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Review

The complexity of mitogen-activated protein kinases (MAPKs) made simple

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Abstract. The mitogen-activated protein kinase (MAPK) pathways are known to be involved in various processes of growth, differentiation and cell death. In spite of their ubiquitous presence and seemingly enormous cross-talk with each other, their action is very specific. This review deals with various aspects of the three different MAPK pathways (ERK, p38 and JNK) and how their specificity is brought about.

Keywords. Mitogen-activated protein kinase, activation, phosphorylation, specificity, ERK, p38, JNK.

Introduction

Mitogen-activated protein kinase (MAPK) signal transduction pathways are among the most widespread mechanisms of eukaryotic cell regulation. All eukaryotic cells possess multiple MAPK pathways that are activated by distinct set of stimuli, allowing the cells to respond coordinately to multiple and divergent inputs. Mammalian MAPKs can be activated by wide variety of stimuli, which include hormones (*e.g.*, insulin), growth factors [*e.g.*, platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF)], inflammatory cytokines of tumor necrosis factor (TNF) family and environmental stresses such as radiation, osmotic shock and ischemic injury. These stimuli may act through different receptor families that are coupled to MAPK pathways such as, receptor tyrosine kinases, G protein-coupled receptors (GPCRs), cytokine receptors and Ser/Thr kinase receptors. Activation of MAPK pathways coordinate diverse cellular activities like gene expression, cell cycle machinery, cellular metabolism, motility, survival, apoptosis, and differentiation. To date, six distinct groups of MAPKs have been characterized in mammals: extracellular regulated kinases (ERK1/2), Jun NH_2 terminal kinases (JNK1/2/3), p38 (p38 α/β/γ/δ), ERK7/8, ERK3/4 and ERK5. There are splice variants for several of the MAPK proteins that increase the diversity of the cascade. The most extensively studied groups are ERK1/2, JNKs and p38 kinases.

ERK 3 and ERK7 are ubiquitously active MAPKs. ERK3/4 does not contain the characteristic Thr-Glu-Tyr (TEY) activation motif and activity of ERK3 is regulated by protein stability. The mechanisms of ERK4 activation are yet unclear and its expression is predominantly localized in brain during development of zebrafish [1]. ERK5, also referred to as big MAP kinase 1 (BMK1), is activated by MEK5 and is known be activated by oxidative stress, hyper-osmolarity and growth factors. Not much information is available on ERK7/8, ERK3/4 and ERK5 and hence they are not discussed in this review.

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General features of MAPK pathways

MAPK modules, activators and effectors

Although each MAPK is unique, they share some common features, and have thus been grouped together in one family. The central three-tiered 'core signaling module' is typical of these pathways. It consists of a set of three evolutionarily conserved, sequentially acting kinases: an MAPK, an MAPK kinase (MAPKK), and an MAPK kinase kinase (MAPKKK). The MAPKKKs, also called MAP3Ks or MEKKs, are Ser/Thr kinases that are activated *via* phosphorylation and/or their interaction with small GTP proteins of Ras/Rho family in response to extracellular stimuli. MAP3K activation leads to phosphorylation and activation of downstream MAPKK, which is a dual specificity kinase and can phosphorylate MAPK on both threonine and tyrosine on a conserved Thr-Xaa-Tyr motif to activate them. Once activated, MAPKs phosphorylate the target substrates on serine or threonine residues only if the amino acid residues are followed by proline. These substrates could be transcription factors, other kinases (MAPK activated kinases or MKs) or proteins like cytoskeletal proteins. Each of these cascades is named after the subgroup of its MAPK component. Of the components of MAPK signaling module that have been identified in human cells, the majority are MAP3Ks (at least 20 genes) followed by MAP2Ks (7 genes) and MAPKs (11 genes) [2].

MAPK-activated protein kinases (MKs), the downstream targets of MAPK, contribute to additional specificity, diversity and amplification in the MAPK cascades. The MK family comprises the p90 ribosomal S6 kinases (RSK 1–4), mitogen and stress activated kinases (MSK 1 and 2), the MAPK-interacting kinases (MNK1 and 2), MAPK-activated protein kinases -2 and -3 (MK2 and MK3 or MAPKAP-K2 and MAPKAP-K3) and MAPK-activated protein kinase 5 (MK-5 or MAPKAP-K5). Among these, RSK family members are exclusively phosphorylated and activated by ERKs. No MKs have yet been assigned for the JNK module.

A few effects of MAPKs have also been observed that do not require their kinase activity. Direct protein-protein interactions are thought to play a role in such effects. The most notable examples are induction of degradation of c-jun by JNK and activation of topoisomerase IIα by ERK [3]. The importance of these protein-protein interactions is now acknowledged and a lot of research is being carried out to identify the domains involved in recognition of activators and effectors of signaling pathway and how such domains impart specificity to the pathway [4–6].

Docking sites

All MAPKs are "proline directed", phosphorylating Ser/Thr residues only if followed immediately by Pro. However, the specificity of MAPKs for their physiological substrates is dictated largely by the presence of binding sites, often substantially different from phosphorylation sites that are specific for distinct MAPK subgroups. Such domains play an important role, not just in selective interaction between components and their activation, but also in increasing the efficiency and specificity of the pathway. The first of such binding sites was described for the JNK substrate, c-jun and was named the δ domain. Other sequences related to the δ domain, called as D domains or D motifs, have been identified in other transcription factors including bZIP, ETS and MAD. These are characterized by a few positively charged residues surrounded by hydrophobic residues and have been demonstrated to increase substrate phosphorylation by MAPKs. These specialized docking motifs are present in upstream activators (MAPKKs), regulators like scaffold proteins (KSR) and phosphatases (PTP-SL and MKPs), and their downstream substrates like transcription factors and MKs.

Another class of MAPK docking site that consists of Phe-Xaa-Phe-Pro sequence was found during the analysis of the transcription factor LIN-1 in *Caenorhabditis elegans* (transcription factor of ETS family) and KSR-1. This domain was termed DEF (docking site for ERK and FXFP) and has been reported to be only recognized by ERK1/2 for efficient phosphorylation of its substrates [7, 8]. Both D and DEF domains can be found on the same protein, *e.g.*, in Elk-1 and KSR. Here they probably act synergistically to strengthen the MAPK interaction.

The regions on MAPKs that might recognize and bind to D domains have also been characterized. Such a conserved C-terminal docking site was named common docking (CD) motif. These comprise acidic and hydrophobic residues that are necessary to establish hydrophobic and electrostatic interactions with the positively charged and hydrophobic residues of D domain [4, 9]. An ERK-specific motif has been identified in ERK and was called the ERK docking (ED) motif that interacts with D domain.

Down-regulation

Protein phosphorylation is regulated by the opposing actions of the phosphatases, and provides an important means of regulating protein function. Since the physiological outcome of MAPK signaling depends on the magnitude and duration of kinase activation [10–12], the regulation by phosphatase plays an important role. In addition, the spatial distribution of MAPK activity can markedly alter the output of

signaling. The factors that influence the spatio-temporal regulation of MAPK signaling are diverse and complex, but because the level of MAP kinases never changes throughout the course of stimulation, dephosphorylation by phosphatases would seem to play a major role in down-regulation of MAPK activity. This process can be mediated by either protein Ser/Thr phosphatases (PPs), protein Tyr phosphatases (PTPases), or by dual specificity phosphatases, generally termed MAPK phosphatases (MKPs). The Ser/Thr phosphatases that dephosphorylate MAPKs include PP2A and PP2C [13]. The PTPases that dephosphorylate MAPKs include three MAPK-specific PTPases namely PTPN5 (or STEP), PTPN7 (or HePTP) and PTPRR (or PTP-SL). They bind to and negatively regulate the activity and cellular localization of members of MAPK family [14]. They can be distinguished from classical PTPases by the presence of a kinase interaction motif (KIM) on the N-terminal side of phosphatase domain [15]. The dephosphorylation of tyrosine residue by PTPases is sufficient to inactivate MAPK, blocking its nuclear translocation. It is not known whether monophosphorylated MAPKs have other biological functions. They themselves are regulated by phosphorylation at multiple sites by kinases, which include ERK, resulting in a feed back regulation. The MKP family comprises of ten dual specificity MAP kinase phosphatases (MKPs) and are highly specific for MAPKs. They have an N-terminal non-catalytic domain (KIM) and a C-terminal catalytic domain [16], and they differentially regulate MAPK isoforms. Based on gene structure, sequence similarity and subcellular localization, the ten MKPs can be divided into three classes [17]. The first comprises the inducible nuclear phosphatases, *i.e.*, DUSP1/MKP-1, DUSP2/PAC-1, DUSP4/MKP-2 and DUSP5. The second group comprises three closely related cytoplasmic phosphatases, DUSP6/MKP-3, DUSP7/MKP-X and DUSP9/MKP-4. The third group consists of MKPs with selectivity towards stress-activated MAPK isoforms, DUSP8, DUSP10/MKP-5 and DUSP16/MKP-7. The substrate specificity of MKPs has been shown to be due to selective binding to their MAPK substrates that then leads to their activation. This has been supported by many studies that showed the selectivity of MKP-3 to ERK1/2 but not to JNK or p38 [18, 19], MKP-5 and MKP-7 to JNK and p38 but not ERK, and MKP-1 regulating all classes of MAPK [20, 21]. This idea was further supported by the studies that showed increase in catalytic activity of MKP after binding to their MAPK substrates [22]. The CD and ED sites in MAPKs as mentioned earlier have been shown to contribute to specific interactions with MKPs apart from various conserved domains in MKPs, like KIM. The importance of MKPs in regulating various functions including immune and metabolic function, stress response and development have been shown by various knockout studies in which defects in innate or adaptive immunity have been observed. Evidence has also accumulated in support of spatial restriction of MKP activity as exemplified by the ability of MKP-1 to inactivate the nuclear as opposed to the cytoplasmic pool of JNK.

Certain MKPs are encoded by genes which are transcriptionally up-regulated by many stimuli that activate MAPK signaling [23, 24] for example the expression of MKP-5 is strongly induced in macrophages exposed to LPS.

Activation and biological response

The diversity of stimuli that activate MAPK and the diversity of their effects require dealing with each MAPK pathway individually. How the varied outcomes, and sometimes opposing outcomes, are achieved by MAPK subgroups is discussed below.

MAPK families – ERK, JNK and p38

ERK1/2 pathway

ERK was the first MAPK to be identified and is the best studied of the mammalian MAPK pathways. The ERK cascade is activated by a large number of extracellular and intracellular stimuli. It is activated strongly by growth factors, serum, and phorbol esters, and, to lesser extent, by ligands of GPCRs, cytokines, osmotic stress and microtubule disorganization [25].

Isoforms

Two isoforms ERK1 and ERK 2 are known which posses 83 % amino acid identity and are expressed to various extents in all tissues.

Upstream kinases and activation mechanism

The signaling *via* this cascade is usually initiated by the activation of cell surface receptors such as tyrosine kinases (RTKs) and GPCRs [26, 27]. The signal is transduced to small G proteins (*e.g.*, Ras) [28], which transmit the signal by recruiting the MAP3K tier like Raf kinases (A-Raf, B-Raf and Raf-1) to the plasma membrane, where they can be activated. The exact mechanism of Raf activation is still elusive but is known to require Ras binding and multiple phosphorylations at the membrane. Other MAP3K components that participate in activation of ERKs under specific conditions are c-Mos, TPL2 and MEKK1/2/3, which act mainly during meiosis, proliferation and stress response, respectively. Activated Raf (or other MAP3K) binds to and phosphorylates the down-

stream dual specificity kinases MEK1 and 2, which in turn phosphorylate ERK1/2 within a conserved Thr-Glu-Tyr motif in their activation loop.

Downstream substrates and function

Upon stimulation, a significant proportion of ERK accumulates in the nucleus [29, 30]. Activated ERKs have been demonstrated to phosphorylate a large number of substrates in all cellular compartments, including various cytosolic and membrane proteins (PLA2, CD120a, Syk and calnexin), nuclear substrates (SRC-1, Pax6, NFAT, Elk-1, MEF-2, c-fos, c-jun, c-myc and STAT3) and cytoskeletal proteins (neurofilaments and paxillin) [31]. Alternatively, the ERKs can transmit the signal further by phosphorylating and activating protein kinases at the MAP-KAPK tier (MKs). Indeed, more than 150 substrates of ERK are known so far and their number is likely to increase. Elk-1, a member of ternary complex factor (TCFs), is the best-studied target of ERK. The phosphorylation of Elk enhances its DNA binding, which then recruits coactivators like CBP and p300, eventually inducing the transactivation of genes. Stimulation of quiescent cells, which do not express c-fos, rapidly increases the expression of c-fos by a mechanism involving Elk-1. Both c-fos mRNA and protein are stabilized by active ERK and the amount of c-fos protein regulates downstream gene expression. Therefore, the proper regulation of c-fos is of major consequence in many cellular processes such as proliferation, differentiation and oncogenic transformation [32]. Similarly, c-myc and c-jun phosphorylation by ERKs is important for regulation of these cellular processes. c-myc is a key regulator of cell proliferation, differentiation and apoptosis and its biological functions are thought to depend in part on its polypeptide-binding partners such as c-jun.

Among the cytoplasmic targets of ERK, phospholipase A2, regulates the production of arachidonic acid in stimulated cells [33]. Among MKs, RSK family members are exclusively activated by ERKs [34, 35]. After activation they can independently translocate into the nucleus and phosphorylate a distinct set of substrates there [29, 30]. However, the activated RSKs have also been found in the cytoplasm, suggesting the existence of their cytosolic targets. Many downstream substrates of ERKs are shared by RSKs and MSKs, such as transcription factors and coactivators such as SRF, CBP, Elk-1 and CREB that participate in immediate early response [36, 37]. Like ERK, immediate early gene products have also been shown to be phosphorylated by RSKs, *e.g.*, c-fos and c-jun. MKs can also regulate the activity of additional kinases, *e.g.*, Myt1, but these are not usually considered to be genuine members of ERK cascade.

Biological response

ERK in cancer: Carcinogenesis and the development of cancer can be said to be a disease of the signaling system. The ERK pathway is deregulated in approximately one third of all human cancers. Many components of this signaling pathway have been linked to cell proliferation. These range from various transcription factors like c-myc, c-fos, SRF, CREB, and AP-1, which control early response genes, to elongation factor eIF4 and activator of RNA pol I, which impact global protein synthesis. The involvement of ras in cancer has been known for a very long time. In the ERK pathway the focal point seems to be the ras/raf motif. Aberration spans the whole pathway beginning from an aberrant receptor like EGFR and ErBB2 overexpression, Ras and Raf mutation to amplification of nuclear targets, most notably myc and AP-1 [38]. The heterodimerization of raf1 with B-raf has been shown to contribute considerably to ERK activity and studies into the mechanism of action of oncogene B-raf have indicated its involvement in cancers [39]. The MKs, RSKs, MNKs and MSKs, which can translocate independently into the nucleus and phosphorylate various transcription factors involved in cell proliferation, further contribute to the induction of immediate early genes and participate in cell proliferation [40]. ERK signaling also plays a role in the disrupting the anti-proliferative effects of ligands such as transforming growth factor-β (TGF-β). For example, activated N-Ras induces the cytoplasmic mislocalization of p27 *via* the Ral-GEF pathway, leading to the disruption of TGF-β-mediated Smad nuclear translocation. Accumulating evidence also suggests that the expression of different feedback inhibitors of the ERK pathway is deregulated in cancer. These include MAP kinase phosphatases (MKPs) and Sprouty family members [41–43].

In addition to cell proliferation, other relevant potential targets downstream of ERK play key roles in angiogenesis, cell migration, invasion and metastasis [44, 45]. ERK signaling may promote a more malignant phenotype by disrupting Rho signaling pathways [46], phosphorylating number of proteins involved in cell migration including MLCK, calpain, FAK and paxillin [47] as well as by regulating the expression of proteases involved in basement membrane degradation [44].

ERK in cell cycle: The ERK1/2 pathway has emerged as a central regulator of cell proliferation by controlling both cell growth and cell cycle progression. Major advances have been made over the last 10 years in identifying the targets of this pathway that are linked to cell cycle machinery. The first demonstration of direct involvement of ERK1/

2 in the mitogenic response was provided by the findings that overexpression of catalytically inactive ERK1 or antisense ERK RNA exerts a dominant negative effect on fibroblast cell proliferation [48]. It was later shown that inhibition of MEK1/2 blocked the growth factor-stimulated global protein synthesis and pyrimidine synthesis [49]. However, it must be noted that persistent and not transient activation of ERK pathway is required for favoring cell growth [11]. Various mechanisms have been proposed to explain the role of ERK1/2 pathway in cell cycle progression. Most important targets are D-type cyclins and c-myc. Activation of the ERK pathway induces the D-type cyclins by up-regulating and activating the transcription factors involved in its synthesis, *e.g.*, AP1 family proteins. It may also control its expression at post-transcriptional level [50, 51]. Activated MEK1 has also been shown to facilitate the formation of cyclinD1-Cdk4 complexes in fibroblasts [52]. Activation of ERK stabilizes c-myc by phosphorylation. c-myc, a transcription factor of the Myc family, which plays an important role in regulating cell growth, cell progression and apoptosis [53], exerts its effects *via* up-regulation of certain genes involved in cell growth such as cyclin D [54], p21 [55] and cdc25A [56], ribosomal proteins and translation factors [57]. Other mechanisms include down-regulation of anti-proliferative genes such as Tob1, Ddit3 and Jun D.

RSK2 has been shown to phosphorylate cyclin-dependent kinase (CDK) inhibitor, p27kip1, thus promoting G_1 progression. RSK2 has also been shown to phosphorylate and inhibit MytI, an inhibitory kinase for Cdc2 (M-phase CDK), thus promoting G_2 progression [58]. RSKs have also been shown to participate in chromatin remodeling by phosphorylating histone H3, resulting in increased transcriptional regulation [59, 60].

ERK in apoptosis: Although the ERK pathway is attributed to survival in most cell types, its activation is now also thought to contribute to apoptosis. Bhat and Zang [61] first reported that inhibition of ERK using the MEK1 inhibitor PD98059 rescues oligodendrocytes from H_2O_2-induced cell death. This observation was subsequently confirmed using different insults in HeLa cells [62], cortical neurons [63] and primary β cells [64]. ERK activation has been shown to be involved in death induced by many other factors such as reactive oxygen species (ROS) [65], *E. coli* toxins [66], and zinc [67], and also by deprivation of survival factors [68]. The mechanisms by which the ERK pathway mediates apoptosis remains poorly understood and seems to occur at different level of signaling. ERK1/2 may act upstream of mitochondrial cytochrome c release and caspase 3 activation as observed in studies with cisplatin-induced apoptosis [69, 70]. Several studies showed decreased Bax and p53 expression after ERK inhibition [71, 72], indicating that ERK may act through up-regulation of Bax and p53, thus inhibiting the action of anti-apoptotic proteins Bcl-2 and Bcl-xl on mitochondria. In addition, ERK has also been shown to phosphorylate p53 directly on Ser15, leading to its enhanced apoptotic role [73]. Some data have also suggested a role of ERK pathway in inducing apoptosis through extrinsic pathway where ERK-mediated up-regulation of TNF-α [74] and activation of caspase 8 [75] have been shown. Promotion of cell death by ERK activation also may result through suppression of Akt-mediated survival signaling [68].

ERK in survival: The ERK pathway is often related to oncogenesis, and the magnitude of ERK activity influences survival of carcinoma cells. High ERK activity reduces the apoptosis rate of colon carcinoma cells and induces cell cycle arrest by up-regulation of CDK inhibitors p21 and p27 [76, 77]. An anti-apoptotic effect of the ERK pathway can be explained partly in terms of its growth-supporting actions. However, direct ERK targets involved in apoptosis have also been discovered. Erhardt et al. [78] have shown that ERK, acting downstream of B-Raf, inhibited cytosolic caspase activation following release of cytochrome c from mitochondria. A second possible target of ERK pathway is the pro-apoptotic Bcl-2 family protein Bad. Bad is known to influence mitochondrial membrane integrity and the release of cytochrome c from mitochondria indirectly, by associating with Bcl-2 and Bcl-xl and inhibiting their anti-apoptotic function [79]. Phosphorylation of Bad by ERK sequesters it in the cytosol, away from mitochondria [80], preventing apoptosis.

RSKs and MSKs have also been shown to regulate survival in proliferating as well as differentiated cells through both transcription-dependent and -independent mechanisms [81]. These effects of MKs are mediated *via* phosphorylation of Bad [81, 82], CREB [83], C/EBPβ [84] and NF-κB [85].

ERK in development and differentiation: Targeting of individual genes has contributed immensely to the knowledge of their roles in development and differentiation. The $ERK^{-/-}$ mice were found to be deficient in thymocyte development [48]. ERK1/2, components of the ERK pathway are necessary for several forms of learning. They also have a role in adipocyte differentiation [86], where deficiency in ERK1 specifically affects adipocyte differentiation and ERK2 its pro-

liferation. However, the deletion of ERK2 isoform is lethal to the mice. ERK2 mutants fail to form the ectoplacental cone and extra-embryonic ectoderm, which give rise to mature trophoblast derivative in fetus [87]. *In vivo* ERK1/2 invalidation leads to different phenotypes. MAPK null phenotypes are listed in Table 1.

RSKs play an important role in development, which is underscored by the fact that defects in rsk2 gene are the cause of Coffin-Lowry syndrome, an X-linked dominant disorder characterized by psychomotor and growth retardation and facial, hand and skeletal malformations [88].

Down-regulation

The inactivation of ERKs is mainly mediated by removal of phosphates from either one, or both the regulatory Thr or Tyr residues of ERKs [89]. This process can be mediated by either protein Ser/Thr phosphatases (PPs) such as PP2A [13], by protein Tyr phosphatases, such as PTP-SL [15] or by dual specificity phosphatases, generally termed MAPK phosphatases (MKPs) [90]. The cytoplasmic subgroup of MKPs, DUSP6/MKP-3, DUSP7/MKP-X and DUSP9/MKP-4 exhibit substrate selectivity towards ERK1 and 2.

JNK pathway

JNKs, originally identified as stress-activated protein kinases (SAPKs) in the livers of the cycloheximide challenged rats [91], were renamed to emphasize their role in phosphorylation and activation of transcription factor c-jun. The JNKs are strongly activated in response to cytokines, UV irradiation, growth factor deprivation, DNA damaging agents and, to lesser extent, by stimulation of some GPCRs, serum, and growth factors [92–94].

Isoforms

The mammalian JNKs are encoded by three distinct genes (JNK1, JNK2 and JNK3). Alternative splicing yields ten different products of 46–55 kDa [95, 96]. While JNK1 and 2 are ubiquitously expressed, JNK3 is restricted to the brain, heart and testis.

Upstream kinases and activation mechanism

The upstream activators for JNK pathway, *i.e.*, MAP2Ks, are MKK4 and MKK7. The diversity of upstream activators of MKK4 and MKK7 allows JNK pathway activation by a large number of external stimuli. Further upstream activators include several MAP3Ks, *i.e.*, MEKK1–4, MLK2 and -3, Tpl-2, DLK, TAO1 and 2, TAK1 and ASK1/2 [97]. Different MAP3Ks are specific for different stimuli, *e.g.*, TAK1 has been shown to be critical for JNK activation in response to inflammatory cytokines (IL-1, TNF-α, TGF-β and lymphotoxin-β) and activation by Toll-like receptors (TLR-3, -4 and -9) [98, 99]. MEKK3 appears to be critical in response to activation by TLR-8 [100]. In addition, The MAP3K isoforms Tpl-2 and MLK-3 have been reported to contribute to TNF-stimulated JNK activation in embryonic fibroblasts [101, 102]. Among the many stimuli that JNK responds to, the exposure to a range of pro-inflammatory cytokines such as TNF-α and IL-1 [103] is typical of these kinases. Furthermore, JNK pathway is also activated in innate immune response following activation of various members of Toll-like receptor family [100, 104]. Stress stimuli, such as withdrawal of growth factors, ER stress, radiation, etc., have been shown to strongly activate JNK pathway [105]. Under these circumstances, JNK activation may influence important cellular consequences, such as alterations in gene expression [106], cell death [107] or altered cellular proliferation [108]. Like ERK1/2, JNK activation requires phosphorylation within a conserved Thr-Pro-Tyr motif in their activation loop.

Downstream substrates and function

Like ERK1/2, the JNKs may relocalize from the cytoplasm to the nucleus following stimulation [109] but not in as significant proportions as ERK. A wide range of nuclear proteins, predominantly transcription factors and nuclear hormone receptors, have been demonstrated to be substrates of JNK. These directly effect the gene expression. Most important and extensively studied nuclear substrate of JNK is c-jun, which when phosphorylated at Ser63 and 73 results in enhancement of AP-1 transcriptional activity. Others include proteins of jun family such as junD, junB, ATF family (*i.e.*, ATF-2), fos family, Elk-1, c-myc, p53, NFAT family, forkhead family, STAT1 and -3, and Pax family proteins [110]. The nuclear hormone receptor proteins activated by JNKs include peroxisome proliferators-activated receptor γ1 (PPARγ1), glucocorticoid receptor, and retinoic acid receptors such as RXR and RARα. Phosphorylation and activation of many non-nuclear substrates of JNK like many scaffold and adaptor proteins (IRS-1, JIP1, JIP3 and 14-3-3) allows modulation of other signaling events in the cell. Many mitochondrial proteins, such as the Bcl-2 family proteins (Bcl-2, Bcl-xl, Bad, Bim and Bax), which have a role in apoptosis, have also shown to be targets of JNK. Cytoskeletal proteins such as Paxillin, microtubule-associated protein 2 and 1B (MAP-2, 1B), and Tau are also phosphorylated by JNKs. Akt, a kinase, has been shown to be a substrate of JNK. JNK-activated MKs have not been found so

far; however, $p90^{RSK}$ has been reported by Zhang et al. [111] to be phosphorylated by JNK after UV treatment.

One very important aspect of JNK is its role in protein degradation [112]. The controlled degradation of proteins provides cells with a handle to control protein activity. Studies with inactive upstream activator of JNK, $MEKK1^{\Delta KD}$, pointed to a role for JNK-dependent events associated with protein degradation [113]. MEKK1 has been shown to act as a E3 ubiquitin ligase [114, 115]. Identification of similarities between the δ domain of c-jun and the domain required for ubiquitination [116] led to studies showing that JNK binding to c-Jun may regulate not only c-Jun activity, but also its stability. JNK was shown to mediate the degradation of other proteins by phosphorylation of E3 ligase Itch [113]. Indeed, JNK plays a role in targeting the ubiquitination and degradation of c-Jun, Jun B and $cFLIP_L$ [117–119]. Docking sequences for JNK have not yet been identified for all substrates of JNKs.

Biological response

JNK in cancer: A role for the JNK pathway in tumorigenesis is supported by the high levels of JNK activity found in several cancer cell lines. JNK activity and phosphorylation of c-jun has been reported to play a critical role in Ras-induced tumorigenesis and Ras and c-jun cooperate in cellular transformation [120, 121]. Nateri et al. [122] showed that ablation of c-jun or mutation of its JNK phosphorylation sites reduced tumor number and size, and prolonged the lifespan. One important function of c-jun appears to be transcriptional repression of p53 [123, 124]. Another example of role of JNK in tumorigenesis has been reported in liver, where it was shown to promote chemically induced hepatocarcinogensis [125].

JNK in apoptosis: A role for JNK in apoptosis is well established [126]; however, the mechanism is controversial and appears to be stimulus and tissue specific [107]. One potential target of pro-apoptotic JNK signaling is the tumor suppressor p53. Binding to JNK was reported to destabilize p53 by promoting ubiquitin-mediated degradation [105, 127]. Conversely, activation of JNK due to stress has been shown to inhibit ubiquitin-dependent degradation of p53 thereby stabilizing it. Another potential target in pro-apoptotic signaling could be c-myc, which is phosphorylated at two sites (Ser62 and Thr71) by JNK. Studies from $JNK^{-/-}$ murine embryo fibroblasts provided a powerful model system for analysis of JNK-induced apoptosis. It was observed that, in such mutants, biochemical defects in stress-induced apoptosis were localized to mitochondria since there was no mitochondrial membrane depolarization and cytochrome c release. Both pro- and anti-apoptotic members of Bcl-2 family have been shown to be substrates of JNK. Anti-apoptotic proteins Bcl-2 and Bcl-xl have been shown to be inhibited through phosphorylation by JNK [128, 129], although there is evidence against the involvement of these proteins as *in vivo* substrates of JNK-induced apoptosis. Bax and 14-3-3σ have been shown to be substrates of JNK *in vivo*, which may account for its pro-apoptotic role [130, 131]. Recent studies have shown the involvement of JNK in degradation of caspase-8 inhibitor $cFLIP_L$ [132]. Another recently identified substrate of JNK, histone 2 variant, H2AX, has been thought to be essential for DNA fragmentation and hence may play a role in JNK-induced apoptosis [133].

Caspase 3 can amplify activation of JNK, as it is able to cleave and activate MEKK1, a kinase upstream of JNK [134].

JNK in survival: Although the role of JNK in apoptosis is well established, it has also been shown to contribute to survival. These opposite effects could be partly due to the duration or magnitude of the activation of the pathway and partly due to activation of other pro-survival pathways. Prolonged activation of JNK has been shown to mediate apoptosis, whereas transient activation has been shown to promote cell survival [135].

JNK in development and differentiation: The homozygous null mice formed by deletion of JNK1 (SAPKγ) had no obvious defect, but displayed a decrease differentiation of CD4 helper T cells (Th cells) [136, 137], decreased adiposity and resistance to high-fat diet because of increase in insulin sensitivity and signaling capacity [138]. The JNK2 (SAPKα) knockout mice were viable and fertile. It has been demonstrated that there was impaired differentiation of mature $CD4^+$ T cells into Th1 effector cells due to a defect in IFN-γ production [139]. JNK1 and 2 isoforms have similar role in T cell differentiation, but only JNK1 is implicated in obesity and response to insulin. The JNK3 (SAPKβ) knockout mice were normal and fertile. Since JNK3 is expressed primarily in the brain, the neuronal cells showed decreased cell death [140] (Table 1).

Down-regulation

The role of protein phosphatases in the regulation of the JNK pathway is poorly understood. However, knockout studies with $Mkp1^{-/-}$ and $Mkp5^{-/-}$ showed the importance of these phosphatases in JNK down-regulation [141, 142].

Table 1. MAPK null phenotypes (Th, T helper cells, IFN, interferon).

Knockouts	Viability	Phenotype	References
ERK $1^{-/-}$	Viable	Defects in thymocyte development Adipocyte differentiation	[48, 86]
$ERK2^{-/-}$	Embryonically lethal	Defects in formation of the ectoplacental cone and extra-embryonic ectoderm	[87]
$JNK1^{-/-}$	Viable	Affects differentiation of CD4 Th cells, increased insulin sensitivity	[136–138]
$JNK2^{-/-}$	Viable	Impaired differentiation of mature $CD4^+$ T cells into Th1 effector cells, less production of IFN-γ	[139]
$JNK3^{-/-}$	Viable	Defective excitotoxicity induced death in neuronal cells	[140]
p38α	Embryonically lethal	Defects in placental angiogenesis and erythropoiesis	[184, 185]

p38 MAPK pathway

p38 homologues have been identified in both low and high eukaryotes. The Hog1 pathway in budding yeast and spc1/sty1 pathway in fission yeast are believed to share an ancestral gene with p38 group of MAPKs. Their role has been implicated in osmoregulation, responses to extracellular stress stimuli and cell cycle events [143, 144]. Like the JNKs, the mammalian p38 s are also activated by environmental stresses and inflammatory cytokines and are inconsistently activated by insulin and growth factors [145, 146]. In almost all instances, the same stimuli that activate JNKs also activate p38 s except in ischemia-reperfusion where only p38 is activated [97].

Isoforms

There are four known isoforms of p38, *i.e.*, α, β, γ and δ. Among these p38α has been extensively studied.

Upstream kinases and activation mechanisms

Like all MAPKs, p38 group kinases are activated by dual kinases, the MKKs, which are called MKK3 and MKK6. There are reports suggesting that different upstream kinases selectively activate p38 isoforms. MKK6 can activate all four isoforms, while MKK3 cannot effectively activate p38β [147]. MKK4 and MKK7 (upstream kinases of JNK) can also activate p38 isoforms [148], indicating that a certain amount of cross-talk does exist between p38 and JNK pathways. Upstream activators of p38 group are more diversified than JNK. This explains its activation with a wide range of stimuli. Several MKKKs (MAP3Ks) have been reported to cause p38 activation. These include MTK1, MLK2/MST, MLK3, DLK, ASK1 and TAK1. They may confer the specificity of response to different stimuli, *e.g.*, JNK pathway in dominant negative mutants of MTK1 was activated by stress signals but not by cytokines [149]. Low molecular weight GTP-binding proteins, like Ras in the Ras/Raf/MEK/ERK pathway, have been shown to play role in p38 activation. These were shown to be proteins belonging to Rho family, *i.e.*, Rac or cdc42 [150, 151]. Chemokines such as fMet-Leu-Phe (fMLP) and platelet-activating factor (PAF) act *via* GPCRs and activate p38 kinases.

Downstream substrates and function

p38 was shown to be present in both the nucleus and cytoplasm of quiescent cells. Some evidence suggests that, following activation, p38 translocates from the cytoplasm to the nucleus [155], but other data indicate that activated p38 is also present in the cytoplasm of stimulated cells [156]. Role of p38 has been extensively studied in immune system [157, 158]. It participates in macrophage and neutrophil functional responses including respiratory burst activity, chemotaxis, granular exocytosis, adherence, and apoptosis and also mediates T cell differentiation and apoptosis by regulating IFN-γ production [159–161].

Activation of p38α has been observed in many systems. Growth factors like granulocyte/macrophage colony-stimulating factor, fibroblast growth factor (FGF), erythropoietin, interleukins, nerve growth factor, insulin-like growth factor (IGF) and PDGF [162–164], were found to trigger p38α activation in certain cell types. Its activation has also been shown in response to stimuli like TGF-β, GPCR agonists [165], a muscarinic agonist, vasoactive peptides [166], heat shock and ischemia reperfusion [167]. Thus, it has been difficult to categorize p38 to any particular kind of response, unlike ERK or JNK that can be identified as proliferative or stress responsive kinases, respectively, albeit loosely.

While the exact mechanisms involved in p38 functions are starting to emerge, activated p38 has been shown to phosphorylate several cellular targets. The target may be a transcription factor leading to change in gene expression or it may be an MK. Apart from these, other targets like cytosolic proteins phospholipase A2, the microtubule-associated protein Tau, keratin 8, Na^+/H^+ exchanger isoforms-1 (NHE-1) have also been reported to be substrates of p38α [168, 169].

Many transcription factors encompassing a broad range of action have been shown to be phosphorylated and subsequently activated by p38. Examples include activating transcription factor 1, 2 and 6 (ATF-1/2/6), SRF accessory protein (Sap1), CHOP (CREB homologous protein or growth arrest DNA damage inducible gene, *i.e.*, GADD 153), p53, Max, C/EBPβ, myocyte enhance factor 2C (MEF2C), MEF2A, MITF1, DDIT3, Elk1, NFAT and high mobility group-box protein (HBP-1) [170].

Among the MKs, MK2 was the first identified substrate for p38α. Both MK2 and MK3 are potently activated by p38 *in vivo* and are implicated in its effects on cytokine biosynthesis and cell migration. They have been shown to activate various substrates including small heat shock protein, and transcription factors ATF, CREB and SRF [171–173]. More recently, MK2 has been found to phosphorylate tristetraprolin (TTP), heterogeneous nuclear ribonucleoprotein (hnRNP) [174] and polyA-binding protein (PABP1) [175]. These proteins are known to destabilize mRNA, hinting at the role for p38 in mRNA stability. MNK1 is another kinase substrate of p38 whose function is thought to reside in translational initiation because of the fact that MNK1 and MNK2 can phosphorylate eIF4F. MSKs have also been shown to be activated by p38 pathway, and share some substrates of the p38 pathway.

Biological response

p38 in cell cycle: The involvement of p38 in cell cycle arrest became apparent when overexpression of p38α in yeast led to significant slowing of proliferation [176]. G1 arrest of NIH3T3 cells, caused by micro injection of p38α, is prevented by kinase dead MKK3, showing the involvement of active p38α. A link between p38 and cell cycle control has been proposed through regulation of substrates like HBP1 and p21 [177].

Many effects of p38 on cell cycle are mediated *via* MSKs, which phosphorylate various substrates like CREB and influence immediate early gene expression.

p38 in apoptosis: Activation of p38 was observed in cells undergoing apoptosis induced by variety of agents. Many chemotherapeutic agents require p38 activity for the induction of apoptosis [178, 179]. Inhibition of p38 activity has been reported to enhance apoptosis in response to DNA damaging agents such as doxorubicin and cisplatin [180]. Studies showing the inhibition of p38 by caspase inhibitors suggested the role of p38 downstream of caspase activation [181]. However, overexpression of MKK6 could induce caspase activity and increased cell death, indicating the role of p38 upstream of caspase activation as well [182]. Another mechanism of inducing apoptosis by p38 in endothelial cells has been shown to be *via* the phosphorylation and down-regulation of Bcl-xl, and by up-regulation of p53 [179].

p38 in cancer: It has been reported that loss of MKK3 and MKK6 resulted in increased proliferation and likelihood of tumorigenic conversion regardless of the cell line or tumor induction agent used [179]. Many further studies showed that p38 functions as a tumor suppressor. These tumor-suppressive effects have been demonstrated to be mediated *via* activation of p53 and p53-dependent apoptosis [183].

p38 in development and differentiation: p38α deletion leads to death during embryogenesis [184]. They die at embryonic day (E) 10.5–11.5 due to defects in placental angiogenesis [185] (Table 1). p38 has also been shown to be involved in erythropoietin expression since hematopoietic profiles of these mice were aberrant [186]. However, p38β, δ and γ knockout mice had no visible defect. The possibility that there is a redundancy in the pathways is being dealt with by targeting multiple genes at the same time. Additionally, tissue-specific knockouts will help to understand their role better.

p38α and β have been implicated in cell differentiation for certain cell types. Differentiation of 3T3-L1 cells into adipocytes and PC12 cells into neurons require p38α and/or β [187, 188]. p38α has been also implicated in muscle development since it is an important transcription factor for many muscle proteins [189].

p38 in inflammation: As has been mentioned previously, the role of p38 MAPK has been found to be critical in immune function. p38 activation is important in inflammatory responses, as in LPS-induced IL production, TNF-induced cytokine production [190], IL-12-mediated inflammatory responses [191]. The activation of p38 pathway plays an essential role in production of pro-inflammatory cytokines (IL-1β, TNF-α and IL-6), induction of enzymes such as cycloxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), induction of VCAM-1 and other adherent proteins along with other inflammation-related molecules [192]. Apart from activating the transcription factors and leading to transactivation of the above-mentioned genes, p38 has been shown to play a role in post-transcriptional and post-translational control of gene expression. The signal-induced stabilization of mRNAs encoding proinflammatory proteins contributes to robust and efficient induction

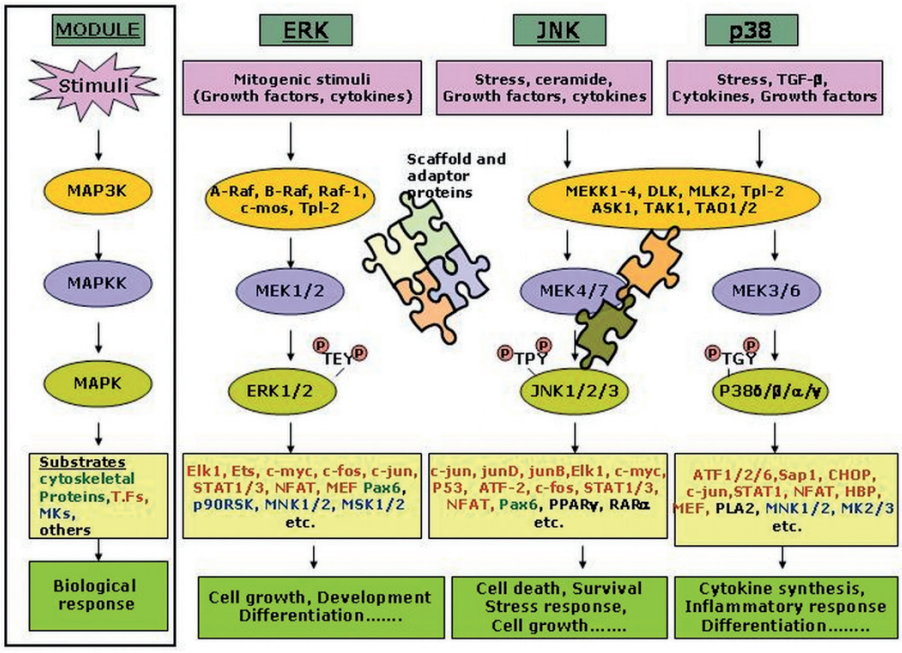

Figure 1. A simplified diagram showing mitogen- and stress-induced activation of mitogen-activated protein kinase (MAPK) pathways. The general architecture is shown in a box and the corresponding components of each of three main MAPK pathways are shown. The individual pathways are named on the third component of each three-tiered module, *i.e.*, extracellular signal-regulated kinase (ERK), 38-kDa stress-activated kinase (p38) and c-Jun N-terminal kinase (JNK); their conserved dual phosphorylation site is also shown. The scaffold and adaptor proteins that facilitate the activation of substrates and allows channeling of the signal into specific cascade are shown (see text for details).

of genes, which was found to be p38 dependent [174, 175].

Down-regulation

In both *in vitro* and transient transfection studies, MKP-1, MKP-4 and MKP-5 can efficiently dephosphorylate p38α and p38β [20]. Interestingly, p38γ and p38δ are resistant to all MKP family members. PP2C, a Ser/Thr phosphatase has also been shown to down-regulate MAPK Hog1 pathway as well as MKK6 and MKK4 [152–154]. Figures 1 and 2 summarize the basic module of MAPK pathways.

Determinants of MAPK specificity

The ability of MAPKs to transmit different, even opposing signals in the same cells raises the question as to how the specificity of the different signals transmitted by the MAPK cascade is regulated. Several mechanisms that participate in this specificity determination have been proposed, including (i) duration and strength of signal, (ii) interaction with various scaffold proteins, (iii) subcellular localization, (iv) presence of several isoforms, at each tier of the cascade, (v) extensive cross-talk and interplay between MAPK cascade and other pathways, and (vi) post-translational modifications other than phosphorylation. All these mechanisms can be best understood using ERK pathway as model system.

Duration and magnitude

The strength and duration of the presence of stimuli can lead to distinct cellular outcomes by altering duration of MAPK activity (Fig. 3). For example, treatment of PC12 cells with nerve growth factor (NGF) induces sustained activation of ERK and causes their differentiation into sympathetic like neurons. By contrast, EGF stimulates transient ERK activation and causes cell proliferation [193]. When the EGF receptor is overexpressed in PC12 cells, ERK activity becomes sustained and cells undergo differentiation in response to EGF [194]. This correlation between the duration of ERK activity and its effects on cellular systems has also been observed in other systems. The duration of ERK activity therefore appears to determine cell fate. Duration-dependent effects have also been observed with the JNK pathway, *e.g.*, in Jurkat cells persistent but not transient activation of JNK led to apoptosis [195].

The regulation of the kinetics of the activation of ERKs has been extensively studied and shown to involve several mechanisms that operate separately in each tier of the MAPK pathway. One mechanism was proposed by Murphy et al. [11], who demonstrated that in fibroblast proliferation, only sustained activation of ERK is able to both induce and stabilize its transactivated immediate early gene (IEG) and gene products, which can then regulate the expression of further downstream genes. Transient activation, on the other hand can not accumulate and maintain enough IEG products to push the cells through S-phase. Some studies have highlighted the role of small

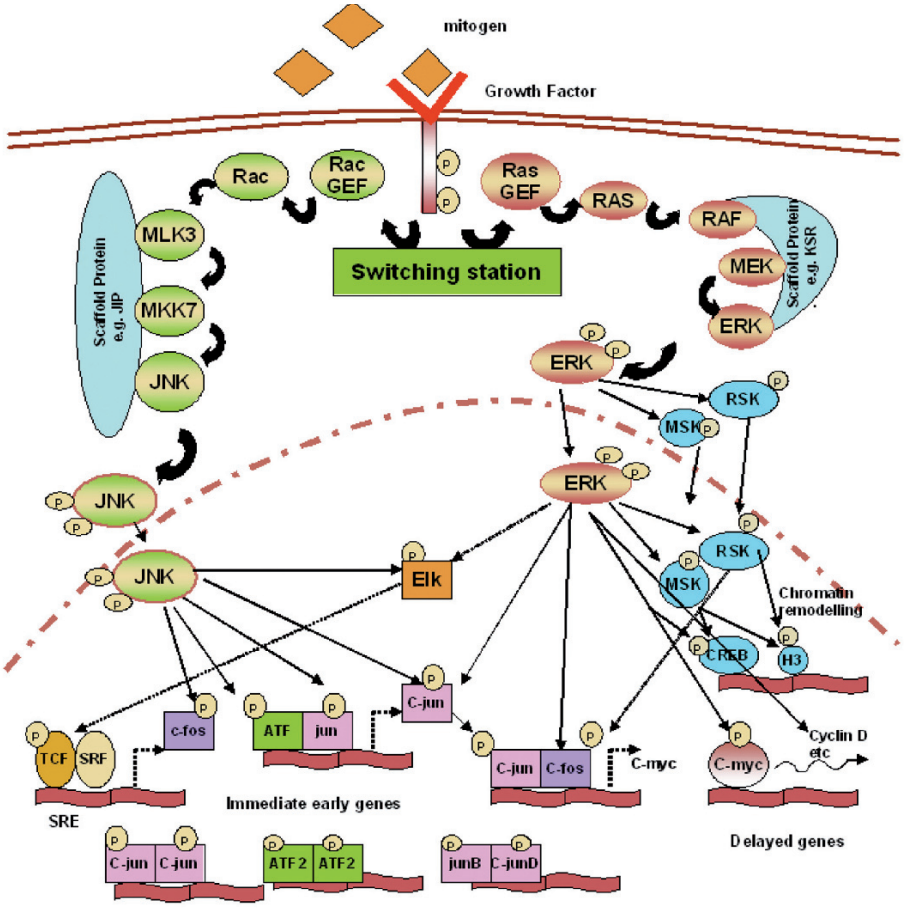

Figure 2. A comprehensive diagram of MAPK signaling and its consequences. Upon stimulation by growth factors, the corresponding receptor tyrosine kinases are activated and stimulate small GTPases of Ras and Rho family. Depending on these upstream proteins, downstream MAPK pathways, ERK or JNK are activated. While Ras stimulates Raf/ERK pathway, Rac stimulates JNK pathway. Scaffold proteins of MAPK pathways can then dictate the rapid and specific MAPK activation as well as its cellular compartmentalization, *e.g.*, scaffold protein KSR forms a molecular complex with ERK and its upstream activators and may transport it to the nucleus, while another scaffold protein paxillin may prevent its entry to nucleus. MAPKs translocated into the nucleus regulate gene expression by phosphorylating the transcription factors ATF, Elk (TCF) and jun proteins. These in turn activate the transcription of immediate early genes such as c-jun and c-fos. C-jun and c-fos can also undergo phosphorylation by the MAPKs. The net result is a new set of activated transcription factors, which can activate transcription of delayed genes such as cyclin D, etc. Apart from acting in concert with ERK, RSKs and MSKs can lead to chromatin remodeling by phosphorylating substrates such as histone H3.

Abbreviations: BMK, big mitogen-activated kinase; CD motif, common docking motif; DEF domain, docking site for ERK and FXFP domain; ED, ERK docking; ED motif, ERK docking motif; EGFR, epidermal growth factor receptor; ERK, extracellular signal regulated kinase; GEF, guanine nucleotide-exchange factor; GPCR, G protein-coupled receptor; JNK, c-Jun N terminal kinase; KSR, kinase suppressor of Ras; MAP2K or MAPKK or MKK or MEK, MAPK kinase; MAP3K or MAPKKK or MKKK or MEKK, MAPK kinase kinase; MAPK, mitogen-activated protein kinases; MKP, MAPK phosphatase; MKs or MAPKAPKs, MAPK-activated kinases; MNK, MAPK-interacting kinases; MP1, MEK partner 1; MSK, mitogen- and stress-activated kinase; NGF, nerve growth factor; p38, stress-activated kinase of 38 kDa; Ras, rat sarcoma viral oncogene; RSK, p90 ribosomal S6 kinase; TCF, ternary complex factor.

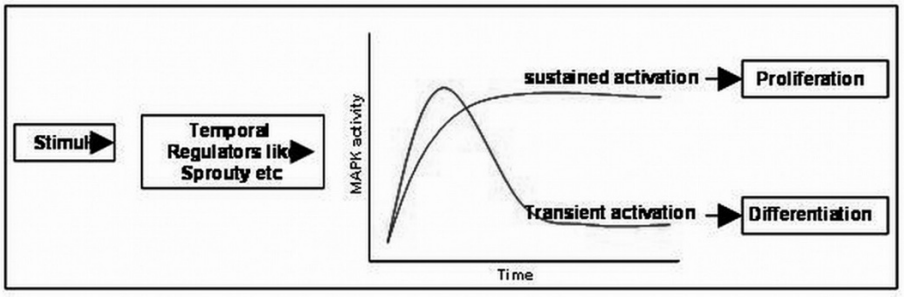

Figure 3. Schematic representation of temporal regulation of MAPK activation leading to differential response.

GTPases [196]. Bhalla et al proposed a positive feedback loop between ERK and PKC signaling resulting in sustained ERK activation [197].
Another temporal regulator of ERK activity is sprouty, a negative feed back inhibitor of the ERK pathway [198]. Sprouty becomes tyrosine phosphorylated in response to growth factor stimulation and inhibits ERK activation. Thus, sprouty represses the later but not initial phase of ERK activation.
Additional mechanisms such as chromatin remodeling may be involved [199], on which studies are underway.

Scaffold proteins and docking sites
The importance of docking sites, which play an important role in increasing the efficiency and fidelity of the MAPK pathway and prevent inappropriate cross-talk, has already been discussed. For instance, JNK1/2 is bound by the N terminus of MKK4 (MAP2K), which also interacts with the catalytic domain of MEKK. Each interaction is disrupted on the activation of downstream kinase. A second mechanism that contributes to the specificity of MAPK cascades is the formation of multiprotein complexes *via* multidomain proteins called as scaffold proteins (Fig. 1). These proteins bring together the components of a single pathway, and insulate the module from activation by irrelevant stimuli and negative regulators like phosphatases. They can also determine the localization of the cascade components and provide better stability to some components of the cascade. By doing so, scaffold proteins induce faster kinetics of activation, modify signaling duration and intensity, secure better interaction between distinct components and modify the cross-talk with other pathways [200]. The importance of scaffold proteins for MAPK signaling was first demonstrated in yeast, where different scaffolds could direct signaling components to regulate distinct processes. Over the past few years, scaffold proteins have also been implicated in regulation of signaling cascades in mammalian cells. For example, the JNK cascade is highly regulated by several distinct scaffold proteins. The JNK interacting protein (JIP) family forms the major class of these proteins, which includes a JNK binding domain (JBD), a src homology domain (SH3) and a phosphotyrosine-binding domain. More than 50 scaffolding and anchoring proteins have been described for ERK pathway [201]. KSR (Kinase suppressor of ras), one of the best studied, is a scaffold protein that interacts with Raf, MEK and ERK, thereby potentiating ERK activation [202]. KSR-null mice show reduced levels of ERK activity, which blocked T cell activation and inhibits tumor development [203]. Other scaffold proteins for ERK pathway play specific roles for the requirement of the cell, *e.g.*, MP1 couples ERK to MEK [204], β-arrestin participates in ERK signaling upon GPCR stimulation [205], and paxillin prevents translocation of ERK to nucleus so as to target it to cytoplasmic substrates.

Subcellular localization
The intracellular distribution of MAPK itself and its components contributes to the specificity of the ERK cascade. The signaling from ERK may vary depending upon its localization in the cell (Fig. 4). For example, MEKK1 colocalizes with the elements of cytoskeleton [206], and cytoskeletal rearrangements may stimulate MEKK1 activity [207, 208]. Such localized JNK activation may allow MEKK1 to modulate cell motility. The cytoplasmic distribution of the components of ERK cascade is dramatically altered upon extracellular stimulation. For example, forcing nuclear localization of an ERK2-MEK1 chimera resulted in an increased transcriptional activity [209], and forcing membrane association of ERKs resulted in an attenuated transcriptional activity [210]. The nuclear and cytoplasmic pool of activated MAPK and MKs can dictate the activation of their downstream targets. The redistribution of activated ERK in cytoplasm or nuclei is governed by many proteins, which include scaffolds, microtubules, phosphatases, and other anchoring proteins. It has also been shown that early exit of ERK from the nuclei results in transient ERK activation [211], while increased accumulation in nucleus leads to sustained activation. Thus, the localization of components of MAPK cascade is a key component in dictating its signaling specificity.

Isoforms
Given the variety of stimuli known to activate the different MAPKs, the diversity of MAP3Ks represents a predominant mechanism by which specificity is achieved in the MAPK cascade. Indeed, as mentioned previously, the MAP3K tier of ERK cascade contains quite a number of components including Raf kinases, c-Mos, Tpl-2, MEKK1 and others that operate under distinct conditions [212]. In total, about 20 MAP3Ks have been characterized. Of these, 6 are known to regulate ERK pathway, at least 12 are known to activate JNK pathway and 9 MAP3Ks regulate the p38 pathway. The greater number of MAP3Ks regulating the stress-activated JNK and p38 pathways is probably indicative of the necessity of cells to be able to respond to many different stress stimuli with the activation of JNK and/or p38. Out of the 6 MAP3Ks known for ERK pathway, 5 are specific for ERK.
Despite the extensive similarity between the MEKs and MAPKs of next tier, there are differences in their

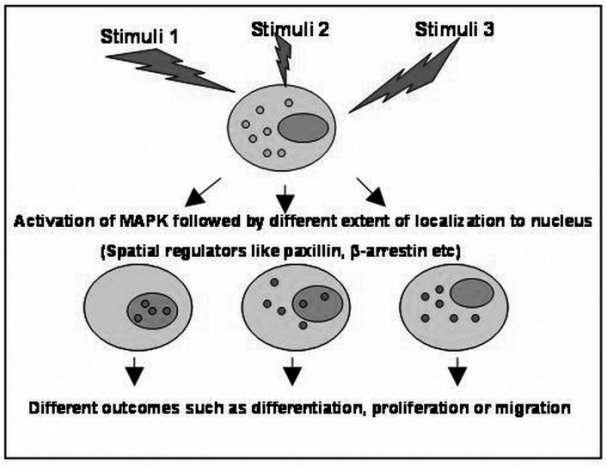

Figure 4. Schematic representation of spatial regulation of MAPK activation leading to differential response.

regulation, as exemplified by the knockout studies of various isoforms [83, 213, 214].

Cross-talk

Biochemical pathways always operate in conjunction with each other and their interplay decides the final outcome. The existence of cross-talk among MAPK pathways themselves is exemplified by the fact that MEK4 can activate both JNK and p38 MAPKs. Previous studies have also shown that pro-survival ERK pathway and pro-apoptotic JNK pathway possibly act in dynamic balance, with the ERK pathway acting to inhibit the JNK pathway or *vice versa* [215, 216].

Many other pathways have also been shown to feed into, inhibit or regulate the MAPK pathways through phosphorylation or dephosphorylation. In addition, many of the signals from other pathways may converge with that of MAPK cascade at downstream components such as transcription factors and kinases [217]. A few examples are Rac1/CDC42-PAK1 cascade, PI3K-PDK1-Akt, mTOR-S6K [218], NIK-IKK-NFκB [219] and CDK pathways.

PKC, activated by PI3K-PDK1-Akt pathway, is known to activate Raf, hence potentiating the ERK pathway. p90 RSK also needs PDK1 phosphorylation to be catalytically active [220]. Rac1 and cdc42, members of the Rho subfamily, are activated by cell adhesion. Activation of the downstream effector PAK1 leads to dynamic changes in cytoskeletal organization by phosphorylating various downstream kinases like LIM kinase and myosin light chain kinase. In addition, PAK1 can also phosphorylate MEK1 in focal adhesion complexes, which is an important step in regulation of cell adhesion and spreading, and converging the integrin and growth factor-mediated signaling. Rac/cdc42 are also known to be potential regulators of p38 pathway [151].

CDKs, key components in the regulation of cell cycle progression, also cross-talk with the MAPK pathway. CDK1 and CDK5 can phosphorylate MEK1 and consequently modify its activity [221].

Other post-translational modifications

Multiple modifications are a normal mode of signal transduction in physiology. Many proteins such as p53 and histones are subjected to various modifications namely phosphorylation, acetylation, SUMOylation, ubiquitination, etc. These post-translational modifications of signaling proteins seem to play important role in transducing the signal by altering the protein stability, duration of activation, localization or protein-protein association. Other modifications of MAPKs cannot be ruled out. Ubiquitination of MAPKs has been shown to alter their localization or degradation [222].

Nitric oxide, recognized as an important signaling molecule, has an ability to modify tyrosine or cysteine residues. JNK has been shown to be *S*-nitrosylated at Cys, resulting in inhibition of its activity [223]. Tyrosine nitration, on the other hand has been shown to activate or inhibit many proteins, including protein kinase C epsilon [224], src tyrosine kinase [225], insulin receptor substrate-1 (IRS-1) [226] and fibrinogen [227]. Since the nitro group bears similarities with a phospho group in terms of charge and bulkiness, it may play an equally important role in signal transduction as phosphorylation. Significant decline in tyrosine phosphorylation of MAPKs in presence of nitric oxide in our studies have been observed without impairment in the function of the macrophages [228]. Extensively nitrated forms of the MAPK were able to activate Elk and jun. Significant ubiquitination of ERK and JNK has also been seen (unpublished results). Depending on the extent of nitrating or nitrosative stress and other factors, nitration and phosphorylation of critical tyrosine residues may be a competitive or cooperative process [229].

Conclusions

The apparent simplicity of the MAPK module belies the cellular functions in which it appears to participate. In fact, it is this simplicity that enables nature to fine tune the whole network. The MAPKs may be few in number, but the numerous MAPKKKs that feed into them and numerous downstream targets that are acted upon such as MKs, diversify the signal and provide specificity. This variability is compounded by the fact that the transcription factors do not act singly

but in combinations and numerous permutations and combinations can be made with just a few transcription factors that could be in various stages of post-translational modifications.

The protein-protein associations of non-catalytic domains of MAPK components further add to the specificity and diversity by allowing the specific interactions with varied affinities. The scaffolds of these kinases may provide specificity in a context-specific and time-specific manner, *e.g.*, JIP2 is known to interact only with rac-specific GEF [230] and hence may direct signaling in a rac-specific manner.

The association of ubiquitin with these scaffolds may contribute to the duration and localization of the MAPK activation.

To date phosphorylation and dephosphorylation supposedly holds the key to most activations and deactivations. How significant the other modifications are, like nitration and nitrosylation, needs to be worked out. How much the diverse post-translational modifications add to the fine tuning is yet to be fully elucidated. These questions can be answered only if we define the interactome for particular stimuli and look at the MAPK pathway in a holistic manner.

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