. Whether PAKs activate the upstream components regulating the stress-activated signalling cascades such as MEKK is not yet known. However, GTP γ S-dependent association of PAK and MEKK1 in Cdc42 complexes has been demonstrated, and in yeast, the PAK equivalent Ste20 activates the MEKK1 homologue Ste11 [60]. Recently, another distinct class of mammalian homologues of Ste20 distantly related to PAKs have been identified in-

cluding the Germinal Centre Kinase (GCK) and upstream kinase-1 (UK-1) [61]. GCK, but not UK-1, specifically activates the JNK pathway. This protein does not appear to require the involvement of a small molecular weight G-protein for activation [61] and therefore may function as an intermediate in the activation of the SAPKs by agents such as anisomycin or TNF that are known to be monomeric G-protein independent (see above).

Cellular Targets of the SAPKs

Various intracellular proteins have been identified as potential targets for the SAPKs and includes a number of transcription factors such as c-jun, ATF2, Elk-1 and CREB [4, 62–65]. These factors participate to overlapping extents in the formation of homo/heterodimer complexes (e.g., c-jun interacts with c-fos in the formation of the AP-1 complex) [see 65–70] which regulate increases in gene expression. Each SAPK may have both specific and common nuclear targets which will necessarily result in an integrated unique pattern of transcriptional activity in response to a given stress stimuli.

As the terminology used in the naming of the SAPKs would suggest, early studies identified c-jun as a substrate for JNKs [4]. Phosphorylation occurs at positions Ser 63 and Ser 73, following binding of JNKs to c-jun at an adjacent amino acid sequence within the N-terminus termed the “delta-region,” and may represent the modifications required for transactivation [4]. Other substrates activated transcription factor 2 (ATF2) [62–64], a member of the cAMP-responsive element (CRE) family of transcription factors, [68] and the Ets-domain transcription factor Elk-1 [65, 66]. These phosphorylation event participate in the assembly of AP-1 [66, 67], SRE [65, 66] and other complexes facilitating the transcription of the relevant promoters.

There is also considerable variation in the relationship between binding and phosphorylation of transcription factors by JNK. In the case of c-jun and ATF2, their respective binding interactions with JNK are requisites for their subsequent phosphorylation [7, 62, 63], whilst for Elk-1 the initial binding of JNK to an analogous adjacent “delta-domain” is not required [7]. Binding of JNKs to other proteins such as Jun B [7] and c-Rel [71] are not followed by phosphorylation and these proteins, therefore, may function as molecular chaperones potentially enabling JNK to interact with and/or phosphorylate other nuclear targets.

Like JNK, p38 MAP kinase may also modulate transcription events by direct phosphorylation and activation of transcription factors. One recently described target is CHOP, a transcription factor of the C/EBP family previously implicated in cell cycle arrest [72]. However, many of the transcriptional events stimulated by p38 MAP kinase may be mediated by the activation of MAPKAP kinase-2, another direct target of p38 MAP kinase (see above; [10, 11]). MAPKAP kinase-2 is able to phosphorylate the same serine residues in both CREB and ATF1 that are targeted by cyclic-AMP dependent protein kinase (PKA) [73, 74]. CREB is phosphorylated by MAPKAP kinase-2 *in vitro* and also in SK-N-MC cells and KB cells following stimulation with agonists known to activate the p38 MAP kinase pathway [73, 74]. Moreover, these events are sensitive to blockade by SB 203580, a specific inhibitor of p38 MAP kinase [12, 13], at concentrations similar to those required for inhibition of MAPKAP kinase-2 activity [73, 74]. Therefore, MAPKAP kinase-2 is likely to regulate the transcription of those genes whose promoters contain CREs or elements sensitive to interactions with homo or heterodimers of the CREB/ATF and AP-1 families [66, 68].

In contrast to studies on gene transcription, there is relatively little information regarding other potential cellular targets of the SAPKs. MAPKAP kinase-2/3 are known to phosphorylate Hsp 27 [75–77], a cytosolic protein thought to stimulate the polymerisation of actin and the subsequent repair of the actin microfilament that is disrupted following cellular stress, thereby aiding cell survival [78]. As p38 MAP kinase is believed to participate in the regulation of glucose transporter function [79] and platelet aggregation [23], it is reasonable that cytoskeletal proteins or other associated intermediates are substrates. Other putative targets include cytosol PLA₂ [80] which was previously ascribed to be a substrate for activated p42/44 MAP kinase [81]. Thus, many of the previously defined intracellular targets of the classical p42/44 MAP kinases may require re-appraisal.

Functional Responses Regulated by Stress-activated Protein Kinases

As the SAPK pathways were elucidated it became apparent that the agents activating these cascades, in particular TNF α , ceramides and UV-radiation, were also well recognised mediators of apoptosis or cell cycle arrest [82, 83]. A strong correlation has now been established between the activation of JNK and apoptosis induced by diverse stimuli including ceramides, Fas-ligation and UV and γ -radiation exposure [14, 15, 84–86]. The use of dominant negative mutants of components of the cascade has confirmed an obligatory role for JNK in cytokine- and ceramide-induced apoptosis in endothelial cells [15], and in neuronal cells where apoptosis is initiated following serum deprivation [87].

Despite the current dogma, however, not all evidence supports a role for JNK in apoptosis or cell cycle arrest. Other studies have shown that while activation of MEKK1 in fibroblasts is required for apoptosis, this response does not

require functional JNK [88]. Furthermore, there is increasing evidence to support a role for JNK in positive regulation of cell proliferation [16, 17]. There is an apparent relationship between the activation of G protein-coupled receptors, by agonists such as AII and carbachol, and increases in DNA synthesis [16, 17]. In non-dividing cells, such as cardiac myocytes, JNK activation is linked to hypertrophy [19]. This pathway may also be activated following ischaemia/reperfusion in both renal [20] and cardiac [21, 22] tissues, suggesting that the activation of JNK may promote cell survival via repair and regrowth. Therefore, the activation of JNK may promote a different proliferative response depending upon both the stimulus and the cell type involved.

Linking p38 MAP kinase activation to a functional response has been hampered by lack of information about its upstream activators or its specific nuclear targets (see above) which has hindered the development of appropriate deletion systems. Much of the information which is available has been derived by the use of the novel compound SB 203580, a specific inhibitor of p38 MAP kinase [12, 13]. Studies utilising this compound have implicated a role for p38 MAP kinase in inflammatory responses including the synthesis of Il-1, TNF α [12] and Il-6 [89], and the induction of enzymes such as COX-2 and iNOS [90, Paul A., Cuenda A., Bryant C. E., Murray J., Chilvers E. R., Cohen P. and Plevin R., unpublished data.]. Additionally, p38 MAP kinase has recently been implicated in the initiation of platelet aggregation [23] and in the regulation of glucose transporter function [79]. These events are associated with changes in the subcellular distribution of proteins and changes in cytoskeletal function and is consistent with the original finding that MAPKAP kinase-2 is associated with adaptive re-arrangement of actin filaments under conditions of heat shock [78].

Less evidence links p38 MAP kinase to cell cycle arrest than JNK. In PC-12 cells, activation of p38 MAP kinase is associated with apoptosis following serum deprivation [90]. However, SB 203580 fails to prevent the cytotoxic effect of TNF α in L929 cells [89] though it enhances the ability of TNF α to induce apoptosis in human neutrophils [Paul A., *et al.*, unpublished data.]. This suggests that either p38 MAP kinase is not involved in promoting apoptosis or is protective against this process. Recently, the physiological roles of iNOS and COX-2 have been extended to include both protection against cell cycle arrest and initiation of apoptosis in different cell types [Paul A. *et al.*, unpublished data, 92], thus it is likely that p38 MAP kinase may regulate cell growth at least by indirect mechanisms through regulation of expression of these enzymes. Whether p38 MAP kinase plays a role in cell growth and division *per se* by directly regulating transcription events associated with cell division, although very likely, remains to be fully determined.

More importantly, it has been implicated that the balance between the classical p42/44 MAP kinase and the SAPK cascades may be critical in determining cellular fate and this may be true in a number of cell systems. However, given the lack of consensus regarding the roles of JNK and

p38 MAP kinase in the regulation of cellular function, it is likely that a balance exists within the stress-kinase cascades themselves and that differential activation of each pathway ultimately determines the functional response and outcome for the cell.

Summary and Future Perspectives

It is now apparent that strong evidence exists for a distinct signal transduction cascade being responsible for mediating the effects of stress and other forms of related stimuli upon all cells. Within this cascade there is evidence for multiple mechanisms of activation of each SAPK pathway by different stimuli, specific nuclear targets for each SAPK homologue and the involvement of the SAPKs in a number of specific cellular functions.

Future studies will undoubtedly address a number of outstanding issues. There is still a large gap in understanding of the very early events responsible for the initiation of the SAPK cascade and the key points of bifurcation within the pathways. Immediate studies are likely to focus upon those events upstream of MKK/MEK6 and the small molecular weight G-proteins. Other key areas which require investigation are the roles of MAP kinase phosphatases in the termination of SAPK signals, "cross talk" regulation of SAPK activity by lipid and cAMP-dependent signalling pathways [93–95], and the potential inhibition of the SAPKs by endogenous non-enzymatic protein inhibitors involved in the cell cycle [96].

Finally, the identification of novel SAPK isoforms (SAPK-3) [97], MAP kinase homologues and MKK/MEK homologues [98, 99, see Figs. 1 and 2] will further increase the complexity of the regulation of these homologous signalling cascades and it is likely that several other parallel cascades will be elucidated. Given the rate of progress in the study of these pathways, we will not have long to wait until these existing gaps in our knowledge are filled.

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