

EDITORIAL

The role of MAP kinases in intracellular signal transduction in bronchial epithelium

The bronchial epithelium is exposed to numerous environmental stimuli which can activate intracellular signalling cascades, leading to altered gene expression and to a wide range of responses e.g. cytokine expression, proliferation and apoptosis [1]. The mitogen-activated protein kinase (MAPK) family of protein kinases are likely to be central to these processes as they are known to regulate intracellular signal transduction in response to many agonists, including growth factors, cytokines, hormones, oxidants and environmental stress factors [2]. MAP kinase signalling pathways are highly conserved and control a diverse range of cellular functions in organisms ranging from yeast to man. In mammals particularly, these have been implicated in the control of cell proliferation, differentiation and apoptosis [3].

Of the five MAP kinase cascades identified to date three have been well characterized. These comprise (i) the extracellular regulated kinases (ERK) 1 and 2 (also known as MAPK1/2), (ii) the c-Jun NH₂-terminal kinases (JNK) 1, 2, 3 (also known as stress activated protein kinases [SAPK]: SAPK1a, SAPK1b, SAPK1c), and (iii) the p38 MAPK α , β , γ and δ isoforms (also known as SAPK2a, SAPK2b, SAPK3/ERK6 and SAPK4) [3]. More recent additions include ERK3, ERK4, ERK5/big mitogen-activated kinase (BMK)/SAPK5 [4]. These are grouped according to their sequence homology, specificity towards upstream activators and different responses to stimuli (Fig. 1).

The MAPKs are proline-directed serine and threonine kinases. They are activated by dual phosphorylation on threonine and tyrosine residues within a thr-x-tyr (TXY) motif located in an activation loop adjacent to the catalytic cleft of the kinase; ERK3 is an exception to this rule, having a ser-glu-gly (SEG) motif [5]. Each subfamily is distinguished by the identity of the amino acid residue (X) within the phosphorylation motif and by the length of their respective activation loops. Once phosphorylated the cytosolic MAPKs can induce ser/thr phosphorylation of cytosolic signalling molecules and cytoskeletal proteins, or they can translocate to the nucleus where they modulate the activity of nuclear transcription factors and kinases. The specific upstream regulators MAPKs are the dual specificity (ser/thr and tyr) MAPK kinases (MKKs or MEKs) which are in turn activated by phosphorylation on

two serine/threonine residues by numerous MAPK kinase kinases (MKKKs). These include Raf family members, transforming growth factor β -activated kinase (TAK1) [6] and MEK kinases (MEKKs). Unlike MAPKs which are specifically recognized by their upstream kinases MKKs, the MKKs themselves can be phosphorylated and activated by several different MKKKs enabling diversity in upstream activation of the MAPK pathways [2].

The MAPK/ERK cascade involving MAPK1 (p44/ERK1) and MAPK2 (p42/ERK2) is the best characterized signalling module and is usually activated in response to mitogens. This cascade is involved in both proliferation and differentiation responses but is only weakly activated in response to stress stimuli. Agonists such as growth factors (EGF), tumour promoters (TPA) and G-protein-coupled receptors (endothelin-1, lysophosphatidic acid, prostaglandin F₂) activate Ras causing recruitment of the serine/threonine kinase, Raf-1, to the membrane where it initiates the MAPK cascade by phosphorylation of the MKK1/2 isoforms. These activated MKKs then induce the dual phosphorylation and activation of MAPK1/2 within the TEY (thr-glu-tyr) motif [7]. The diverse targets of MAPK1/2 include Sos1, cytosolic phospholipase A₂ (cPLA₂), cytoskeletal proteins, p90S6 kinase (RSK) (also known as MAPK-activating protein kinase, MAPKAP-K1), MAPK interacting kinases (MNK1/2), the p62 ternary complex factor (TCF) Elk1 and signal transducers and activators of transcription (STATs). Phosphorylation of cytosolic signalling components may be involved in activation (e.g. cPLA₂), feedback regulation (Sos1) or cross-regulation of other pathways. Activation of transcription factors such as Elk1 enables binding to specific regulatory elements such as the serum response element (SRE) in the c-Fos promoter to initiate c-Fos gene expression [8]. In this way, extracellular signals are conveyed to the nucleus via MAPK1/2 where they modulate the activity of transcription factors and co-ordinate the appropriate mitogenic or differentiation response by the induction of gene expression.

JNK/SAPKs and p38 MAPK are the two major cascades activated in response to cellular stress such as heat and osmotic shock, lipopolysaccharide (LPS), UV radiation, pro-inflammatory cytokines (IL-1 β , TNF α) and inhibitors of translation (cyclohexamide) but respond only weakly to growth factors and phorbol esters. Inflammatory cytokine receptors activate the cascade via the Rho family of small GTPases (Rho, Rac and Cdc42) and p21 Ras-activated

Correspondence: S. M. Puddicombe, University Medicine, Southampton General Hospital, Southampton, SO16 6YD, U.K.

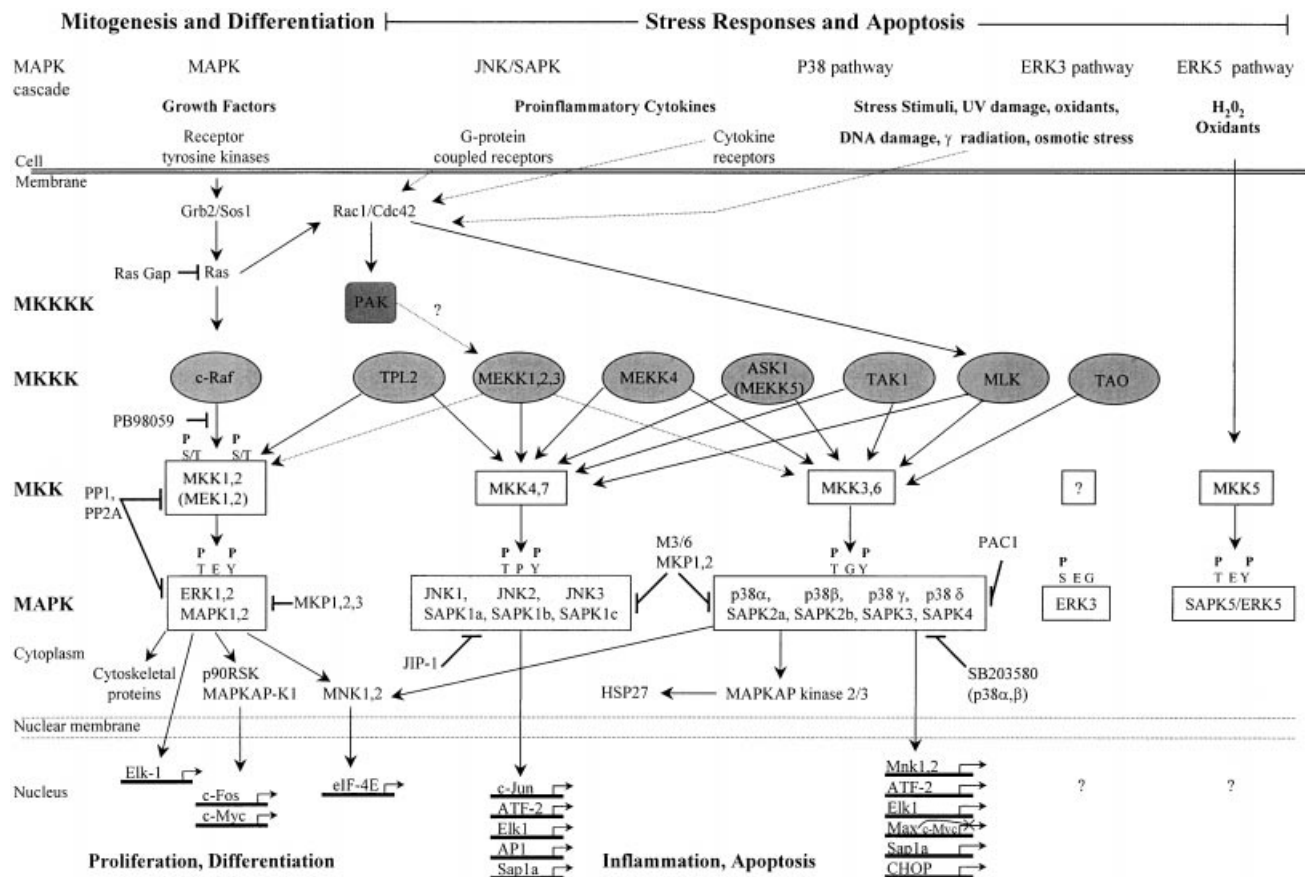


Fig. 1. A scheme representing the direct (solid arrows) and indirect (dotted arrows) activation of the various MAP kinase modules by extracellular stimuli such as growth factors, pro-inflammatory cytokines and osmotic stress. Inhibitors of pathway intermediates are indicated with blunt ended arrows ($\perp$). The specific phosphorylation motif for each MAPK module is also indicated

kinase (PAK) [9] leading to activation of MEKK1-4. However, all of the kinases involved in the activation of MEKK1-4 have not been clearly defined as both osmotic shock and UV radiation stimuli appear to act independently of Rho/Rac [2]. MEKK1-4 phosphorylate MKK7/4 by serine phosphorylation which enables them to activate JNK/SAPK by dual phosphorylation in the motif TPY [10]. Activated JNK kinases can phosphorylate transcription factors such as Jun, Elk-1, Sap1a and activating transcription factor-2 (ATF2) which initiate c-fos and c-jun gene expression and the formation of activator protein-1 (AP-1). In the p38 MAPK cascade the MKKKs that activate MKK3 and MKK6 include TAK1, MLKs (mixed lineage kinases), and TAO (thousand and one kinase). Dual phosphorylation of p38 MAPK by MKK3/6 in the motif TGY activates several cellular kinases, as well as transcription factors, such as CHOP (or Gadd153), ATF2, Max, Elk-1, Sap1a, but not Jun.

Although the MAPKs have been divided according to sequence and activation by mitogens or stress responses,

there is some overlap and the distinction is not clear cut. For example, ERK5/SAPK5, shows most sequence similarity to the ERKs, with a TEY phosphorylation motif, but is activated by MKK5 in response to stress stimuli, such as oxidants and osmotic stress [11]. Furthermore, some mitogens have been implicated in the activation of both JNK/SAPK and p38 cascades, whilst some pro-inflammatory cytokines can activate MEKK1 regulating both MAPK1/2 via MKK1/2, and JNK cascades via MKK4/7 [3]. Thus more than one MAPK pathway can be activated by an individual stimulus and any cross-talk between these cascades will ultimately influence the individual cellular response.

Each MAPK cascade demonstrates specificity towards their MKK upstream activators enabling responses to their specific stimuli. This selectivity can be further enhanced by a number of additional mechanisms that facilitate signalling specificity. For example, studies in yeast suggest that 'scaffold' proteins form multi-enzyme complexes comprising components of a MAPK module. The selective

interactions of these scaffold proteins appear to control the specific MAPK cascades initiated, prevent activation by irrelevant stimuli, facilitate rapid passage of the signal and prevent unwanted cross-talk between pathways [12]. Although it is unclear whether scaffold proteins exist in mammalian cells, likely candidates include JNK-interacting protein 1 (JIP-1) which interacts selectively with components of the JNK pathway and MP-1 which binds MKK1 and ERK1 [13,14]. In addition to activating mechanisms, negative feedback loops involving dual specificity MAPK phosphatases, serine/threonine phosphatases and protein tyrosine phosphatases provide alternative mechanisms for selective regulation of MAPK pathways. In this way, the balance of activated kinases and phosphatases can determine the duration of MAPK activation and influence nuclear retention.

In general ERK activation facilitates cell proliferation whereas JNK and p38 are associated with inflammation and survival or apoptosis. Thus, cyclin D1 is transcriptionally regulated by MKK/ERK and provides a link between the ERK pathway and entry into S-phase through regulation of cyclin/cdk activity. In contrast, p38 MAPK activation inhibits the transcription of cyclin D1 preventing cell cycle progression [15]. In lung epithelial cells ASK1, a MEKK that activates JNK and p38, induces apoptosis [16] whereas, in other cells, apoptosis induced by $\text{TNF}\alpha$ is dependent on inhibition of the JNK/p38 pathways [17–20]. The role of stress activated kinases in cell survival is probably linked to activation of specific kinases such as MAPKAP kinase 2/3 which can phosphorylate heat shock protein hsp27, an F actin-capping protein that stabilizes the actin cytoskeleton [21]. Insight into the pleiotropic response of cells to extracellular stimuli has come from studies of MEKK1. Targeted disruption of MEKK1 results in a greater apoptotic response to microtubule disruption and hyperosmolarity, indicating a role for MEKK1 in cell survival [22]. However, other studies have shown that MEKK1 can be rendered pro-apoptotic by cleavage with caspase-3. This releases MEKK1 from cell membranes amplifies caspase activation and induces apoptosis [23]. Thus, there is a dual role for MEKK1 in cell survival and apoptosis which is controlled by caspase cleavage.

In addition to activation of JNK and p38, cytokines such as $\text{IL-1}\beta$ and $\text{TNF}\alpha$ are potent activators of nuclear factor kappa B ($\text{NF}\kappa\text{B}$) leading to inflammatory responses. These pathways do not act independently of each other, as a number of interactions have been demonstrated which are synergistic and result in optimal expression of specific inflammatory genes. The relationship between the JNK pathway and $\text{NF}\kappa\text{B}$ activation is shown in Fig. 2 where TRAF2 or TRAF6 are responsible both for the activation of MEKK1 and $\text{NF}\kappa\text{B}$ -inducing kinase (NIK) which activates $\text{I}\kappa\text{B}$ kinases ($\text{IKK}\alpha/\beta$). Furthermore, MEKK1 has been

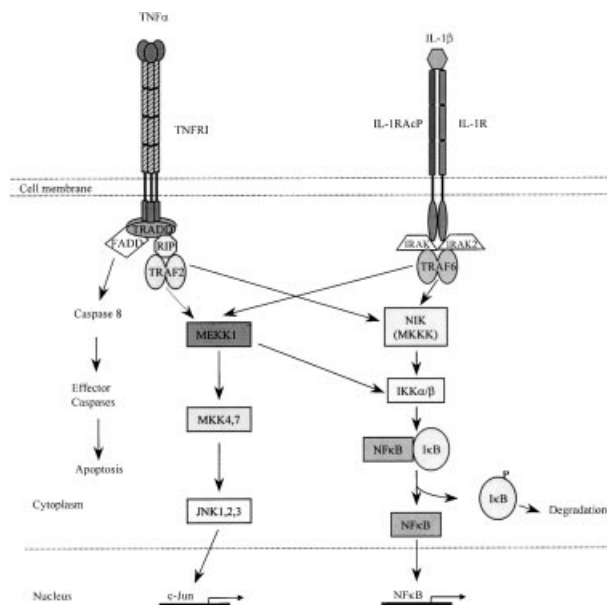


Fig. 2. The activation of $\text{NF}\kappa\text{B}$ and JNK/AP-1 by the pro-inflammatory cytokines $\text{TNF}\alpha$ and $\text{IL-1}\beta$. $\text{TNF}\alpha$ -activated receptors recruit TRADD (TNFR-associated death domain), RIP (receptor-interacting protein) and TRAF2 (TNFR-associated factor 2) whilst $\text{IL-1}\beta$ activated IL-1R and its accessory protein IL-1RAcP can recruit IRAKs (IL-1R associated kinases) which bind to TRAF6. Both TRAF2 and TRAF6 induce the phosphorylation and activation of MEKK1 which initiates the JNK cascade and to the induction of c-Jun transcription. In addition to MEKK1, the TRAFs activate NIK ($\text{NF}\kappa\text{B}$ -inducing kinase, another MKKK) and both of these MKKKs can phosphorylate the $\text{I}\kappa\text{B}$ kinases (IKK). Activation of IKK leads to the phosphorylation of $\text{I}\kappa\text{B}$ and the release of active $\text{NF}\kappa\text{B}$ which can translocate to the nucleus to induce gene expression. Furthermore, the activated TNF receptor can recruit FADD (Fas-associated death domain) which can induce apoptosis via activation of caspase 8 and other caspases

shown to directly activate $\text{I}\kappa\text{B}$ kinases [24]. Other interactions are also evident at the transcriptional level where physical associations between $\text{NF}\kappa\text{B}$ and AP-1 synergize to induce gene expression [25]. Thus MAPK stress pathways are intimately related with $\text{NF}\kappa\text{B}$ -dependent gene expression and may be important therapeutic targets for controlling cytokine expression during inflammatory processes by non-steroid-dependent mechanisms.

The study of MAPK modules has been greatly facilitated by the availability of two cell permeable MAPK inhibitors, PD098059 (a flavone compound) [26] and SB203580 (a pyridinyl imidazole compound) [27]. At low micromolar concentrations, PD098059 binds to the inactive form of MKK1 preventing its activation by Raf-1; it also prevents activation of MKK2, but is 10 times less effective. PD098059 does not inhibit phosphorylated MKK1 or any

other protein kinase tested. As no substrates other than ERK1/2 have been identified for MKK1, inhibitory effects of PD098059 are interpreted as indicative of roles of ERK1/2 in regulation of a specific response and a number of physiological roles for MKK/ERKs have been identified [reviewed in 28]. SB203580 was developed as an inhibitor of LPS-induced synthesis of IL-1 and TNF α in monocytes [29]. When used at sub-micromolar concentrations SB203580 selectively abolishes the activity of p38a/p38b but not ERK1/2, JNK or SAPK3/4 [30]. However, at $\approx$ 10–100-fold higher doses, SB203580 has been reported to inhibit JNK2 isoforms. Therefore, it is essential to demonstrate that the observed effects occur at the same concentration of SB203580 that blocks phosphorylation of an authentic physiological substrate. SB203580 has been shown to inhibit p38 activation and IL-6 and granulocyte macrophage-colony stimulating factor (GM-CSF) transcription in TNF α -stimulated fibroblasts, IL-8 transcription in IL-1-stimulated monocytes and translation of IL-1 and TNF α in LPS-stimulated monocytes [29,31,32]. Although, SB203580 does not affect NF κ B activation by TNF α , it has been shown to inhibit NF κ B-dependent gene transcription. The mechanism of this inhibition has not been determined but could occur at the level of a NF κ B co-activator or the basal transcription machinery or chromatin [28].

In asthma, the bronchial epithelium exhibits a 'stressed' phenotype with enhanced expression of a variety of cytokines and chemokines including GM-CSF, IL-1 β , IL-8, IL-5, IL-6 and eotaxin [1,33–36]. Expression of these cytokines indicates that the bronchial epithelium is actively contributing to the inflammatory process in asthma, rather than playing a passive role in which it becomes the target of the ongoing inflammatory response. The involvement of stress-activated MAPK pathways in regulation of cytokine gene expression in asthma is suggested by the fact that asthmatic bronchial epithelium shows enhanced expression of c-fos [37,38], and that SB203580 has been shown to inhibit release of IL-8 from bronchial epithelial cells [39,40]. The paper by Hashimoto *et al.* [41] in this issue extends these observations by exploring the involvement of the p38 MAPK cascade in production of RANTES and GM-CSF. In this study, TNF α , IL-1 β or PAF treatment of monolayer cultures of bronchial epithelial cells caused a dose-dependent increase in RANTES and GM-CSF production. The treated cultures also showed evidence of enhanced serine phosphorylation of MKK3 and MKK6, the upstream activators of p38 MAPK. Increased p38 MAPK activity was confirmed by immunoprecipitation and evaluation in an *in vitro* kinase assay using an ATF-2 fusion protein as substrate. Pretreatment of cells with SB203580 was shown to cause a dose-dependent inhibition of cytokine release with complete inhibition occurring at 10 μ M/L. The same concentration of SB203580 also

completely abolished activation of p38 MAPK, suggesting that the p38 MAPK pathway may be involved in regulation of the expression of RANTES and GM-CSF in bronchial epithelial cells. However, as the authors acknowledge, the involvement of other pathways involving JNK isoforms cannot be excluded in view of the relatively high single concentration of SB203580 used in the phosphorylation studies, as compared with the known potency of the drug against p38 MAPK. However, the ability of SB203580 to block cytokine release from bronchial epithelial cells is of considerable interest with respect to treatment of asthma and other inflammatory airway diseases. SB203580 has low toxicity *in vivo* and in animal models appears to be efficacious for the treatment of chronic inflammatory diseases such as rheumatoid arthritis [27]. Its precise mechanism of action in bronchial epithelial cells, particularly its influence on NF κ B-dependent and independent gene transcription, deserves further attention.

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S. M. PUDDICOMBE

D. E. DAVIES

University Medicine

Southampton General Hospital

Southampton SO16 6YD

UK