

Review

Regulatory mechanisms of mitogen-activated kinase signaling

Y. Zhang* and C. Dong

Department of Immunology, MD Anderson Cancer Center, Unit 906, 7455 Fannin, Houston, Texas 77030 (USA), Fax: +1 713 563 0604, e-mail: ylzhang@mdanderson.org

Received 11 January 2007; received after revision 31 May 2007; accepted 5 July 2007
Online First 30 August 2007

Abstract. MAP kinases (MAPKs) are evolutionarily conserved regulators that mediate signal transduction and play essential roles in various physiological processes. There are three main families of MAPKs in mammals, whose functions are regulated by activators, inactivators, substrates and scaffolds, which together form delicate signaling cascades in response to different extracellular or intracellular stimulation.

MAPK signaling is tightly regulated so that optimal biological activities are achieved and health is maintained. However, how the specificity of the signaling flow along each cascade is achieved is still relatively unclear. In this review, we summarize recent advances in understanding the regulation of MAPK cascades and the roles of MAP kinases and their regulators in development and in immune responses.

Keywords. MAPKs, docking interaction, scaffold proteins, MAPK phosphatases.

Introduction

Three main families of MAP kinases (MAPKs) exist in mammalian species, grouped by their structures and functions: the extracellular signal-regulated protein kinases (ERKs), the p38 MAPK, and the c-Jun NH₂-terminal kinases (JNKs) [1–4]. All the MAPKs consist of a Thr-X-Tyr (TXY) motif within their activation loop. The phosphorylation of both threonine and tyrosine within the activation loop is essential and sufficient for their activation. The ERK family contains a TEY (Thr-Glu-Tyr) activation motif [5]. Members of this family could be further divided into two subgroups: 1) the classic MAPKs that consist primarily of a kinase domain such as ERK1 and ERK2; and 2) the large MAPKs such as ERK3, ERK5, ERK7, and ERK8 that consist of both a kinase domain and a C-terminal domain and range in size from 60 to greater than 100 kDa [6]. The p38 family

has a TGY activation motif and includes α , β , γ , δ , and ERK6, which is a newly identified member [6, 7]. There are three members in the JNK family which all contain Thr-Pro-Tyr (TPY) in their activation motif [8]. The expressions of JNK1 and JNK2 have been found to be ubiquitous, while the expression of JNK3 is brain-specific.

MAPKs respond to a broad range of extracellular and intracellular changes (Fig. 1). Cooperating with other signal transduction pathways, MAPKs transduce these signals into alterations in gene expression and regulation of cell function. In this review, we will focus on recent development on how MAPK pathways achieve their signaling specificity and how they are negatively regulated.

* Corresponding author.

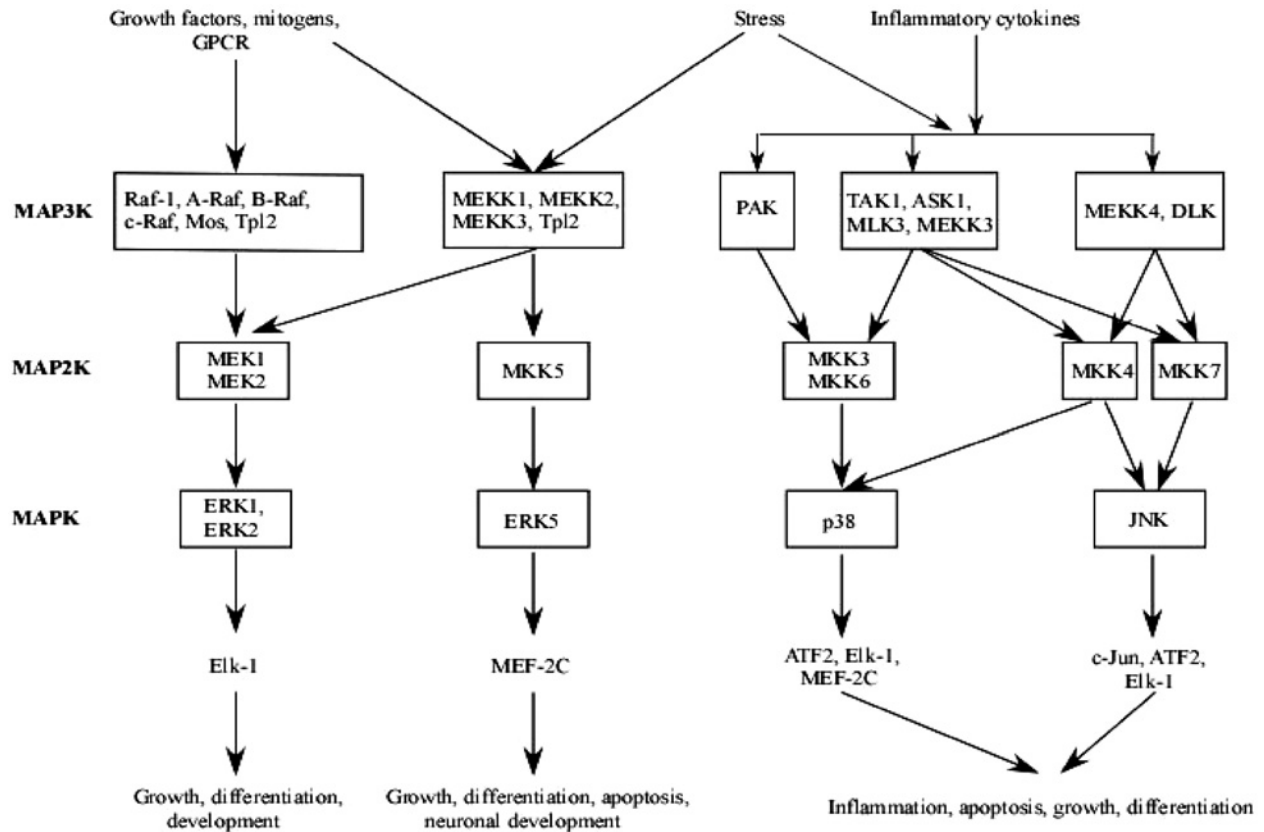


Figure 1. Mammalian MAP kinase pathways. The activation of MAPK signaling pathways is achieved through a core triple kinase cascade. MAPKs are activated by dual phosphorylation on Thr and Tyr caused by specific MKKs. The MKKs are activated, in turn, by MKKKs.

Signaling specificity regulation of MAPKs by docking and scaffolding

Docking interactions regulate the specificity and efficiency of MAPK signal transduction

Multiple mechanisms present in the MAPK signaling pathways ensure the fidelity and efficiency of the signaling flow. These mechanisms include the interaction between the kinase catalytic domain and the substrate phosphor-acceptor site, docking interactions between members of a particular MAPK cascade, and scaffold proteins. Docking interaction is commonly shared among the members of an MAPK cascade when they interact with their corresponding partners. Docking motifs are characterized as a short peptide with a few charged or hydrophobic residues. The so-called docking interaction happens when one member of the MAPK cascade interact with its corresponding partners through electrostatic and/or hydrophobic interactions. These docking interactions are essential for the efficiency of signaling and contribute to a certain degree to the signaling specificity. In this section, we will focus on how different members of MAPK cascades use different docking domains to interact with their cognate binding partners to regu-

late the specificity and efficiency of signal transduction.

Docking interaction between MAP3Ks and MAP2Ks

It is well known that a larger number of MAP kinase kinase kinases (MAP3Ks) are present to activate a more limited number of MAP kinase kinases (MAP2Ks; seven in mammalian species), which leads to the activation of a specific MAPK (Fig. 1). The specific MAP3K-MAP2K interaction is thought to be a fate-determining event [9]. Docking interaction is one of the mechanisms that contribute signaling specificity at this stage. The docking interaction between MAP3K and MAP2K and its contribution to signaling specificity was first appreciated in mating yeast. The two MAP3Ks, Ssk2/Ssk22, in the HOG MAPK pathway, which is activated by external high osmolarity, transfer the signal only to MAP2K Pbs2. It was found that a docking domain in the N-terminus of Pbs2 and its specific interaction with Ssk2/Ssk22 determine the signaling specificity at this point [9]. In mammalian species, a docking domain, termed DVD (domain for versatile docking) site, was also found to be commonly present in the C-terminus of MAP2Ks right after their kinase domains (Table 1)

[10]. It is composed of about 20 amino acids and is critical for the interaction of MAP2Ks with their corresponding MAP3Ks and for the activation of MAP2Ks by their MAP3Ks. For example, in human MKK6, the C-terminal DVD site consisting of 24 amino acids is required for its binding to the kinase domain of all its MAP3Ks, including MTK1, TAK1, ASK1, and TAO2. Synthetic oligopeptides of this domain competitively inhibited the activation of MKK6. When this domain was mutated, activation of MKK6 by its MAP3Ks in response to various stimulations, including UV irradiation, osmotic stress, translation inhibition, oxidative stress, and cytokine stimulation, was impaired [10]. This DVD site was also commonly present in other human MAP2Ks with similar function, including MKK3, MKK4, MKK7, and MEK1, indicating that the DVD sites in mammalian MAP2Ks are essential for their binding and activating by their MAP3Ks.

The DVD sites of mammalian MAP2Ks were also found to contribute to the specificity of signal transmission. For instance, MKK4 could be phosphorylated by both MEKK1 and MTK1, whereas MKK7 could be phosphorylated only by MEKK1. It was found that the DVD sites of MKK4 could bind both MEKK1 and MTK1, whereas that of MKK7 could bind to only MEKK1, indicating that the DVD domain is an important determinant of the specific MAP3K-MAP2K interaction [10]. However, after switching of the DVD domain of MKK7 to that of MKK4, MKK7 could no longer be phosphorylated by either MEKK1 or MTK1, indicating that the DVD-mediated docking interaction only partially contributed to the signaling. To our knowledge, the corresponding regions in MAP3Ks of DVD sites have not been identified, although some regions, such as the sub-domain VIII in MEKK1, have been found to be involved in the specific interaction with its substrates [11]. Further studies will be needed to elucidate the mechanisms of docking interaction between MAP3Ks and MAP2Ks.

Docking interactions between MAP2Ks and MAPKs

Once activated by their corresponding MAP3Ks, the MAP2Ks will make contact with their cognate MAPKs to phosphorylate and activate them. Two MAP2Ks, MEK1 and MEK2, are responsible for ERK activation; MKK4 and MKK7, and MKK3 and MKK6, are for JNK and p38 activation, respectively; whereas MKK5 is responsible for the activation of ERK5 (Fig. 1) [12, 13]. It had been found that a docking motif called D domain, also known as D-site or DEJL motif, other than DVD site in MAP2Ks contributed to the specific interactions of some MAP2Ks with their cognate MAPKs. The D domain

normally contains a cluster of basic residues (2–3 R/K residues), followed by two conserved hydrophobic residues in the arrangement of Φ_A -X- Φ_B (Φ_A and Φ_B are hydrophobic residues) with 1–6 residues in between [14] (Table 1). This D domain actually was first identified in Ste7, the yeast MAP2K in Kss1 and Fus3 MAPK pathways [15]. It was further found to be evolutionarily conserved from yeast to humans in certain classes of the MAP2Ks, including MEK1, MEK2, MKK3b, MKK4, and MKK7 [15–20]. The D domains in these MAP2Ks mediate their binding to their cognate MAPKs and are important for their proper functions. For example, mutations of the D domain in MEK1 inhibited its binding to ERK, its ability to exclude ERK from nucleus, and its feedback phosphorylation by ERK [16]. Synthetic D domain peptide of MEK1 inhibited both MEK1 phosphorylation of ERK2 and the feedback phosphorylation of MEK1 by ERK2 [16, 21]. Similar functions of D domains in MKK3b and MKK6 in mediating their binding and function on p38, and MKK4 on JNK, have been confirmed [18, 19]. In the case of MKK7, there are three D domains instead of one in its N terminus working together to mediate its binding to JNK [20]. However, the contributions of D domains to signaling specificity in different MAP2Ks may vary. For instance, the synthetic D domains of MEK1 and MEK2 bound only to ERK and only inhibited the binding and phosphorylation of ERK by MEK1 and MEK2, whereas the D domain peptide of MKK4 inhibited not only the binding and phosphorylation of JNK by MKK4 but also the binding and phosphorylation of ERK2 by MEK2 [19], indicating the presence of other specificity-determining mechanisms between MAP2Ks and MAPKs.

Unlike other MAP2Ks, the ERK5 activator MEK5 α contains a unique docking domain which comprises a cluster of acidic residues in its N terminus (Table 1) [22]. This docking domain mediates its binding to both MEKK2 and ERK5, its activator and target, to facilitate the activation of ERK5 cascade.

CD and ED sites in determining binding specificity of MAPKs with activators and substrates

The first identified docking domain in MAPKs is called common docking domain or CD domain because it is present in all members of MAPKs and is evolutionarily conserved from yeast, *Caenorhabditis elegans*, *Drosophila*, and plants, to human; MAPKs use this same docking domain to interact with their activators, inactivators, and substrates [23]. The CD domain, comprising a cluster of negatively charged amino acids, is located in the C-terminal region right after their kinase domain (Table 1) [23]. Mutational studies demonstrated that the acidic amino acids are

Table 1. Docking domains in MAPK cascades.

Members of MAPK cascades	Docking domain	Structure or representative sequences	Interaction with	References
MAP2Ks	DVD (domain for versatile docking) site, located at the C-terminal side of catalytic domain	<p>MKK6</p> <p>1 53 Kinase domain DVD site 311 334</p> <p>HPFFTLHESKGTDVASFVKLILGD</p>	MAP3Ks	Takekawa, M. et al., 2005
	D domain (also known as D-site, or DEJF motif), contains a Cluster of basic residues followed by two conserved hydrophobic residues located at its N-terminus	<p>MKK6</p> <p>1 18 DVD site 311 334</p> <p>D domain MSQSKGKKRNPLKIPKE</p> <p>Basic residues Φ_A-X-Φ_B</p>	MAPKs	Enslin, H. et al., 2000
	Docking domain of MEK5, located in its N-terminus	<p>MEK5α</p> <p>61EYEDGD68 448</p>	MEKK2 and ERK5	Seyfried, J. et al., 2005
MAPKs	CD (common docking) domain comprises a Cluster of negatively charged amino acids, located in the C-terminal region right after their kinase domain	<p>ERK2</p> <p>159 172 Kinase domain CD domain 316 329</p> <p>LEQYYDPSDEPVAE</p>	MAP2Ks, MKPs, Substrates, etc.	Tanoue, T. et al., 2000, 2001
	ED site is also composed of a group of negatively charged amino acids, located at the opposite of CD domain to the kinase domain	<p>ERK2</p> <p>159 172 Kinase domain CD domain 316 329</p> <p>ED site LLNTTCDLKICDFG</p>	MAP2Ks, MKPs, Substrates, etc.	Tanoue, T. et al., 2001
MAPK substrates	D domain (also known as D-site, or DEJF motif); see description above	<p>c-Jun</p> <p>32 51 D domain 51</p> <p>KILKQSMTLNLADPVGSLKP</p>	JNK	Dai, T. et al., 1995; Vinciguerra, M. et al., 2004
	DEF domain (docking site for ERK) or FX(F/Y)P motif	<p>c-Fos</p> <p>311 336 380 DEF domain 380</p> <p>LCTPVVTCPTSCCTYTSSVFVTYPEE</p>	ERK	Jacobs, D. et al., 1999; Murphy, L. et al., 2002; Vinciguerra M. et al., 2004
	some ERK Substrates contain both D domain and DEF motif	<p>JunD</p> <p>48MLKKDALTLSLAE60 D domain 341</p> <p>DEF domain 112LVTTTPTSTQFLYPKV127</p>	JNK, ERK	Vinciguerra, M. et al., 2004
Scaffold proteins and MKPs	the D domain in JNK scaffold JIP-1 was first identified as JNK-binding domain (JBD)	<p>JIP-1</p> <p>144 163 D domain 660</p> <p>PGTGSGDYRPKRPTLLNLF</p>	JNK	Dickens, M. et al., 1997
	the N-terminus of MKP-1 has a positive-hydrophobic-positive motif which includes the D domain and is for docking to CD and ED domains in p38 and JNK	<p>MKP-1</p> <p>51 75 D domain 336 345 367 DEF domain 367</p> <p>IVRRRAKAGAMGLEHIVPNAELRGRL Basic residues Φ_A-X-Φ_B</p> <p>TTVFNFPVSI</p>	JNK, p38	Tanoue, T. et al., 2002
	DEF domain of MKP-1 in its C-terminus is essential for ERK-directed ubiquitination and proteolysis		ERK	Lin, Y. and Yang, J., 2006

crucial for the docking interaction with some of their binding partners but not fully responsible for their binding with other partners or for their binding specificity [23, 24]. For instance, mutations of negatively charged amino acids in the ERK2 CD domain, Asp321–Asp324, and mutations of Asp313–Asp315–Asp316 in p38 α CD domain greatly impaired the binding of ERK2 and p38 α with their corresponding partners and their enzymatic activities, respectively [23]. However, the mutation of Ser 323 in the CD domain of ERK2 to an aspartic acid, which changed the ERK2 CD domain sequence to a p38 α CD sequence, did not change its docking specificity. Furthermore, mutation of the three aspartic amino acids in the CD domain of p38 α did not fully block its binding with MAPKAPK-3/3pk, one of its substrates [24]. Together, these data indicated the presence of other specificity determinants in MAPKs, which led to the finding of another docking domain named ED site (Table 1) [24]. The ED site of p38 α is also composed of a group of negatively charged amino acids and was closed to the CD domain in steric structure. Mutation of this ED site alone reduced the binding and activation of p38 α toward 3pk. When both ED and CD sites were mutated, the binding ability of p38 α to 3pk was almost completely lost, and its ability to phosphorylate 3pk was further decreased compared with the single mutant, indicating that both the ED and CD sites contribute to its docking specificity [24]. However, the contribution of CD and ED sites to docking specificity may vary with different substrates. For instance, the ED of the p38 α site contributes more to regulation of the docking specificity toward 3pk than does the CD site, whereas the CD site is the main specificity determinant toward MSK2; both the CD and ED sites are equally important for determining the docking specificity toward PRAK [24].

Docking domains in MAPK substrates

Different types of docking sites have been identified in MAPK substrates. One docking domain present in MAPK substrates is the D domain, which is also present in MAP2Ks. The D domain was actually first identified in the bZip transcription factor c-Jun [25, 26]. It is essential for efficient and specific phosphorylation of c-Jun by JNK. The D domain was later found to be conserved among various MAPK substrates. It not only contributes to the signaling specificity from MAPKs to their substrates but is also required for the activation and function of these substrates through mediating binding events rather than catalysis [27–29]. For instance, the MEF2 subfamily of MADS-box transcription factors MEF2A and MEF2C is preferentially phosphorylated and activated by the p38 subfamily members p38 α and

p38 β [2, 29]. Deletion of the D domains from both MEF2A and MEF2C greatly reduced their phosphorylation by p38 and their transcriptional activities. A synthesized peptide that corresponds to the D domains of MEF2A acted as an inhibitor of p38 α and p38 β -mediated phosphorylation of MEF2A [29]. Mutagenesis study showed that although residues throughout the D domain contributed to its function, the hydrophobic residues in its C-terminus (L273, V275, and I277 in MEF2A) are particularly important [28]. The D domains in MEF2A and MEF2C also determine their specificity for p38 α and p38 β . Fusion of the D domain from MEF2A to Elk-1 and c-Jun, which either does not or responds poorly to p38, converts them to be good targets of both p38 α and p38 β by directing the phosphorylation of the physiologically relevant sites [29]. On the other hand, fusion of Elk-1 (a JNK and ERK substrate) D domain could only convert MEF2A into a JNK target but not ERKs [29]. Similarly, fusion of D domain from SAP-1, which is targeted by p38 α , p38 β , and ERK, with D domain-deleted MEF2A could only restore its ability to be a p38 substrate but not an ERK substrate. Therefore, other factors in Elk-1 and SAP-1 determine their ability as ERK substrates.

Another docking domain called DEF domain or FX(F/Y)P motif was then identified in both ERK and p38 α substrates (Table 1) [30–32]. It was first identified in the Ets family transcription factor LIN-1 in *C. elegans* and was further found to be an evolutionarily conserved docking motif that mediated ERK binding to ETS proteins [30,31]. This motif, like the D domain, contributes to signaling efficiency and specificity. For instance, it has been shown that ERK substrate c-Fos could function as a molecular sensor for ERK signal duration due to the presence of its DEF domain [33]. In response to transient ERK signal, c-Fos was not phosphorylated and was degraded quickly. In response to sustained ERK activation, initial phosphorylation of the c-Fos in its C terminus resulted in the exposure of its DEF domain and the docking interaction with ERK, thereby helping to increase the efficiency of ERK-regulated activation and to contribute to the following biological outcomes, such as S phase entry of the cells.

Because two different docking domains are present in various ERK substrates, ERK substrates can therefore be divided into three classes based on the presence of DEF domain or D domain or both [31]. The DEF motif was found to be present in these ERK substrates, which can be targeted by more than one subgroup of MAPKs [32]. Its primary sequences contain the necessary information to mediate binding to ERK. However, the maximum ERK response may require the cooperation of both D and DEF domains.

For instance, JunD contains both D and DEF domains and can be activated by both ERK and JNK MAPKs [34–36]. The D domain alone mediated its response to JNK-specific activation, whereas D and DEF domains functioned additively in response to ERK-mediated activation [36]. Introduction of the DEF domain into c-Jun, which does not respond to ERK activation, enabled it to be responsive to transient activation of ERK signaling [36]. In the case of Elk-1, which also contains both D and DEF domains, although mutation of either D domain or DEF domain reduced its transcriptional activity significantly in response to ERK activation, mutation of both the D and DEF domain completely eliminated its transcriptional activity [32,37].

It had been shown that MAPKs such as ERK2 interact with the D domain and the DEF domain using different regions, thereby mediating the activation of different substrates in response to different stimulations [38]. Residues from 185–261, which is proximal to the activation lip in ERK2, were found to be essential for interaction with the DEF domain, whereas residues 316 and 319 in the CD domain mediated the interaction with the D domain [23,38]. Mutational study indicated that downstream branches of ERK2 signaling can be selectively inhibited by specific mutations in either of these two regions without blocking total pathway activity [39]. For instance, mutations in the DEF domain binding pocket prevent activation of DEF domain-containing substrates such as c-Fos but not D domain-containing substrates such as RSK, and vice versa.

Because the docking interaction between ERK and its substrate determines their individual signal specificity, the disrupting of interactions between one substrate with ERK would not affect ERK interacting with other substrates. Targeting these particular interactions might lead to the identification of specific compounds for a particular signal branch of ERK. For instance, by targeting different regions in the CD and ED domains of ERK, Hancock et al. identified two small molecular ERK inhibitors that affect ERK-mediated Rsk-1 activation and ERK-mediated Elk-1 activation, respectively [40]. Therefore, further study of docking interactions between members of MAPK cascades and their mechanisms could lead to the finding of new pharmaceutical targets for MAPK-related diseases.

Docking domains, such as D domain and DEF domain, have been found not only in MAP2Ks and MAPK substrates but also in other MAPK interacting molecules, such as scaffold proteins and MKPs (Table 1). The sequence similarity of the D domain in different MAPK-interacting molecules supports the notion that they bind to the same region in their

corresponding MAPKs. There is evidence suggesting that various D domain-containing interacting molecules of one MAPK compete for the same docking site in that MAPK. For example, peptides derived from D domains of MEK1 and MEK2 not only inhibited MEK-mediated ERK phosphorylation but also inhibited ERK-dependent Elk-1 phosphorylation, indicating that the D domains of MEK1 and MEK2 compete with that of Elk-1 to bind to the ERK docking site [19]. It was also found that MKK4 and JIP1 compete with each other to bind to JNK3, whereas MKK4 and JIP1 compete with JNK2 substrates such as c-Jun and ATF2 for binding to JNK2 [19].

Scaffold proteins assemble functional MAPK modules

Scaffold proteins had been identified as organizers of the MAPK signaling pathway. Members in one particular MAPK pathway may be brought together by a scaffold protein to assemble into an MAPK module in response to a particular stimulation. Therefore, scaffold proteins play a critical role in the signaling specificity of MAPKs. They also can provide spatial and temporal control of MAPK signaling [41]. The elegant studies on yeast MAPK scaffold proteins clearly demonstrate that scaffold proteins determine the signaling specificity of MAPKs in yeast. For instance, in the budding yeast *Saccharomyces cerevisiae*, the mating and high-osmolarity response MAPK pathways share some components such as Ste11. The Ste20p (MAP4K)-Ste11p (MAP3K)-Ste 7p (MAP2K)-Fus 3p (MAPK) pathway is responsible for mating signal transduction, which is activated by peptide mating pheromones (Fig. 2) [42, 43], whereas the Sho1 branch of the high-osmolarity response pathway, which includes Ste20p (MAP4K)-Ste11p(-MAP3K)-Pbs2p(MAP2K)-Hog1p (MAPK), mediates the response to osmotic stress [44, 45]. The same MAP3K Ste11p is used by these two distinct MAPK pathways, yet with no crosstalk. Scaffold proteins Ste5p for the mating pathway and Pbs2 for the Sho1 pathway were found to be the determinants for their respective signaling specificities [44, 46]. By interacting with multiple components, including Ste11p, Ste7p, and Fus3p with separable binding sites, Ste5p not only creates a signaling module specific for the activation of Fus3p but also positively regulates its activation (Fig. 2) [46]. A similar function is assumed by Pbs2, which is also MAP2K in the high-osmolarity glycerol (Hog) signaling pathway [44, 47, 48]. As a scaffold protein, Pbs2p binds to its upstream activator Sho1p and its downstream components Ste11p and Hog1p to assemble a specific signaling module to regulate response to high extracellular

osmolarity [44]. As an MAP2K, it directly phosphorylates Hog1p. The critical roles of Ste5p and Pbs2p in the maintenance of specificity of the mating and HOG1 pathways, respectively, were further demonstrated by the studies from the groups led by Pryciak and Lim [49, 50]. By using protein fusions to force the common component Ste11p to associate preferentially with a subset of its possible binding partners, signaling became confined to a particular pathway [49]. For instance, the fusing of Ste11p to Ste5p and Ste7p resulted in Fus3-dependent signaling; whereas fusing it to Pbs2 caused it to function best in the HOG pathway and to become less available for other pathways. By creating a hybrid scaffold, Park et al. further showed that a new MAPK pathway with non-natural input-output properties could be made from combinations of preexisting kinases. A diverter scaffold was made by fusing the scaffolds Ste5p and Pbs2p and mutagenically destroying interactions with the downstream mating output (Ste7p) and the upstream osmolarity input (the osmosensor Sho1) [50]. Upon pheromones peptide stimulation, cells bearing this diverter scaffold did not mate but could survive on high high-osmolarity only in the presence of pheromone peptide. Therefore, scaffolds Ste5p and Pbs2p play essential roles in the efficiency and specificity of the mating signaling and HOG pathways, respectively, by selectively binding to specific components, restricting them to the site of pathway activation, and segregating them from their participation in other pathways [49–54].

In the mammalian MAPK signaling pathways, many scaffold protein candidates have been identified to date. Biochemical and genetic studies have indicated that three members of the JIP protein family are scaffold proteins of the JNK pathway. Several molecules, including KSR and MP1, are ERK scaffold proteins. Proteins such as CNK1 and β -arrestin may also act as scaffolds in multiple signaling pathways. To date, few molecules have been identified as scaffolds for the p38 pathway. In the next section, we will focus on these molecules that are involved in JNK, ERK, and multiple signaling pathways.

JIP family connects JNK signaling modules to signal receptors in the cell membrane

The JNK-interaction proteins (JIPs) as scaffold proteins for the JNK pathway have been identified by Davis and his colleagues and are relatively well studied [55–57]; four members of this family, JIP1, JIP2, JIP3, and JIP4, have been identified. Among them, JIP1 and JIP2 share high sequence homology and similar binding partners. Both JIP1 and JIP2 selectively bind to mixed-lineage protein kinase (MLK) groups of MAP3Ks (including DLK, MLK2,

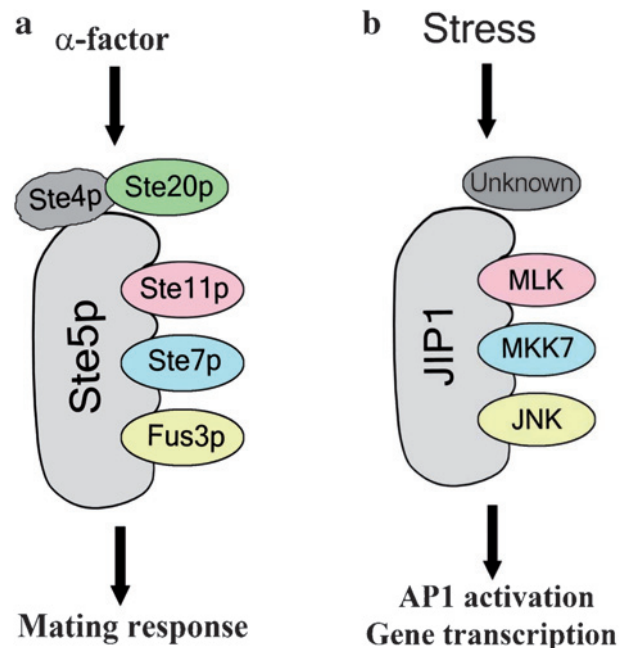


Figure 2. Scaffold proteins assemble functional MAPK modules. (a) In budding yeast *S. cerevisiae*, in response to a mating signal, the scaffold protein Ste5 interacts with Ste4p through docking interaction, recruits MAP3K Ste11, MAP2K Ste7 and MAPK Fus3, thereby segregating the common MAP3K Ste11 from other MAPK pathways, such as the high-osmolarity pathway. (b) In mammalian species, the JIP family member JIP1 binds to MLK groups of MAP3Ks DLK, MLK, and MLK3, MAP2K MKK7, and JNK MAPK to facilitate JNK activation in response to stress, such as excitotoxic stress and anoxic stress in neurons.

and MLK3), and MAP2K MKK7 with their C-terminal regions and interact with JNK using their N-terminal regions [56,57]. When co-expressed with MLK3 or MKK7, JIP1 enhanced JNK activity, whereas mutated JIP1 with deletion of JNK binding domain failed to do so, indicating that JIP1 enhances JNK activation by the MLK-MKK7 signaling pathway [37]. Furthermore, JIP1 also interacts with one of the three mammalian Ste20p-related proteins, hematopoietic progenitor kinase-1 (HPK1) [37], one of the JNK pathway activators [58], indicating that JIP1 could selectively associated the JNK module with upstream components. The role of JIP1 in regulation of MAPK signaling in vivo had been studied using gene knock-out mouse models. Study of JIP1 knockout mice demonstrated that JIP1 was a critical component of the JNK signaling pathway in neurons and positively regulated JNK activation in response to excitotoxic stress and anoxic stress (Fig. 2b) [59]. After JIP1 was knocked out, JNK activation was markedly attenuated in hippocampal neurons upon kainite treatment and oxygen-glucose deprivation. Furthermore, the neurons exhibited increased resistance to the apoptotic effects of these two treatments.

In addition to facilitating JNK activation through binding to components of the JNK pathway, JIP1 had also been found to regulate JNK signaling under certain circumstances by binding to other signaling molecules such as Akt and Notch, probably to prevent harmful activation of JNK [60, 61]. In neurons, Akt1 directly binds to JIP1 and inhibits the ability of JIP1 to assemble active JNK signaling complexes (with MLK3, MKK7, and JNK), therefore inhibiting JIP1-mediated JNK activity [60]. The downregulation of JNK by Akt1 attenuates excitotoxic neuronal death. Kim et al. showed that Notch1 physically associated with JIP1 through the JNK binding domain, thereby inhibiting the interaction between JIP1 and JNK to prevent JIP1-mediated JNK activation and cell death under stresses such as glucose deprivation [61].

The second member of the JIP family, JIP2, not only has a binding preference similar to JIP1 but also shares similar subcellular localization [57]. Both of them are located in the cytoplasm, and both can form homo-oligomers or hetero-oligomers with each other *in vivo*. Furthermore, both JIP1 and JIP2 bind to MAPK phosphatase 7 (MKP7) and M3/6, which target JNK, and the binding of MKP7 blocks the MLK3-mediated JNK activity enhancement [62]. Whether JIP1 and JIP2 are functionally redundant or have specific individual function in mediating JNK signaling still awaits clarification.

Some studies have indicated that JIP2 is a scaffold protein for the p38 pathway instead of JNK and link the p38 MAPK pathway to upstream signaling [63]. Buchsbaum et al. showed that JIP2 brought the MLK3-MKK3-p38 α module to Tiam1 and Ras-GRF1, two guanine nucleotide exchange factors (GEFs) that activate the Rac GTPase [63]. The binding of Tiam1 and Ras-GRF1 to JIP2 increases the binding efficiency of JIP2 to p38 signaling components and enhances p38 activity. Although the precise function of interaction between Tiam1/Ras-GRF1 and JIP2 remains unclear, it was suggested that this interaction contributes signaling specificity by coupling Rac activation to one of its many downstream target proteins [63]. A study by Robidoux et al. indicated that JIP2 probably assembled a p38 signaling complex including MKK3 and p38 α to mediate PKA-induced p38 activation and promote uncoupling protein 1 (UCP1) expression during cold-induced nonshivering thermogenesis [64]. However, further studies are needed to identify the exact function of JIP2 *in vivo*.

In addition, both JIP1 and JIP2 interact with other non-JNK signaling molecules, including kinesin light chain, apolipoprotein E receptor 2, p190 rhoGEF, and amyloid precursor protein [65–67]. However, whether the interactions of JIP1 and JIP2 with these

molecules would lead to specific JNK activations, and the biological consequences of these interactions, still need to be further elucidated.

JIP3, also known as JNK/stress-activated protein kinase-associated protein 1 (JSAP1), has no significant sequence homology with JIP1 and JIP2. It had been shown that JSAP1/JIP3 binds to different MAP3Ks, including MEKK1, MLK3, and ASK1 [2, 55, 68]. Its N-terminal region is important for the association with these MAP3Ks [2, 55]. JSAP1/JIP3 directly binds to MKK7 with its central region (residues 410–815) [55]. The interaction of JSAP1/JIP3 with SEK1/MKK4 was activation-induced, since the unstimulated MKK4 bound to MEKK1 and only the activated form of MKK4 was found to be associated with JSAP1/JIP3 [2]. JSAP1/JIP3 is thought to be a JNK3 scaffold protein for two reasons. First, although JSAP1/JIP3 bound to all three JNK isoforms, it exhibited higher binding affinity to JNK3 than to the other two isoforms [2, 55]; second, its expression in mouse tissues is similar to that of JNK3 [55]. Ichijo et al. have shown that JSAP/JIP3 not only functioned as a scaffold protein but also dynamically participated in signal transduction by forming a stimulation-dependent functional complex in the ASK1-JNK module in cells in response to H₂O₂ stimulation [68]. Upon H₂O₂ stimulation, ASK1 was activated [69]. The activated ASK1 then phosphorylated JSAP1/JIP3, which led to the recruitment of SEK1/MKK4, MKK7, and JNK3 by JSAP1/JIP3 to assemble a signaling complex and ultimately resulted in the enhancement of JNK3 activity [68].

Like JIP1 and JIP2, JIP3 can also link the JNK signaling module to its upstream activators. In macrophages, JIP3 is associated with TLR4 and is specifically involved in LPS-mediated JNK activation [70]. The cytoplasmic domain of TLR4 is essential for its association with JIP3, and its association with JIP3 is specific since neither JIP1 nor JIP2 was associated with TLR4 [56,57,70]. MEKK1 and TAK1 have been shown to be the essential MAP3Ks for LPS-induced JNK activation [71, 72]. In macrophages, MEKK1, but not TAK1, was found to be constitutively associated with JIP3 [70]. JIP3 was also found to be associated not only with TLR4 but also with TLR2 and 9, indicating its involvement in TLR2 and TLR9 signaling [70]. Therefore, JIP3 probably plays a role in immune responses to microbial infection, but this possibility needs to be further elucidated using technologies such as mouse gene knockout.

Except for linking JNK components to TLRs, JIP3s have been also found to link JNK signaling module to other upstream activators to mediate specific JNK activation. Takino et al. showed that JIP3 cooperated with FAK to regulate JNK activation and cell migra-

tion in response to fibronectin (FN) [73, 74]. FN stimulation of cells induces tyrosine phosphorylation of endogenous FAK and JIP3 [73]. The N-terminal (1–343 amino acid residues) of JIP3 then interacts with FAK to form a stable complex to mediate JNK activation through the module formed by JIP3 [73]. It had been found that JIP3 not only facilitates JNK activation, but also inhibits ERK activation by isolating it from its activators. Using its C-terminal region (residues 1054–1305), JIP3 bound to Raf-1 and MEK1, the ERK activators, to prevent Raf-1 from phosphorylating MEK1 and thereby inhibiting ERK activation [2, 75].

All these studies demonstrate that as a JNK scaffold protein, JIP3 recruits different components to assemble a functional signaling module and connects the module to other cellular components to mediate its activation in response to various stimulations.

A new JIP3-related protein named JIP4, which shares many of the properties of JIP3 such as binding to JNK and to the light chain of the microtubule motor protein kinesin-1, was identified recently [76]. Although it binds to JNK, JIP4 neither binds to JNK activators such as MLK3 and MKK7 nor activates JNK signaling. Instead, it is a p38 scaffold candidate based on the fact that it interacts with p38 isoforms (including p38 α and p38 β) and potentiates p38 activation (which requires MKK3 and MKK6). However, the function of JIP4 still needs to be further explored.

ERK scaffold proteins

Among the several ERK scaffold protein candidates, only kinase suppressor of Ras (KSR) has been confirmed by genetic studies as a bona fide scaffold for the Ras/Raf/MEK/ERK signaling pathway to facilitate ERK activation in mammals [33, 77]. It was first identified in *C. elegans* and *Drosophila melanogaster* in a search for regulatory molecules of the Ras/ERK pathway [78–80]. Nguyen et al. showed that KSR $^{-/-}$ MEF and T cells had reduced activation-induced ERK phosphorylation [33]. Upon TCR activation, KSR $^{-/-}$ T cells were defective in activation, proliferation, and function. Furthermore, a ternary complex containing KSR, MEK, and ERK present in wild-type cells was lost in the knockout cells, indicating that KSR assembled a functional module to facilitate ERK activation. The KSR-deficient mice generated by the Kolesnick group exhibited a disorganized hair follicle phenotype that closely resembles that found in EGFR (epidermal growth factor receptor)-deficient mice [77, 81], indicating the connection between EGFR and KSR-mediated module.

The scaffold function of KSR has been found to be regulated by its two binding partners, C-TAK1 and 14-

3-3. Two KSR1 sites, Ser297 and Ser392, were found to be essential for its association with 14-3-3 [82]. C-TAK1 constitutively associates with the N-terminus of KSR1, and together with nm23-H1, phosphorylates KSR1 at Ser392, which together with Ser297 mediates the binding of 14-3-3 to KSR1 [83, 84]. The binding of 14-3-3 with KSR1 is critical to retain KSR1 in the cytosol of quiescent cells to avoid unnecessary activation of ERK. Upon growth factor stimulation, Ser392 was dephosphorylated, which led to the colocalization of KSR1-mediated signaling complex with activated Ras and Raf-1 at the plasma membrane, thereby facilitating the activation of MEK and ERK [83].

By mediating ERK signaling, KSR1 is involved in multiple physiological events. Lozano et al. showed that v-Ha-ras-mediated skin cancer signaling through the Raf-1/MAPK cascade required KSR1 [77]. Fusello et al. showed that deficiency of KSR resulted in inhibition of ERK activation in response to stress and inflammatory cytokines including TNF α (tumor necrosis factor α) and IL (interleukin)-1, and they found that KSR $^{-/-}$ mice were resistant to K/BxN serum-induced arthritis [85]. Shalin et al. showed that deficiency of KSR in the hippocampus resulted in ERK activation defect specifically in response to PKC-dependent stimuli, which led to deficits in associative learning and theta burst stimulation-induced LTP of the animals [86]. KSR was also found to be involved in adipogenesis [87].

Another possible ERK scaffold protein, MP1, was identified as an MEK partner in a yeast hybridization system [88]. It was found that MP1 selectively bound to MEK1 and ERK to form a ternary complex and facilitated the interaction of the two enzymes. The interaction of MP1 with MEK1 and ERK1 enhanced their activation.

The scaffold function of MP1 is associated with p14, a MP1 binding partner [89,90] that was found to be a highly conserved peripheral member protein enriched in the cytoplasmic face of the late endosomal/lysosomal compartment. p14 interacted with MP1 to recruit MAPK members, including MEK1 and ERK, to modulate ERK signaling [89]. Teis et al. further showed that the binding of p14 with MEK1 and ERK requires MP1, while localization of MP1 at the late endosomes was mediated by p14 [90]. p14 and MP1 cooperatively and specifically promoted ERK activation on endosomes. Pullikuth et al. showed that MP1 and p14 specifically regulated PAK1-dependent ERK activation during adhesion and cell spreading, therefore linking the scaffold function to a particular biological function [91]. Upon EGF stimulation, MP1/p14 associated with PAK1 through its C-terminal to mediate MEK1 phosphorylation by PAK1 and ERK activation and to suppress Rho and ROCK

function, thereby allowing focal adhesion remodeling and member protrusion during spreading on fibronectin. These data indicated that in association with p14, MP1 directs a specific pool of ERK to the cellular compartment and facilitates its activation and function.

IQGAP1, a multidomain molecule, was also identified as scaffold for the ERK pathway [92,93]. The IQ domain of IQGAP1 is necessary for its association with MEK, whereas the WW domain is required for its binding to ERK [92, 93]. Both overexpression and knockdown of IQGAP1 inhibited MEK and ERK activation in response to EGF stimulation [92, 93].

Scaffold proteins involved in multiple signaling pathways

Several molecules could be scaffold proteins of multiple MAPK pathways. β -arrestin 2 is one such molecule which was involved in the signaling of the JNK and ERK pathways. JNK2 and JNK3 were identified as β -arrestin 2 binding partners in a yeast two-hybridization system and β -arrestin 2 was found to specifically associate with JNK3 in vivo [3]. It was found that β -arrestin 2 bound to ASK1 with its N-terminal, bound to JNK3 with its C-terminal, and associated with MKK4 indirectly through ASK1 and JNK3 to assemble a signaling module. Upon agonist stimulation of GPCRs, β -arrestin 2 mediated activation of a specific pool of JNK3 and colocalized the activated JNK3 on the endosomal vesicles [3]. Therefore, β -arrestin 2 not only facilitates but also spatially regulates JNK3 activation in response to GPCR stimulation. However, the targets of this specific pool of JNK3 and its biological effect are still unknown.

Luttrell et al. found that upon stimulation of AT1aR, activated ERK2 formed multi-protein complexes containing AT1aR, β -arrestin 2, and the components of the ERK pathway (including cRaf-1 and MEK1) in endosomal vesicles [94]. Kobayashi et al. showed that β_2 -adrenergic receptor (β_2 AR), one member of GPCRs (G-protein-coupled receptors), mediated ERK nuclear translocation through β -arrestin 2 [95]. β_2 AR activation increased both cytoplasmic and nuclear ERK phosphorylation. The C-terminal regions of β_2 AR interacted with β -arrestin 2 to enhance phospho-ERK nuclear translocation. In contrast to the role of β -arrestin 2 in β_2 AR-mediated ERK nuclear translocation, it was found that β -arrestin 2 retained activated phospho-ERK in the cytosol upon stimulation of angiotensin type 1A receptor, another member of GPCRs [96]. Together, these studies indicated that β -arrestin 2 assembled an ERK signaling module to mediate ERK activation in response to various GPCR stimulations and directed activated

ERK to a specific cellular compartment. However, the role of these specific pools of activated ERK still needs to be studied.

Another molecule, CNK1, has also been found to be a scaffold protein for both the JNK and ERK pathways. CNK was first identified in *Drosophila* as the connector enhancer of KSR (CNK), containing multidomains to associate with different components of Ras cascade to act on several signaling pathways downstream of Ras and upstream or in parallel to Raf [97–99]. In mammals, three CNK members have been identified; of the three, only CNK1 is expressed ubiquitously [100, 101]. It was found that CNK1 associated with two Rho-specific GEFs, Net1 and p115RhoGEF, and components of the JNK pathway (including MLK2, MLK3, and MKK7) to promote Rho-dependent JNK activation [102]. CNK1 was also found to bind activated Src and preactivated Raf-1 to mediate Src-dependent Raf-1 activation [103]. Knockdown of CNK1 with siRNA (small interfering RNA) inhibited ERK activation in response to VEGF (vascular endothelial growth factor) stimulation, suggesting that CNK1 was a scaffold protein that mediated Raf-1 activation and thereby facilitated ERK activation [103]. Other identified CNK1 interacting factors include AT2 receptor and Ras effector Ral-GDS [102, 104]. Therefore, mammalian CNK1 is probably a scaffold protein involved in different MAPK pathways. However, its biological functions await further study.

Overall, scaffold proteins in mammalian species not only associate components of a single MAPK pathway to promote its activation but also can direct signaling modules to a specific subcellular compartment to regulate a special pool of MAPK activation and can connect the signaling modules to upstream activators or to stimuli receptors in the cell membrane to channel the signaling flow. However, the majority of the studies of mammalian scaffold proteins are still limited to *ex vivo* investigations. Their functions in physiological context still need to be explored.

MAPK phosphatases (MKPs) as negative regulators of MAPKs

The activation of MAPKs, the magnitude and duration of their activation, and their inactivation are critical in mediating appropriate physiological responses. Therefore, negative regulation of MAPKs plays essential roles in the MAPK signaling pathway.

Inactivation of MAPKs by MAPK phosphatases

Important advances have recently been made in our understanding of the negative regulation of MAPKs.

One family of proteins, named MAPK phosphatases (MKPs) or dual specificity phosphatases (DUSPs), have been defined as negative regulators of MAP kinases [105–107]. They are identified by their common structural features: a C-terminal catalytic domain containing a highly conserved signature motif HCXXXXXR and two Cdc25-like domains. They inactivate MAPKs through dephosphorylation of threonine and/or tyrosine residues within the signature sequences -Thr-X-Tyr- located in the activation loop of MAPKs.

The inactivation of MAPKs by MKPs is a two-step catalytic reaction [108]. This inactivation begins with nucleophilic attack by the thiolate anion of cysteine in the signature motif of MKPs on the phosphorus atom of phosphotyrosine within MAPKs. Meanwhile, an aspartate in the general acid loop of MKPs donates a proton to the phenolic oxygen atom of phosphotyrosine, which results in the formation of a transient MKP-phosphate intermediate and the release of dephosphorylated MAPK [109]. In the next step, the same aspartate in the general acid loop accepts a proton from a water molecule and the resulting hydroxyl anion attacks the phosphorus atom of the cysteinyl-phosphate intermediate leading to release of inorganic phosphate and regeneration of a thiolate anion at the active site cysteine in the free enzyme. The dephosphorylation of threonine is believed to be a mechanism similar to the dephosphorylation of tyrosine [108].

Thirteen members of this family had been characterized so far, with differential substrate specificities and distinct subcellular localizations and modes of regulation (Table 2). For instance, *in vitro* studies indicated that VHR and MKP-3 are highly specific for ERK; MKP-1, DUSP2, MKP-5, MKP-7, and VH5 have preference for p38/JNK; and MKP-2 and MKP-4 showed a similar preference to the three MAPKs. However, their substrate specificities *in vivo* could be different from their *in vitro* specificities. For example, *in vitro* studies indicated that the MKP-5 substrate preference is as p38~JNK>>ERK [110, 111]. One study using MKP-5 knockout mice showed that MKP-5 is a JNK phosphatase *in vivo* [112]. MKP-5 knockout T cells have increased JNK activity but not increased p38 and ERK activities. Another example of a discrepancy between their substrate specificities *in vivo* and *in vitro* came from the study of Pac-1/DUSP2. Pac-1 had been shown to preferentially dephosphorylate p38 and ERK *in vitro* [113]. Mouse gene knockout study showed that both Pac-1-deficient macrophages and mast cells had reduced p38 and pERK phosphorylation upon activation [114]. On the other hand, the lower affinity sub-

strate *in vitro*, JNK, showed a greater level of activity in knockout cells compared with wild-type cells, indicating that Pac-1 actually is a JNK phosphatase *in vivo*. The reduced ERK activity in Pac-1-deficient cells is probably due to the negative crosstalk between the JNK and ERK pathway [114]. Therefore, their substrate preferences of MKPs need verification using gene knockout animal models. The difference in their substrates *in vitro* and *in vivo* could be due to the fact that other factors such as specific scaffold proteins and docking interaction are involved in regulating MAPK signaling *in vivo*.

MKPs in development

In *Drosophila*, the JNK phosphatase puckered plays a critical role in embryogenesis [115]. Mutations in the *puc* gene resulted in a failure in dorsal closure during *Drosophila* embryogenesis. In *C. elegans*, animals defective in VHP-1, a JNK phosphatase, are arrested during larval development [116]. In vertebrates, MKPs have also been shown to be involved in embryogenesis. For instance, during chicken embryogenesis, the expression of *MKP3* was tightly controlled by fibroblast growth factor 8 (FGF8) to spatially regulate ERK activation [117, 118]. Ecotopic expression of MKP-3 resulted in embryo development defects such as AER disruption, defects in neural plate development, and limb bud truncation [117, 118]. In mammalian species, the only evidence to date of MKP involvement in development comes from the study of MKP-4/DUSP9, which is essential for placental development [119]. The MKP-4-deficient mice died in utero between 8 and 10.5 days postcoitum due to a failure of labyrinth development, which is correlated with the expression pattern of MKP-4 in the trophoblast giant cell and labyrinth of the placenta. However, this study failed to link this phenotype to the role of MKP-4 as a negative regulator of MAPKs because no abnormal activation of MAPKs was detected in the placental tissue of the deficient embryos [119].

MKPs in innate and adaptive immune responses

There are two types of immune responses, innate and adaptive, in vertebrate animals. The innate immune system detects the presence and the nature of infection, provides the first line of host defense, and controls the initiation and determination of the effector class of the adaptive immune response. A specific immune response, such as the production of antibodies against a particular pathogen, is known as adaptive immune response, because it occurs during the lifetime of an individual as an adaptation to infection with that pathogen. Many signal transduc-

Table 2. Mammalian MAPK phosphatases.

Name	Substrate specificity	Other names	Subcellular localization	Human gene localization	Phenotype of knockout mice
VHR	ERK >> JNK ~ p38	DUSP3		17q21	
MKP-2	ERK ~ JNK ~ p38	DUSP4, VHS2, PYT1	nuclear	8p11-12	
MKP-3	ERK >> JNK ~ p38	DUSP6, PYST1	cytosolic	12q22-q23	
MKP-4	ERK ~ JNK ~ p38	DUSP9, PYST3	nuclear/cytosolic	Xq28	embryonic lethal due to a failure of labyrinth development
MKP-6	ERK ~ JNK >> p38	DUSP14, MKP-L		17q12	
VH5	JNK ~ p38 >> ERK	DUSP8, M3/6	nuclear/cytosolic	11p15.5	
Pac-1	JNK >> p38 ~ ERK (?)	DUSP2	nuclear	2q11	decreased pro-inflammatory cytokine productions by macrophages and mast cells; resistant to K/BxN serum-induced arthritis
MKP-5	JNK >> p38 >> ERK	DUSP10	nuclear/cytosolic	1q32	enhanced innate and adaptive immune responses; resistant to MOG-induced EAE; fatal immune response to 2nd LCMV infection
MKP-7	JNK ~ p38 >> ERK	DUSP16	cytosolic	12p12	
MKP-1	p38 ~ JNK >> ERK	DUSP1, HVH1, CL100	nuclear	5q35	enhanced innate immune responses; resistant to LPS-induced hepatic shock; increased CIA-induced arthritis disease; resistant to diet-induced obesity
DSP2	p38 ~ JNK >> ERK	LMW-DSP2			
VH3	N.D.	DUSP5, B23	nuclear	10q25	
PYST2	N.D.	DUSP7, B59, MKP-X	cytosolic	3p21	

The MKPs are subgrouped according to their substrate specificities.

tion pathways participate in both innate and adaptive immune responses. The MAPK signaling cascades play essential regulatory roles in both innate and adaptive immune responses [120].

MKP5 plays a pivotal role in both innate and adaptive immune responses

MAPKs play critical role in both innate and adaptive immune responses [120]. As negative regulators of MAPKs, the role of MKPs in immune responses has been under extensive study recently, especially after one member, MKP-5, had been shown to have a important role in both innate and adaptive immune responses [112]. Using gene knockout mice, MKP-5 was found as an important negative regulator of innate inflammatory cytokine production. Macrophages from MKP-5-deficient mice produced a higher level of inflammatory cytokines in response to various TLR (toll-like receptor) ligands and live *Listeria* infection. Following injection of a sub-lethal dose of LPS (lipopolysaccharide), MKP-5-deficient animals had a

greater serum concentration of TNF α compared with wild-type controls. Dendritic cells from MKP-5-deficient animals displayed increased CD4⁺ and CD8⁺ T cell priming capability. In adaptive immune responses, MKP-5 regulates T cell activation and function differently. On the one hand, MKP-5 acts as a positive regulator of T cell activation and proliferation, since MKP-5-deficient T cells proliferated poorly upon activation. On the other hand, MKP-5 is a negative regulator of T cell effector function. Both CD4⁺ and CD8⁺ T cells produced significantly higher levels of effector cytokines than their wild-type control. Therefore, MKP-5 has a principal function in both innate and adaptive immune responses.

Other MKPs in innate and adaptive immune responses

So far, the roles of two more MKP members, MKP-1 and Pac-1, in addition to MKP-5, in immune responses have been addressed using gene knockout models. The role of MKP-1 in innate immunity, especially in regulating macrophage cytokine production, has been

extensively studied by several groups. It was found that different types of macrophages, including bone marrow-derived macrophages, peritoneal macrophages, and alveolar macrophages, produced significantly increased pro-inflammatory cytokines such as TNF α and IL-6 in response to various TLR activation [121–123]. MKP-1 also negatively regulates the production of IL-10, an anti-inflammatory cytokine, in macrophages [121, 122]. The role of MKP-1 in the regulation of dendritic cell cytokine production has also been addressed [121]. Similar to MKP-1-deficient macrophages, MKP-1-deficient DCs produced increased levels of both pro-inflammatory cytokines (including TNF α and IL-6) and anti-inflammatory cytokine (IL-10). Therefore, MKP-1 is a negative regulator of both pro-inflammatory and anti-inflammatory cytokines in innate immunity. Furthermore, MKP-1 also has a role in endotoxin tolerance or LPS hyporesponsiveness [124], a phenomenon in which pre-treatment of cells with LPS leads to an attenuated response to a subsequent LPS stimulation. Peritoneal macrophages from MKP-1 knockout mice are not fully tolerized by LPS compared with wild-type mice, and the role of MKP-1 in the regulation of endotoxin tolerance is most probably through regulation of p38 activation [124]. Because innate immunity controls adaptive immune responses and MKP-1 plays a critical regulatory role in it, most probably MKP-1 also has a role in regulating adaptive immunity. However, data on the role of MKP-1 in adaptive immune responses are still lacking.

In mice, the expression of Pac-1 in macrophages was rapidly induced by activation [114], indicating its possible role in regulating macrophage function. Indeed, macrophages from Pac-1-deficient mice produced a lower level of pro-inflammatory cytokines, including TNF α , IL-6 and IL-12, compared with wild-type controls. Similarly, mast cells from Pac-1 knockout mice had a lower production of inflammatory cytokines such as TNF α and IL-6. Together, these data indicated that Pac-1 actually plays a positive regulatory role in inflammatory cytokine production of both macrophages and mast cells [114].

Together, these studies showed that the three members of the MKP family all played an indispensable role in regulating MAPKs during immune responses and played an important regulatory role in innate and adaptive immune responses. The *in vivo* function of the majority of MKP members still needs to be addressed. Because there are more numbers of MKPs than MAPKs, one may expect that some of them may be functionally redundant.

MKPs involved in various inflammatory and metabolic disorders

Because MKPs play a critical role in regulating inflammatory cytokine production in both innate and adaptive immunity, it is reasonable to argue that disturbance in their function could be related to various inflammatory diseases. Studies of various MKP gene knockout animals have proved this point. The role of MKP-5 in EAE, a murine model of human multiple sclerosis, has been studied by our group [112]. The MKP-5-deficient mice displayed more resistance than the wild-type controls, as shown by slower disease onset and lower disease severity to MOG-induced EAE. Cell infiltration of the central nervous system indicated that there were significant fewer CD4⁺ cells in the MKP-5 knockout animals than in the wild-type controls, which is consistent with the positive role of MKP-5 in CD4⁺ T cell activation. Considering the critical role of JNK in regulating T cell activation and function and the role of MKP5 as a negative regulator of JNK in T cells, it is possible that MKP-5 is also involved in other T cell-mediated autoimmune diseases such as rheumatoid arthritis. We are currently investigating this possibility.

In rheumatoid arthritis, the deficiency of Pac-1 resulted in protection of the mice from 'K/BxN' serum-induced disease, indicating that Pac-1 actually promotes inflammatory response *in vivo* [114]. MKP-1, on the other hand, probably plays an opposite role in this disease because MKP-1-deficient mice had increased disease incidence and severity in a collagen-induced arthritis model [122].

Several MKP members, including MKP-1, MKP-3, and MKP-4, were found to be involved in metabolic syndrome [20, 125–127]. The role of MKP-1 in metabolic syndrome has been studied using MKP-1-deficient mice [20]. The MKP-1-deficient mice were resistant to diet-induced obesity owing to enhanced energy expenditure. Although ERK, JNK, and p38 activities were all increased in insulin-responsive tissues as compared with wild-type mice, insulin signaling was not disturbed [20]. In the liver, the activities of both p38 and JNK but not ERK were found to be enhanced in the MKP-1-deficient mice compared with wild-type mice, which resulted in enhanced hepatic lipid metabolism. ERK, JNK, and p38 were all hyper-activated in the skeletal muscle of MKP-1-deficient mice as compared with the wild-type mice, which led to increased mitochondrial respiration. It was further found that JNK in the nucleus but not in the cytosol exhibited increased activity, which is consistent with nuclear localization of MKP-1 and the unimpaired insulin-mediated glucose homeostasis [20, 125]. This study demonstrated that MKP-1 regulates fatty acid metabolism and energy expenditure but not

glucose homeostasis by negative regulation of the nuclear pool of JNK, p38, and ERK [20].

MKP-3 and MKP-4 were identified as novel genes that antagonize insulin action on the transcription of *PEPCK*, a key enzyme in hepatic gluconeogenesis [125, 126, 128]. It was further found that the expression of MKP-4 was upregulated in insulin-responsive tissues in the obese state. Overexpression of MKP-4 inhibited adipocyte differentiation and insulin-regulated glucose disposal, indicating the possible role of MKP-4 in the pathogenesis of insulin resistance [126]. Similarly, the expression of MKP-3 was also increased in liver and white adipose tissue but not in muscle from obese mice [126]. Overexpression of MKP-3 opposed the effect of insulin on suppressing *PEPCK* transcription, increased hepatic glucose production and activated gluconeogenic gene expression, indicating that MKP-3 plays a role in modulating the hepatic gluconeogenic program [126]. However, the roles of MKP-3 and MKP-4 in the regulation of insulin signaling and in metabolic syndrome *in vivo* still need further study.

Together, these studies demonstrated that MKPs, like their targets, are involved in main fundamental aspects of cell physiology and in the pathogenesis of a broad range of inflammatory and autoimmune diseases. MKPs could be new therapeutic targets for these diseases. Studies on their individual roles in immune responses and in regulating various inflammatory diseases are still limited, however, and data on the function of human MKPs are still rare. Because there are many more MKPs than MAPKs, it is reasonable to hypothesize that some of them may be redundant in regulating MAPK function. Therefore, much still needs to be learned to fully understand their roles in regulating immune responses *in vivo*.

Regulation of MKPs

The inactivation of MAPKs is critical for the optimal function of MAPKs in immune responses. As major negative regulators of MAPKs, the induction and inactivation of MKPs should also be tightly regulated. A limited number of studies have investigated the signaling mechanisms that regulate MKP expression during immune response. Studies of MKP-1 and MKP-3 expression have demonstrated that negative feedback control of MKP transcription by their target pathways is one such mechanism. In innate immune cells, it had been shown that MKP-1 could be rapidly induced by various TLR activations in macrophages through MyD88 and TRIF signaling. However, the induction of MKP-1 protein started after 30–45 min after TLR activation and peaked at 60 min [129, 130], which is delayed compared with the activation of MAPKs, indicating the presence of a feedback control

mechanism of MKP-1 expression by MAPKs. Using p38 inhibitor and siRNA strategies, Hu et al. showed that inhibition of p38 markedly reduced MKP-1 protein expression in response to LPS and PGN (peptidoglycan) stimulation in macrophages [130]. Wang et al. showed that MKP-1 expression was induced in response to heat shock and that inhibition of p38 activity inhibited MKP-1 expression at both the transcriptional and post-transcriptional levels [131]. Collectively, these studies indicated that activation of p38 regulates MKP-1 expression and thereby controls the magnitude and inactivation of MAPKs in innate immunity.

MKP-3 expression in response to FGF stimulation was also found to be regulated by its target, ERK [121]. ERK inhibition suppressed MKP-3 expression, whereas ectopic expression of MKP-3 inhibited endogenous MKP-3 transcription. However, how p38 activation regulates MKP-1 expression and ERK activation regulates MKP-3 expression is still not clear. Epigenetic regulation study could be used to address how MKP-1 is regulated by p38 during immune responses.

It has been found that the transcriptional activities of two other MKP members, Pac-1 and DUSP5, were regulated by p53 under certain stress conditions [132, 133]. The study from Barrett's group showed that p53 regulated *Pac-1/DUSP2* gene expression during apoptosis mediated by oxidative damage and nutritional stress [132]. Under these stress conditions, activated p53 binds to a palindromic binding site in the Pac-1 promoter region to activate Pac-1 gene transcription. Pac-1 then inhibits MAPK activation to promote apoptosis. DUSP5 is also one of the target genes of p53, and its expression is regulated by p53 [133]. In response to adriamycin-induced DNA damage, p53 bound to the promoter region of *DUSP5* to regulate its expression.

These studies collectively indicate that multiple mechanisms are involved in the regulation of MKP expression in response to different stimuli. Further studies are warranted to determine the detailed mechanisms of MKP gene regulation.

Following an MAPK activation event, once the stimuli are removed, MAPK activation gradually returns to its basal level due to the action of MKPs. The action of MKPs then should be turned off. Ubiquitin-mediated proteolysis is probably a common mechanism to degrade MKPs at the end of a MKP activation event. For instance, in response to LPS stimulation, MKP-1 expression was downregulated to its basal level after 3–4 h [121]. The downregulation of MKP-1 protein levels is most probably due to ubiquitin-mediated proteolysis. Lin et al. had shown that activation of ERK promoted MKP-1 ubiquitination

and degradation in various mammalian cell lines to sustain ERK activation [132]. The activated ERK docked to the MKP-1 DEF motif to phosphorylate its Ser²⁹⁶/Ser³²³ and to promote MKP-1 binding to SCF^{Skp2} ubiquitin ligase, which led to MKP-1 proteolysis in H293 T cells [121].

The stability of another MKP member, MKP-7, is also mediated by ubiquitin-mediated proteolysis and regulated by ERK [70, 134]. Activated ERK bound to the C-terminal region of MKP-7 and phosphorylated its Ser-446, which resulted in the increased stability of MKP-7, probably by blocking the interaction with an E3 ligase in these regions [70, 134]. *In vitro* studies showed that in addition to MKP-1 and MKP-7, the degradation of MKP-2 was also due to ubiquitin-mediated proteolysis [135]. Therefore, ubiquitin-mediated protein degradation probably is a common mechanism for the turnover of MKPs during immune responses. However, information on how MKPs are induced and on turnover is still scarce.

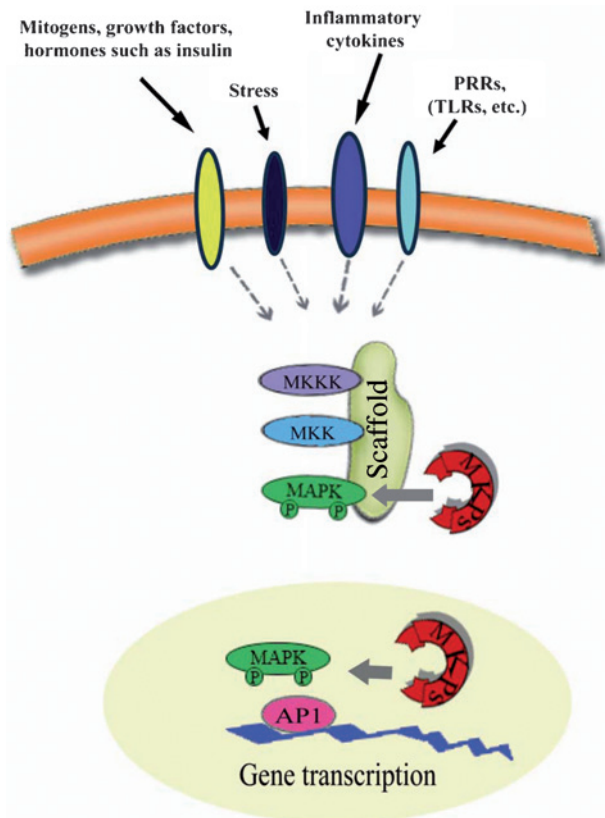


Figure 3. Regulation of MAPKs in immune responses. In response to various stimulation, components of MAPK pathways, including MAP3Ks, MAP2Ks, MAPKs, and MKPs, are assembled into a dynamic signaling complex mediated by scaffold proteins and docking interaction.

Conclusion

MAPKs play fundamental roles in regulating cell responses to internal and external changes. Multiple mechanisms act cooperatively to regulate the initiation, the strength, the duration, and the specificity of their signaling flow so that an optimal biological outcome can be achieved and the health of the body can be maintained (Fig. 3). Errors at any single checkpoint within MAPK signaling could lead to disturbed activation of MAPKs and therefore pathological outcomes such as inflammatory and autoimmune diseases. Understanding how signaling specificity is ensured in each point of the pathways could lead to the discovery of new pharmaceutical targets. To date, many of the studies on components, such as scaffold proteins and MKPs, have been limited to *in vitro* investigation or studies of cell lines. More *in vivo* studies, such as those using gene knockout models, will further our understanding of functions of these components and could therefore lead to advances in the fight against various inflammatory and metabolic diseases.

Acknowledgements. We thank many colleagues and collaborators for their help and discussion. This work is supported in part by grants from the National Institute of Health (C.D.). Y.L. is an Odyssey Scholar supported by the Odyssey Program and the Cockrell Foundation at The University of Texas M. D. Anderson Cancer Center, and C.D. receives an Investigator award from the Cancer Research Institute, an Arthritis Investigator award of the Arthritis Foundation and a Trust Fellowship from the M.D. Anderson Cancer Center.

- Schaeffer, H. J. and Weber, M. J. (1999) Mitogen-activated protein kinases: specific messages from ubiquitous messengers. *Mol. Cell. Biol.* 19, 2435 – 2444.
- Zhao, M., New, L., Kravchenko, V. V., Kato, Y., Gram, H., di Padova, F., Olson, E. N., Ulevitch, R. J., and Han, J. (1999) Regulation of the MEF2 family of transcription factors by p38. *Mol. Cell. Biol.* 19, 21 – 30.
- McDonald, P. H., Chow, C. W., Miller, W. E., Laporte, S. A., Field, M. E., Lin, F. T., Davis, R. J. and Lefkowitz, R. J. (2000) Beta-arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3. *Science* 290, 1574 – 1577.
- Chang, L. and Karin, M. (2001) Mammalian MAP kinase signalling cascades. *Nature* 410, 37 – 40.
- Cobb, M. H. and Goldsmith, E. J. (1995) How MAP kinases are regulated. *J. Biol. Chem.* 270, 14843 – 14846.
- Kuo, W. L., Duke, C. J., Abe, M. K., Kaplan, E. L., Gomes, S. and Rosner, M. R. (2004) ERK7 expression and kinase activity is regulated by the ubiquitin-proteasome pathway. *J. Biol. Chem.* 279, 23073 – 23081.
- Lechner, C., Zahalka, M. A., Giot, J. F., Moller, N. P. and Ullrich, A. (1996) ERK6, a mitogen-activated protein kinase involved in C2C12 myoblast differentiation. *Proc. Natl. Acad. Sci. USA* 93, 4355 – 4359.
- Kyriakis, J. M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E. A., Ahmad, M. F., Avruch, J. and Woodgett, J. R. (1994) The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* 369, 156 – 160.
- Tatebayashi, K., Takekawa, M. and Saito, H. (2003) A docking site determining specificity of Pbs2 MAPKK for

- Ssk2/Ssk22 MAPKKs in the yeast HOG pathway. *EMBO J.* 22, 3624 – 3634.
- 10 Takekawa, M., Tatebayashi, K. and Saito, H. (2005) Conserved docking site is essential for activation of mammalian MAP kinase kinases by specific MAP kinase kinase kinases. *Mol. Cell* 18, 295 – 306.
 - 11 Tu, Z., Mooney, S. M. and Lee, F. S. (2003) A subdomain of MEKK1 that is critical for binding to MKK4. *Cell. Signal.* 15, 65 – 77.
 - 12 Zhou, G., Bao, Z. Q. and Dixon, J. E. (1995) Components of a new human protein kinase signal transduction pathway. *J. Biol. Chem.* 270, 12665 – 12669.
 - 13 Kato, Y., Kravchenko, V. V., Tapping, R. I., Han, J., Ulevitch, R. J. and Lee, J. D. (1997) BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C. *EMBO J.* 16, 7054 – 7066.
 - 14 Holland, P. M. and Cooper, J. A. (1999) Protein modification: docking sites for kinases. *Curr. Biol.* 9, R329 – 331.
 - 15 Bardwell, L. and Thorner, J. (1996) A conserved motif at the amino termini of MEKs might mediate high-affinity interaction with the cognate MAPKs. *Trends Biochem. Sci.* 21, 373 – 374.
 - 16 Xu, B., Stippec, S., Robinson, F. L. and Cobb, M. H. (2001) Hydrophobic as well as charged residues in both MEK1 and ERK2 are important for their proper docking. *J. Biol. Chem.* 276, 26509 – 26515.
 - 17 Bardwell, A. J., Flatauer, L. J., Matsukuma, K., Thorner, J. and Bardwell, L. (2001) A conserved docking site in MEKs mediates high-affinity binding to MAP kinases and cooperates with a scaffold protein to enhance signal transmission. *J. Biol. Chem.* 276, 10374 – 10386.
 - 18 Enslin, H., Brancho, D. M. and Davis, R. J. (2000) Molecular determinants that mediate selective activation of p38 MAP kinase isoforms. *EMBO J.* 19, 1301 – 1311.
 - 19 Ho, D. T., Bardwell, A. J., Abdollahi, M. and Bardwell, L. (2003) A docking site in MKK4 mediates high affinity binding to JNK MAPKs and competes with similar docking sites in JNK substrates. *J. Biol. Chem.* 278, 32662 – 32672.
 - 20 Wu, J. J., Roth, R. J., Anderson, E. J., Hong, E. G., Lee, M. K., Choi, C. S., Neuffer, P. D., Shulman, G. I., Kim, J. K. and Bennett, A. M. (2006) Mice lacking MAP kinase phosphatase-1 have enhanced MAP kinase activity and resistance to diet-induced obesity. *Cell Metab.* 4, 61 – 73.
 - 21 Xu, B., Wilsbacher, J. L., Collisson, T. and Cobb, M. H. (1999) The N-terminal ERK-binding site of MEK1 is required for efficient feedback phosphorylation by ERK2 in vitro and ERK activation in vivo. *J. Biol. Chem.* 274, 34029 – 34035.
 - 22 Seyfried, J., Wang, X., Kharebava, G. and Tournier, C. (2005) A novel mitogen-activated protein kinase docking site in the N terminus of MEK5 α organizes the components of the extracellular signal-regulated kinase 5 signaling pathway. *Mol. Cell. Biol.* 25, 9820 – 9828.
 - 23 Tanoue, T., Adachi, M., Moriguchi, T. and Nishida, E. (2000) A conserved docking motif in MAP kinases common to substrates, activators and regulators. *Nat. Cell Biol.* 2, 110 – 116.
 - 24 Tanoue, T., Maeda, R., Adachi, M. and Nishida, E. (2001) Identification of a docking groove on ERK and p38 MAP kinases that regulates the specificity of docking interactions. *EMBO J.* 20, 466 – 479.
 - 25 Adler, V., Polotskaya, A., Wagner, F. and Kraft, A. S. (1992) Affinity-purified c-Jun amino-terminal protein kinase requires serine/threonine phosphorylation for activity. *J. Biol. Chem.* 267, 17001 – 17005.
 - 26 Hibi, M., Lin, A., Smeal, T., Minden, A. and Karin, M. (1993) Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev.* 7, 2135 – 2148.
 - 27 Seidel, J. J. and Graves, B. J. (2002) An ERK2 docking site in the Pointed domain distinguishes a subset of ETS transcription factors. *Genes Dev.* 16, 127 – 137.
 - 28 Barsyte-Lovejoy, D., Galanis, A. and Sharrocks, A. D. (2002) Specificity determinants in MAPK signaling to transcription factors. *J. Biol. Chem.* 277, 9896 – 9903.
 - 29 Yang, S. H., Galanis, A. and Sharrocks, A. D. (1999) Targeting of p38 mitogen-activated protein kinases to MEF2 transcription factors. *Mol. Cell. Biol.* 19, 4028 – 4038.
 - 30 Jacobs, D., Beitel, G. J., Clark, S. G., Horvitz, H. R. and Kornfeld, K. (1998) Gain-of-function mutations in the *Caenorhabditis elegans* lin-1 ETS gene identify a C-terminal regulatory domain phosphorylated by ERK MAP kinase. *Genetics* 149, 1809 – 1822.
 - 31 Jacobs, D., Glossip, D., Xing, H., Muslin, A. J. and Kornfeld, K. (1999) Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase. *Genes Dev.* 13, 163 – 175.
 - 32 Fantz, D. A., Jacobs, D., Glossip, D. and Kornfeld, K. (2001) Docking sites on substrate proteins direct extracellular signal-regulated kinase to phosphorylate specific residues. *J. Biol. Chem.* 276, 27256 – 27265.
 - 33 Nguyen, A., Burack, W. R., Stock, J. L., Kortum, R., Chaika, O. V., Afkarian, M., Muller, W. J., Murphy, K. M., Morrison, D. K., Lewis, R. E. et al. (2002) Kinase suppressor of Ras (KSR) is a scaffold which facilitates mitogen-activated protein kinase activation in vivo. *Mol. Cell. Biol.* 22, 3035 – 3045.
 - 34 Gallo, A., Cuozzo, C., Esposito, I., Maggiolini, M., Bonfiglioglio, D., Vivacqua, A., Garramone, M., Weiss, C., Bohmann, D. and Musti, A. M. (2002) Menin uncouples Elk-1, JunD and c-Jun phosphorylation from MAP kinase activation. *Oncogene* 21, 6434 – 6445.
 - 35 Yazgan, O. and Pfarr, C. M. (2002) Regulation of two JunD isoforms by Jun N-terminal kinases. *J. Biol. Chem.* 277, 29710 – 29718.
 - 36 Vinciguerra, M., Vivacqua, A., Fasanella, G., Gallo, A., Cuozzo, C., Morano, A., Maggiolini, M. and Musti, A. M. (2004) Differential phosphorylation of c-Jun and JunD in response to the epidermal growth factor is determined by the structure of MAPK targeting sequences. *J. Biol. Chem.* 279, 9634 – 9641.
 - 37 Yang, S. H., Whitmarsh, A. J., Davis, R. J. and Sharrocks, A. D. (1998) Differential targeting of MAP kinases to the ETS-domain transcription factor Elk-1. *EMBO J.* 17, 1740 – 1749.
 - 38 Lee, T., Hoofnagle, A. N., Kabuyama, Y., Stroud, J., Min, X., Goldsmith, E. J., Chen, L., Resing, K. A. and Ahn, N. G. (2004) Docking motif interactions in MAP kinases revealed by hydrogen exchange mass spectrometry. *Mol. Cell* 14, 43 – 55.
 - 39 Dimitri, C. A., Dowdle, W., MacKeigan, J. P., Blenis, J. and Murphy, L. O. (2005) Spatially separate docking sites on ERK2 regulate distinct signaling events in vivo. *Curr. Biol.* 15, 1319 – 1324.
 - 40 Hancock, C. N., Macias, A., Lee, E. K., Yu, S. Y., Mackerell, A. D., Jr. and Shapiro, P. (2005) Identification of novel extracellular signal-regulated kinase docking domain inhibitors. *J. Med. Chem.* 48, 4586 – 4595.
 - 41 Morrison, D. K. and Davis, R. J. (2003) Regulation of MAP kinase signaling modules by scaffold proteins in mammals. *Annu. Rev. Cell Dev. Biol.* 19, 91 – 118.
 - 42 Sprague, G. F., Jr. (1998) Control of MAP kinase signaling specificity or how not to go HOG wild. *Genes Dev.* 12, 2817 – 2820.
 - 43 Garrington, T. P. and Johnson, G. L. (1999) Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr. Opin. Cell Biol.* 11, 211 – 218.
 - 44 Posas, F. and Saito, H. (1997) Osmotic activation of the HOG MAPK pathway via Ste11p MAPKKK: scaffold role of Pbs2p MAPKK. *Science* 276, 1702 – 1705.
 - 45 Posas, F., Witten, E. A. and Saito, H. (1998) Requirement of STE50 for osmotic stress-induced activation of the STE11 mitogen-activated protein kinase kinase in the high-

- osmolarity glycerol response pathway. *Mol. Cell. Biol.* 18, 5788 – 5796.
- 46 Elion, E. A. (2001) The Ste5p scaffold. *J. Cell Sci.* 114, 3967 – 3978.
- 47 O'Rourke, S. M. and Herskowitz, I. (2002) A third osmosensing branch in *Saccharomyces cerevisiae* requires the Msb2 protein and functions in parallel with the Sho1 branch. *Mol. Cell. Biol.* 22, 4739 – 4749.
- 48 Seet, B. T. and Pawson, T. (2004) MAPK signaling: Sho business. *Curr. Biol.* 14, R708 – 710.
- 49 Harris, K., Lamson, R. E., Nelson, B., Hughes, T. R., Marton, M. J., Roberts, C. J., Boone, C. and Pryciak, P. M. (2001) Role of scaffolds in MAP kinase pathway specificity revealed by custom design of pathway-dedicated signaling proteins. *Curr. Biol.* 11, 1815 – 1824.
- 50 Park, S. H., Zarrinpar, A. and Lim, W. A. (2003) Rewiring MAP kinase pathways using alternative scaffold assembly mechanisms. *Science* 299, 1061 – 1064.
- 51 Madhani, H. D. and Fink, G. R. (1998) The riddle of MAP kinase signaling specificity. *Trends Genet.* 14, 151 – 155.
- 52 Elion, E. A. (1998) Routing MAP kinase cascades. *Science* 281, 1625 – 1626.
- 53 Pryciak, P. M. and Huntress, F. A. (1998) Membrane recruitment of the kinase cascade scaffold protein Ste5 by the Gbetagamma complex underlies activation of the yeast pheromone response pathway. *Genes Dev.* 12, 2684 – 2697.
- 54 Yashar, B., Irie, K., Printen, J. A., Stevenson, B. J., Sprague, G. F., Jr., Matsumoto, K. and Errede, B. (1995) Yeast MEK-dependent signal transduction: response thresholds and parameters affecting fidelity. *Mol. Cell. Biol.* 15, 6545 – 6553.
- 55 Kelkar, N., Gupta, S., Dickens, M. and Davis, R. J. (2000) Interaction of a mitogen-activated protein kinase signaling module with the neuronal protein JIP3. *Mol. Cell. Biol.* 20, 1030 – 1043.
- 56 Whitmarsh, A. J., Cavanagh, J., Tournier, C., Yasuda, J. and Davis, R. J. (1998) A mammalian scaffold complex that selectively mediates MAP kinase activation. *Science* 281, 1671 – 1674.
- 57 Yasuda, J., Whitmarsh, A. J., Cavanagh, J., Sharma, M. and Davis, R. J. (1999) The JIP group of mitogen-activated protein kinase scaffold proteins. *Mol. Cell. Biol.* 19, 7245 – 7254.
- 58 Boomer, J. S. and Tan, T. H. (2005) Functional interactions of HPK1 with adaptor proteins. *J. Cell Biochem.* 95, 34 – 44.
- 59 Whitmarsh, A. J., Kuan, C., Kennedy, N., Kelkar, N., Haydar, T., Mordes, J., Appel, M., Rossini, A., Jones, S., Flavell, R. et al. (2001) Requirement of the JIP1 scaffold protein for stress-induced JNK activation. *Genes Dev.* 15, 2421 – 2432.
- 60 Kim, A. H., Yano, H., Cho, H., Meyer, D., Monks, B., Margolis, B., Birnbaum, M. J. and Chao, M. V. (2002) Akt1 regulates a JNK scaffold during excitotoxic apoptosis. *Neuron* 35, 697 – 709.
- 61 Kim, J. W., Kim, M. J., Kim, K. J., Yun, H. J., Chae, J. S., Hwang, S. G., Chang, T. S., Park, H. S., Lee, K. W., Han, P. L. et al. (2005) Notch interferes with the scaffold function of JNK-interacting protein 1 to inhibit the JNK signaling pathway. *Proc. Natl. Acad. Sci. USA* 102, 14308 – 14313.
- 62 Willoughby, E. A., Perkins, G. R., Collins, M. K. and Whitmarsh, A. J. (2003) The JNK-interacting protein-1 scaffold protein targets MAPK phosphatase-7 to dephosphorylate JNK. *J. Biol. Chem.* 278, 10731 – 10736.
- 63 Buchsbaum, R. J., Connolly, B. A. and Feig, L. A. (2002) Interaction of Rac exchange factors Tiam1 and Ras-GRF1 with a scaffold for the p38 mitogen-activated protein kinase cascade. *Mol. Cell. Biol.* 22, 4073 – 4085.
- 64 Robidoux, J., Cao, W., Quan, H., Daniel, K. W., Moukdar, F., Bai, X., Floering, L. M. and Collins, S. (2005) Selective activation of mitogen-activated protein (MAP) kinase kinase 3 and p38alpha MAP kinase is essential for cyclic AMP-dependent UCP1 expression in adipocytes. *Mol. Cell. Biol.* 25, 5466 – 5479.
- 65 Verhey, K. J., Meyer, D., Deehan, R., Blenis, J., Schnapp, B. J., Rapoport, T. A. and Margolis, B. (2001) Cargo of kinesin identified as JIP scaffolding proteins and associated signaling molecules. *J. Cell. Biol.* 152, 959 – 970.
- 66 Meyer, D., Liu, A. and Margolis, B. (1999) Interaction of c-Jun amino-terminal kinase interacting protein-1 with p190 rho-GEF and its localization in differentiated neurons. *J. Biol. Chem.* 274, 35113 – 35118.
- 67 Stockinger, W., Brandes, C., Fasching, D., Hermann, M., Gotthardt, M., Herz, J., Schneider, W. J. and Nimpf, J. (2000) The reelin receptor ApoER2 recruits JNK-interacting proteins-1 and -2. *J. Biol. Chem.* 275, 25625 – 25632.
- 68 Matsuura, H., Nishitoh, H., Takeda, K., Matsuzawa, A., Amagasa, T., Ito, M., Yoshioka, K. and Ichijo, H. (2002) Phosphorylation-dependent scaffolding role of JSAP1/JIP3 in the ASK1-JNK signaling pathway. *J. Biol. Chem.* 277, 40703 – 40709.
- 69 Tobiume, K., Matsuzawa, A., Takahashi, T., Nishitoh, H., Morita, K., Takeda, K., Minowa, O., Miyazono, K., Noda, T. and Ichijo, H. (2001) ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. *EMBO Rep.* 2, 222 – 228.
- 70 Matsuguchi, T., Masuda, A., Sugimoto, K., Nagai, Y. and Yoshikai, Y. (2003) JNK-interacting protein 3 associates with Toll-like receptor 4 and is involved in LPS-mediated JNK activation. *EMBO J.* 22, 4455 – 4464.
- 71 Kopp, E., Medzhitov, R., Carothers, J., Xiao, C., Douglas, I., Janeway, C. A. and Ghosh, S. (1999) ECSIT is an evolutionarily conserved intermediate in the Toll/IL-1 signal transduction pathway. *Genes Dev.* 13, 2059 – 2071.
- 72 Ninomiya-Tsuji, J., Kishimoto, K., Hiyama, A., Inoue, J., Cao, Z. and Matsumoto, K. (1999) The kinase TAK1 can activate the NIK-I kappaB as well as the MAP kinase cascade in the IL-1 signalling pathway. *Nature* 398, 252 – 256.
- 73 Takino, T., Yoshioka, K., Miyamori, H., Yamada, K. M. and Sato, H. (2002) A scaffold protein in the c-Jun N-terminal kinase signaling pathway is associated with focal adhesion kinase and tyrosine-phosphorylated. *Oncogene* 21, 6488 – 6497.
- 74 Takino, T., Nakada, M., Miyamori, H., Watanabe, Y., Sato, T., Gantulga, D., Yoshioka, K., Yamada, K. M. and Sato, H. (2005) JSAP1/JIP3 cooperates with focal adhesion kinase to regulate c-Jun N-terminal kinase and cell migration. *J. Biol. Chem.* 280, 37772 – 37781.
- 75 Kuboki, Y., Ito, M., Takamatsu, N., Yamamoto, K. I., Shiba, T. and Yoshioka, K. (2000) A scaffold protein in the c-Jun NH2-terminal kinase signaling pathways suppresses the extracellular signal-regulated kinase signaling pathways. *J. Biol. Chem.* 275, 39815 – 39818.
- 76 Kelkar, N., Standen, C. L. and Davis, R. J. (2005) Role of the JIP4 scaffold protein in the regulation of mitogen-activated protein kinase signaling pathways. *Mol. Cell. Biol.* 25, 2733 – 2743.
- 77 Lozano, J., Xing, R., Cai, Z., Jensen, H. L., Trempus, C., Mark, W., Cannon, R. and Kolesnick, R. (2003) Deficiency of kinase suppressor of Ras1 prevents oncogenic ras signaling in mice. *Cancer Res.* 63, 4232 – 4238.
- 78 Kornfeld, K., Hom, D. B. and Horvitz, H. R. (1995) The *ksr-1* gene encodes a novel protein kinase involved in Ras-mediated signaling in *C. elegans*. *Cell* 83, 903 – 913.
- 79 Sundaram, M. and Han, M. (1995) The *C. elegans ksr-1* gene encodes a novel Raf-related kinase involved in Ras-mediated signal transduction. *Cell* 83, 889 – 901.
- 80 Therrien, M., Chang, H. C., Solomon, N. M., Karim, F. D., Wassarman, D. A. and Rubin, G. M. (1995) KSR, a novel protein kinase required for RAS signal transduction. *Cell* 83, 879 – 888.
- 81 Hamilton, M. and Wolfman, A. (1998) Oncogenic Ha-Ras-dependent mitogen-activated protein kinase activity requires signaling through the epidermal growth factor receptor. *J. Biol. Chem.* 273, 28155 – 28162.
- 82 Cacace, A. M., Michaud, N. R., Therrien, M., Mathes, K., Copeland, T., Rubin, G. M. and Morrison, D. K. (1999) Identification of constitutive and ras-inducible phosphoryla-

- tion sites of KSR: implications for 14-3-3 binding, mitogen-activated protein kinase binding, and KSR overexpression. *Mol. Cell Biol.* 19, 229 – 240.
- 83 Muller, J., Ory, S., Copeland, T., Piwnica-Worms, H. and Morrison, D. K. (2001) C-TAK1 regulates Ras signaling by phosphorylating the MAPK scaffold, KSR1. *Mol. Cell* 8, 983 – 993.
- 84 Hartsough, M. T., Morrison, D. K., Salerno, M., Palmieri, D., Ouatas, T., Mair, M., Patrick, J. and Steeg, P. S. (2002) Nm23-H1 metastasis suppressor phosphorylation of kinase suppressor of Ras via a histidine protein kinase pathway. *J. Biol. Chem.* 277, 32389 – 32399.
- 85 Fusello, A. M., Mandik-Nayak, L., Shih, F., Lewis, R. E., Allen, P. M. and Shaw, A. S. (2006) The MAPK scaffold kinase suppressor of Ras is involved in ERK activation by stress and proinflammatory cytokines and induction of arthritis. *J. Immunol.* 177, 6152 – 6158.
- 86 Shalin, S. C., Hernandez, C. M., Dougherty, M. K., Morrison, D. K. and Sweatt, J. D. (2006) Kinase suppressor of Ras1 compartmentalizes hippocampal signal transduction and subserves synaptic plasticity and memory formation. *Neuron* 50, 765 – 779.
- 87 Kortum, R. L., Costanzo, D. L., Haferbier, J., Schreiner, S. J., Razidlo, G. L., Wu, M. H., Volle, D. J., Mori, T., Sakae, H., Chaika, N. V., Chaika, O. V. and Lewis, R. E. (2005) The molecular scaffold kinase suppressor of Ras 1 (KSR1) regulates adipogenesis. *Mol. Cell Biol.* 25, 7592 – 7604.
- 88 Schaeffer, H. J., Catling, A. D., Eblen, S. T., Collier, L. S., Krauss, A. and Weber, M. J. (1998) MP1: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade. *Science* 281, 1668 – 1671.
- 89 Wunderlich, W., Fialka, I., Teis, D., Alpi, A., Pfeifer, A., Parton, R. G., Lottspeich, F. and Huber, L. A. (2001) A novel 14-kilodalton protein interacts with the mitogen-activated protein kinase scaffold mp1 on a late endosomal/lysosomal compartment. *J. Cell Biol.* 152, 765 – 76.
- 90 Teis, D., Wunderlich, W. and Huber, L. A. (2002) Localization of the MP1-MAPK scaffold complex to endosomes is mediated by p14 and required for signal transduction. *Dev. Cell* 3, 803 – 814.
- 91 Pullikuth, A., McKinnon, E., Schaeffer, H. J. and Catling, A. D. (2005) The MEK1 scaffolding protein MP1 regulates cell spreading by integrating PAK1 and Rho signals. *Mol. Cell Biol.* 25, 5119 – 5133.
- 92 Roy, M., Li, Z. and Sacks, D. B. (2004) IQGAP1 binds ERK2 and modulates its activity. *J. Biol. Chem.* 279, 17329 – 17337.
- 93 Roy, M., Li, Z. and Sacks, D. B. (2005) IQGAP1 is a scaffold for mitogen-activated protein kinase signaling. *Mol. Cell Biol.* 25, 7940 – 7952.
- 94 Luttrell, L. M., Roudabush, F. L., Choy, E. W., Miller, W. E., Field, M. E., Pierce, K. L. and Lefkowitz, R. J. (2001) Activation and targeting of extracellular signal-regulated kinases by beta-arrestin scaffolds. *Proc. Natl. Acad. Sci. USA* 98, 2449 – 2454.
- 95 Kobayashi, H., Narita, Y., Nishida, M. and Kurose, H. (2005) Beta-arrestin2 enhances beta2-adrenergic receptor-mediated nuclear translocation of ERK. *Cell Signal.* 17, 1248 – 1253.
- 96 Wei, H., Ahn, S., Barnes, W. G. and Lefkowitz, R. J. (2004) Stable interaction between beta-arrestin 2 and angiotensin type 1A receptor is required for beta-arrestin 2-mediated activation of extracellular signal-regulated kinases 1 and 2. *J. Biol. Chem.* 279, 48255 – 48261.
- 97 Therrien, M., Wong, A. M. and Rubin, G. M. (1998) CNK, a RAF-binding multidomain protein required for RAS signaling. *Cell* 95, 343 – 353.
- 98 Douziech, M., Roy, F., Laberge, G., Lefrancois, M., Armenogod, A. V. and Therrien, M. (2003) Bimodal regulation of RAF by CNK in *Drosophila*. *EMBO J.* 22, 5068 – 5078.
- 99 Therrien, M., Wong, A. M., Kwan, E. and Rubin, G. M. (1999) Functional analysis of CNK in RAS signaling. *Proc. Natl. Acad. Sci. USA* 96, 13259 – 13263.
- 100 Yao, I., Hata, Y., Ide, N., Hirao, K., Deguchi, M., Nishioka, H., Mizoguchi, A. and Takai, Y. (1999) MAGUIN, a novel neuronal membrane-associated guanylate kinase-interacting protein. *J. Biol. Chem.* 274, 11889 – 11896.
- 101 Ohtakara, K., Nishizawa, M., Izawa, I., Hata, Y., Matsushima, S., Taki, W., Inada, H., Takai, Y. and Inagaki, M. (2002) Densin-180, a synaptic protein, links to PSD-95 through its direct interaction with MAGUIN-1. *Genes Cells* 7, 1149 – 1160.
- 102 Jaffe, A. B., Aspenstrom, P. and Hall, A. (2004) Human CNK1 acts as a scaffold protein, linking Rho and Ras signal transduction pathways. *Mol. Cell Biol.* 24, 1736 – 1746.
- 103 Ziegas, A., Moelling, K. and Radziwill, G. (2005) CNK1 is a scaffold protein that regulates Src-mediated Raf-1 activation. *J. Biol. Chem.* 280, 24205 – 24211.
- 104 Fritz, R. D. and Radziwill, G. (2005) The scaffold protein CNK1 interacts with the angiotensin II type 2 receptor. *Biochem. Biophys. Res. Commun.* 338, 1906 – 1912.
- 105 Camps, M., Nichols, A. and Arkinstall, S. (2000) Dual specificity phosphatases: a gene family for control of MAP kinase function. *FASEB J.* 14, 6 – 16.
- 106 Theodosiou, A. and Ashworth, A. (2002) MAP kinase phosphatases. *Genome Biol.* 3, REVIEWS3009.
- 107 Farooq, A. and Zhou, M. M. (2004) Structure and regulation of MAPK phosphatases. *Cell Signal.* 16, 769 – 779.
- 108 Denu, J. M. and Dixon, J. E. (1998) Protein tyrosine phosphatases: mechanisms of catalysis and regulation. *Curr. Opin. Chem. Biol.* 2, 633 – 641.
- 109 Zhao, Y. and Zhang, Z. Y. (2001) The mechanism of dephosphorylation of extracellular signal-regulated kinase 2 by mitogen-activated protein kinase phosphatase 3. *J. Biol. Chem.* 276, 32382 – 32391.
- 110 Theodosiou, A., Smith, A., Gillieron, C., Arkinstall, S. and Ashworth, A. (1999) MKP5, a new member of the MAP kinase phosphatase family, which selectively dephosphorylates stress-activated kinases. *Oncogene* 18, 6981 – 6988.
- 111 Tanoue, T., Moriguchi, T. and Nishida, E. (1999) Molecular cloning and characterization of a novel dual specificity phosphatase, MKP-5. *J. Biol. Chem.* 274, 19949 – 19956.
- 112 Zhang, Y., Blattman, J. N., Kennedy, N. J., Duong, J., Nguyen, T., Wang, Y., Davis, R. J., Greenberg, P. D., Flavell, R. A. and Dong, C. (2004) Regulation of innate and adaptive immune responses by MAP kinase phosphatase 5. *Nature* 430, 793 – 797.
- 113 Ward, Y., Gupta, S., Jensen, P., Wartmann, M., Davis, R. J. and Kelly, K. (1994) Control of MAP kinase activation by the mitogen-induced threonine/tyrosine phosphatase PAC1. *Nature* 367, 651 – 654.
- 114 Jeffrey, K. L., Brummer, T., Rolph, M. S., Liu, S. M., Callejas, N. A., Grumont, R. J., Gillieron, C., Mackay, F., Grey, S., Camps, M. et al. (2006) Positive regulation of immune cell function and inflammatory responses by phosphatase PAC-1. *Nat. Immunol.* 7, 274 – 283.
- 115 Martin-Blanco, E., Gampel, A., Ring, J., Virdee, K., Kirov, N., Tolkovsky, A. M. and Martinez-Arias, A. (1998) puckered encodes a phosphatase that mediates a feedback loop regulating JNK activity during dorsal closure in *Drosophila*. *Genes Dev.* 12, 557 – 570.
- 116 Mizuno, T., Hisamoto, N., Terada, T., Kondo, T., Adachi, M., Nishida, E., Kim, D. H., Ausubel, F. M. and Matsumoto, K. (2004) The *Caenorhabditis elegans* MAPK phosphatase VHP-1 mediates a novel JNK-like signaling pathway in stress response. *EMBO J.* 23, 2226 – 2234.
- 117 Kawakami, Y., Rodriguez-Leon, J., Koth, C. M., Buscher, D., Itoh, T., Raya, A., Ng, J. K., Esteban, C. R., Takahashi, S., Henrique, D. et al. (2003) MKP3 mediates the cellular response to FGF8 signalling in the vertebrate limb. *Nat. Cell Biol.* 5, 513 – 519.
- 118 Eblaghie, M. C., Lunn, J. S., Dickinson, R. J., Munsterberg, A. E., Sanz-Ezquerro, J. J., Farrell, E. R., Mathers, J., Keyse, S. M., Storey, K. and Tickle, C. (2003) Negative feedback

- regulation of FGF signaling levels by Pyst1/MKP3 in chick embryos. *Curr. Biol.* 13, 1009 – 1018.
- 119 Christie, G. R., Williams, D. J., Macisaac, F., Dickinson, R. J., Rosewell, I. and Keyse, S. M. (2005) The dual-specificity protein phosphatase DUSP9/MKP-4 is essential for placental function but is not required for normal embryonic development. *Mol. Cell. Biol.* 25, 8323 – 8333.
- 120 Dong, C., Davis, R. J. and Flavell, R. A. (2002) MAP kinases in the immune response. *Annu. Rev. Immunol.* 20, 55 – 72.
- 121 Zhao, Q., Wang, X., Nelin, L. D., Yao, Y., Matta, R., Manson, M. E., Baliga, R. S., Meng, X., Smith, C. V., Bauer, J. A. et al. (2006) MAP kinase phosphatase 1 controls innate immune responses and suppresses endotoxin shock. *J. Exp. Med.* 203, 131 – 140.
- 122 Salojin, K. V., Owusu, I. B., Millerchip, K. A., Potter, M., Platt, K. A. and Oravec, T. (2006) Essential role of MAPK phosphatase-1 in the negative control of innate immune responses. *J. Immunol.* 176, 1899 – 1907.
- 123 Zhao, Q., Shepherd, E. G., Manson, M. E., Nelin, L. D., Sorokin, A. and Liu, Y. (2005) The role of mitogen-activated protein kinase phosphatase-1 in the response of alveolar macrophages to lipopolysaccharide: attenuation of proinflammatory cytokine biosynthesis via feedback control of p38. *J. Biol. Chem.* 280, 8101 – 8108.
- 124 Nimah, M., Zhao, B., Denenberg, A. G., Bueno, O., Molken- tin, J., Wong, H. R. and Shanley, T. P. (2005) Contribution of MKP-1 regulation of p38 to endotoxin tolerance. *Shock* 23, 80 – 87.
- 125 Xu, H., Yang, Q., Shen, M., Huang, X., Dembski, M., Gimeno, R., Tartaglia, L. A., Kapeller, R. and Wu, Z. (2005) Dual specificity MAPK phosphatase 3 activates PEPCK gene transcription and increases gluconeogenesis in rat hepatoma cells. *J. Biol. Chem.* 280, 36013 – 36018.
- 126 Xu, H., Dembski, M., Yang, Q., Yang, D., Moriarty, A., Tayber, O., Chen, H., Kapeller, R. and Tartaglia, L. A. (2003) Dual specificity mitogen-activated protein (MAP) kinase phosphatase-4 plays a potential role in insulin resistance. *J. Biol. Chem.* 278, 30187 – 30192.
- 127 Bazuine, M., Carlotti, F., Tafrechí, R. S., Hoeben, R. C. and Maassen, J. A. (2004) Mitogen-activated protein kinase (MAPK) phosphatase-1 and -4 attenuate p38 MAPK during dexamethasone-induced insulin resistance in 3T3-L1 adipocytes. *Mol. Endocrinol.* 18, 1697 – 1707.
- 128 Hanson, R. W. and Reshef, L. (1997) Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. *Annu. Rev. Biochem.* 66, 581 – 611.
- 129 Shepherd, E. G., Zhao, Q., Welty, S. E., Hansen, T. N., Smith, C. V. and Liu, Y. (2004) The function of mitogen-activated protein kinase phosphatase-1 in peptidoglycan-stimulated macrophages. *J. Biol. Chem.* 279, 54023 – 54031.
- 130 Hu, J. H., Chen, T., Zhuang, Z. H., Kong, L., Yu, M. C., Liu, Y., Zang, J. W. and Ge, B. X. (2007) Feedback control of MKP-1 expression by p38. *Cell Signal.* 19, 393 – 400.
- 131 Wong, H. R., Dunsmore, K. E., Page, K. and Shanley, T. P. (2005) Heat shock-mediated regulation of MKP-1. *Am. J. Physiol. Cell Physiol.* 289, C1152 – 1158.
- 132 Yin, Y., Liu, Y. X., Jin, Y. J., Hall, E. J. and Barrett, J. C. (2003) PAC1 phosphatase is a transcription target of p53 in signalling apoptosis and growth suppression. *Nature* 422, 527 – 531.
- 133 Ueda, K., Arakawa, H. and Nakamura, Y. (2003) Dual-specificity phosphatase 5 (DUSP5) as a direct transcriptional target of tumor suppressor p53. *Oncogene* 22, 5586 – 5591.
- 134 Katagiri, C., Masuda, K., Urano, T., Yamashita, K., Araki, Y., Kikuchi, K. and Shima, H. (2005) Phosphorylation of Ser-446 determines stability of MKP-7. *J. Biol. Chem.* 280, 14716 – 14722.
- 135 Torres, C., Francis, M. K., Lorenzini, A., Tresini, M. and Cristofalo, V. J. (2003) Metabolic stabilization of MAP kinase phosphatase-2 in senescence of human fibroblasts. *Exp. Cell Res.* 290, 195 – 206.

To access this journal online:
<http://www.birkhauser.ch/CMLS>
