

REVIEW ARTICLE

Mechanisms and functions of p38 MAPK signalling

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The p38 MAPK (mitogen-activated protein kinase) signalling pathway allows cells to interpret a wide range of external signals and respond appropriately by generating a plethora of different biological effects. The diversity and specificity in cellular outcomes is achieved with an apparently simple linear architecture of the pathway, consisting of a core of three protein kinases acting sequentially. In the present review, we dissect the molecular mechanisms underlying p38 MAPK functions, with special emphasis on the activation and regulation of the

core kinases, the interplay with other signalling pathways and the nature of p38 MAPK substrates as a source of functional diversity. Finally, we discuss how genetic mouse models are facilitating the identification of physiological functions for p38 MAPKs, which may impinge on their eventual use as therapeutic targets.

Key words: cell regulation, mitogen-activated protein kinase (MAPK), p38, phosphorylation, signalling, stress response.

INTRODUCTION

Cells need to be constantly aware of changes in the extracellular milieu to respond accordingly, so they have developed sophisticated mechanisms to receive signals, transmit the information and orchestrate the appropriate responses. Signal transduction mechanisms heavily rely on post-translational modifications of proteins, among which phosphorylation plays a major role. Eukaryotic cells contain a wide repertoire of protein kinases (518 in human cells), many of them poorly characterized, but, on the basis of current knowledge, the kinases referred to as MAPKs (mitogen-activated protein kinases) seem to be involved in most signal transduction pathways. Such extensive knowledge of MAPK biological functions may be due at least in part to the availability of inhibitors for the several MAPK families that usually function as parallel pathways in any given cell. In the present review, we provide an overview of the p38 MAPK pathway, which is strongly activated by stress, but also plays important roles in the immune response as well as in the regulation of cell survival and differentiation (reviewed in [1–4]). We focus on the components and regulatory mechanisms of this pathway, referring to recent reviews for detailed information on specific topics.

p38 MAPKs

The first member of the p38 MAPK family was independently identified by four groups as a 38 kDa protein (p38) that was rapidly phosphorylated on tyrosine in response to LPS (lipopolysaccharide) stimulation [5], as a target of pyridinylimidazole drugs [CSBP (cytokine-suppressive anti-inflammatory drug-binding protein)] that inhibited the production of pro-inflammatory cytokines [6], and as an activator [RK (reactivating kinase)] of MAPKAP-K2/MK2 (MAPK-activated protein kinase 2) in cells stimulated with heat shock, arsenite or IL (interleukin)-1 [7,8]. This protein was found to be the homologue of *Saccharomyces cerevisiae* Hog1, an important regulator of the osmotic response, and is now referred to as p38 α (MAPK14). Additional p38 MAPK family members, which are approx. 60 % identical in their amino acid sequence, were subsequently cloned and named p38 β (MAPK11), p38 γ [SAPK (stress-activated protein kinase) 3, ERK (extracellular-signal-regulated kinase) 6 or MAPK12] and p38 δ (SAPK4 or MAPK13) [9–14] (Figure 1). The four p38 MAPKs are encoded by different genes and have different tissue expression patterns, with p38 α being ubiquitously expressed at significant levels in most cell types, whereas the others seem to be expressed in a more tissue-specific manner; for example, p38 β in brain,

Abbreviations used: Ago2, Argonaute 2; ARE, AU-rich element; ASK1, apoptosis signal-regulating kinase 1; ATF, activating transcription factor; BAF60, BRG1-associated factor 60; CDK, cyclin-dependent kinase; C/EBP, CCAAT/enhancer-binding protein; c-IAP1/2, cellular inhibitor of apoptosis 1/2; CREB, cAMP-response-element-binding protein; CSBP, cytokine-suppressive anti-inflammatory drug-binding protein; DDB2, damaged-DNA-binding complex 2; D domain, docking domain; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; FADD, Fas-associated death domain; FGFR1, fibroblast growth factor receptor 1; FLIPs, short isoform of FLICE (FADD-like interleukin 1 β -converting enzyme)-inhibitory protein; GRK2, G-protein-coupled receptor kinase 2; GSK, glycogen synthase kinase; hDlg, human discs large; HePTP, haemopoietic tyrosine phosphatase; IKK, I κ B (inhibitor of nuclear factor κ B) kinase; IL, interleukin; JNK, c-Jun N-terminal kinase; JIP, JNK-interacting protein; JLP, JNK-associated leucine zipper protein; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MAP2K, MAPK kinase; MAP3K, MAP2K kinase; MCP-1, monocyte chemoattractant protein 1; MEF, myocyte enhancer factor; MEKK, MAPK/ERK kinase kinase; MK, MAPK-activated protein kinase; MKK, MAPK kinase; MKP, MAPK phosphatase; MLK3, mixed-lineage kinase 3; MSK, mitogen- and stress-activated kinase; MyoD, myogenic differentiation factor D; NF- κ B, nuclear factor κ B; OGT, O-GlcNAc transferase; p38IP, p38 α -interacting protein; PKD, protein kinase D; PP, protein phosphatase; SAP, synapse-associated protein; SAPK, stress-activated protein kinase; SRCAP, Snf2-related CREB-binding protein-activator protein; SRF, serum-response factor; TCR, T-cell receptor; TGF, transforming growth factor; TAK1, TGF β -activated kinase 1; TAB1, TAK1-binding protein 1; TNF, tumour necrosis factor; TACE, TNF α -converting enzyme; TRAF, TNF-receptor-associated factor; TTSS, type III secretion system; Ubc13, ubiquitin-conjugating enzyme 13; USF1, upstream stimulatory factor 1.

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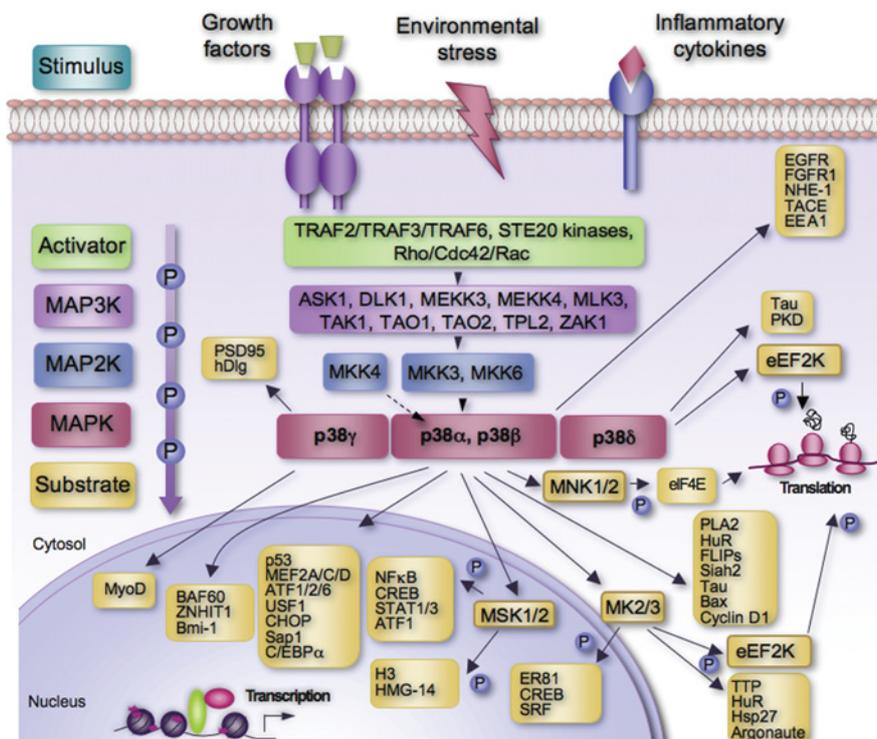


Figure 1 The p38 MAPK pathway

Different stimuli such as growth factors, inflammatory cytokines or a wide variety of environmental stresses can activate p38 MAPKs. A number of representative downstream targets, including protein kinases, cytosolic substrates, transcription factors and chromatin remodellers, are shown. CHOP, C/EBP-homologous protein; DLK1, dual-leucine-zipper-bearing kinase 1; EEA1, early-endosome antigen 1; eEF2K, eukaryotic elongation factor 2 kinase; eIF4E, eukaryotic initiation factor 4E; HMG-14, high-mobility group 14; NHE-1, Na⁺/H⁺ exchanger 1; PLA2, phospholipase A₂; PSD95, postsynaptic density 95; Sap1, SRF accessory protein 1; STAT, signal transducer and activator of transcription; TAO, thousand-and-one amino acid; TPL2, tumour progression loci 2; TTP, tristetraprolin; ZAK1, leucine zipper and sterile- α motif kinase 1; ZNHIT1, zinc finger HIT-type 1.

p38 γ in skeletal muscle and p38 δ in endocrine glands. p38 MAPK family members have overlapping substrate specificities, albeit some differences have been reported, with particular substrates being better phosphorylated by p38 α and p38 β than p38 γ and p38 δ or vice versa [4]. The genetic ablation of specific p38 MAPK family members has also demonstrated the existence of functional redundancy. For example, the osmotic shock-induced phosphorylation of SAP (synapse-associated protein) 97/hDlg (human discs large) is usually mediated by p38 γ , but, in the absence of this kinase, other p38 MAPKs can perform this function [15].

Several alternatively spliced isoforms of p38 α have been also described. Mxi2 is identical with p38 α in amino acids 1–280, but has a unique C-terminus of 17 amino acids. This isoform displays reduced binding to p38 MAPK substrates, whereas it can bind to ERK1/2 MAPKs and regulates their nuclear import [16]. Another isoform is Exip, which has a unique 53-amino-acid C-terminus, is not phosphorylated by the usual p38 MAPK-activating treatments and has been reported to regulate the NF- κ B (nuclear factor κ B) pathway [17]. Finally, CSBP1 differs from p38 α (CSBP2) only in an internal 25-amino-acid sequence [6], but its contribution to p38 MAPK signalling is unclear.

The structure of p38 α has been solved by X-ray crystallography. As for other MAPKs, the structural topology consists of two distinct lobes, forming the catalytic groove between them. In addition, p38 α has been co-crystallized with inhibitors such as SB203580 [18] and substrates such as MK2 [19,20], and these studies have provided useful information to understand the mechanisms underlying p38 α function and inhibition. The

three-dimensional structure of p38 β is highly similar overall to that of p38 α ; however, there are some differences in the relative orientation of the N- and C-terminal domains that cause a reduction in the size of the p38 β ATP-binding pocket. This difference in size between the two pockets could perhaps be exploited to achieve selectivity of ATP-competitive inhibitory compounds [21]. The structure of phosphorylated p38 γ has been also determined, and the conformation of the activation loop was found to be almost identical with the one in active ERK2. However, unlike ERK2, the structure of activated p38 γ does not reveal a dimer interface. This observation is supported by studies indicating that activated p38 γ exists as a monomer in solution, suggesting that not all activated MAPKs form dimers [22].

CANONICAL PATHWAY OF ACTIVATION

As in many other protein kinases, the activation of MAPKs requires phosphorylation on a flexible loop termed the phosphorylation lip or activation loop. These phosphorylations induce conformational reorganizations that relieve steric blocking and stabilize the activation loop in an open and extended conformation, facilitating substrate binding. p38 MAPKs are activated by dual phosphorylation in the activation loop sequence Thr-Gly-Tyr. In response to appropriate stimuli, threonine and tyrosine residues can be phosphorylated by three dual-specificity MKKs/MAP2Ks (MAPK kinases) (Figure 1). MKK6 can phosphorylate the four p38 MAPK family members, whereas MKK3 activates p38 α , p38 γ and p38 δ , but not p38 β .

Both MKK3 and MKK6 are highly specific for p38 MAPKs [14,23]. In addition, p38 α can be also phosphorylated by MKK4, an activator of the JNK (c-Jun N-terminal kinase) pathway [24,25]. The relative contribution of different MAP2Ks to p38 α activation depends on the stimulus, but also on the cell type, because of variations in MAP2K expression levels among cell types [23,25]. In addition, several studies including genetic analysis in mice have demonstrated functional differences between MKK3 and MKK6, as discussed in [26]. Depending on the stress stimulus, MKK3 and MKK6 also contribute to different extents to the activation of other p38 MAPK family members [27].

MAP2Ks are activated by phosphorylation in two conserved serine/threonine sites of the activation loop. The kinase domain of constitutively active MKK6, with aspartate mutations replacing the two phosphorylation sites, has been crystallized recently, revealing an auto-inhibited dimer [28]. Intriguingly, this mutant kinase appears to adopt an inactive conformation despite the presence of phosphomimetic mutations [28]. Several MAP3Ks (MAP2K kinases) have been shown to trigger p38 MAPK activation, including ASK1 (apoptosis signal-regulating kinase 1), DLK1 (dual-leucine-zipper-bearing kinase 1), TAK1 [TGF (transforming growth factor) β -activated kinase 1], TAO (thousand-and-one amino acid) 1 and 2, TPL2 (tumour progression loci 2), MLK3 (mixed-lineage kinase 3), MEKK (MAPK/ERK kinase kinase) 3 and MEKK4, and ZAK1 (leucine zipper and sterile- α motif kinase 1) (Figure 1). Some MAP3Ks that trigger p38 MAPK activation can also activate the JNK pathway. Upstream of the cascade, the regulation of MAP3Ks is more complex, involving phosphorylation by STE20 family kinases and binding of small GTP-binding proteins of the Rho family as well as ubiquitination-based mechanisms. The diversity of MAP3Ks and their regulatory mechanisms provide the ability to respond to many different stimuli and to integrate p38 MAPK activation with other signalling pathways [29].

Specific MAP3Ks have sometimes been linked to particular stimuli. For example, in *Drosophila* cells, MEKK1 controls the activation of p38 MAPKs by UV or peptidoglycan, whereas heat-shock-induced activation signals through both MEKK1 and ASK1, and maximal activation of p38 MAPKs by hyperosmolarity requires four MAP3Ks [30]. In mammalian cells, ASK1 plays a key role in the activation of p38 α by oxidative stress [31]. The underlying mechanism involves ASK1 dimerization and autophosphorylation, which is facilitated by the oxidation-mediated release of ASK1-binding proteins such as thioredoxin [32].

The TRAF [TNF (tumour necrosis factor)-receptor-associated factor] family of E3 ubiquitin ligases has a critical function in TAK1 activation, which usually mediates the activation of p38 MAPK induced by cytokine receptors. This has been attributed to the role of the Lys⁶³-linked polyubiquitin chains as scaffolds for the assembly of complexes required for kinase activation. Recently, TRAF6 has been shown to be required for the activation of p38 MAPKs (and JNKs) by TGF β receptors [33,34]. TRAF6 interacts with TGF β receptors, and this interaction is necessary for the TGF β -induced auto-ubiquitination of TRAF6, promoting its association with and subsequent activation of TAK1 [33,34]. Importantly, TRAF6 regulates the activation of TAK1 independently of both the canonical Smad pathway and the kinase activity of the TGF β receptor. Of note, TAK1 is also an important activator of the NF- κ B anti-apoptotic pathway, besides MAPKs, in response to TNF α stimulation.

Activation of MAP3Ks such as MEKK1 or TAK1 by members of the TNF receptor family such as CD40 is thought to require assembly of multiprotein complexes at receptor intracellular domains. However, a recent report has showed that complex

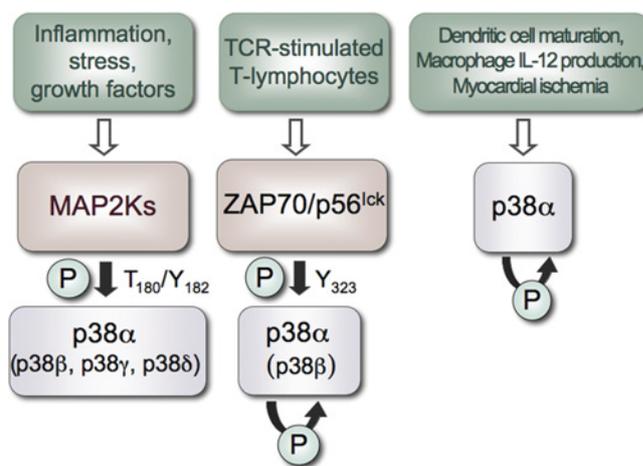


Figure 2 Mechanisms of p38 MAPK activation

Most stimuli activate the canonical pathway, which involves MAP2K-mediated phosphorylation of p38 MAPKs on a threonine and a tyrosine residue of the activation loop. An alternative mechanism of p38 MAPK activation operates in T-lymphocytes and involves tyrosine phosphorylation of p38 α , which results in its autophosphorylation on the activation loop. In some cases, p38 α appears to be activated by autophosphorylation, which might be stimulated by its association with proteins such as TAB1, independently of both MAP2Ks and tyrosine phosphorylation. ZAP70, ζ -chain-associated protein kinase of 70 kDa.

assembly at the receptor only primes MAP3Ks for activation, whereas activation of the kinase cascade is actually delayed until the complex is released into the cytoplasm [35]. The idea is that CD40 is membrane-associated in non-stimulated B-lymphocytes, and receptor engagement induces trimerization and recruitment of TRAF2, TRAF3, c-IAP1/2 (cellular inhibitor of apoptosis 1/2) and Ubc13 (ubiquitin-conjugating enzyme 13), which is followed by the recruitment of IKK [I κ B (inhibitor of NF- κ B) kinase] γ and MEKK1. The complex is stabilized by interactions between IKK γ and MEKK1, and Lys⁶³-linked polyubiquitin chains catalysed by TRAF2 and Ubc13. c-IAP1/2 catalyse Lys⁴⁸-linked polyubiquitination of TRAF3 whose proteasomal degradation results in translocation of the receptor-assembled signalling complex into the cytosol where MEKK1 is activated and in turn activates downstream components of MAPK cascades [35]. This illustrates how MAP3K activation is subjected to elaborate regulatory mechanisms, which probably facilitate both signal fine-tuning and cross-talk with other pathways.

ALTERNATIVE MECHANISMS OF ACTIVATION

The canonical p38 MAPK-activation pathway involves MAP2K-catalysed phosphorylation of threonine and tyrosine residues in the activation loop, which induces conformational changes that enhance both binding to substrates and catalytic activity of p38 MAPKs. In fact, gene-targeting experiments in mice have demonstrated that MKK3 and MKK6 play major roles in p38 α activation [25].

However, non-canonical mechanisms of p38 α (and probably p38 β) activation have been also described (Figure 2). One is apparently specific to antigen receptor [TCR (T-cell receptor)]-stimulated T-lymphocytes. This involves phosphorylation of p38 α on Tyr³²³ by the TCR-proximal tyrosine kinases ZAP70 (ζ -chain-associated protein kinase of 70 kDa) and p56^{lck}, which leads to p38 α autophosphorylation on the activation loop and, as a consequence, increases its kinase activity towards substrates [36]. GADD (growth-arrest and DNA-damage-inducible protein) 45 α

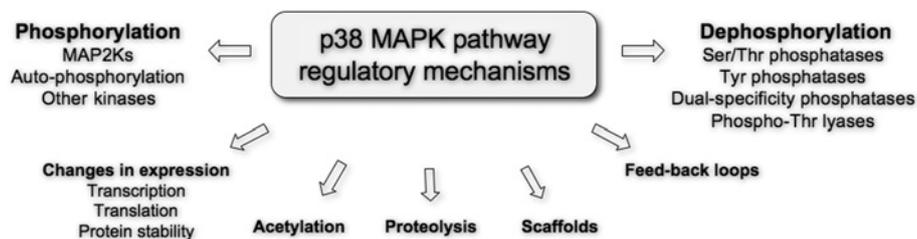


Figure 3 Mechanisms involved in p38 MAPK regulation

p38 MAPK activity is mainly controlled by phosphorylation–dephosphorylation mechanisms, but a number of additional regulatory mechanisms have been also reported.

appears to act as an endogenous inhibitor of the alternative p38 α -activation pathway in T-cells, by binding to p38 α and preventing Tyr³²³ phosphorylation [37]. The importance of the non-canonical TCR-mediated activation pathway compared with the canonical MAP2K-mediated mechanism has been investigated by generating p38 α -knockin mice in which Tyr³²³ was replaced by phenylalanine. These mice are viable and fertile, suggesting a tissue-restricted use of Tyr³²³ phosphorylation as an activation mechanism. However, in T-cells from these mice, p38 α is not activated upon TCR stimulation, confirming the requirement for the alternative pathway based on Tyr³²³ phosphorylation. The failure to activate p38 α in T-cells from the knockin mice results in a modest delay in cell-cycle entry, but once this point has been passed, cells apparently undergo normal division. Moreover, IFN γ (interferon γ) production is decreased in TCR-activated knockin T-lymphocytes. Thus the Tyr³²³-mediated non-canonical pathway is the physiological means of p38 α activation through the TCR and appears to be necessary for normal Th1 function, but not Th1 generation [38].

An additional alternative pathway of p38 α activation involves TAB1 (TAK1-binding protein 1), which can bind to p38 α , but not to other p38 MAPK family members, and induces p38 α autophosphorylation in the activation loop [39,40]. The direct activation of p38 α by TAB1 using purified proteins has been difficult to reproduce [41], although there is evidence suggesting that a TAB1-dependent mechanism probably involving autophosphorylation might contribute to p38 α regulation during myocardial ischaemia and in some functions of myeloid cells [42–45].

Finally, a third non-canonical MAP2K-independent mechanism for p38 MAPK activation has been proposed to operate upon down-regulation of the protein kinase Cdc7, which induces an abortive S-phase leading to p38 α -mediated apoptosis in HeLa cells. However, the underlying mechanism is unclear [46].

INACTIVATION OF THE PATHWAY

Down-regulation of p38 MAPKs is critical to achieve transient activation and to regulate signal intensity, which in turn results in specific outcomes. Termination of the kinase catalytic activity involves the activity of several phosphatases that target the activation loop threonine and tyrosine residues, including generic phosphatases that dephosphorylate serine/threonine residues, such as PP (protein phosphatase) 2A and PP2C, or tyrosine residues, such as STEP (striatal enriched tyrosine phosphatase), HePTP (haemopoietic tyrosine phosphatase) and PTP-SL (protein tyrosine phosphatase SL) (Figure 3). The activity of these phosphatases would lead to the formation of monophosphorylated MAPK forms, whose biological functions are not clear. Biochemical studies have shown that p38 α phosphorylated on

both Thr¹⁸⁰ and Tyr¹⁸² is 10–20-fold more active than p38 α phosphorylated only on Thr¹⁸⁰, whereas p38 α phosphorylated on Tyr¹⁸² alone is inactive [47]. Moreover, a p38 α mutant that cannot be tyrosine-phosphorylated (Y182F) shows some catalytic activity *in vitro* and is able to activate reporter genes in cells, although to a much lower extent than wild-type p38 α , suggesting that, whereas Thr¹⁸⁰ is necessary for catalysis, Tyr¹⁸² may be required for auto-activation and substrate recognition [48].

There is good evidence for p38 α activity down-regulation by Wip1, a serine/threonine phosphatase of the PP2C family that can be transcriptionally up-regulated by p53. In response to UV radiation, Wip1 mediates a negative-feedback loop acting on the p38 α /p53 pathway, which has been proposed to be important during the recovery phase of the damaged cells [49]. Wip1 can dephosphorylate numerous target proteins other than p38 α , including several tumour suppressors, which probably accounts for its important oncogenic activity [50].

The tyrosine phosphatase HePTP has been shown to control β_2 -adrenergic receptor-induced activation of p38 MAPK in B-lymphocytes, which in turn contributes to IgE production. In particular, β_2 -adrenergic receptor stimulation induces the PKA (protein kinase A)-dependent phosphorylation of HePTP on Ser²³, which inactivates HePTP and releases bound p38 MAPK, making it available for phosphorylation by MAP2Ks and subsequent IgE up-regulation [51].

A family of pathogenic effectors of the specialized TTSS (type III secretion system) conserved in plant and animal Gram-negative bacteria, including *Shigella* OspF, *Salmonella* SpvC and *Pseudomonas syringae* HopA11, can remove the phosphate from the phosphothreonine residue in the activation loop and inactivate MAPKs in the host cells. These enzymes show high phosphothreonine lyase activity towards p38 α . Structural studies of SpvC indicate that recognition of the phosphotyrosine residue followed by insertion of the threonine phosphate into an arginine pocket places the phosphothreonine residue into the enzyme active site. This requires conformational flexibility suggesting that the pThr-Gly-pTyr motif of p38 MAPKs is a likely physiological substrate [52].

The activity of MAPKs can be also regulated by a family of DUSPs (dual-specificity phosphatases)/MKPs (MAPK phosphatases), which dephosphorylate both phosphotyrosine and phosphothreonine residues. Most MKPs include a docking domain that mediates the interaction with the MAPK substrate and a dual-specific phosphatase domain. Moreover, binding of MAPK to the MKP docking domain increases its phosphatase activity. MKPs 1, 4, 5 and 7 can dephosphorylate p38 α and p38 β in addition to JNK MAPKs. Importantly, some MKPs are transcriptionally up-regulated by stimuli that activate MAPK signalling, and are thought to play an important role limiting the extent of MAPK activation [53].

Induction of MKP1 appears to be a common mechanism used by several extracellular stimuli to protect from apoptosis [54,55] or to suppress pro-inflammatory cytokine expression [56], via p38 MAPK activity down-regulation. MKP1 is known to be regulated transcriptionally as well as by phosphorylation and ubiquitin-mediated proteolysis [57]. A recent report shows that MKP1 may be also regulated by acetylation [58]. When RAW macrophages are stimulated with LPS, MKP1 becomes acetylated on Lys⁵⁷ by p300. Acetylation of this residue, which is located within the substrate-binding domain, promotes MKP1 interaction with p38 MAPK, resulting in dephosphorylation of p38 MAPK. Interestingly, MKP1-knockout mice do not respond to the inflammation-reducing effect of HDAC (histone deacetylase) inhibitors, suggesting that acetylation of MKP1 inhibits innate immune signalling [58].

Genetic analysis has assigned specific functions to some MKPs acting on p38 MAPKs. For example, MKP5 has a role in the protection against LPS-induced and oxidant-mediated tissue injury by controlling p38 MAPK activation in neutrophils. MKP5 regulates p38 MAPK activation, which in turn can phosphorylate Ser³⁴⁵ of mouse p47^{phox}, a component of the NADPH oxidase complex. Thus MKP5 inactivation increases p38 MAPK-mediated phosphorylation of p47^{phox}, resulting in NADPH oxidase priming and superoxide production at inflammatory sites. This MKP5 function cannot be substituted for by other MKPs, such as MKP1, that have similar substrate specificity [59].

SCAFFOLDS AND OTHER REGULATORY MECHANISMS

The organization of MAPK cascades involves binary interactions between the kinases as well as scaffolding proteins that interact simultaneously with several components. Importantly, there is evidence indicating that scaffolds not only physically link MAPK cascade components, but may allosterically regulate the phosphorylation events required for MAPK activation too. For example, a domain of the scaffold protein Ste5 has been reported to increase the rate of phosphorylation of the Fus3 MAPK by the Ste7 MAP2K in yeast [60].

One of the p38 MAPK scaffolds is OSM (osmosensing scaffold for MEKK3), which forms a complex with Rac, MEKK3 and MKK3, and regulates p38 α activation in response to osmotic shock [61]. In addition, the JIP (JNK-interacting protein) family members JIP2 and JIP4 have been reported to regulate p38 MAPK activation [62,63].

Scaffolds have also been implicated in the regulation of cell differentiation by p38 MAPKs. Upon induction of muscle or neuronal differentiation, the cell-surface receptor of the Ig superfamily Cdo binds directly to the scaffold protein JLP (JNK-associated leucine zipper protein), and via JLP to p38 α and p38 β . In addition, Cdo binds to the Cdc42 scaffold protein Bnip-2. Formation of a Cdo-Bnip2-Cdc42 complex facilitates activation of Cdc42, which in turn triggers signals leading to the phosphorylation and activation of the Cdo/JLP-bound p38 α and p38 β . p38 MAPKs then phosphorylate E proteins, facilitating their heterodimerization with myogenic or neurogenic bHLH (basic helix-loop-helix) factors that will induce the gene-expression programmes required for myogenesis or neurogenesis respectively [64,65].

A number of additional regulatory mechanisms have been reported to control p38 MAPK signalling (Figure 3). p38 MAPKs and their activators are thought to be regulated mainly by phosphorylation and dephosphorylation of the activation-loop residues (Figure 3), but changes in expression levels of the cascade components, involving both transcriptional and post-

transcriptional mechanisms, have been also described. Thus p38 γ expression is up-regulated during myogenesis [66], and the expression levels of MKK3 and MKK6 have been reported to change upon CD4⁺ T-cell stimulation [67]. Moreover, the c-Abl tyrosine kinase has been shown to mediate the cisplatin-induced activation of the p38 MAPK pathway by a mechanism that is independent of its tyrosine kinase activity, but involves increased stability of the MKK6 protein [68]. The levels of MKK4 protein also increase in senescent human fibroblasts through enhanced translation, which seems to be regulated by four microRNAs (*miR-15b*, *miR-24*, *miR-25* and *miR-141*) that target the 5' and 3' untranslated regions of the *MKK4* mRNA [69]. In addition, the E3 ubiquitin ligase Itch promotes MKK4 protein degradation in cells exposed to high concentrations of sorbitol [70].

The stability of p38 α can be regulated by association with its substrates MK2 and MK3. Thus MK2/MK3-double-knockout cells display significantly reduced levels of p38 α [71] and, interestingly, MK2 levels are also decreased in p38 α -deficient cells [72]. The molecular mechanism responsible for the reciprocal regulation of these protein kinases is most likely to be related to stabilizing protein-protein interactions. These observations should be taken into account when using gene-specific ablation to assign functions to these kinases.

An additional mechanism that can regulate p38 MAPK activity is the phosphorylation on Thr¹²³ [73], a residue located at the docking groove for substrates and regulators. This phosphorylation decreases the association of p38 MAPK with its activator MKK6, as well as the ability of p38 MAPK to bind to and phosphorylate substrates. Interestingly, Thr¹²³ can be phosphorylated *in vitro* by the G-protein-coupled receptor kinase GRK2 [73], suggesting that one of the GRK2 functions could be to limit p38 MAPK activity levels in cells.

MAP2Ks can be also modulated by several mechanisms, including anthrax lethal toxin-mediated proteolytic cleavage, which renders them inactive [74]. In addition, acetylation of the MAP2K activation-loop residues, which precludes their activating phosphorylation, has been proposed to occur during the infection of Gram-negative bacterial pathogens. For example, the TTSS effector protein YopJ of *Yersinia* can acetylate and inactivate the host MAP2Ks [75].

SUBCELLULAR LOCALIZATION

In contrast with other MAPKs, p38 α has no nuclear localization signal and has been detected in both the nucleus and the cytoplasm of non-stimulated cells. However, the subcellular localization upon activation is controversial. Some evidence indicates that, following activation, p38 α translocates from the cytoplasm to the nucleus [76], but there is also evidence showing that, in response to specific stimuli, p38 α preferentially accumulates in the cytosol [77]. The discrepancy could be due to the analysis of different pools of p38 α , as it is conceivable that p38 α molecules may be located in different subcellular compartments as well as bound to different partners. For example, the re-localization of activated p38 α to the cytoplasm has been ascribed to nuclear export in association with its substrate MK2.

A recent study postulates that p38 α nuclear translocation could be relevant for the regulation of G₂/M cell-cycle arrest and to promote DNA repair, since p38 α translocates to the nucleus upon activation by stimuli that induce DNA double-strand breaks, but not other stimuli [78]. Such translocation does not require p38 α catalytic activity, but it is induced by a conformational change triggered by the phosphorylation on Thr¹⁸⁰ and Tyr¹⁸² at the activation loop [78]. Since phosphorylation of p38 α in response

to DNA damage, but not in response to other stimuli, promotes nuclear accumulation, it is plausible that nuclear shuttling is also specifically induced by DNA damage. Therefore selective nuclear transport of p38 α would require both its phosphorylation and active nuclear shuttling. Alternatively, DNA damage signals could release p38 α from docking molecules such as MK2 or TAB1 that are known to retain p38 α in the cytosol [79,80], perhaps due to conformational changes induced upon DNA damage.

KINASE-INDEPENDENT FUNCTIONS

The functions of p38 MAPKs are normally associated with the phosphorylation of substrates on Ser-Pro or Thr-Pro motifs, although occasional reports have shown the phosphorylation of non-proline-directed sites by p38 MAPKs [41,81]. Intriguingly, p38 MAPKs may also have kinase-independent roles, which are thought to be due to the binding to targets in the absence of phosphorylation. An example of such kinase-independent functions is the regulation of the multifunctional ATF (activating transcription factor)/CREB (cAMP-response-element-binding protein) protein ATF1 by the p38 MAPK Spc1 during hotspot recombination in *Schizosaccharomyces pombe*. Several ATF1 functions are regulated by Spc1-mediated phosphorylation. However, a phosphorylation-deficient mutant of ATF1 was found to be fully proficient for hotspot recombination, and further characterization suggests that Spc1 may regulate ATF1 binding to chromosomes during meiotic recombination without affecting ATF1 phosphorylation [82]. In *S. cerevisiae*, the p38 MAPK Hog1 is also recruited to chromatin as a component of transcription complexes, and some of its gene-expression-regulatory functions in response to osmotic stress might not require Hog1 kinase activity. For example, the Hot1 transcription factor can be phosphorylated by Hog1 and regulates a subset of Hog1-responsive genes, but mutation of all of the Hog1 phosphorylation sites in Hot1 affects neither the kinetics nor the levels of expression of stress-induced genes [83,84].

p38 α has been also proposed to regulate the proliferation of HeLa cells in a kinase-independent manner, on the basis of the different effects of RNAi (RNA interference)-mediated p38 α down-regulation compared with chemical inhibitors as well as on the ectopic expression of a kinase-negative p38 α mutant [85]. However, the relevance of these observations and the mechanism involved remain to be elucidated. In addition, a kinase-independent function of p38 α has been suggested in the context of ischaemia-induced stress in brain. Protein O-GlcNAcylation catalysed by the OGT (O-GlcNAc transferase) enzyme is regulated by p38 α , and, although OGT does not seem to be phosphorylated by p38 α , their interaction increases upon p38 α activation induced by glucose deprivation. This interaction may regulate OGT activity by recruiting it to specific targets such as neurofilament H, stimulating its O-GlcNAcylation [86]. Likewise, p38 γ has been suggested to play a phosphorylation-independent function in Ras-induced transformation of rat intestinal epithelial cells, on the basis of the observation that K-Ras induces p38 γ expression without increasing its phosphorylation, and that p38 γ down-regulation impairs K-Ras-induced transformation. The mechanism underlying such a putative kinase-independent function of p38 γ remains unknown [87].

Taken together, these results suggest that p38 MAPKs may be able to bind to some proteins and modulate their functions without phosphorylating them. This may involve structural modification of the targets, changes in their subcellular location or competition with their binding to other proteins.

SUBSTRATE RECOGNITION

The ability of MAPKs to phosphorylate some substrates is influenced by the presence of binding sites (usually referred to as docking domains or D domains), which are different from the serine/threonine phospho-acceptor sites. D domains are characterized by the presence of both positively charged and hydrophobic residues, and are present not only in MAPK substrates such as transcription factors and MKs, but also in regulatory proteins such as upstream MAP2Ks, scaffold proteins and phosphatases [88,89]. This implies that the ordered and sequential use of interaction domains should be strictly regulated for accurate MAPK signalling [90]. Docking interactions can be regulated by phosphorylation either in the D domain of the substrate or in the interacting region of p38 MAPK [91]. Importantly, docking interactions not only regulate the association between cascade components, but may also induce allosteric conformational changes that expose the activation loop influencing the strength and duration of MAPK signalling [60].

Several regions of MAPKs have been shown to participate in docking interactions, including the CD (common docking) and ED (ERK docking) motifs, which contain acidic and hydrophobic residues, as well as a docking groove identified by crystallographic studies of p38 α in complex with D domain-containing peptides derived from the substrate MEF (myocyte enhancer factor) 2A and the upstream kinase MKK3b [92]. In the case of MKs, variations in the D domain sequence have been reported to determine the binding specificity of ERK and p38 MAPKs [93]. It has been also reported that the selectivity of the interaction between MAP2Ks and MAPKs is such that within-pathway interactions are in general preferred by about an order of magnitude over non-cognate interactions [94]. Of note, p38 γ is unique among MAPKs in that it has a C-terminal sequence which docks directly to PDZ domains of several proteins, such as α_1 -syntrophin, SAP90/PSD95 (postsynaptic density 95) and the scaffold protein SAP97/hDlg, and phosphorylation of these proteins by p38 γ is dependent on its binding to the PDZ domains [15,95].

Although it is clear that docking interactions enhance the efficiency and specificity of target phosphorylation by MAPKs, it seems that some substrates do not have docking domains. Therefore other mechanisms are likely to operate to facilitate the efficient phosphorylation of these substrates *in vivo*. This adds a new level of regulation in MAPK signalling, as it opens up the possibility of selectively interfering with the phosphorylation of particular sets of substrates. Along this line, the p38 α chemical inhibitor CMPD1 has been reported to selectively prevent the *in vitro* phosphorylation of MK2 by p38 α without affecting the phosphorylation of ATF2. The exact mechanism of substrate-selective inhibition by CMPD1 has not been elucidated, although CMPD1 neither competes with ATP nor interferes with the binding of p38 α to MK2. It has been suggested that CMPD1 binding in the active-site region of p38 α may somehow affect the optimal positioning of substrates and cofactors, resulting in selective inhibition of p38 α kinase activity [96].

SUBSTRATES AND FUNCTIONS

It has been estimated that MAPKs may have approx. 200–300 substrates each. Accordingly, p38 MAPKs have been reported to phosphorylate a broad range of proteins, both *in vitro* and *in vivo*. Much of the information about p38 MAPK substrates comes from the use of chemical inhibitors such as SB203580. Recent studies based on targeted deletion of several p38 MAPK

pathway components are allowing a better definition of the *bona fide* p38 MAPK substrates.

On the basis of their functions, p38 α substrates comprise protein kinases implicated in different processes, nuclear proteins, including transcription factors and regulators of chromatin remodelling, and a heterogeneous collection of cytosolic proteins that regulate processes as diverse as protein degradation and localization, mRNA stability, endocytosis, apoptosis, cytoskeleton dynamics or cell migration (Figure 1).

Protein kinases

Several kinases activated by the p38 MAPK pathway are involved in the control of gene expression at different levels (Figure 1). MSK (mitogen- and stress-activated kinase) 1 and 2 can directly phosphorylate and activate transcription factors such as CREB, ATF1, the NF- κ B isoform p65 and STAT (signal transducer and activator of transcription) 1 and 3, but can also phosphorylate the nucleosomal proteins histone H3 and HMG-14 (high-mobility group 14) [97]. MSK1 and MSK2 play important roles in the rapid induction of immediate-early genes in response to stress or mitogenic stimuli, either by inducing chromatin remodelling or by recruiting the transcription machinery [98]. MSK2 can also control the transcriptional activity of p53 in a kinase-independent manner [99]. In contrast with MSK1 and MSK2, which preferentially activate transcription, MK2 and MK3 participate in the control of gene expression mostly at the post-transcriptional level, by phosphorylating the ARE (AU-rich element)-binding proteins TTP (tristetraprolin) and HuR, and by regulating eEF2K (eukaryotic elongation factor 2 kinase), which is important for the elongation of mRNA during translation. Nevertheless, some transcription factors, such as E47, ER81, SRF (serum-response factor) and CREB, are also phosphorylated by MK2 [4,93,100]. Finally, MNK1 and MNK2 regulate protein synthesis by phosphorylating the initiation factor eIF4E (eukaryotic initiation factor 4E) [101].

In addition, p38 MAPK-activated kinases can phosphorylate many other targets involved in diverse cellular processes. Notably, MK2 is well known to play an important role in actin filament remodelling by phosphorylating Hsp27 (heat-shock protein 27) [100]. A recent report has also proposed the implication of MK2 in the regulation of RNA-mediated gene silencing by cellular stress. MK2 was found to phosphorylate *in vitro* the Ago2 (Argonaute 2) protein on Ser³⁸⁷, which was identified as the major Ago2 phosphorylation site in cells, and mutation of Ser³⁸⁷ to alanine impairs Ago2 localization to the RNA-containing granules termed processing bodies [102].

Cytoplasmic substrates

A large number of cytosolic proteins can be phosphorylated by p38 MAPKs, including phospholipase A₂, the microtubule-associated protein tau, NHE-1 (Na⁺/H⁺ exchanger 1), cyclin D1, CDK (cyclin-dependent kinase) inhibitors, Bcl-2 family proteins, growth factor receptors or keratins (reviewed in [2,4,100]).

The p38 MAPK pathway is an important regulator of protein turnover. For example, FLIP_s {short isoform of FLICE [FADD (Fas-associated death domain)-like IL-1 β -converting enzyme]-inhibitory protein} is an inhibitor of TNF-induced apoptosis whose proteasome-mediated degradation is regulated by p38 MAPK phosphorylation. Infection of macrophages with *Mycobacterium tuberculosis* triggers ROS (reactive oxygen species)-dependent activation of both c-Abl and the MAP3K ASK1, leading to the phosphorylation of FLIP_s on Tyr²¹¹ and Ser⁴

by c-Abl and p38 MAPK respectively. These phosphorylations facilitate the interaction between FLIP_s and the E3 ubiquitin ligase c-Cbl, leading to proteasomal degradation of FLIP_s, which in turn allows procaspase 8 to interact with FADD and initiate apoptosis [103]. In a similar way, p38 MAPKs phosphorylate the ubiquitin ligase Siah2 on Thr²⁴ and Ser²⁹, regulating its activity towards PHD3 (prolyl hydroxylase 3) [104]. Phosphorylation by p38 α also leads to the stabilization of Siah2 under conditions of high cell density, which in turn down-regulates Sprouty2, facilitating the release of the Sprouty2-bound c-Cbl. This leads to the ubiquitination and degradation of the EGFR (epidermal growth factor receptor), shutting off mitogenic signals and allowing the establishment of cell–cell contact inhibition [105].

A recent report has proposed that p38 α inhibits the lysosomal degradation pathway of autophagy by interfering with the intracellular trafficking of the transmembrane protein Atg9. This would be mediated by p38IP (p38 α -interacting protein), which was found to bind to Atg9, facilitating starvation-induced Atg9 trafficking and autophagosome formation [106]. How exactly p38 α might regulate the p38IP–Atg9 interaction and whether this is the only link between the p38 MAPK pathway and autophagy remains to be elucidated.

Another function of p38 α is to regulate the endocytosis of membrane receptors by different mechanisms that impinge on the small GTPase Rab5 [107,108]. For example, endocytosis of the μ -opioid receptor, a key regulator of opioid pharmacological effects, is controlled by the p38 α -dependent phosphorylation of the Rab5 effector EEA1 (early endosome antigen 1A) [108]. In addition, clathrin-mediated EGFR internalization induced by inflammatory cytokines and UV irradiation depends on p38 α -mediated phosphorylation of EGFR itself as well as of Rab5 effectors [109].

Ectodomain shedding of transmembrane proteins is regulated by p38 MAPKs as well. In response to inflammatory stimuli, p38 MAPKs phosphorylate the membrane-associated metalloprotease TACE (TNF α -converting enzyme)/ADAM17 (a disintegrin and metalloproteinase domain 17) on Thr⁷³⁵, located in its cytoplasmic domain. Such phosphorylation is required for TACE-mediated ectodomain shedding of TGF α family ligands, which results in the activation of EGFR signalling and cell proliferation [110].

Additional examples of p38 MAPK substrates are the FGFR1 (fibroblast growth factor receptor 1) and the ARE-binding and mRNA-stabilizing protein HuR. FGFR1 can be translocated from the extracellular space into the cytosol and nucleus of target cells, and regulates processes such as rRNA synthesis and cell growth. FGFR1 translocation requires p38 MAPK activation, which phosphorylates the C-terminal tail of FGFR1 on Ser⁷⁷⁷. Interestingly, the mutation S777A abolishes FGFR1 translocation, whereas phosphomimetic mutants bypass the requirement for active p38 MAPK for translocation [111]. In the case of HuR, a new direct link with p38 MAPK has been established recently in the G₁ cell-cycle arrest induced by γ -radiation [112]. When cells are irradiated, p38 α is transiently activated and phosphorylates HuR on Thr¹¹⁸, which results in HuR cytoplasmic accumulation and enhanced binding to the p21^{Cip1} mRNA. HuR binding increases p21^{Cip1} mRNA stability, therefore allowing the expression of the appropriate p21^{Cip1} protein levels required for G₁ cell-cycle arrest [112].

Nuclear substrates

Many transcription factors are phosphorylated and activated by p38 MAPKs in response to different stimuli. Classical examples include ATF1, 2 and 6, Sap1 (SRF accessory protein 1), CHOP [C/EBP (CCAAT/enhancer-binding protein)-homologous

protein], p53 and MEF2C and MEF2A [100,113]. Recent results have established a role for p38 α in the regulation of lineage choices during myelopoiesis through phosphorylation of C/EBP α on Ser²¹ [114]. A core network of 16 transcription factors has also been recently proposed to mediate the regulation by p38 α of human squamous carcinoma cell quiescence [115]. In addition, p38 MAPK-dependent phosphorylation of the transcription factor USF1 (upstream stimulatory factor 1) on Thr¹⁵³ has been reported to facilitate acetylation, which in turn changes the gene regulatory properties of USF1 [116].

p38 MAPKs are emerging as important modulators of gene expression by regulating chromatin modifiers and remodellers. The promoters of several genes involved in the inflammatory response, such as IL-6, IL-8, IL-12p40 and MCP-1 (monocyte chemoattractant protein 1), display a p38 MAPK-dependent enrichment of histone H3 phosphorylation on Ser¹⁰ in LPS-stimulated myeloid cells. This phosphorylation enhances the accessibility of the cryptic NF- κ B-binding sites marking promoters for increased NF- κ B recruitment. Importantly, H3 phosphorylation is not carried out directly by p38 MAPK, but more likely through MSK1 [117]. In addition, phosphorylation of histone H3 by the p38 MAPK pathway also contributes to the chromatin relaxation status necessary for nuclear excision repair factor assembly in response to UV-induced DNA lesions. Rapid activation of the p38 MAPK pathway in response to UV radiation helps to enhance the DDB2 (damaged-DNA-binding complex 2) ubiquitination, probably through DDB2 phosphorylation. Consequent DDB2 degradation facilitates the recruitment of XPC (xeroderma pigmentosum complementation group C) needed for continuation of the repair process [118].

The targeting of the Ash2L-containing methyltransferase complexes to specific genes during muscle differentiation is another example of the implication of p38 MAPK in epigenetic changes. In this case, Ash2L recruitment requires p38 MAPK-mediated phosphorylation of MEF2D on Thr³⁰⁸ and Thr³¹⁵, which is essential for histone H3 Lys⁴ modification and subsequent gene expression [119]. Moreover, a recent study has shown that p38 MAPKs may regulate the phosphorylation of polycomb group proteins, transcriptional repressors that are recruited to specific DNA sequences through recognition of modified histones [120].

In *S. cerevisiae*, the p38 MAPK Hog1 is recruited to the promoters of regulated genes through its association with transcription factors, which is important to stimulate Pol II (RNA polymerase II) as well as for recruitment of the Rpd3 histone deacetylase and SAGA (Spt-Ada-Gcn5-acetyltransferase) histone acetylase complexes, resulting in transcription initiation. Hog1 also mediates the recruitment of the RSC (chromatin structure remodelling) complex of the SWI/SNF family to osmoresponsive genes, which is required for the nucleosome rearrangements found in osmoresponsive genes in response to high osmolarity [84]. The importance of p38 MAPK signalling for the function of SWI/SNF chromatin remodellers has also been demonstrated in higher eukaryotes. Thus, during muscle differentiation, p38 MAPK is required for the association between MyoD (myogenic differentiation factor D) and the ATPase subunits of the SWI/SNF complex BRG1 and BRM. Moreover, the SWI/SNF subunit BAF60 (BRG1-associated factor 60) is phosphorylated by p38 MAPK *in vitro*, although the functional relevance of this phosphorylation has yet to be established. It is worth mentioning that several BAF60 isoforms have been implicated in the interactions between the SWI/SNF complex and transcription factors. In addition, BRG1 can interact with both MyoD and Pbx on the myogenin promoter [121].

An additional type of chromatin remodelling process involves the exchange of the canonical histone H2A for the H2A.Z

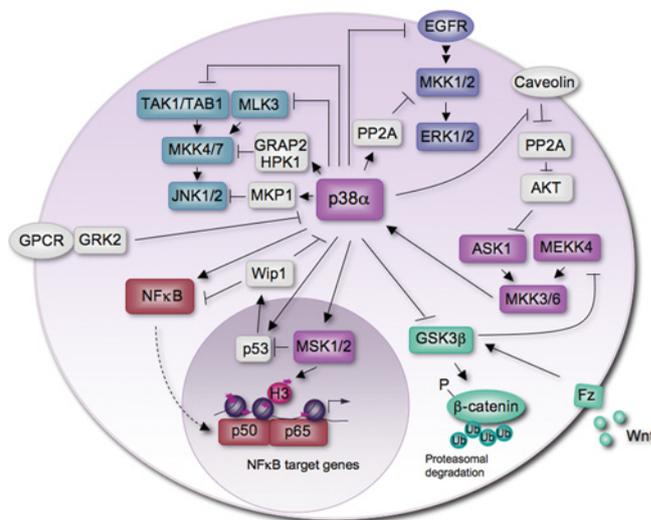


Figure 4 Cross-talk between p38 MAPKs and other signalling pathways

The interplay between p38 α and several signalling pathways including JNK, ERK1/2, NF- κ B, Wnt/GSK3 β , Akt and G-protein-coupled receptors (GPCRs) is shown. Fz, Frizzled; GRAP2, Grb2-related adaptor protein 2; H3, histone H3; HPK1, haemopoietic progenitor kinase 1; Ub, ubiquitin.

variant, which is present in the nucleosomes close to transcription start sites and has been associated with transcription activation [122,123]. The exchange of histone H2A.Z is catalysed by the SRCAP (Snf2-related CREB-binding protein-activator protein) chromatin-remodelling complex [124]. The SRCAP subunit ZNHIT1 (zinc finger HIT-type 1) or p18^{Hamlet} is a substrate of p38 α and p38 β , which mediates p53-dependent transcriptional responses to genotoxic stress [125,126]. In response to DNA damage, p38 α phosphorylates the p18^{Hamlet} protein in at least four threonine residues, increasing its stability. Phosphorylated p18^{Hamlet} enhances the transcription of several p53-dependent genes, such as *Noxa*, involved in the apoptotic response. From a mechanistic point of view, p18^{Hamlet} is necessary for the UV- or cisplatin-induced recruitment of p53 to the *Noxa* promoter, and p18^{Hamlet} binding to the promoter is also stimulated by DNA damage. More recently, p18^{Hamlet} has been implicated in muscle differentiation. In particular, p18^{Hamlet} is required for the incorporation of histone H2A.Z into the myogenin promoter, which is a crucial event for the initiation of the myogenic transcriptional programme. Differentiation conditions induce the phosphorylation of p18^{Hamlet} by p38 MAPK, and overexpression of the wild-type p18^{Hamlet}, but not of a non-phosphorylatable mutant, suffices to initiate myogenesis. Moreover, p38 MAPK-mediated phosphorylation of p18^{Hamlet} appears to be necessary for assembly of the SRCAP complex. These results suggest a mechanism by which p38 MAPK signals are converted into chromatin structural changes, thereby facilitating transcriptional activation during mammalian cell differentiation [127].

CROSS-TALK WITH OTHER SIGNALLING PATHWAYS

Cross-talk between signalling pathways is a common theme in cell regulation, which usually depends on cell context and plays an important role in fine-tuning biological responses. There are many examples that illustrate the cross-talk between different MAPK pathways (Figure 4). One such example is the inhibition of the ERK1/2 pathway by p38 MAPKs. In normal cells, p38 MAPK signalling causes rapid inactivation of the ERK1/2 pathway

mediated by PP2A. In particular, p38 MAPK activity stimulates the physical association between PP2A and the MKK1/2–ERK1/2 complex, leading to MKK1/2 dephosphorylation by PP2A. In addition, direct interaction between ERK1/2 and p38 MAPKs might also inhibit ERK phosphorylation [128]. The interplay between the JNK and p38 MAPK pathways is also broadly documented both in cell lines and in animal models. Although multiple stimuli can simultaneously activate both pathways, JNK and p38 MAPK activation have antagonistic effects in many cases. From a mechanistic point of view, the p38 MAPK pathway can negatively regulate JNK activity at the level of MAP3Ks, either by phosphorylating MLK3 or the TAK1 regulatory subunit TAB1. p38 α can also antagonize JNK signalling by cell-type-specific mechanisms such as the regulation of the dual-specificity phosphatase MKP1 in myoblasts, or the proteins GRAP2 (Grb2-related adaptor protein 2) and HPK1 (haemopoietic progenitor kinase 1) in fetal haemopoietic cells (reviewed in [129]).

The p38 MAPK pathway can positively regulate NF- κ B activity by different mechanisms, including chromatin remodelling through Ser¹⁰ phosphorylation of histone H3 at NF- κ B-dependent promoters such as IL-8 and MCP-1 [117], or by impinging on IKK or the p65 subunit in a direct or indirect manner [130,131] (Figure 4). A recent study has shown that the Wip1 phosphatase directly dephosphorylates p65 negatively modulating NF- κ B-dependent transcription of genes such as TNF α [132]. In addition, Wip1 can also dephosphorylate p38 α (see above), and therefore may interfere indirectly with the positive effect of the p38 MAPK pathway on NF- κ B signalling [132].

A link between the p38 MAPK and Wnt/ β -catenin pathways has been reported recently (Figure 4). β -Catenin is normally phosphorylated by GSK (glycogen synthase kinase) 3 β , which targets β -catenin for ubiquitination and proteasome-mediated degradation. Wnt stimulation suppresses GSK3 β activity, leading to elevation of cytosolic and nuclear β -catenin levels, which turn on the genes necessary for embryonic development, patterning and cellular proliferation. Phosphorylation on Ser⁹ by Akt is the best-characterized mechanism for GSK3 β inhibition. However, p38 α also inactivates GSK3 β by direct phosphorylation of the C-terminal residue Ser³⁸⁹, leading to β -catenin accumulation. This non-canonical p38 MAPK-dependent phosphorylation of GSK3 β seems to occur primarily in the brain and thymocytes, and provides a potential mechanism for p38 MAPK-mediated survival in specific tissues [133]. On the other hand, GSK3 β has been reported to inhibit p38 MAPK (and JNK) activation by phosphorylating the MAP3K MEKK4 [134].

The p38 MAPK and Akt pathways have been proposed to interplay at different levels (Figure 4). Several reports have shown that Akt inhibits the activation of p38 α , for example by phosphorylation of ASK1 on Ser⁸³ [135], a mechanism that would contribute to anti-apoptotic effects of Akt2 [136]. On the other hand, p38 α may negatively modulate Akt activity, independently of PI3K (phosphoinositide 3-kinase), by regulating the interaction between caveolin-1 and PP2A through a mechanism dependent on cell attachment [137]. Finally, genetic ablation of p38 α suppresses Akt activation in mouse macrophages, which has been proposed to regulate macrophage apoptosis *in vivo* [138]. The underlying mechanism might involve MK2-mediated activation of Akt via phosphorylation on Ser⁴⁷³ [139].

SIGNAL INTENSITY AND DURATION

The strength of signalling pathway activation is known to play a key role in the cellular responses. The duration of the signal is also a very important determinant. For example, transient activation of the ERK1/2 cascade induces PC12 cells to proliferate, whereas

sustained activation of the same MAPK pathway induces PC12 cell differentiation [140].

The p38 MAPK pathway can be activated at different levels by diverse stimuli, which in turn correlates with various cellular outcomes. Thus stress responses usually trigger high and sustained p38 MAPK activation, whereas homeostatic functions tend to be associated with transient and lower p38 MAPK activity levels [108]. It is therefore expected that the amount of p38 MAPK activity should determine the networks of substrates being phosphorylated, which in turn would impinge on the cellular response. This may be one of the mechanistic explanations for the sometimes opposing effects observed upon p38 MAPK activation. For example, there is evidence that strong p38 MAPK activation is likely to engage apoptosis, whereas lower levels of p38 MAPK activity tend to be associated with cell survival (reviewed by [141]).

Consistent with these ideas, chronic exposure to stresses, such as UV light, oxidative stress or DNA-damaging agents, can induce cellular senescence. Interestingly, sustained activation of p38 MAPK by stable expression of a constitutively active form of MKK6 (MKK6EE) is sufficient to induce a permanent and irreversible G₁ cell-cycle arrest with both morphological and biochemical features of senescence [142]. Enforced activation of p38 MAPK by MKK6EE also leads to growth arrest and terminal differentiation in rhabdomyosarcoma cells. However, p38 MAPK activation by inflammatory cytokines do not promote differentiation in these cells, possibly because p38 MAPK is only transiently activated, but maybe also because of the activation of parallel pathways, such as JNK, which antagonize differentiation. These observations support the idea that persistent p38 MAPK activation is required to induce myogenic differentiation [143].

It is worth mentioning that negative-feedback mechanisms contribute to fine-tuning p38 MAPK activity levels. For example, p38 α regulates *MKK6* mRNA stability in non-stimulated cells, potentially limiting the intensity and duration of the signal [144]. Also, p38 α phosphorylates TAB1, leading to inhibition of the MAP3K TAK1, which may be operational in the inflammatory response to control activation of TAK1-regulated signalling pathways, such as p38 MAPKs, JNKs and NF- κ B [41]. Finally, p38 α induces the up-regulation, usually at the transcriptional level, of phosphatases such as Wip1 and MKPs, which in turn can potentially inactivate p38 MAPKs (see above).

PHYSIOLOGICAL FUNCTIONS

Studies carried out during the last few years using gene-targeting in mice have provided important information regarding the regulation of the p38 MAPK pathway and its functions *in vivo* (Figure 5). Most of the studies have focused on p38 α , establishing its implication in tissue homeostasis and several pathologies from inflammation and the immune response to cancer, heart and neurodegenerative diseases (reviewed in [4,129]). Mice with a constitutive deletion of p38 α die during embryonic development owing to a defect in placental organogenesis [145–147]. Interestingly, MKK3/MKK6-double-knockout mice also die during embryogenesis owing to a placental defect [25], whereas mice deficient only in MKK3 or MKK6 are viable, but show defects in the immune response [67,148]. The roles of p38 α in adult mice have been investigated using conditional alleles. Inactivation of p38 α significantly affects lung homeostasis, reflecting the importance of this pathway in the co-ordination of proliferation and differentiation in lung epithelial cells [149,150]. Of note, p38 α ablation results in enhanced proliferation of other types of primary cells, including cardiomyocytes, hepatocytes and haemopoietic cells [150,151]. This effect may be mediated

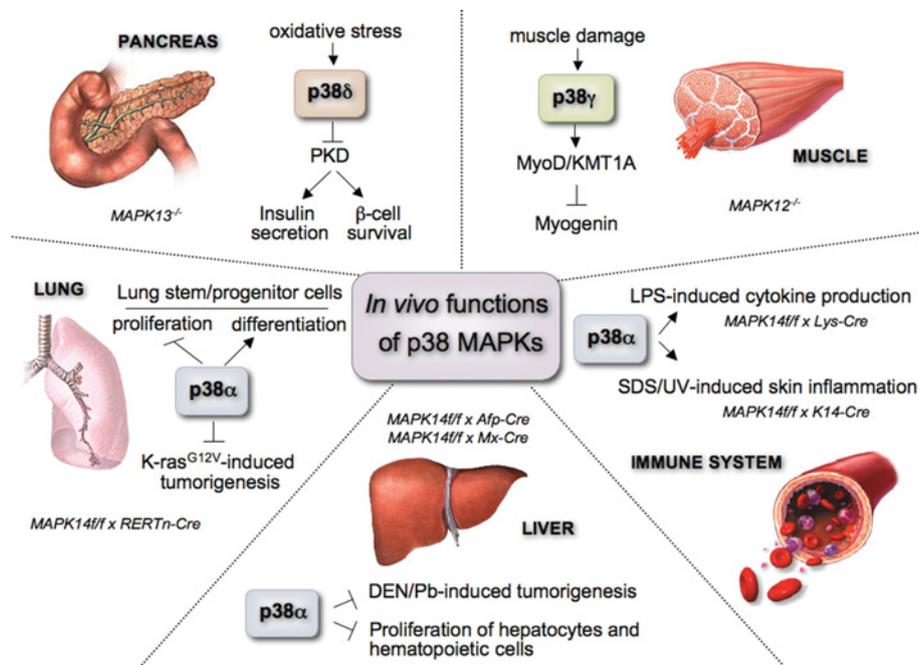


Figure 5 Physiological roles of p38 MAPKs

In vivo functions of the different p38 MAPK family members are shown. Genetically modified mouse models are indicated in italics.

by EGFR down-regulation [149] or by negative regulation of the JNK/c-Jun pathway [150] depending on cell types. Specific deletion of p38 α in myeloid or epithelial cells has also provided evidence for the physiological implication of this pathway in cytokine production and inflammatory responses [152,153]. Moreover, p38 α -deficient mice are sensitized to K-Ras^{G12V}-induced lung tumorigenesis [149] or chemically induced liver cancer [150,154], supporting the function of p38 α as a tumour suppressor *in vivo*. The implication of p38 MAPKs in age-related degenerative diseases has been also suggested on the basis of the expression in mice of a dominant-negative allele of p38 α with the activation-loop phosphorylation sites mutated (T180A and Y182F). The pancreatic islets of these mice show enhanced proliferation and regeneration, which correlates with attenuated age-induced expression of CDK inhibitors when compared with wild-type littermates of the same age [155].

In contrast with p38 α , mice deficient in p38 β , p38 γ or p38 δ are viable and were initially reported to have no obvious phenotypes [15,156]. Functional redundancy due to overlapping substrate specificity of different p38 MAPKs combined with the ubiquitous and high-level expression of p38 α may contribute, at least partially, to the lack of overt phenotypes upon deletion of other family members. However, recent reports have highlighted the implication of p38 δ and p38 γ in metabolic diseases, cancer and tissue regeneration (Figure 5), further strengthening the interest in this pathway for the development of new therapeutic strategies. In one study [157], p38 δ has been shown to regulate insulin secretion as well as survival of pancreatic β -cells. Mice lacking p38 δ display improved glucose tolerance owing to enhanced insulin secretion from pancreatic β -cells, which correlates with the activation of PKD (protein kinase D) owing to the lack of p38 δ -mediated inhibitory phosphorylation. Moreover, p38 δ -null mice are protected against high-fat-feeding-induced insulin resistance and oxidative stress-mediated β -cell failure. Therefore

the p38 δ /PKD pathway integrates regulation of the insulin-secretory capacity and survival of pancreatic β -cells, pointing to a pivotal role for this pathway in diabetes. Interestingly, p38 δ -deficient mice have also reduced susceptibility to skin carcinogenesis [158]. Regarding p38 γ , studies in mice have suggested its role in blocking the premature differentiation of satellite cells, a skeletal muscle stem cell population that participates in adult muscle regeneration [159]. In particular, p38 γ promotes the association between the transcription factor MyoD and the histone methyltransferase KMT1A, which act together in a complex to repress the premature expression of myogenin. Paradoxically, p38 α and p38 γ appear to play opposite roles in skeletal muscle differentiation, since p38 α activity is well known to promote skeletal muscle differentiation at different levels [121].

INHIBITORS AND THERAPEUTIC APPLICATIONS

p38 α is an interesting pharmaceutical target because of its important role in inflammatory diseases such as psoriasis, arthritis or chronic obstructive pulmonary disease. Pyridinyl-imidazole drugs such as SB203580 were the first p38 MAPK inhibitors to be identified that bind competitively at the ATP-binding pocket, and have been widely used to study p38 MAPK functions (reviewed in [26,160,161]). At low concentrations, these compounds inhibit p38 α and p38 β , but not p38 γ and p38 δ (reviewed in [162]). The molecular basis for this specificity was found to rely on the interaction of the inhibitors with several amino acids near the ATP-binding pocket of the kinase. In particular, Thr¹⁰⁶ of p38 α and p38 β is replaced by methionine in p38 γ and p38 δ , precluding inhibitor binding. In fact, replacing Thr¹⁰⁶ with a more bulky residue (such as methionine) abolishes SB203580 binding to p38 α , whereas replacing Met¹⁰⁶ in p38 γ and p38 δ with threonine enhances their sensitivity to the drug [163,164]. Nevertheless,

in spite of their relatively high specificity for p38 α and p38 β , SB203580 has been reported to target additional proteins, usually at higher concentrations [165,166].

Over the years, a large number of structurally diverse p38 α and p38 β inhibitors have been developed with both enhanced potency and specificity [26,161,167]. Most of these inhibitors are ATP competitors, but a new class of inhibitors has also been reported that function allosterically, such as BIRB796, leading to a conformational reorganization in the kinase that prevents ATP binding and activation [168]. In addition, crystallographic and computational analyses of human p38 α have revealed the potential interest of the C-terminal cap, formed from an extension to the kinase fold, unique to the MAPK and CDK families and GSK3. This C-terminal domain pocket has been predicted to have flexibility for potentially binding different-shaped compounds [169]. Given the similarity in the ATP-binding site of different kinases, developing small-molecule inhibitors that target other regions of the kinase might result in less off-target effects.

Although p38 MAPK inhibitors with diverse chemical structures have good anti-inflammatory effects in pre-clinical animal models, they have repeatedly failed in clinical trials, mainly due to side effects such as liver and neural problems [170]. The extent to which p38 α inhibitor toxicities might be due to off-target effects or to the broad physiological roles of p38 α is unclear. An interesting strategy to avoid toxicity would be the use of drug-delivery vehicles such as the polymer PCADK (polycyclohexane-1,4-diyacetone dimethylene ketol) that maintain therapeutic concentrations of p38 MAPK inhibitors within specific organs, as has been shown for the treatment of cardiac dysfunction following myocardial infarction [171].

The discovery of pathological conditions in which p38 MAPKs are involved supports the potential therapeutic interest of modulating the activity of these kinases. A number of potent and specific p38 α inhibitors are already available, and the challenge is now to find the best therapeutic use for them. New approaches to inhibit the p38 α pathway are also being explored [26,161,172]. In addition, the development of specific inhibitors against other p38 MAPK family members is emerging as an attractive possibility, for example to treat diabetes or to induce muscle regeneration.

CONCLUDING REMARKS

Many reports have established the implication of p38 MAPKs in numerous biological processes, including the responses to stress and inflammation, as well as the regulation of proliferation, differentiation and survival in particular cell types. There is also a lot of information on the pathway components and regulatory mechanisms. However, very little is known about *in vivo* functions, and detailed molecular information on how this signalling pathway regulates particular cellular processes is still scarce. Outstanding questions that should be addressed by future studies include (i) the extent of functional redundancy and interplay between p38 MAPK family members, (ii) how cross-talk with other signalling pathways contributes to context-specific responses, (iii) the identification of *bona fide* substrates that are responsible for specific functions and how p38 MAPKs can modulate some proteins without phosphorylating them, and (iv) the physiological and pathological roles of this signalling pathway. The application of systems biology approaches and high-throughput genomic and proteomic technologies may provide valuable insights into these questions. Furthermore, the use of genetically modified mice to modulate the activity of this signalling pathway in a time- and tissue-specific manner as well as to express at physiological levels p38 MAPK proteins

with particular mutations will be very useful to elucidate *in vivo* functions. The combined information obtained from mechanistic studies and animal models should also help to identify patient populations who could benefit most from p38 MAPK-inhibiting drugs, while minimizing their side effects. Hopefully, the knowledge generated will soon translate into new therapeutic opportunities by targeting p38 MAPK signalling.

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