Current Biology, Vol. 13, R886–R888, November 11, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.cub.2003.10.053

MAP Kinase Modules: Many Roads Home

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All known MAP kinase cascades have a simple three tier linear architecture; yet despite their diverse range of inputs, they provide exquisitely precise and sensitive responses. Recent studies have shown that differential use of pathway components enhances pathway specificity, facilitates signal integration and confers output selectivity.

MAP kinases are a family of enzymes that coordinate diverse cellular programs in response to signals relayed from the extracellular milieu. They are components of signalling pathways that have a common architecture, in which the MAP kinases themselves are the terminal members of a three-component linear kinase cascade. The MAP kinases are activated by dual phosphorylation on a conserved Thr-Xxx-Tyr motif in their activation loop by an upstream MAP kinase kinase (MAP2K). MAP2Ks are themselves activated by dual phosphorylation by MAP2K kinases (MAP3Ks). Confusingly, these enzymes have various alternative names, which are summarised in Table 1.

Three families of MAP kinases have been identified: the extracellular signal regulated protein kinases, ERK1 and ERK2; the c-Jun NH2 terminal kinases, JNK1, JNK2 and JNK3; and the p38 MAP kinases, α, β, δ and γ. A handful of other MAP kinases, including ERK5 and ERK3, have been discovered that are less well understood and may have divergent properties [1,2]. Each of the MAP kinase families has at least two cognate MAP2Ks and multiple MAP3Ks. MAP2Ks display remarkable substrate specificity for their MAP kinases; several recognize only one or two MAP kinases and few if any other substrates. In contrast, MAP3Ks have the capacity to activate multiple MAP kinase cascades (Figure 1). The multiple mammalian MAP3Ks and MAP2Ks may have arisen because mammalian MAP kinases must sense a panoply of ligands and hence have evolved multiple components sensitive to ligand subsets.

A recent study by Brancho *et al.* [3], who have investigated how MAP kinases are activated by diverse upstream pathway components and the consequences of these choices, has shed new light on the features of MAP kinase cascades that provide them their remarkable response specificity.

Signaling Specificity: Multiple Means to One End

How do these simply organized MAP kinase cascades respond to ligands that activate different cellular programs? Several mechanisms have been elucidated that confer signaling specificity. For example, scaffolding proteins have been identified for each MAP kinase family [4]. These proteins enhance signaling fidelity by sequestering the kinases and insulating them from inappropriate activation. An additional layer of regulation arises from stimulus-selective phosphorylation of scaffolds which determines the components that can bind to them [5]. The presence of distinct subcellular pools of kinases, often created by selective interaction with scaffolds, limits their access to specific regulators and substrates [6,7].

MAP kinases have specificity-determining motifs to which bind scaffold proteins, activators and effectors. They have a docking groove and acidic common docking (CD) and Glu-Asp (ED) pockets which are distinct from their active sites [8–10]. Complementing these MAP kinase motifs, many of the proteins they interact with — MAP kinase substrates, MAP2Ks, phosphatases and scaffolds — have a docking (D) domain, composed of basic and hydrophobic residues [8,11]. The crystal structures of a p38 MAP kinase bound to peptides based on the D domains of the p38 activator MKK3b and a substrate MEF2A show that both D domains bind to the same docking groove on p38, although in the opposite orientation [10]. The binding of the D domain peptides causes distinct conformational changes in p38, leading to the hypothesis that activators and effectors use the same site on a MAP kinase to regulate the enzyme in different ways. Specificity also comes from the differential use of MAP3Ks and MAP2Ks, depending on the extracellular cue.

Stimuli Couple to Subsets of Kinase Components

The characterization of several MAP3Ks that have the capacity to activate MAP kinases from more than one MAP kinase family has provoked a reconsideration of cascade architecture. Why are there multiple MAP3Ks for any given MAP kinase? Some ligands may stimulate a single MAP3K. Other ligands may activate more than one MAP3K, causing a more complex response through parallel activation of pathways and amplification of the signal to a pathway accessed by more than one MAP3K.

These potential mechanisms were evaluated by studying the requirement for MAP3Ks in the activation of the JNK pathway in *Drosophila* S2 cells [12]. In these cells, loss of the TGFβ-activated kinase (TAK1), a MAP3K, completely abrogated JNK activation by lipopolysaccharide. On the other hand, osmotic stresses used four MAP3Ks to activate JNK. Lipopolysaccharide acts predominantly through a single receptor, TLR4, while osmotic stresses conscript diverse mechanisms including growth factor receptor transactivation [13].

MAP2Ks May Be Called Up For Overtime Duty

p38s and JNKs are activated by numerous stress stimuli — p38s by the MAP2Ks MKK3/6 and JNKs by MKK4/7 [14]. MKK4 can also activate p38 *in vitro*, but

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Table 1. Alternative acronyms for components of MAP kinase cascades.

Kinase	Acronym	Example
MAP kinase kinase kinase	MAP3K, MEKK, MKKK	MEKK1-4 Ste20 homologs
MAP kinase kinase	MAP2K, MKK, MEK	MKK3, MEK3, MKK6, MEK6
MAP kinase	MAPK	ERK1/2, p38α,β,γ,δ, JNK1–3

the physiological significance has been unclear. Recently, Brancho *et al.* [3] re-examined the requirements for MKK3, MKK4 and MKK6 in p38 activation by different stimuli, using gene disruption and RNA inferference (RNAi) [3]. Their studies revealed that, in spite of a differential requirement for MKK3 and MKK6, both were essential for activation of p38 by TNFα. Intriguingly, they found that fibroblasts lacking both MKK3 and MKK6 showed residual UV-activation of p38 which was dependent on MKK4. This provided the first evidence for a physiological role of MKK4 in a p38 pathway.

Agents that engender global changes in cell homeostasis, such as UV and osmotic shock, may do so by harnessing multiple MAP2Ks and MAP3Ks in different pathways to generate a pleiotropic response. Consistent with the linkage of MAP2Ks to p38 as a function of stimulus, Brancho *et al.* [3] also showed that ligands which activate p38 use the upstream activators of p38 differentially to achieve the desired response. This feature is mirrored in the JNK pathway, where it has been shown that the JNK activators anisomycin and heat shock preferentially use MKK4 over MKK7 [15], but proinflammatory cytokines use predominantly MKK7 [16].

With all the ligands tested, however, maximum activation required two MAP2Ks: MKK3 and MKK6 for p38, and MKK4 and MKK7 for JNK. The requirement for both MAP2Ks has been attributed to the selective recognition of individual phosphorylation sites by these MAP2Ks: *in vitro* MKK4 preferentially catalyzes phosphorylation of the activation loop tyrosine, while MKK7 phosphorylates the threonine [16,17]. The fact that these MAP kinases are still activated in the absence of

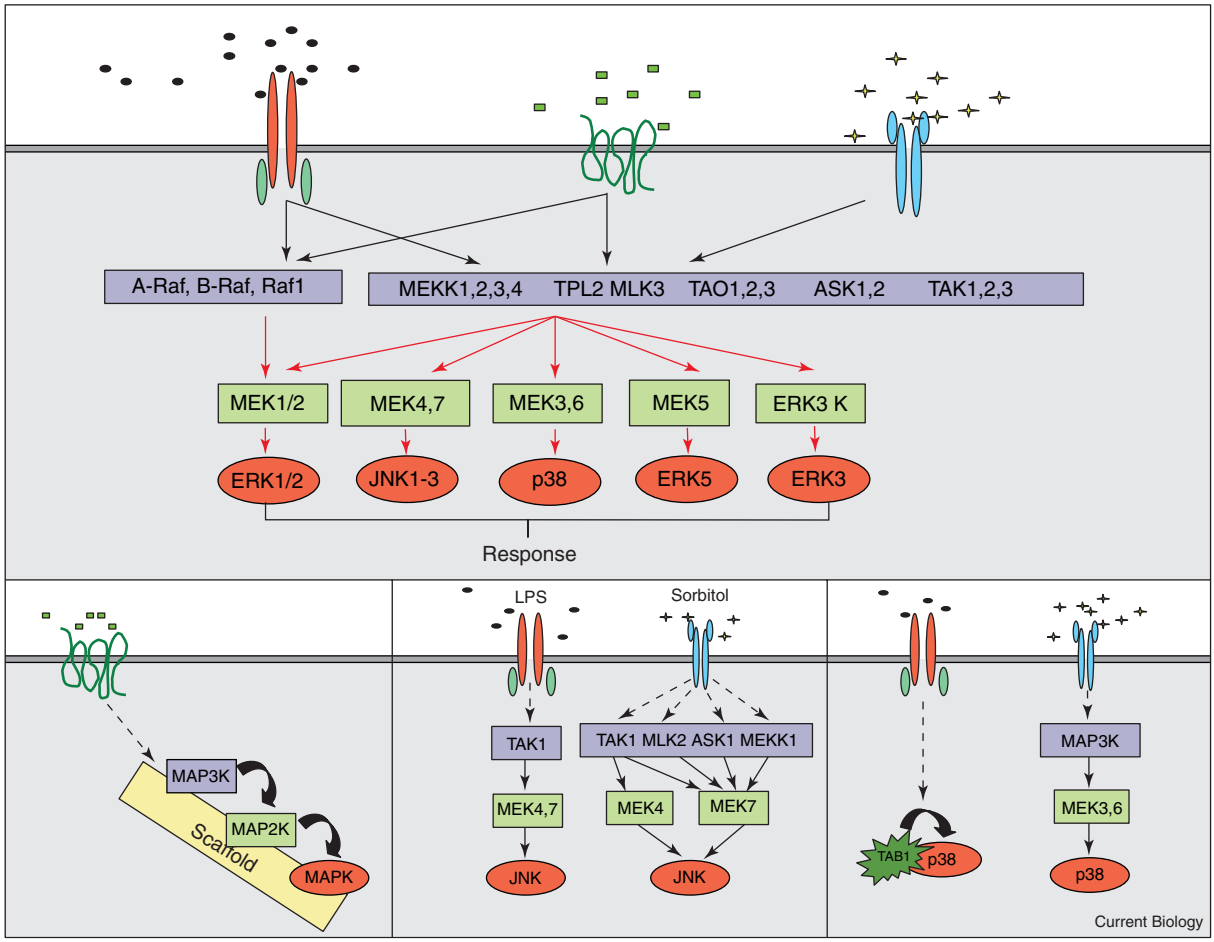

Figure 1. Building specificity into MAP kinase cascades.

MAP kinase cascades use multiple methods to achieve specific responses to diverse stimuli. These include using scaffolds, the differential use of MAP2Ks and MAP3Ks, and the use of proteins such as TAB1 which activates MAP kinases independently of MAP2Ks.

individual MAP2Ks suggests, either that each MAP2K can catalyze both phosphorylations, or that autophosphorylation contributes to activation. MAP kinase autophosphorylation can occur on activation loop sites.

MAP2K-Independent Activation of MAP Kinases

The predominant mode of MAP kinase activation is by dual phosphorylation by MAP2Ks on the activation loop sites. Nevertheless, MAP2K-independent activation has been reported for p38 via the TAK1 binding protein TAB1 [18]. TAB1 enhances p38 autophosphorylation in a stimulus-specific manner in the absence of MKK3 and MKK6. Activation of p38 by ischemia as a consequence of myocardial injury, for example, reportedly occurs by TAB1-induced p38 autophosphorylation [19]. Scaffolds may more generally take an active part in kinase activation than previously recognized. The motor-binding protein TPX2 has been shown to promote activation of the Aurora A kinase by promoting its autophosphorylation [20].

In conclusion, these studies provide insight into the myriad mechanisms that create specificity from the three kinase module. Signals initiated by diverse extracellular ligands induce ligand-specific re-wiring of kinase components via scaffolding proteins and distinct binding motifs to produce signal-specific, custom-made signal transduction cascades.

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