

Review Article

The p38 signal transduction pathway Activation and function

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Abstract

The p38 signalling transduction pathway, a Mitogen-activated protein (MAP) kinase pathway, plays an essential role in regulating many cellular processes including inflammation, cell differentiation, cell growth and death. Activation of p38 often through extracellular stimuli such as bacterial pathogens and cytokines, mediates signal transduction into the nucleus to turn on the responsive genes. p38 also transduces signals to other cellular components to execute different cellular responses. In this review, we summarize the characteristics of the major components of the p38 signalling transduction pathway and highlight the targets of this pathway and the physiological function of the p38 activation. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

The response of cells to extracellular stimuli is in part mediated by a number of intracellular kinase and phosphatase enzymes [1]. The mitogen-activated protein (MAP) kinases are members of discrete signalling cascades, which are focal points for diverse extracellular stimuli, and function to regulate fundamental cellular processes. Four distinct subgroups within the MAP kinase family have been described. These include (1) extracellular signal-regulated kinases (ERKs), (2) c-jun N-terminal or stress-activated protein kinases (JNK/SAPK), (3) ERK5/big MAP kinase 1 (BMK1), and (4) the p38 group of protein kinases. Within this area of research, the activation of ERKs has been extensively described and characterised as a central component of the signal transduction pathways, stimulated by growth-related stimuli [2,3]. The JNK group of protein kinases are activated in response to a number of cellular stresses, including high osmolarity and oxidation [4]. The ERK5/BMK1 MAP kinase signalling pathway regulates serum-induced early gene expression [5]. The p38 group kinases have been found to be involved in inflammation, cell growth, cell differentiation, the cell cycle, and cell death [6]. It is clear, then, that the p38 pathway

shares many similarities with the other MAP kinase cascades. The purpose of this review, however, is to highlight the unique characteristics of the p38 group of kinases, the components of this kinase cascade as well as the activation of this pathway and the biological consequences of its activation.

2. Properties of the p38 group of MAP kinase members

p38 α (or simply p38) was first isolated as 38-kDa protein, which was rapidly tyrosine phosphorylated in response to LPS stimulation [7,8]. Molecular cloning of the protein revealed that it is an MAP kinase family member [8]. p38 (termed RK and p40) was identified as an upstream kinase of MAP kinase-activated protein kinase-2 (MAPKAPK-2 or M2) in IL-1 or arsenite-stimulated cells [9,10]. p38 was also purified and its cDNA cloned as a molecule that binds pyridinyl imidazole derivatives (which inhibit the production of proinflammatory cytokines) and was termed cytokine suppressive antiinflammatory drugs binding protein (CSBP) [11]. Three p38 homologues, p38 β [12], p38 γ (or ERK6, SAPK3) [13–15], and p38 δ (or SAPK4) [16,17], were cloned in mammals. The p38 α and p38 β genes are ubiquitously expressed [12]. However, p38 γ and δ are differentially expressed in different tissues. p38 γ is predominantly expressed in skeletal muscle [13,14] and p38 δ is enriched in lung, kidney, testis, pancreas, and small in-

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Table 1
Properties of p38 group MAP kinase members

p38 isoforms	Other names	No. of amino acids	Size of mRNA (kb)	Apparent MW (kDa)	Sensitivity to SB 203580
p38	p38 α , CSBP, MPK2, RK Mxi2	360	3.5	38	+
p38 β	p38-2, p38 β_2	364	2.5	39	+
p38 γ	ERK6, SAPK3	367	2.0	43	–
p38 δ	SAPK4	366	1.8	40	–

testine [17]. p38 γ expression was reported to be induced during muscle differentiation and p38 δ expression was shown to be developmentally regulated [13,18]. An up-regulation of the expression of p38 isoforms was observed in the inflammatory cell lineages [19]. Sequence comparisons revealed that each p38 isoform has more than 60% identity within this group, but only 40 to 45% to the other MAP kinase family members. Table 1 shows the properties of the p38 group of MAP kinase members.

All the known MAP kinases can be categorised by the sequence of the canonical dual phosphorylation site Thr-Xaa-Tyr (TXY) in a regulatory loop between kinase subdomains VII and VIII [20]. All the p38 group kinases have the Thr-Gly-Tyr (TGY) dual phosphorylation motif; however, ERK1/2 and ERK5/BMK1 possess a Thr-Glu-Tyr (TEY) motif and JNK/SAPK group has a Thr-Pro-Tyr (TPY) motif. The Xaa residue in the dual phosphorylation motif, as well as the length of the loop, influences p38 substrate specificity. In addition to this, the length of the loop plays a major role in controlling auto-phosphorylation [21]. In contrast, modification of the loop structure alone does not change the selectivity of the upstream MAPK kinases [21]. The crystallographic structure of p38 provides insight into the mechanism by which activator and substrate specificity is achieved [22–24]. Structural analysis also shows that the p38 specific inhibitor, SB203580, is bound in the ATP pocket and, thereby, specifically inhibits its enzymatic activity [25]. There are several other inhibitors, such as pyrroles, that act similarly to SB203580 to exert a potent antiinflammatory effect *in vitro* and *in vivo* [26,27].

3. Regulation of the p38 signalling pathway

3.1. Extracellular stimuli

p38 homologues have been identified and cloned in both low and high eukaryotic species, including fly, frog, and yeast [10,28–30]. The Hog1 pathway [29] in budding yeast and the Spc1/Sty1 pathway in fission yeast [30] are believed to share an ancestral gene with p38 group kinases. Their role has been implicated in osmoregulation, responses to extracellular stress stimuli, and cell-cycle events [29–31]. Mammalian p38s are also activated by environmental stresses [8–11,32]. Since mammalian p38 was identified in studies designed

to understand signalling pathways during inflammation [8], extensive data of p38 regulation have been developed in immune systems. p38 activation has been observed in inflammatory responses, as in LPS-treated macrophages [8], TNF-stimulated endothelial cells [33], IL-17-stimulated chondrocytes [34], IL-18-stimulated U1 monocytic cell line [35], human platelets stimulated with thrombin [36], and chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (fMLP) or phorbol myristate acetate (PMA)-treated human neutrophils [37,38].

In the past few years, intensive study has been done regarding the activation of p38 α in many other systems. Growth factors like granulocyte macrophage colony-stimulating factor (GM-CSF) [39], fibroblast growth factor (FGF) [40], erythropoietin [41], IL-3 [39,41], IL-2, IL-7 [42], nerve growth factor (NGF) [43,44], insulin-like growth factor (IGF) [45], vascular endothelial growth factor (VEGF) [46], and platelet-derived growth factor (PDGF) [47] were found to trigger p38 α activation in certain cell types. p38 α activation has also been observed in response to several other stimuli, including transforming growth factor (TGF)- β [48], G-protein-coupled receptor agonists [49,50], a muscarinic agonist [51], vasoactive peptides [52,53], cholecystokinin [54], heat shock [55], cell stretching [56], and ischemia/reperfusion [57]. Thus, a variety of signalling events are able to trigger the activation of p38 pathway. Table 2 lists some stimuli reported in recent years that lead to the activation of p38 α . It should be clarified that the activation of p38 α is not only dependent on stimulus, but also dependent on cell type. For example, insulin can stimulate p38 in 3T3-L1 adipocytes [58], but downregulates p38 activity in chick forebrain neuron cells [59].

Although the other three p38 group members, p38 β , p38 γ , and p38 δ have been cloned for a couple of years, there is not much information concerning their activation in different cells under different conditions. Several groups of researchers have shown that the four p38 group members display similar activation profiles by using transiently expressed epitope-tagged p38 isoforms [12,16,18,60]. However, differences have been observed in the kinetics and in the level of activation of these isoforms. There are reports suggesting that distinct upstream kinases selectively activate p38 isoforms. MKK3 cannot effectively activate p38 β like MKK6 does [61]; p38 δ can be activated not only by MKK3 and MKK6,

Table 2
Extracellular stimuli of p38

Stimuli	Cell type	Functional outcome	Reference
Pathogens			
LPS	RAW264.7, monocyte, THP-1		Han et al. [8]
Soluble staphylococcal peptidoglycan (pPGN)	Raw264.7	H ₂ O ₂ production	Dziarski et al. [142]
Plasma-opsinized <i>Staphylococcus aureus</i> (S-SA)	Human PMN		McLeish et al. [143]
Superantigen staphylococcal enterotoxin B (SEB)	HA-1.70	TNF- α production	Schafer et al. [144]
Macrophage-activating lipopeptide (MALP)-2	Human monocyte, macrophage, RAW264.7	IL-1, TNF production	Garcia et al. [145]
Mycoplasma fermentas lipid-associated membrane proteins (L-AMPP)	THP-1, PMN	IL-8 production	Marie et al. [146]
Echovirus1 (EV1)	HOSpa ₂ AW	junB expression	Huttunen et al. [147]
Murine hepatitis virus strain 3 (MHV-3)	Peritoneal exudative macrophage (PEM)	Macrophage prothrombinase fgl-2 production	McGilvray et al. [148]
Sindbis virus (SV)	Vero		Nakatsue et al. [149]
Simian immunodeficiency virus	Jurkatt		Popik et al. [150]
Herpes simplex virus type-1 (HSV-1)	Baby hamster kidney (BHK) cell		Zachos et al. [151]
Clostridium botulinum C ₃ toxin	Rat-1	c-Jun expression	Beltman et al. [152]
Cytokines			
TNF- α	L5178, human chondrocyte, HUVEC, neutrophil		Pietersma et al. [33]; Moriguchi et al. [71]
IL-1	NIH313, human chondrocyte, MRC-5, KB		Freshny et al. [9]
IL-2, IL-7	Kit-225, CT6	Proliferation	Crawley et al. [42]
IL-17	chondrocyte	iNOS, Cyclooxygenase (COX)-2 and IL-6 production	Shalom-Barak et al. [34]
IL-18	U1	HIV type I and IL-8 production	Shapiro et al. [35]
TLL1	Bovine pulmonary artery endothelial cell (BPAEC)	Apoptosis	Yue et al. [135]
TNF- α and GM-CSF	PMN	Respiratory burst response?	McLeish et al. [153]
Growth factors			
TGF β	Neutrophil	Chemotaxis, actin organization	Moriguchi et al. [71]; Wang et al. [48]
GM-CSF	Mast cell	Development of hemopoietic cell	Foltz et al. [39]
CSF-1	Mast cell	Development of hemopoietic cell	Foltz et al. [39]
EPO	Hemopoietic progenitor cell	Development of hemopoietic cell	Foltz et al. [39]; Nagata et al. [41]
Steel locus factor (SLF)	Mast cell	Development of hemopoietic cell	Foltz et al. [39]
FGF	SK-N-MC	Regulate gene expression at the CRE	Tan et al. [40]
IGF	SH-SY5Y, PC12	Anti-apoptosis, CREB-dependent gene expression	Cheng et al. [45]; Pugazhenthi et al. [154]

(continued)

Table 2
Continued

Stimuli	Cell type	Functional outcome	Reference
NGF	PC12	Differentiation	Morooka et al. [43]; Xing et al. [44]
NGF withdrawal	PC12	Apoptosis	Kummer et al. [126]
PDGF	Airway smooth muscle cell	Proliferation	Pyne et al. [47]
VEGF	HUVEC	Actin organization, migration	Rousseau et al. [46]
bFGF and forskolin	F9	CCK transcription	Hansen et al. [155]
Stress environment			
Heat shock	Hela		Moriguchi et al. [71]
Hyperosmolarity	L5178, KB, monocyte, PC12, 293		Clerk et al. [52]
Hypoosmolarity	Human intestine 407, 293		Kumar et al. [17]
Ultraviolet	CH3 10T1/2, Hela, TK, NIH3T3, vascular endothelium, KB, PC12		Hazzalin et al. [107]
Oxygen radical metabolites (H ₂ O ₂ , menadione)	Macrophage		Ogura et al. [72]
Stretch	Cardiac myocyte	Hypertrophy	Kudoh et al. [56]
Cyclic stretch	Mesangial cell	Proliferation	Ingram et al. [156]
Hypoxia	Bovine pulmonary artery fibroblast	Repair	Scott et al. [157]
Ischemia/reperfusion	Perfused heart		Bogyevitch et al. [57]
As, V, Zn, and Cd	BEAS, 9L rat brain tumor cell		Samet et al. [158]; Hung et al. [159]
Arsenite	KB, PC12, Hela, 293	ERK activation	Ludwig et al. [160]
Others			
CD40 cross-linking	B cell line	NF-κB activation, CD54/ICAM expression, B cell proliferation	Craxton et al. [124]
B cell antigen receptor cross-linking	B cell line		Hashimoto et al. [161]
Okadaic acid	fibroblast	Collagenase-1 (MMP-1) expression	Westermarek et al. [162]
SKF38393 (D1 dopamine receptor agonist)	SKNMC human neuroblastoma cell		Zhen et al. [163]
Phenylephrine	Rat-1 cell		Alexandrov [164]
Norepinephrine	PC12	Differentiation	Williams et al. [49]
Isoproterenol	rat epididymal fat cell		Moule et al. [165]
Carbachol	Airway smooth muscle cell	HSP phosphorylation	Larsen et al. [51]
Angiotensin II	Vascular smooth muscle cell	Proliferation, Na ⁺ /H ⁺ exchange	Ushio-Fukai et al. [53]; Kusuhabara et al. [166]
Endothelin-1	Cardiac myocyte	Hypertrophy	Clerk et al. [52]
Thrombin	CCL39, platelet		Saklatvala et al. [36]
Three dimensional collagen	Dermal fibroblast	Collagenase-3 (MMP-13) expression	Ravanti et al. [167]

(continued)

Table 2
Continued

Stimuli	Cell type	Functional outcome	Reference
PAF	CHO		Zang et al. [83]
Synthetic oligonucleotides (CpG DNA)	B cell, monocyte, macrophage, dendritic cell		Yi et al. [168]; Hacker et al. [169]
Adenosine	Rat myoblast H9C2 perfused rat heart	Ischemic preconditioning?	Haq et al. [170]; Nagarkatti et al. [171]
Ceramide (C ₂ , C ₆ and SMase)	184B5/HER	COX-2 production	Subharamaiah et al. [172]
MAHMA-NO (NO donor)	Rat mesangial cell		Huwiler et al. [173]
S-nitrosoglutathione (GSNO)	RAW264.7		Callsen et al. [174]
CD473 (retinoid)	HL-60R		Zhang et al. [175]
PMA	Neutrophil		El Benna et al. [38]
Calphostin-C	Human glioma cell		Ozaki et al. [176]
Anisomycin	CH3 10T1/2, KB, PC12, HeLa, 293		Kumar et al. [17]
Sodium salicylate	FS-4	Apoptosis	Schwenger et al. [133]
Taxol	RAW264.7		Lee et al. [177]
Glutamate	Rat cerebellar granule cell	Apoptosis	Kawasaki et al. [134]
Gonadotropin-releasing hormone (GnRH)	aT3-1 cell		Roberson et al. [178]
Cholecystokinin (CCK)	Rat pancreatic acinus	Actin organization	Schafer et al. [54]

but also by JNK kinases, MKK4 and MKK7 [18]. These data suggest that the activation of p38 isoforms can be both specifically regulated through different regulators and coactivated by the same upstream regulators. In addition to these various forms of regulation, the difference in tissue distribution and/or expression pattern during development may also be instrumental in the functional diversity of these isoforms.

3.2. Upstream kinases responsible for p38 activation

Like all MAP kinases, p38 group kinases are activated by dual kinases, the MAP kinase kinases (MKKs). Despite the conserved dual phosphorylation sites of this group of MAP kinases, selective activation of different isoforms by distinct MKKs was observed. For example, MKK6, which is 80% homologous to the isoform MKK3, can activate all four p38 isoforms, whereas MKK3 preferentially activates only p38 α , p38 γ , and p38 δ [62]. Activation of p38 α and p38 δ by MKK4 has been reported [16]. MKK7 has also been reported to activate p38 δ [18]. This suggests that substrate selectivity may be a reason why each MKK has a distinct function. The constitutively active form of MKK6, MKK6(E), is much more efficient than MKK3(E) in inducing apoptosis of Jurkat T cell [63]. Moreover, when expressed in cardiac myocytes, MKK3(E) has more prominent apoptotic effects than MKK6(E) [64]. Multiple splicing variants have been cloned for MKK3, MKK4, MKK6, and MKK7. The long form of each of these MKKs is the predominant form within most cells and exerts stronger enzymatic activity than the shorter forms [65].

3.3. Further upstream activators

The signalling pathways upstream of the MKK/p38 pathway are further diversified, which may explain why the p38 pathway can be activated by various stimuli. Several MKK kinases (MAP3K) have been reported to cause p38 activation. These include MTK1, MLK2/MST [15,66,67], MLK3/PTK/SPRK [68], DLK/MUK/ZPK [67,69], ASK1/MAPKKK5 [70], and TAK1 [71]. Overexpression of these MAP3Ks leads to activation of both the p38 and JNK pathways, which may be the reason why p38 and JNK are often coactivated. However, specific activation of p38 and JNK has been observed, implying that there is specific activation of the p38 pathway at this level [72]. In addition to the differential effects the MAP3Ks may have downstream, different MAK3Ks may also function to mediate different upstream signals. For example, MTK1 may only mediate stress signals but not cytokines. This is demonstrated by the fact that the dominant negative MTK1 mutant only inhibits activation of the p38 pathway by environmental stress (osmotic shock, UV, and anisomycin), but not by the cytokine TNF [66]. A group of stress-induced proteins GADD 45 $\alpha/\beta/\gamma$, which are able to bind to the N-terminal domain of MTK1, might be involved in regulation of MTK1 activation [73].

Considering the important role Ras is known to play in upstream events initiating activation of the Raf/MEK/MAP kinase pathway, much interest was raised on low molecular weight GTP-binding proteins in propagating signals for other MAP kinase pathways. With the use of cotransfection techniques, the Rho family low molecular weight GTP-binding proteins, Rac and Cdc42, were identified as potential regulators of the p38 pathway [74,75]. These molecules are likely to serve as critical intermediates. This is demonstrated by the fact that dominant negative Rac or Cdc42 was reported to inhibit p38 activity in response to IL-1 [75], muscarinic activation, and heteromeric G-protein $\beta\gamma$ subunit complexes [76]. Since MLK1, MLK2, and MLK3 each contain a potential Rac/Cdc42 GTPase-binding (CRIB) motif, Rac/Cdc42 may directly activate MAP3K [68,77].

Evidence has also accumulated in support of an involvement by members of a family of serine/threonine protein kinases called p21-activated kinases (PAKs). These kinases are the mammalian homologues of Ste20 (the upstream activator of Ste11; yeast MAP3K) in yeast. Three related enzymes termed PAK1 (α PAK), PAK2 (γ PAK), and PAK3 (β PAK) were shown to be activated by binding to Cdc42 and Rac in vitro [75, 78,79]. Furthermore, dominant negative, catalytically inactive PAK inhibits the p38 activation by IL-1, Rac, and Cdc42 [74,75,80]. More recently, Mst1, another mammalian homologue of Ste20, was identified as an activator of MKK6, p38, MKK7, and JNK in cotransfection assays [81].

Not only are small G-proteins involved in the p38 signal cascade, but the large G-proteins are also involved. Interaction between chemokines such as fMet-LeuPhe (fMLP) and PAF with their respective G-protein-coupled receptors can lead to p38 activation [82]. Regulators of G protein signalling (RGS) proteins were also shown to be involved in G-protein-dependent p38 activation [83].

4. Downregulation of the p38 signalling pathway

Under physiological conditions, MAP kinase activation is often transient. Because the level of MAP kinases never changes throughout the course of stimulation, dephosphorylation by phosphatases would seem to play a major role in the downregulation of MAP kinase activity. A group of dual phosphatase has been identified and cloned. MAP kinase phosphatase (MKP)-1 (or CL100/3CH134) is the archetypal member of this gene family and has activity for several MAP kinases, such as ERK, JNK, and p38 [84]. Nine other mammalian dual-specificity phosphatases have been identified, several of which are under tight transcriptional control and display distinct tissue, cell, and subcellular expression patterns [85,86]. In both in vitro and transient transfection studies, MKP-1, MKP-4, and MKP-5 can efficiently de-

phosphorylate p38 α and p38 β [87,88]. Interestingly, p38 γ and p38 δ are resistant to all MKP family members. This may be a mechanism to differentially regulate p38 isoforms. Although most dual-specificity phosphatases including MKP-1 are highly inducible in response to mitogenic and/or stress stimuli, at least one member of this group of phosphatases, MKP-3, is constitutively expressed [89]. The activity of this phosphatase is regulated by the binding of ERK2 to the noncatalytic amino-terminus of MKP-3 [90]. This suggests that the regulatory mechanism and function of MKP-3 may be different from that of the other phosphatases.

In addition to dual phosphatase, studies in yeast indicated that other types of phosphatase, such as protein tyrosine phosphatase (PTPase) and a serine/threonine protein phosphatase type 2C (PP2C), have important roles in downregulating the MAP kinase HOG1 pathway [91–93]. By screening a human cDNA library for clones that could block the activation of the yeast HOG1 pathway, the human protein phosphatase PP2C α was identified. Studies using a mammalian system showed that PP2C α also negatively regulates the human MKK6 and MKK4 in vitro and in vivo [94]. Thus, different phosphatases function at different levels to inactivate MAP kinase cascades.

5. Downstream substrates of p38 group MAP kinases

5.1. Protein kinase substrates of p38

MAP kinase-activated protein kinase 2 (MAPKAP-K2 or M2) was the first identified p38 α substrate. In vitro phosphorylation of M2 by p38 α activates M2. In vivo activation is inhibited by SB203580, a specific inhibitor of p38 α and p38 β [9,10,95]. Subsequently, a closely related protein kinase, M3 (or 3pk), was also found to be a substrate of p38 α [95]. Moreover, activated M2 and 3 phosphorylate various substrates including small heat shock protein 27 (HSP27) [96], lymphocyte-specific protein 1 (LSP1) [97], cAMP response element-binding protein (CREB) [40], ATF1 [40], SRF [98], and tyrosine hydroxylase [99].

Recently several other protein kinases were also identified as downstream substrates of p38 α or p38 β . These include:

1. MAP Kinase Interaction Protein Kinase, MNK1, but not its isoform MNK2, was found to bind to p38 and ERK1. Moreover, it was demonstrated in vitro that MNK1 could be phosphorylated by ERK1 and p38, but not by JNK [100,101]. Since MNK1 and 2 can phosphorylate eukaryotic initiation factor-4E (eIF-4E) in vitro, it suggests a potential link between MAP kinase activation and translational initiation [100].
2. p38 regulated/activated kinase, PRAK, is a stress-activated protein kinase that can be activated by

p38 α and p38 β [102]. Recent results indicate that PRAK is a preferred substrate for p38 β (our unpublished results). Our data also indicate that HSP27 is a substrate for PRAK [102].

- Mitogen- and stress-activated kinase, MSK (RSK-B or RLPK), is a protein that is activated by both stress and mitogen [103]. Interestingly, a co-transfection assay revealed that p38 α can activate MSK [103,104]. However, in vitro coupled kinase assay did not show an activation of MSK by p38 α [105]. This suggests that p38 α may act indirectly to activate MSK. It has also been shown that CREB and histone 2B are potential substrates of MSK [103–105].

Given the aforementioned data, it would seem that the substrates of p38 are instrumental in diversifying and amplifying p38 signals. This is further proven by the fact that M2 appears to act on a translational level. Gene disruption of M2 reveals that M2 is required for translational activation of TNF α and IFN γ genes [106].

5.2. Transcription factors activated by p38

Several transcription factors have been shown to be phosphorylated and subsequently activated by p38 α . These transcription factors include activating transcription factor-2 (ATF-2), ATF-1, SRF accessory protein 1 (Sap1), CHOP (growth arrest and DNA damage inducible gene 153, or GADD153), p53, C/EBP β , myocyte enhance factor 2C (MEF2C), and MEF2A [32,40,107–113].

The various transcription factors have unique modes of action. By themselves, ATF-1 interact with the cAMP responsive element (CRE) [114]. Phosphorylation of p53 by p38 α may play a role in p53-dependent transcription [113]. An important *cis*-element, AP-1 binding site, appears to be influenced by the p38 pathway in several different mechanisms. ATF2, a substrate of p38, can form heterodimers with Jun family transcription factors and thereby directly associate with AP-1 binding site. p38 can phosphorylate Sap-1a, a component of Ternary Complex Factor (TCF), which binds to serum responsive elements (SRE). Since it is well established that induction of *c-fos*, a component of AP-1, is SRE-dependent, by involving *c-fos* upregulation, the p38 pathway indirectly regulates AP-1 activity. p38 is likely to be involved in *c-Jun* expression by regulating MEF2A and 2C activity [112]. Participation in *c-Jun* induction may be another way by which the p38 pathway regulates AP-1 activity. It is known that ERK and JNK mediate another component of the TCF called Elk-1. This allows for the possibility of the coordinated participation of the three MAP kinases in the regulation of *c-fos* expression. However, this Sap-1a-mediated *c-fos* SRE stimulation does not occur in RK13 cells [109], suggesting that signalling via p38 and Sap-1a is restricted, thus ensuring its specificity.

Another group of transcription factors that may fall under the control of p38 is the C/EBP family of transcription factors. CHOP 10 (Gadd 153), a member of the C/EBP family, is known to be involved in the regulation of cell growth and differentiation. In vitro assays reveal that CHOP 10 is phosphorylated by p38. In vivo assays further support the notion of such interaction as the p38 inhibitor, SB203580, abolishes the stress-induced phosphorylation of CHOP 10 [110]. Another member of the C/EBP transcription family, C/EBP β , was shown to be a substrate of p38 α , hinting at a role in adipocytes differentiation [115].

As described earlier, many of the transcriptional events stimulated by p38 MAP kinase may also be mediated by the activation of M2, RSK-B and MSK1, direct targets of p38 (see above).

5.3. Other types of substrates for p38

Several other proteins including cPLA2 and Na⁺/H⁺ exchanger isoform-1 have been reported to be substrates for p38 α [116,117]. Stathmin was recently reported to be a substrate for p38 α [118]. These data suggested that the p38 pathway has a variety of functions.

6. Genes regulated by the p38 pathway

The use of inactive and constitutively active mutants of MKK3 and 6 as well as the use of the p38 inhibitor, SB203580, has greatly elucidated how p38 functions to regulate different genes. The expression of many cytokines, transcription factors, and cell surface receptors was found to be coordinated by p38. Table 3 summarises the genes that were reported to be regulated by the p38 pathway. In the future, the full identification of p38 regulated genes will be a great help to the understanding of this pathway.

7. Biological consequences of p38 activation

7.1. p38 and inflammation

Evidence to support the importance of the p38 pathway in inflammation comes from several sources. The activation of the p38 pathway plays an essential role in: (1) production of proinflammatory cytokines such as IL-1 β , TNF- α and IL-6 [119]; (2) induction of enzymes such as COX-2 [120], which controls connective tissue remodelling in pathological condition; (3) expression of an intracellular enzyme such as iNOS [121,122], which regulates oxidation; (4) induction of adherent proteins such as VCAM-1 and many other inflammatory related molecules [123]. In addition to these, the p38 pathway plays a regulatory role in the proliferation and differentiation of cells of the immune system. p38 participates in GM-CSF, CSF, EPO, and CD40-induced cell proliferation and/or differentiation [33,124].

Table 3
p38-regulated genes

Genes	Cell type	Stimuli	References
c-jun, c-fos	aT3-1	GnRH	Robertson et al. [178]
junB	HOSp ₂ AW	EV1	Huttunen et al. [147]
IL-1, TNF	Human monocyte, macrophage, RAW264.7	MALP-2	Garcia et al. [145]
IL-6	Human fibroblast-like synovioocyte, rat renal mesangial cell	IL-1	Miyazawa et al. [179]; Guan et al. [120]
IL-8	THP-1, PMN, human bronchial endothelial cell	LAMPf, hyperosmolarity	Manie et al. [146]; Hashimoto et al. [161]
MCP-1	Endothelial cell	TNF- α	Goebeler et al. [180]
VCAM-1	Endothelial cell	TNF- α	Piesterma et al. [33]
iNOS	Chondrocyte, oligodendrocyte	IL-17, TNF + INF γ	Shalom-Barak et al. [34]; Bhat et al. [181]
PPAR γ	3T3-L1		Engelman et al. [115]
Cyclooxygenase (COX)-2	Human monocyte 184B5/HER, HeLa	LPS, IL-1, SMase	Niirio et al. [182]; Subbaramaiah et al. [169]
Collagenase-1 (MMP-1)	Fibroblast	Okadaic acid	Westermarck et al. [161]
Collagenase-3 (MMP-13)	Human skin fibroblast	Three-dimensional collagen	Ravanti et al. [164]
HIV-LTR	HeLa	IL-1, TNF, UV, and osmotic stress	Kumar et al. [183]
Fgl-2	Macrophage	MHV-3	McGilvray et al. [148]
Brain natriuretic peptide (BNP)	Neonatal rat ventricular myocyte	IL-1b	He et al. [184]
CD23	U937	IL-4	Marshall et al. [185]
CKK	F9	bFGF + forskolin	Hansen et al. [154]
Phosphoenolpyruvate carboxy-kinase-cytosolic	Hepatoma cell	Arsenite	Cheong et al. [181]
Cyclin D1 (Negatively)	CCL39		Lavote et al. [139]
LDL receptor (Negatively)	HepG2	IL-1 and TNF	Kumar et al. [183]

The role of the p38 pathway in inflammatory-related diseases was studied in several animal models. Inhibition of p38 by SB203580 can reduce mortality in a murine model of endotoxin-induced shock and inhibit the development of mouse collagen-induced arthritis and rat adjuvant arthritis [27]. Furthermore, a recent study showed that SB220025, which is a more potent p38 inhibitor, caused a significant dose-dependent decrease in vascular density of the granuloma [125]. These results suggested that p38 or the components of the p38 pathway can be a therapeutic target for inflammatory disease.

7.2. p38 and apoptosis

Ample evidence suggests a correlation between the activation of the p38 pathway and apoptosis. Such a correlation is based on the concomitant activation of p38 and apoptosis induced by a variety of agents including NGF withdrawal and Fas ligation [126–128]. Central to the apoptotic execution pathway is a family of cysteine proteases, termed caspases, and are expressed as inactive zymogens [129,130]. Caspase inhibitors can block p38 activation by Fas cross-linking, indicating that this pathway may function downstream of caspase activation [128,131]. However, overexpression of dominant active MKK6b can also induce caspase activity and cell death [63,132]. These results suggest that the p38 pathways may function both upstream and downstream of caspases in the apoptotic response. The mechanism by which this pathway might influence caspase activity is unknown.

p38 α is activated in Jurkat T-cells during Fas ligation; however, its activity is not required for apoptosis [63,128]. On the other hand, SB203580 can block sodium salicylate-induced FS-4 fibroblast apoptosis [133], glutamate-induced cerebellar granule cell apoptosis [134], serum depletion induced Rat-1 cell death [126], NGF withdrawal-induced PC12 cell apoptosis [126], and TL1-induced bovine pulmonary artery endothelial cell apoptosis [135]. Therefore, the involvement of p38 in apoptosis is cell type- and stimulus-dependent.

7.3. p38 in the cell cycle

The involvement of p38 α in cell growth became apparent when it was noticed that overexpression of p38 α in yeast led to significant slowing of proliferation [136]. A slower proliferation of cultured mammalian cells was observed when the cells were treated with p38 α / β inhibitor, SB203580. Recently, Takenaka reported that p38 α was activated in mammalian cultured cells when the cells were arrested in M phase by disruption of the spindle with nocodazole [137]. Activation of p38 α appears to be involved in the spindle assembly checkpoint of somatic cell cycles. G1 arrest of NIH3T3 cells caused by microinjection of Cdc42 is p38 α -dependent [138]. Disruption of fission yeast p38 cascades, Wis1-Spc/Sty1, lead to cell size enlargement and cell division arrest,

suggesting that the Wis1-Spc1 MAP kinase cascade is linked to the G2/M cell cycle control mechanism [30]. It is possible that p38 α activity exerts a positive effect at certain stages of cell cycle and an inhibitory effect at others. The involvement of p38 α in cyclin D1 expression [139] and tertiary complex factor regulation may be the link between p38 α activity and cell cycle progression.

7.4. p38 and cardiomyocyte hypertrophy

Because p38 is a stress-activated kinase, its activation and function has been studied in cardiomyocyte hypertrophy. Both p38 α and p38 β activity were increased during the progression of hypertrophy. Activation of the p38 pathway by the introduction of constitutively active MKK6- and MKK3-elicited hypertrophic responses, including an increase in cell size, enhanced sarcomeric organisation and elevated atrial natriuretic factor expression. Furthermore, p38 α and p38 β appear to have different functions in cardiomyocytes. p38 β seems to be more potent in inducing hypertrophy, whereas p38 α appears to be more important in cardiomyocyte apoptosis [64].

7.5. p38 and cell differentiation

p38 α and/or p38 β were found to play an important role in cell differentiation for several different cell types. The differentiation of 3T3-L1 cells into adipocytes and the differentiation of PC12 cells into neurons both require p38 α and/or β [43,115]. The p38 pathway was found to be necessary and sufficient for SKT6 differentiation into haemoglobinised cells as well as C2C12 differentiation into myotubes [140]. The transcription factors C/EBP, CREB, and MEF2C were suggested to be downstream of the p38 pathway and would participate in the process of differentiation mentioned above.

8. Discussion

Due to the brevity of this review, it is impossible to cover all the research done on the p38 signal cascade. However, certain encompassing conclusions may be drawn concerning how p38 operates as a signal transduction mediator. p38 is activated by both stress and mitogen stimuli in a cell specific manner. The p38 group of kinases can directly or indirectly target various proteins to control transcription and translation. This kinase also activates other kinases and consequently regulates cellular responses. Close to a hundred genes were found to be regulated through the p38 signalling pathway. Since the p38 signalling pathway was implicated in a variety of cellular responses, including inflammation, cell cycle, cell death, and cell differentiation, emphasis should be placed on how p38 functions in a specific cell type.

Significant progress has been made in understanding the structure and function of the p38 group of MAP ki-

nases. Nevertheless, many questions regarding the regulation and function of this group of kinases remain unsolved. The activity of p38 α has been proven to be instrumental in cytokine gene expression. This is demonstrated by the fact that inhibitors of p38 block TNF α , IL-1, and IL-6 expression. The role of p38 becomes less clear when we consider the influence of various transcription factors on cytokine expression. Some of the transcription factor substrates of p38 described above were predicted to be influenced by TNF transcription. However, mutation of the *cis*-elements, predicted to interact with these transcription factors, has little or no effect on the p38-mediated TNF α promoter activity. The mechanism by which the p38 pathway regulates gene expression is still largely unknown. Furthermore, there is evidence that illustrates the distinct functions of different p38 isoforms. In vitro substrate specificity of different p38 isoforms has been observed. Identification of the physiological substrate of each p38 isoform as well as specific definitions of the role of each p38 group member stands as a challenge for future studies.

Regulation of the p38 pathway does not appear to be an isolated cascade. Many different upstream signals can lead to p38 activation, and divergent signalling pathways downstream of p38 have been observed. These findings are supported by recent work with knockout mice. Gene disruption of p38 α is lethal, while mice deficient in MKK3 (a p38 activator) [141] or M2 (a p38-activated kinase) [106] were viable. Macrophages from MKK3-deficient mice had a defect in IL-12 transcription, while those from M2-deficient mice had reduced TNF production, which is believed to be controlled at the translational level. Such in vivo complexity indicates that the p38 signalling pathway is not a stepwise chain reaction. Each component in the pathway may transmit the signal to downstream target(s) as well as interact with other cellular component(s) to coordinate the cellular process, such as feedback mechanisms. Knockouts of different components of the p38 pathway yielded different outcomes, indicating that each element in this pathway should be evaluated individually as a drug target. Because this signalling pathway is not an isolated event in vivo, future work in this field should pay more attention to the interaction between different pathways and the balance/regulation among the signalling events.

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References

- [1] Hunter T. *Cell* 1995;80:225–36.
- [2] Cobb MH, Boulton TG, Robbins DJ. *Cell Regul* 1991;2:965–78.
- [3] Sugden PH, Clerk A. *Cell Signal* 1997;9:337–51.
- [4] Ip YT, Davis RJ. *Curr Opin Cell Biol* 1998;10:205–19.
- [5] Kato Y, Kravchenko VV, Tapping RI, Han J, Ulevitch RJ, Lee JD. *EMBO J* 1997;16:7054–66.
- [6] New L, Han J. *Trends in Cardiovascular Medicine* 1998;8:220–8.
- [7] Han J, Lee JD, Tobias PS, Ulevitch RJ. *J Biol Chem* 1993;268:25009–14.
- [8] Han J, Lee J-D, Bibbs L, Ulevitch RJ. *Science* 1994;265:808–11.
- [9] Freshney NW, Rawlinson L, Guesdon F, Jones E, Cowley S, Hsuan J, Saklatvala J. *Cell* 1994;78:1039–49.
- [10] Rouse J, Cohen P, Trigon S, Morange M, Alonso-Llamazares A, Zamanillo D, Hunt T, Nebreda AR. *Cell* 1994;78:1027–37.
- [11] Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, McNulty D, Blumenthal MJ, Heys JR, Landvatter SW, Strickler JE, McLaughlin MM, Siemens IR, Fischer SM, Livi GP, White JR, Adams JL, Young PR. *Nature* 1994;373:46.
- [12] Jiang Y, Chen C, Li Z, Guo W, Gegner JA, Lin S, Han J. *J Biol Chem* 1996;271:17920–6.
- [13] Lechner C, Zahalka MA, Giot J-F, Moler NPH, Ullrich A. *Proc Natl Acad Sci USA* 1996;93:4355–9.
- [14] Li Z, Jiang Y, Ulevitch RJ, Han J. *Biochem Biophys Res Commun* 1996;228:334–40.
- [15] Cuenda A, Dorow DS. *Biochem J* 1998;333:11–5.
- [16] Jiang Y, Gram H, Zhao M, New L, Gu J, Feng L, Padova F, Ulevitch R, Han J. *J Biol Chem* 1992;333:30122–8.
- [17] Kumar S, McDonnell PC, Gum RJ, Hand AT, Lee JC, Young PR. *Biochem Biophys Res Commun* 1997;235:533–8.
- [18] Hu MC, Wang YP, Mikhail A, Qiu WR, Tan TH. *J Biol Chem* 1999;274:7095–102.
- [19] Hale KK, Trollinger D, Rihaneck M, Manthey CL. *J Immunol* 1999;162:4246–52.
- [20] Hanks SK, Hunter T. *FASEB J* 1995;9:576–96.
- [21] Jiang Y, Li Z, Schwarz EM, Lin A, Guan K, Ulevitch RJ, Han J. *J Biol Chem* 1997;272:11096–102.
- [22] Wang Z, Harkins PC, Ulevitch RJ, Han J, Cobb MH, Goldsmith EJ. *Proc Natl Acad Sci USA* 1997;94:2327–32.
- [23] Pav S, White DM, Rogers S, Crane KM, Cywin CL, Davidson W, Hopkins J, Brown ML, Pargellis CA, Tong L. *Protein Sci* 1997;6:242–5.
- [24] Wilson KP, Fitzgibbon MJ, Caron PR, Griffith JP, Chen W, McCaffrey PG, Chambers SP, Su MS-S. *J Biol Chem* 1996;271:27696–700.
- [25] Tong L, Pav S, White DM, Rogers S, Crane KM, Cywin CL, Brown ML, Pargellis CA. *Nat Struct Biol* 1997;4:311–6.
- [26] de Laszlo SE, Visco D, Agarwal L, Chang L, Chin J, Croft G, Forsyth A, Fletcher D, Frantz B, Hacker C, Hanlon W, Harper C, Kostura M, Li B, Luell S, MacCoss M, Mantlo N, O'Neill EA, Orevillo C, Pang M, Parsons J, Rolando A, Sahly Y, Sidler K, Widmer WR, O'Keefe SJ. *Bioorg Med Chem Lett* 1998;8:2689–94.
- [27] Badger AM, Bradbeer JN, Votta B, Lee JC, Adams JL, Griswold DE. *J Pharmacol Exp Ther* 1996;279:1453–61.
- [28] Han SJ, Choi KY, Brey PT, Lee WJ. *J Biol Chem* 1998;273:369–74.
- [29] Brewster JL, de Valoir T, Dyer ND, Winter E, Gustin MC. *Science* 1993;259:1760–3.
- [30] Shiozaki K, Russell P. *Nature* 1995;378:739–43.
- [31] Shiozaki K, Russell P. *Genes Dev* 1996;10:2276–88.
- [32] Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, David RJ. *J Biol Chem* 1995;270:7420–6.
- [33] Pietersma A, Tilly BC, Gaestel M, de Jong N, Lee JC, Foster JF, Sluiter W. *Biochem Biophys Res Commun* 1997;230:44–8.
- [34] Shalom-Barak T, Quach J, Lotz M. *J Biol Chem* 1988;273:27467–73.
- [35] Shapiro L, Puren AJ, Barton HA, Novick D, Peskind RL, Shenkar R, Gu Y Su, MS, Dinarello CA. *Proc Natl Acad Sci USA* 1998;95:12550–5.
- [36] Saklatvala J, Rawlinson L, Waller RJ, Sarsfield S, Lee JC, Morton LF, Barnes MJ, Farndale RW. *J Biol Chem* 1996;271:6586–9.
- [37] Krump E, Sanghera JS, Pelech SL, Furuya W, Grinstein S. *J Biol Chem* 1997;272:937–44.
- [38] El Benna J, Han J, Park JW, Schmid E, Ulevitch RJ, Babior BM. *Arch Biochem Biophys* 1996;334:395–400.
- [39] Foltz IN, Lee JC, Young PR, Schrader JW. *J Biol Chem* 1997;272:3296–301.
- [40] Tan Y, Rouse J, Zhang A, Cariati S, Cohen P, Comb MJ. *EMBO J* 1996;15:4629–42.
- [41] Nagata Y, Moriguchi T, Nishida E, Todokoro K. *Blood* 1997;90:929–34.
- [42] Crawley JB, Rawlinson L, Lali FV, Page TH, Saklatvala J, Foxwell BM. *J Biol Chem* 1997;272:15023–7.
- [43] Morooka T, Nishida E. *J Biol Chem* 1998;273:24285–8.
- [44] Xing J, Kornhauser JM, Xia Z, Thiele EA, Greenberg ME. *Mol Cell Biol* 1998;18:1946–55.
- [45] Cheng HL, Feldman EL. *J Biol Chem* 1998;273:14560–5.
- [46] Rousseau S, Houle F, Landry J, Huot J. *Onc* 1997;15:2169–77.
- [47] Pyne NJ, Pyne S. *Cell Signal* 1997;9:311–7.
- [48] Wang W, Zhou G, Hu MCT, Yao Z, Tan TH. *J Biol Chem* 1997;272:22771–5.
- [49] Williams NG, Zhong H, Minneman KP. *J Biol Chem* 1998;273:24624–32.
- [50] Yamauchi J, Nagao M, Kaziro Y, Itoh H. *J Biol Chem* 1997;272:27771–7.
- [51] Larsen JK, Yamboliev IA, Weber LA, Gerthoffer WT. *Am J Physiol* 1997;273:930–40.
- [52] Clerk A, Michael A, Sugden PH. *J Cell Biol* 1998;142:523–35.
- [53] Ushio-Fukai M, Alexander RW, Akers M, Griendling KK. *J Biol Chem* 1998;273:15022–9.
- [54] Schafer C, Ross SE, Bragado MJ, Groblewski GE, Ernst SA, Williams JA. *J Biol Chem* 1998;273:24173–80.
- [55] Cuenda A, Cohen P, Buee-Scherrer V, Goedert M. *EMBO J* 1997;16:295–305.
- [56] Kudoh S, Komuro I, Hiroi Y, Zou Y, Harada K, Sugaya T, Takekoshi N, Murakami K, Kadowaki T, Yazaki Y. *J Biol Chem* 1998;273:24037–43.
- [57] Bogoyevitch MA, Gillespie-Brown J, Ketterman AJ, Fuller SJ, Ben-Levy R, Ashworth A, Marshall CJ, Sugden PH. *Circ Res* 1996;79:162–73.
- [58] Sweeney G, Somwar R, Ramlal T, Volchuk A, Ueyama A, Klip A. *J Biol Chem* 1999;274:10071–8.
- [59] Heidenreich KA, Kummer JL. *J Biol Chem* 1996;271:9891–4.
- [60] Cuenda A, Cohen P, Buee-Scherrer V, Goedert M. *EMBO J* 1997;16:295–305.
- [61] Enslin H, Raingeaud J, Davis RJ. *J Biol Chem* 1998;273:1741–8.
- [62] Keesler GA, Bray J, Hunt J, Johnson DA, Gleason T, Yao Z, Wang SW, Parker C, Yamane H, Cole C, Lichenstein HS. *Protein Expr Purif* 1998;14:221–8.
- [63] Huang S, Jiang Y, Li Z, Nishida E, Mathias P, Lin S, Ulevitch RJ, Nemerow GR, Han J. *Immunity* 1997;6:739–49.
- [64] Wang Y, Huang S, Sah VP, Ross JJ, Brown JH, Han J, Chien KR. *J Biol Chem* 1998;273:2161–8.
- [65] Han J, Wang X, Jiang Y, Ulevitch RJ, Lin S. *FEBS Lett* 1997;403:19–22.
- [66] Takekawa M, Posas F, Saito H. *EMBO J* 1997;16:4973–82.

- [67] Hirai SX, Katoh M, Terada M, Kyriakis JM, Zon LI, Rana A, Avruch J, Ohno S. *J Biol Chem* 1997;272:15167–73.
- [68] Tibbles LA, Ing YL, Kiefer F, Chan J, Iscove N, Woodgett JR, Lassar NJ. *EMBO J* 1996;15:7026–35.
- [69] Fan G, Merritt SE, Kortzenjann M, Shaw PE, Holzman LB. *J Biol Chem* 1996;271:24788–93.
- [70] Ichijo H, Nishida E, Irie K, ten Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K, Gotoh Y. *Science* 1997;275:90–4.
- [71] Moriguchi T, Kuroyanagi N, Yamaguchi K, Gotoh Y, Irie K, Kano T, Shirakabe K, Muro Y, Shibuya H, Matsumoto K, Nishida E, Hagiwara M. *J Biol Chem* 1996;271:13675–9.
- [72] Ogura M, Kitamura M. *J Immunol* 1998;161:3569–74.
- [73] Takekawa M, Saito H. *Cell* 1998;95:521–530.
- [74] Zhang S, Han J, Sells MA, Chernoff J, Knaus UG, Ulevitch RJ, Bokoch GM. *J Biol Chem* 1995;270:23934–6.
- [75] Bagrodia S, Derijard B, Davis RJ, Cerione RA. *J Biol Chem* 1995;270:27995–8.
- [76] Coso OA, Teramoto H, Simonds WF, Gutkind JS. *J Biol Chem* 1996;271:3963–6.
- [77] Nagata KI, Puls A, Futter C, Aspenstrom P, Schaefer E, Nakata T, Hirokawa N, Hall A. *EMBO J* 1998;17:149–58.
- [78] Martin GA, Bollag G, McCormick F, Abo A. *EMBO J* 1995;14:1970–8.
- [79] Manser E, Leung T, Salihuddin H, Zhao Z, Lim L. *Nature* 1994;367:40–6.
- [80] Knaus UG, Morris S, Dong HJ, Chernoff J, Bokoch GM. *Science* 1995;269:221–3.
- [81] Graves JD, Gotoh Y, Draves KE, Ambrose D, Han DK, Wright M, Chernoff J, Clark EA, Krebs EG. *EMBO J* 1998;17:2224–34.
- [82] Nick JA, Avdi NJ, Young SK, Knall C, Gerwins P, Johnson GL, Worthen GS. *J Clin Invest* 1997;99:975–86.
- [83] Zhang Y, Neo SY, Han J, Yaw LP, Lin SC. *J Biol Chem* 1999;274:2851–7.
- [84] Sun H, Charles CH, Lau LF, Tonks NK. *Cell* 1993;75:487–93.
- [85] Misra-Press A, Rim CS, Yao Y, Roberson MS, Stork PJS. *J Biol Chem* 1995;270:14587–96.
- [86] Martell KJ, Seasholtz AF, Kwak SP, Clemens KK, Dixon JE. *J Neurochem* 1995;65:1823–33.
- [87] Camps M, Nichols A, Gillieron C, Antonsson B, Muda M, Chabert C, Boschert U, Arkinstall S. *Science* 1998;280:1262–5.
- [88] Muda M, Theodosiou A, Rodrigues N, Boschert U, Camps M, Gillieron C, Davies K, Ashworth A, Arkinstall S. *J Biol Chem* 1996;271:27205–8.
- [89] Groom LA, Sneddon AA, Alessi DR, Dowd S, Keyse SM. *EMBO J* 1996;15:3621–2.
- [90] Muda M, Theodosiou A, Gillieron C, Smith A, Chabert C, Camps M, Boschert U, Rodrigues N, Davies K, Ashworth A, Arkinstall S. *J Biol Chem* 1998;273:9323–9.
- [91] Maeda T, Wurgler-Murphy SM, Saito H. *Nature* 1994;369:242–5.
- [92] Posas F, Wurgler-Murphy SM, Maeda T, Witten EA, Thai TC, Saito H. *Cell* 1996;86:865–75.
- [93] Wurgler-Murphy SM, Maeda T, Witten EA, Saito H. *Mol Cell Biol* 1997;17:1289–97.
- [94] Takekawa M, Maeda T, Saito H. *EMBO J* 1998;17:4744–52.
- [95] McLaughlin MM, Kumar S, McDonnell PC, Van Horn S, Lee JC, Livi GP, Young PR. *J Biol Chem* 1996;271:8488–92.
- [96] Stokoe D, Engel K, Campbell DG, Cohen P, Gaestel M. *FEBS* 1992;313:307–13.
- [97] Huang CK, Zhan L, Ai Y, Jongstra J. *J Biol Chem* 1997;272:17–9.
- [98] Heidenreich O, Neininger A, Schratt G, Zinck R, Cahill MA, Engel K, Kotlyarov A, Kraft R, Kostka S, Gaestel M, Nordheim A. *J Biol Chem* 1999;274:14434–43.
- [99] Thomas G, Haavik J, Cohen P. *Eur J Biochem* 1997;247:1180–9.
- [100] Waskiewicz AJ, Flynn A, Proud CG, Cooper JA. *EMBO J* 1997;16:1909–20.
- [101] Fukunaga R, Hunter T. *EMBO J* 1997;16:1921–33.
- [102] New L, Jiang Y, Zhao M, Liu K, Zhu W, Flood LJ, Kato Y, Parry GC, Han J. *EMBO J* 1998;17:3372–84.
- [103] Deak M, Clifton AD, Lucocq LM, Alessi DR. *EMBO J* 1998;17:4426–41.
- [104] Pierrat B, Correia JS, Mary JL, Tomas-Zuber M, Lesslauer W. *J Biol Chem* 1998;273:29661–71.
- [105] New L, Zhao M, Li Y, Bassett WW, Feng Y, Ludwig S, Padova FD, Gram H, Han J. *J Biol Chem* 1999;274:1026–32.
- [106] Kotlyarov A, Neininger A, Schubert C, Eckert R, Birchmeiner C, Volk HD, Gaestel M. *Nature Cell Biol* 1999;1:94–7.
- [107] Hazzalin CA, Cano E, Cuenda A, Barratt MJ, Cohen P, Mahadevan LC. *Curr Biol* 1996;6:1028–31.
- [108] Whitmarsh AJ, Yang SH, Su MS, Sharrocks AD, Davis RJ. *Mol Cell Biol* 1997;17:2360–71.
- [109] Janknecht R, Hunter T. *EMBO J* 1997;16:1620–7.
- [110] Wang XZ, Ron D. *Science* 1996;272:1347–9.
- [111] Han J, Jiang Y, Li Z, Kravchenko VV, Ulevitch RT. *Nature* 1997;386:296–9.
- [112] Zhao M, New L, Kravchenko VV, Kato Y, Gram H, di Padova F, Olson EN, Ulevitch RJ, Han J. *Mol Cell Biol* 1999;19:21–30.
- [113] Huang C, Ma WY, Maxiner A, Sun Y, Dong Z. *J Biol Chem* 1999;274:12229–35.
- [114] Rehfuess RP, Walton KM, Loriaux MM, Goodman RH. *J Biol Chem* 1991;266:18431–4.
- [115] Engelman JA, Lisanti MP, Scherer PE. *J Biol Chem* 1998;273:32111–20.
- [116] Kramer RM, Roberts EF, Um SL, Borsch-Haubold AG, Watson SP, Fisher MJ, Jakubowski JA. *J Biol Chem* 1996;271:27723–9.
- [117] Kusuhara M, Takahashi E, Peterson TE, Abe J, Ishida M, Han J, Ulevitch R, Berk BC. *Circ Res* 1998;83:824–31.
- [118] Parker CG, Hunt J, Diener K, McGinley M, Soriano B, Kessler GA, Bray J, Yao Z, Wang XS, Kohno T, Lichenstein HS. *Biochem Biophys Res Commun* 1998;249:791–6.
- [119] Perregaux DG, Dean D, Cronan M, Connelly P, Gabel CA. *Mol Pharmacol* 1995;48:433–42.
- [120] Guan Z, Buckman SY, Pentland AP, Templeton DJ, Morrison AR. *J Biol Chem* 1998;273:12901–8.
- [121] Badger AM, Cook MN, Lark MW, Newman-Tarr TM, Swift BA, Nelson AH, Barone FC, Kumar S. *J Immunol* 1998;161:467–73.
- [122] Da Silva J, Pierrat B, Mary JL, Lesslauer W. *J Biol Chem* 1997;272:28373–80.
- [123] Pietersma A, Tilly BC, Gaestel M, de Jong N, Lee JC, Koster JF, Sluiter W. *Biochem Biophys Res Commun* 1997;230:44–8.
- [124] Craxton A, Shu G, Graves JD, Saklatvala J, Krebs EG, Clark EA. *J Immunol* 1998;161:3225–6.
- [125] Jackson JR, Bolognese B, Hilleagass L, Kassiss S, Adams J, Griswold DE, Winkler JD. *J Pharmacol Exp Ther* 1998;284:687–92.
- [126] Kummer JL, Rao PK, Heidenreich KA. *J Biol Chem* 1997;272:20490–4.
- [127] Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. *Science* 1995;270:1326–31.
- [128] Juo P, Kuo CJ, Reynolds SE, Konz RF, Raingeaud J, Davis RJ, Biemann H-P, Blenis J. *Mol Cell Biol* 1997;17:24–35.
- [129] Henkart PA. *Immunity* 1996;4:195–201.
- [130] Fernandes-Alnemri T, Armstrong RC, Krebs J, Srinivasula SM, Wang L, Bullrich F, Fritz LC, Trapani JA, Tomaselli KJ, Litwack G, Alnemri ES. *Proc Natl Acad Sci USA* 1996;93:7464–9.
- [131] Cahill MA, Peter ME, Kischkel FC, Chinnaiyan AM, Dixit VM, Krammer PH, Nordheim A. *Onc* 1996;13:2087–96.
- [132] Cardone MH, Salvesen GS, Widmann C, Johnson G, Frisch SM. *Cell* 1997;90:315–23.
- [133] Schwenger P, Bellosta P, Vietor I, Basilico C, Skolnik EY, Vilcek J. *Proc Natl Acad Sci USA* 1997;94:2869–73.
- [134] Kawasaki H, Morooka T, Shimohama S, Kimura J, Hirano T, Gotoh Y, Nishida E. *J Biol Chem* 1997;272:18518–21.

- [135] Yue TL, Ni J, Romanic AM, Gu JL, Keller P, Wang C, Kumar S, Yu GL, Hart TK, Wang X, Xia Z, DeWolf WE, Feuerstein GZ. *J Biol Chem* 1999;274:1479–86.
- [136] Ziegler-Heitbrock HWL, Blumenstein M, Kafferlein E, Kieper K, Petersmann I, Endres S, Flegel WA, Northoff H, Reithmuller G, Haas JG. *Immunology* 1992;75:264–8.
- [137] Takenaka K, Moriguchi T, Nishida E. *Science* 1998;280:599–602.
- [138] Molnar A, Theodoras AM, Zon LI, Kyriakis JM. *J Biol Chem* 1997;272:13229–35.
- [139] Lavoie JN, L'Allemain G, Brunet A, Mulelr R, Pouyssegur J. *J Biol Chem* 1996;271:20608–16.
- [140] Zetser A, Gredinger E, Bengal E. *J Biol Chem* 1999;274:5193–200.
- [141] Lu HT, Yang DD, Wysk M, Gatti E, Mellman I, Davis RJ, Flavell RA. *EMBO J* 1999;18:1845–57.
- [142] Dziarski R, Jin Y-P, Gupta D. Differential activation of extracellular signal-regulated kinase (ERK) 1, ERK2, p38, and c-Jun NH2-terminal kinase mitogen-activated protein kinases by bacterial peptidoglycan. *J Infect Dis* 1996;174:777–85.
- [143] McLeish KR, Klein JB, Coxon PY, Head KZ, Ward RA. Bacterial phagocytosis activates extracellular signal-regulated kinase and p38 mitogen-activated protein kinase cascades in human neutrophils. *J Leukoc Biol* 1998;64:835–44.
- [144] Schafer PH, Wang L, Wadsworth SA, Davis JE, Siekierka JJ. T cell activation signals upregulate p38 mitogen-activated protein kinase activity and induce TNF-alpha production in a manner distinct from LPS activation of monocytes. *J Immunol* 1999;162:659–68.
- [145] Garcia J, Lemercier B, Roman-Roman S, Rawadi G. A Mycoplasma fermentans-derived synthetic lipopeptide induces AP-1 and NF-kappaB activity and cytokine secretion in macrophages via the activation of mitogen-activated protein kinase pathways. *J Biol Chem* 1998;273:34391–8.
- [146] Marie C, Roman-Roman S, Rawadi G. Involvement of mitogen-activated protein kinase pathways in interleukin-8 production by human monocytes and polymorphonuclear cells stimulated with lipopolysaccharide or Mycoplasma fermentans membrane lipoproteins. *Infect Immun* 1999;67:688–93.
- [147] Huttunen P, Hyypia T, Vihinen P, Nissinen L, Heino J. Echovirus 1 infection induces both stress- and growth-activated and mitogen-activated protein kinase pathways and regulates the transcription of cellular immediate-early genes. *Virology* 1998;250:85–93.
- [148] McGilvray ID, Lu Z, Wei AC, Dackiw AP, Marshall JC, Kapus A, Levy G, Rotstein OD. Murine hepatitis virus strain 3 induces the macrophage prothrombinase fgl-2 through p38 mitogen-activated protein kinase activation. *J Biol Chem* 1998;273:32222–9.
- [149] Nakatsue T, Katoh I, Nakamura S, Takahashi Y, Ikawa Y, Yoshinaka Y. Acute infection of Sindbis virus induces phosphorylation and intracellular translocation of small heat shock protein HSP27 and activation of p38 MAP kinase signaling pathway. *Biochem Biophys Res Commun* 1998;253:59–64.
- [150] Popik W, Pitha PM. Early activation of mitogen-activated protein kinase kinase, extracellular signal-regulated kinase, p38 mitogen-activated protein kinase, and c-Jun N-terminal kinase in response to binding of simian immunodeficiency virus to Jurkat T cells expressing CCR5 receptor. *Virology* 1998;252:210–17.
- [151] Zachos G, Clements B, Conner J. Herpes simplex virus type 1 infection stimulates p38/c-Jun N-terminal mitogen-activated protein kinase pathways and activates transcription factor AP-1. *J Biol Chem* 1999;274:5097–103.
- [152] Beltman J, Erickson JR, Martin GA, Lyons JF, Cook SJ. C3 toxin activates the stress signaling pathways, JNK and p38, but antagonizes the activation of AP-1 in rat-1 cells. *J Biol Chem* 1999;274:3772–80.
- [153] McLeish KR, Klein JB, Coxon PY, Head KZ, Ward RA. Bacterial phagocytosis activates extracellular signal-regulated kinase and p38 mitogen-activated protein kinase cascades in human neutrophils. *J Leukoc Biol* 1998;64:835–44.
- [154] Pugazhenthii S, Boras T, O'Connor D, Meintzer MK, Heidenreich KA, Reusch JE. Insulin-like growth factor I-mediated activation of the transcription factor cAMP response element-binding protein in PC12 cells. Involvement of p38 mitogen-activated protein kinase-mediated pathway. *J Biol Chem* 1999;274:2829–37.
- [155] Hansen TV, Rehfeld JF, Nielsen FC. Mitogen-activated protein kinase and protein kinase A signaling pathways stimulate cholecystokinin transcription via activation of cyclic adenosine 3',5'-monophosphate response element-binding protein. *Mol Endocrinol* 1999;13:466–75.
- [156] Ingram AJ, Ly H, Thai K, Kang M, Scholey JW. Activation of mesangial cell signaling cascades in response to mechanical strain. *Kidney Int* 1999;55:476–85.
- [157] Scott PH, Paul A, Belham CM, Peacock AJ, Wadsworth RM, Gould GW, Welsh D, Plevin R. Hypoxic stimulation of the stress-activated protein kinases in pulmonary artery fibroblasts. *Am J Respir Crit Care Med* 1998;158:958–62.
- [158] Samet JM, Graves LM, Quay J, Dailey LA, Devlin RB, Ghio AJ, Wu W, Bromberg PA, Reed W. Activation of MAPKs in human bronchial epithelial cells exposed to metals. *Am J Physiol* 1998;275:L551–8.
- [159] Hung JJ, Cheng TJ, Lai YK, Chang MD. Differential activation of p38 mitogen-activated protein kinase and extracellular signal-regulated protein kinases confers cadmium-induced HSP70 expression in 9L rat brain tumor cells. *J Biol Chem* 1998;273:31924–31.
- [160] Lu HT, Yang DD, Wysk M, Gatti E, Mellman I, Davis RJ, Flavell RA. Defective IL-12 production in mitogen-activated protein (MAP) kinase kinase 3 (Mkk3)-deficient mice. *EMBO J* 1999;18:1845–57.
- [161] Hashimoto A, Okada H, Jiang A, Kurosaki M, Greenberg S, Clark EA, Kurosaki T. Involvement of guanosine triphosphatases and phospholipase C-gamma2 in extracellular signal-regulated kinase, c-Jun NH2-terminal kinase, and p38 mitogen-activated protein kinase activation by the B cell antigen receptor. *J Exp Med* 1998;188:1287–95.
- [162] Westermark J, Holmstrom T, Ahonen M, Eriksson JE, Kahari VM. Enhancement of fibroblast collagenase-1 (MMP-1) gene expression by tumor promoter okadaic acid is mediated by stress-activated protein kinases Jun N-terminal kinase and p38. *Matrix Biol* 1998;17:547–57.
- [163] Zhen X, Uryu K, Wang HY, Friedman E. D1 dopamine receptor agonists mediate activation of p38 mitogen-activated protein kinase and c-Jun amino-terminal kinase by a protein kinase A-dependent mechanism in SK-N-MC human neuroblastoma cells. *Mol Pharmacol* 1998;54:453–8.
- [164] Alexandrov A, Keffel S, Goepel M, Michel MC. Stimulation of alpha1A-adrenoceptors in Rat-1 cells inhibits extracellular signal-regulated kinase by activating p38 mitogen-activated protein kinase. *Mol Pharmacol* 1998;54:755–60.
- [165] Moule SK, Denton RM. The activation of p38 MAPK by the beta-adrenergic agonist isoproterenol in rat epididymal fat cells. *FEBS Lett* 1998;439:287–90.
- [166] Kusuvara M, Takahashi E, Peterson TE, Abe J, Ishida M, Han J, Ulevitch R, Berk BC. p38 Kinase is a negative regulator of angiotensin II signal transduction in vascular smooth muscle cells: effects on Na⁺/H⁺ exchange and ERK1/2. *Circ Res* 1998;83:824–31.
- [167] Ravanti L, Heino J, Lopez-Otin C, Kahari VM. Induction of collagenase-3 (MMP-13) expression in human skin fibroblasts by three-dimensional collagen is mediated by p38 mitogen-activated protein kinase. *J Biol Chem* 1999;274:2446–55.
- [168] Yi AK, Krieg AM. Rapid induction of mitogen-activated protein kinases by immune stimulatory CpG DNA. *J Immunol* 1998;161:4493–7.

- [169] Hacker H, Mischak H, Miethke T, Liptay S, Schmid R, Sparwasser T, Heeg K, Lipford GB, Wagner H. CpG-DNA-specific activation of antigen-presenting cells requires stress kinase activity and is preceded by non-specific endocytosis and endosomal maturation. *EMBO J* 1998;17:6230–40.
- [170] Haq SE, Clerk A, Sugden PH. Activation of mitogen-activated protein kinases (p38-MAPKs, SAPKs/JNKs and ERKs) by adenosine in the perfused rat heart. *FEBS Lett* 1998;434:305–8.
- [171] Nagarkatti DS, Sha'afi RI. Role of p38 MAP kinase in myocardial stress. *J Mol Cell Cardiol* 1998;30:1651–64.
- [172] Subbaramaiah K, Chung WJ, Dannenberg AJ. Ceramide regulates the transcription of cyclooxygenase-2. Evidence for involvement of extracellular signal-regulated kinase/c-Jun N-terminal kinase and p38 mitogen-activated protein kinase pathways. *J Biol Chem* 1998;273:32943–9.
- [173] Huwiler A, Pfeilschifter J. Nitric oxide stimulates the stress-activated protein kinase p38 in rat renal mesangial cells. *J Exp Biol* 1999;202:655–60.
- [174] Callsen D, Brune B. Role of mitogen-activated protein kinases in S-nitrosoglutathione-induced macrophage apoptosis. *Biochemistry* 1999;38:2279–86.
- [175] Zhang Y, Huang Y, Rishi AK, Sheikh MS, Shroot B, Reichert U, Dawson M, Poirer G, Fontana JA. Activation of the p38 and JNK/SAPK mitogen-activated protein kinase pathways during apoptosis is mediated by a novel retinoid. *Exp Cell Res* 1999;247:233–40.
- [176] Ozaki I, Tani E, Ikemoto H, Kitagawa H, Fujikawa H. Activation of stress-activated protein kinase/c-Jun NH2-terminal kinase and p38 kinase in calphostin C-induced apoptosis requires caspase-3-like proteases but is dispensable for cell death. *J Biol Chem* 1999;274:5310–17.
- [177] Lee LF, Li G, Templeton DJ, Ting JP. Paclitaxel (Taxol)-induced gene expression and cell death are both mediated by the activation of c-Jun NH2-terminal kinase (JNK/SAPK). *J Biol Chem* 1998;273:28253–60.
- [178] Roberson MS, Zhang T, Li HL, Mulvaney JM. Activation of the p38 mitogen-activated protein kinase pathway by gonadotropin-releasing hormone. *Endocrinology* 1999;140:1310–18.
- [179] Miyazawa K, Mori A, Miyata H, Akahane M, Ajisawa Y, Okudaira H. Regulation of interleukin-1beta-induced interleukin-6 gene expression in human fibroblast-like synoviocytes by p38 mitogen-activated protein kinase. *J Biol Chem* 1998;273:24832–38.
- [180] Goebeler M, Kilian K, Gillitzer R, Kunz M, Yoshimura T, Brocker EB, Rapp UR, Ludwig S. The MKK6/p38 stress kinase cascade is critical for tumor necrosis factor-alpha-induced expression of monocyte-chemoattractant protein-1 in endothelial cells. *Blood* 1999;93:857–65.
- [181] Bhat NR, Zhang P, Bhat AN. Cytokine induction of inducible nitric oxide synthase in an oligodendrocyte cell line: role of p38 mitogen-activated protein kinase activation. *J Neurochem* 1999;72:472–8.
- [182] Niuro H, Otsuka T, Ogami E, Yamaoka K, Nagano S, Akahoshi M, Nakashima H, Arinobu Y, Izuhara K, Niho Y. MAP kinase pathways as a route for regulatory mechanisms of IL-10 and IL-4 which inhibit COX-2 expression in human monocytes. *Biochem Biophys Res Commun* 1998;250:200–5.
- [183] Kumar S, Orsin MJ, Lee JC, McDonnell PC, Debouck C, Young PR. Activation of the HIV-1 long terminal repeat by cytokines and environmental stress requires an active CSBP/p38 MAP kinase. *J Biol Chem* 1996;271:30864–9.
- [184] He Q, LaPointe MC. Interleukin-1beta regulation of the human brain natriuretic peptide promoter involves Ras-, Rac-, and p38 kinase-dependent pathways in cardiac myocytes. *Hypertension* 1999;33:283–9.
- [185] Marshall LA, Hansbury MJ, Bolognese BJ, Gum RJ, Young PR, Mayer RJ. Inhibitors of the p38 mitogen-activated kinase modulate IL-4 induction of low affinity IgE receptor (CD23) in human monocytes. *J Immunol* 1998;161:6005–13.