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The MEK Kinases MEKK4/Ssk2p Facilitate Complexity in the Stress Signaling Responses of Diverse Systems

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Abstract The mammalian JNK/p38 MAP kinase kinase kinase MEKK4 and the *Saccharomyces cerevisiae* Ssk2p are highly homologous. MEKK4 can replace all of the known functions of Ssk2p in yeast, including functioning in the high osmolarity glycerol (HOG) MAPK pathway and the recently described actin recovery pathway. MEKK4 and Ssk2p share a number of conserved domains and appear to be activated by a similar mechanism. Binding of an activating protein to the N-terminal region alleviates auto-inhibition and causes the kinase to auto-phosphorylate, resulting in activation. In this review we will examine the role of the MAP kinase kinase kinase isoform Ssk2p/MEKK4 in the adaptation of both yeast and mammalian systems to specific external stimuli. Recent work has provided a wealth of information about the activation, regulation, and functions of these MEKK kinases to extra-cellular signals. We will also highlight evidence supporting a role for MEKK4 in mediating actin recovery following osmotic shock in mammalian cells. J. Cell. Biochem. 101: 34–43, 2007.

Key words: *SSK2*; MEKK4; MTK1; osmotic stress; MAP kinase pathway; actin

Eukaryotic stress-activated mitogen-activated protein kinase (SAPK) pathways have evolved to respond to a multitude of extra-cellular stresses, such as physical (UV radiation, osmotic variability, heat), chemical (pH, oxidative stress), and biological (tissue or organ stressors, DNA damage, cytokines, pheromones) stresses [Kyriakis and Avruch, 2001]. SAPK pathways, which have been highly conserved throughout evolution, relay signals through a sequential cascade of proteins that begins with the activation of a MEK kinase, also called a MAP kinase kinase kinase (MAPKKK). The MAPKKK phosphorylates and activates an intermediate MAP kinase kinase (MKK) (MKK3, MKK4, MKK6, and/or MKK7, for example), which in turn phosphorylates and activates a MAP kinase (such as JNK or p38). Activated MAP kinases elicit the appropriate cellular response through the phosphorylation of numerous proteins including transcription factors. This simple architecture has been recycled throughout nature to build a myriad of parallel pathways that fine tune the cellular responses to diverse stress stimuli.

Surprisingly, parallel SAPK pathways use many of the same signaling intermediates. The JNK and p38 cascades, for example, share the MAPKKK MEKK2, 3, and 4 as well as the intermediate MKK4 [Kyriakis and Avruch, 2001]. To establish and preserve specificity of signaling, nature has evolved a number of regulatory mechanisms including compartmentalization (scaffolding and localization), the use of activating or inhibitory protein interactions, and tissue-specific expression of pathway components. Recent work has significantly advanced our understanding of another mechanism of specificity, the use of multiple MAPKKK isoforms in a single pathway [Schlesinger et al., 1998; Hagemann and Blank, 2001; Uhlik et al., 2004]. In this review we will examine the role of the MAPKKKs Ssk2p/MEKK4 in the adaptation of both yeast and mammalian systems to specific external stimuli. The mammalian p38 pathway employs both MEKK3 and MEKK4 to phosphorylate and activate MKK6 [Deacon and

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Blank, 1997; Takekawa et al., 1997], while the fungal p38-equivalent high osmolarity glycerol (HOG) pathway uses Ssk2p, Ssk22p, and Ste11p to phosphorylate the MEK Pbs2p (Fig. 1) [Maeda et al., 1994; Maeda et al., 1995; Posas and Saito, 1997]. Rather than simply increasing signal or providing redundancy, the individualized functions of each of these MEKK kinases provide for specificity and complexity in the cellular response to specific stressors.

Studies in the yeast model have been very useful for understanding the architecture of signaling pathways, in particular the importance of scaffolds for signaling specificity and efficiency. In particular, our understanding of the regulation of MEKK4 has been greatly aided by studying this human protein when it is expressed in yeast and comparing this regulation to its yeast cognate kinase Ssk2p.

IDENTIFICATION OF *SSK2* AND MEKK4

The HOG pathway, which is analogous to the p38 MAP pathway in mammalian cells, senses osmotic stress and induces cellular changes which result in adaptation and resumption of growth (for a review see [Hohmann, 2002]). Adaptation involves the expression of osmoregulatory and stress response genes as mediated by the MAP kinase Hog1p. Among other changes, Hog1p activation results in the synthesis, retention and resulting accumulation of glycerol, the major osmolyte of yeast, which restores a higher internal osmolarity between the cell and its environment. Ssk2p was first identified as an extragenic suppressor of a lethal *sln1Δ* mutant [Maeda et al., 1994]. Sln1p is one of the two membrane-bound proteins that mediate activation of the HOG pathway in response to osmotic stress, the other being Sho1p. These proteins transmit signals through two separate branches of the pathway that converge on the MAPKK Pbs2p. In the Ssk2p branch of the HOG pathway, Sln1p serves as an osmosensor that responds to changes in turgor pressure [Reiser et al., 2003]. When the cell is exposed to stress, Sln1p detects a shift in turgor pressure resulting in the inhibition of a histidine phospho-relay that normally keeps Ssk1p inactive; inhibition of this phospho-relay activates Ssk1p. Activated Ssk1p then binds and activates the MEK kinases Ssk2p and Ssk22p [Maeda et al., 1995; Posas and Saito, 1998]. Ssk2p is one of the three MEKK kinases in the HOG pathway, the other two being Ste11p and Ssk22p (Fig. 1) [Maeda et al., 1995; Posas and Saito, 1998]. More recently, it has been appreciated that Ssk2p has a specific function, not shared with the other MAPKKKs of the HOG pathway, in facilitating recovery of the actin cytoskeleton from osmotic

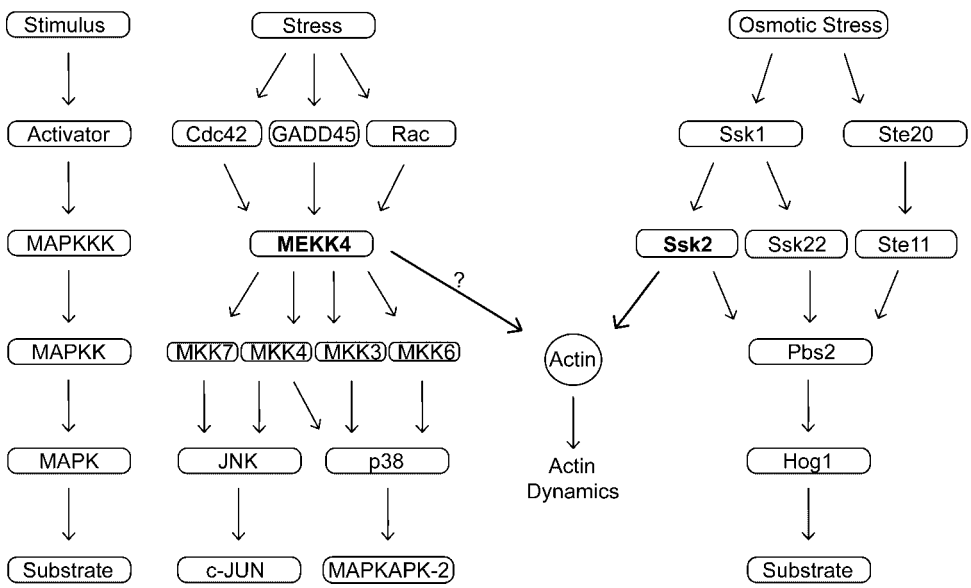

Fig. 1. Comparison of the JNK/p38 MAPK pathway in mammals and yeast. Both pathways sense extracellular stress and transmit signal through a traditional MAPK signaling pathway. Although MEKK4 is able to mediate actin recovery in yeast, this role has yet to be assigned to the kinase in mammalian systems.

stress [Yuzyuk et al., 2002]. Ssk2p is homologous to MEKK4, especially in the kinase domain (38% identity) and this homology belies a surprising degree of functional conservation as will be described below.

Mammalian MEKK4 (MEK kinase 4, also known as KIAA0213, MTK1, MAP3K4, and MAPKKK4 in mammals and D-MEKK1 in *Drosophila*) was first identified from a murine fibroblast cDNA library using degenerate primers to isolate homologues of the yeast MAPKKK *STE11* [Gerwins et al., 1997]. MEKK4 was independently cloned from a human cDNA library as a protein kinase that could rescue the osmo-sensitivity of yeast defective for both branches of the HOG pathway (an *ssk2Δ*, *ssk22Δ*, *sho1Δ* strain) (see Fig. 1) [Takekawa et al., 1997]. Both studies revealed two isoforms of MEKK4, α and β (Fig. 2A). The human isoform α contains 49 amino acids (1175–1223) not found in the β isoform [Gerwins et al., 1997; Takekawa et al., 1997]. Similarly, the mouse isoform α contains a 52 amino acid insertion (1162–1213). The role of MEKK4 as a mediator of the JNK pathway has been established by numerous studies [Fanger et al., 1997; Gerwins et al., 1997; Takekawa et al., 1997; Chan-Hui and Weaver, 1998]. Studies in *Drosophila*, mice, and human cell lines have also established MEKK4 as a signaling protein of the p38 MAPK pathway [Takekawa et al., 1997; Chan-Hui and Weaver, 1998; Inoue et al., 2001; Abell et al., 2005; Aissouni et al., 2005; Zhuang et al., 2006]. MEKK4's traditional role is to transmit signal through the pathway by phosphorylating downstream MKK (Fig. 1). Similar to other MEKK kinases, MEKK4 interacts with multiple MKK kinases which function in both the p38 and JNK pathways. MKK3, MKK4, and MKK6 have all been shown to be phosphorylation targets of MEKK4 [Gerwins et al., 1997; Takekawa et al., 1997; Chan-Hui and Weaver, 1998; Takekawa and Saito, 1998; Inoue et al., 2001; Mita et al., 2002; Derbyshire et al., 2005; Takekawa et al., 2005].

THE DOMAIN STRUCTURES OF MEKK4 AND Ssk2p

Many MAPKKKs are regulated by mechanisms that alleviate auto-inhibition through phosphorylation, cleavage, or binding of an activator to the auto-inhibitory domain [Cardone et al., 1997; Posas and Saito, 1998; Zenke et al., 1999]. For example, both MEKK4 and Ssk2p are constitutively activated when their N-terminal domain is deleted, indicating there is an inhibitory function within the N-terminal domains of both proteins [Maeda et al., 1995; Takekawa et al., 1997]. The inhibitory domain of MEKK4 (located between residues 253 and 332) was mapped in yeast by deleting regions and looking for constitutive activation as measured by complementation of the osmo-sensitive phenotype of an *ssk2Δ/ssk22Δ/ste11Δ* strain. An observed two-hybrid interaction between the N- and C- termini of MEKK4 suggested that a physical interaction between these domains was part of the auto-inhibitory mechanism. The interaction with the N-terminus required a ~100-residue region just upstream of the C-terminal kinase domain. Consistently, mutations in the regions involved in the inhibitory interaction, around residue 534 of the N-terminal domain and around residue 1,300 of the C-terminus (just upstream of the kinase domain) resulted in constitutive activation and prevented the two-hybrid interaction [Mita et al., 2002].

Further studies in yeast identified another important regulatory domain of Ssk2p and MEKK4 termed the localization domain (the LD domain; see Fig. 2B). The LD domain is a region of Ssk2p (aa 426–465) with a high degree of homology to MEKK4 (78%). Deletion of the LD domain of Ssk2p resulted in a serious localization defect when yeast cells were exposed to osmotic stress as well as an inability to facilitate the actin specific functions of Ssk2p [Yuzyuk et al., 2002]. The LD domain of MEKK4 (aa 264–303) is located within the inhibitory region of the protein (aa 253–553) and is also required for MEKK4 to perform the actin specific functions of the MAPKKK when expressed in yeast. The functional importance of this domain in the mammalian stress response has not yet been confirmed.

MEKK4 contains a number of additional motifs that could be involved in regulatory protein–protein interactions including a proline-rich region, a pleckstrin homology (PH) domain, and a modified Cdc42/Rac interactive (CRIB) domain. Although not much is known about the functional importance of the proline-rich region and the PH domains of MEKK4, the CRIB domain has been suggested to play a role in activation of the JNK pathway

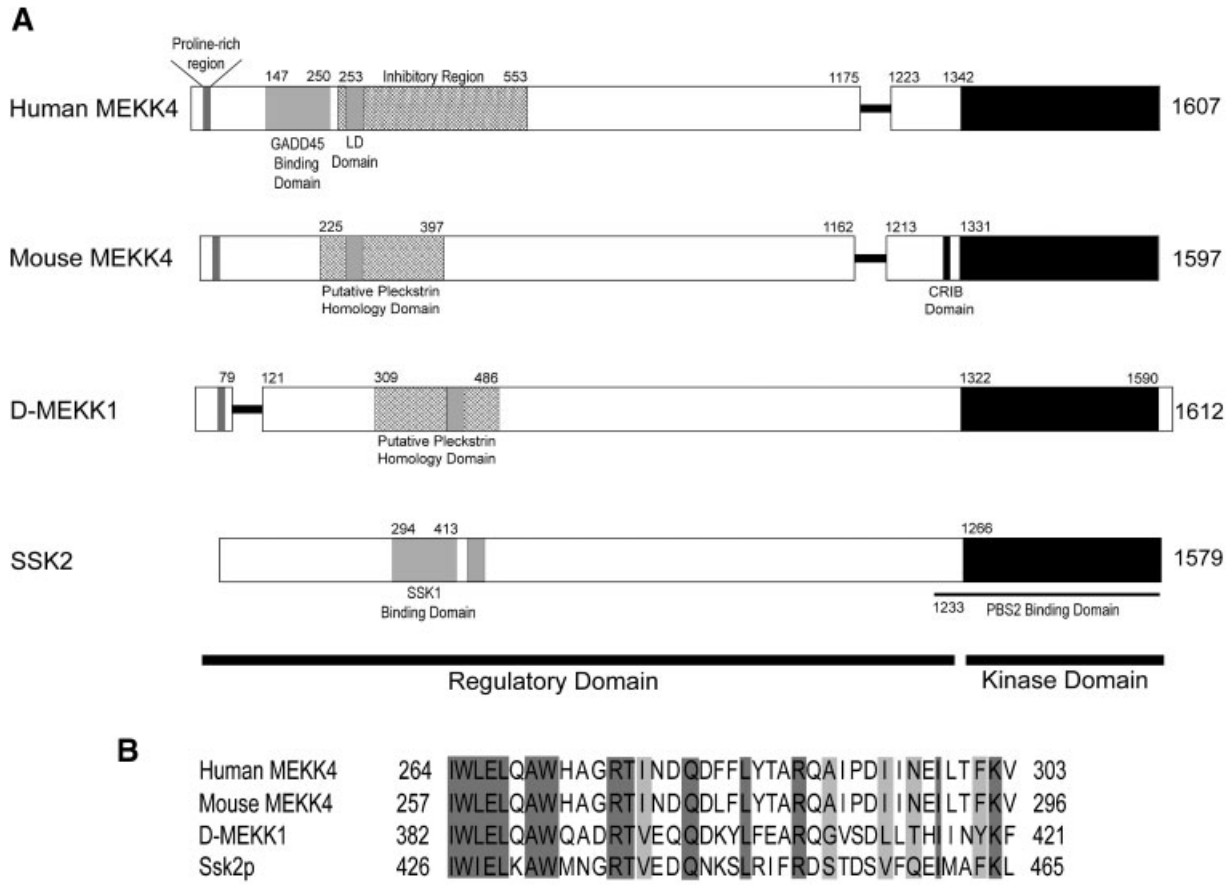

Fig. 2. Comparison of the Ssk2p/MEKK4 kinase in humans, mice, *Drosophila*, and *S. cerevisiae*. **A**: The domain structure of the four proteins is remarkably similar, including a N-terminal inhibitory/activator-binding region, the LD domain (sequences shown in **B**), and the highly homologous kinase domain. The Drosophila, mouse, and human proteins have two isoforms (alpha and beta) created by alternate splicing.

[Fanger et al., 1997]. Constitutively active Cdc42 or Rac stimulates the JNK pathway, but a kinase dead allele of MEKK4 blocks that activation. Indeed, MEKK4 binds directly to Cdc42 and Rac in both the GDP and GTP states. Interestingly, deletion of the CRIB domain of MEKK4 diminishes but does not abolish Cdc42 binding [Fanger et al., 1997; Gerwins et al., 1997].

ACTIVATION OF Ssk2p/MEKK4 KINASE ACTIVITY

Transmission of extra-cellular stimuli through the relevant MAPK signaling pathways requires activation of MEKK4 and Ssk2p by relieving auto-inhibition of kinase activity. As mentioned above, Ssk1p is the terminal member of a phospho-relay and is the upstream activator of Ssk2p [Posas and Saito, 1998]. Under normal osmotic conditions, the transmembrane osmo-sensor Sln1p is kept in an active state and transfers a phosphate to the carrier protein Ypd1p, who in turn donates the phosphate to the receiver domain of Ssk1p [Maeda et al., 1994; Posas et al., 1996]. In response to changes in turgor pressure induced by hyper-osmotic stress, the phospho-relay is inhibited and Ssk1p becomes de-phosporylated by phosphatases. The de-phosphorylated Ssk1p binds to the N-terminal auto-inhibitory domain of Ssk2p and Ssk22p, activating their auto-phosphorylation on a conserved threonine residue. The activated Ssk2/22p kinases are then free to phosphorylate their downstream target, the MAP kinase kinase Pbs2p.

A highly analogous mechanism is employed to control the activation of MEKK4. Members of the growth arrest and DNA damage-inducible family of proteins have been shown to alleviate

auto-inhibition of MEKK4 by binding the N-terminus of the kinase. Gadd45β and Gadd45γ were cloned from a human placenta cDNA library using a two-hybrid screen for proteins that interact with full-length MEKK4 [Takekawa and Saito, 1998]. Subsequent analysis revealed that Gadd45α, the third member of the GADD45 family, also bound MEKK4. The GADD proteins have been proposed to play a role in activation of the p38 and JNK pathways in response to cellular stresses [Hildesheim et al., 2002; Bulavin et al., 2003; Zerbini et al., 2004]. Consistently, expression of the three GADD proteins is induced by a variety of environmental stresses including methyl methanesulfonate (MMS), γ-irradiation, UV, H_2O_2, and the antibiotic anisomycin [Takekawa and Saito, 1998].

The GADD45 binding domain (GADD45BD) of human MEKK4 maps to residues 147–250 [Takekawa and Saito, 1998]. Although this sequence is not conserved in the yeast homologue *SSK2*, it is similar to the Ssk1p-binding domain of Ssk2p (residues 294–413) [Posas and Saito, 1998], suggesting an analogous mechanism of regulation. Full-length MEKK4, which alone is unable to complement the osmo-sensitivity of an *ssk2Δ ssk22Δ ste11Δ* strain due to auto-inhibition of the kinase, was co-expressed with a cDNA library in yeast to identify activators that allowed it to function similarly to the N-terminal truncation, which is able to complement the osmo-sensitivity [Mita et al., 2002]. Gadd45α and β were identified in this screen and subsequent analysis revealed Gadd45γ to have the same ability.

When Gadd45β or γ were co-expressed with MEKK4 in COS-7 cells the kinase's activity, measured in an in vitro assay using MKK6 as the substrate, was significantly increased [Takekawa and Saito, 1998]. However, Gadd45α upregulated MEKK4's kinase activity to a lesser degree. Gadd45α, β, or γ expression alone caused an increase in the activation of both the p38 and JNK pathways, but not the ERK pathway [Takekawa and Saito, 1998; Aissouni et al., 2005]. Similar to the previous assay, Gadd45α was not able to activate either pathway at a level comparable to β or γ. The activation of the p38 pathway by Gadd45γ expression could be suppressed by co-expression of an N-terminal fragment of MEKK4 (MEKK4ΔK, residues 1–1342), suggesting that the GADD45 proteins bind and activate MEKK4 in the p38 and JNK pathways. The in vitro kinase assays and the in vivo activation assays mentioned above suggest that human MEKK4 has much less affinity for Gadd45α compared to the β and γ isoforms. This implies varying roles for the GADD proteins in regulating MEKK4 functions.

Recent evidence has suggested that Ssk2p is also activated by an Ssk1p-independent mechanism [Yuzyuk et al., 2002; Yuzyuk and Amberg, 2003]. As mentioned above, Ssk2p facilitates actin recovery from an osmotic insult. This aspect of Ssk2p function does not require the normal activator Ssk1p. Furthermore, we have been able to measure normal Ssk2p kinase activity from cells lacking the *SSK1* gene. Given the functional conservation of MEKK4, as measured in yeast, it seems likely that this alternative activation pathway is also conserved.

Ssk2p/MEKK4 MONITOR THE INTEGRITY OF THE ACTIN CYTOSKELETON AND REGULATE ITS RECOVERY FROM OSMOTIC STRESS

Re-polarization of the actin cytoskeleton by Ssk2p is an important example of the roles that MEKK4 and Ssk2p play outside of the typical MAP kinase cascade to fine tune the response of the cell to external stresses. Like all cells, the yeast *Saccharomyces cerevisiae* maintains an internal hyperosmotic environment to utilize the inward-directed push of water. When exposed to higher external osmolarity, the cells experience hyperosmotic shock leading to rapid water depletion and cell shrinkage. In addition to activating the HOG MAPK pathway, hyperosmotic shock rapidly causes the actin cytoskeleton to disassemble and depolarize leading to arrested cell growth. After approximately, 1 h, the actin cytoskeleton is re-polarized and the yeast re-enter the cell cycle [Chowdhury et al., 1992]. De-polymerization of the actin cytoskeleton in response to osmotic stress causes Ssk2p to bind actin with a 1:1 stoichiometry and concomitantly alter its intracellular distribution [Yuzyuk et al., 2002]. Within minutes of osmotic stress, Ssk2p re-localizes to growth sites such as the mother-bud neck and the cortex of small buds. The LD of Ssk2p (a region with high homology to MEKK4) is required for this translocation. Deletion of this domain also interrupts the Ssk2p–actin interaction suggesting that the actin interaction, in part,

functions to regulate kinase localization. Surprisingly, neither the re-localization or the actin-binding activity of Ssk2p requires a functional HOG MAP kinase pathway; deletion of the known upstream activator (Ssk1p) nor the downstream effector (Pbs2p) had any effect on these functions [Yuzyuk et al., 2002]. Once at growth sites, the kinase activity of Ssk2p promotes recovery of a polarized actin cytoskeleton through a yet-to-be-determined mechanism. However, our lab does have some preliminary evidence implicating the actin filament nucleating formin, Bni1p, in Ssk2p-facilitated actin recovery (manuscript under review). The only kinase previously known to bind directly to actin is c-Abl [Van Etten et al., 1994] but the cellular function of this interaction is unclear. Therefore, Ssk2p is one of the first examples of actin directly regulating a protein kinase and suggests that Ssk2p has an unprecedented role in monitoring the integrity of the actin cytoskeleton. In addition to this monitoring role, Ssk2p also has an equally unprecedented function in regulating polarized actin assembly.

Research into the yeast mating MAP kinase pathway gave us the first insight into the importance of scaffolding proteins (Ste5p in the case of the mating pathway) in facilitating efficient signaling but more importantly preventing cross-talk between related kinases of different pathways [Elion, 2001]. Similarly, the cytoskeletal aspects of Ssk2p function are also facilitated by a yeast scaffolding protein called Spa2p. Spa2p is a member of a 12S complex termed the polarisome which controls cell polarity by directing localized assembly of actin filaments at sites of polarized growth through the polarized activation of the yeast formins Bni1p and Bnr1p [Sheu et al., 1998]. Ssk2p interacts with Spa2p in response to osmotic stress, and deletion of the scaffold prevents proper re-localization of the kinase under stress conditions and actin recovery [Yuzyuk and Amberg, 2003]. Interestingly, over-expression of Ssk2p can suppress the actin recovery defects of a *spa2Δ* strain, suggesting that the scaffolding activity of Spa2p functions to concentrate Ssk2p with its cytoskeleton relevant substrate(s) which could include the Spa2p binding partner, the formin Bni1p. Importantly, this kinase-mediated actin recovery pathway has been conserved through evolution. When MEKK4 is expressed in yeast, it too is regulated by osmotic stress both in the ability to bind actin and re-localize to sites of polarized growth in association with the scaffold protein Spa2p [Yuzyuk and Amberg, 2003]. Most impressively, MEKK4 is also able to complement the actin recovery defects of an *ssk2Δ* strain. These studies support the conclusion that MEKK4 is capable of regulating the actin cytoskeleton in mammalian cells. Although this has not yet been determined, such a finding will shed a great deal of insight into the regulation of the mammalian actin cytoskeleton in response to cellular stresses.

MORE ON THE FUNCTION AND REGULATION OF MEKK4 IN MAMMALIAN CELLS

Like most signaling proteins, MEKK4 contains a number of independent domains or 'modules' in addition to the catalytic domain. These modules, which are often genetically interchangeable, mediate interactions with regulatory proteins and are believed to contribute to efficient evolvability [Bhattacharyya et al., 2006]. Independent modules also impart greater specificity upon signaling proteins involved in more than one signaling pathway [Remenyi et al., 2006].

In mammalian cells, MEKK4 interacts with a number of scaffolding proteins that influence the response of the kinase to extra-cellular stimuli. Axin is a DIX (disheveled-axin)-domain scaffold in the Wnt signaling pathway that when over-expressed activates the JNK MAPK pathway through a direct interaction with MEKK1 and MEKK4 [Zhang et al., 1999; Luo et al., 2003]. Interestingly, MEKK1 and MEKK4 compete for Axin binding employing entirely different regions of Axin, suggesting that cellular conditions may dictate which kinase is selectively allowed to bind [Luo et al., 2003]. Another DIX-domain protein, Ccd1 (coiled-coil-DIX1), forms an additional level of Axin/MEKKK regulation through two separate mechanisms. Ccd1 can bind Axin directly to prevent the MEKK1 interaction or it can bind MEKK4 to attenuate its interaction with Axin [Wong et al., 2004].

A second scaffolding protein, TRAF4, which binds the kinase domain of MEKK4, also helps mediate the normal roles of MEKK4 in the cell [Abell and Johnson, 2005]. TNF receptor-associated factors (TRAF) are cytoplasmic adaptor proteins that, when recruited to the

cytoplasmic domain of receptors, function as scaffolds to mediate signals leading to the activation of the NF-κB and JNK pathways [Arch et al., 1998]. Recent work has suggested that MEKK4 and TRAF4 are involved in the process of neurulation [Abell and Johnson, 2005; Chi et al., 2005]. Neural tube defects (NTD), which are the result of improper closure of the neural tube, are the second most prevalent form of birth defects, occurring in about 1 in 1,000 pregnancies worldwide [Copp et al., 2003]. Currently, alterations in cell proliferation, cell survival (mis-regulated apoptosis), and the actin cytoskeleton are believed to be the leading causes of NTDs [Juriloff and Harris, 2000]. Generation of MEKK4−/− mice revealed that the protein is not essential for embryonic survival, but >80% of mice exhibited NTDs and died shortly after birth [Chi et al., 2005]. Mice that were homozygous for kinase inactive MEKK4 ($MEKK4^{K1361R}$) also showed a remarkable increase in the number of NTDs [Abell et al., 2005]. Both studies suggested that MEKK4 plays a role in downregulating apoptosis during neurulation via the p38 MAPK pathway.

Mediator proteins facilitate another form of MEKK4 regulation. CIN85, first identified as a Cbl-interacting protein [Take et al., 2000], is a non-catalytic adaptor molecule that regulates the assembly of signaling complexes involved in cell growth, development, and survival [Dikic and Giordano, 2003]. CIN85 mediates interactions with other proteins through three SH3 domains that recognize the consensus sequence PxxxPR and through a proline-rich region able to bind other proteins containing SH3 domains [Szymkiewicz et al., 2002; Dikic and Giordano, 2003]. A search for proteins containing the PxxxPR motif found that murine MEKK4 contains three of these motifs [Aissouni et al., 2005]. Human MEKK4, on the other hand, has only two of the potential CIN85-binding motifs. A mutant version of murine MEKK4 lacking all three PxxxPR domains (MEKK4-3R) completely abolished the CIN85 interaction and was used to examine the physiological relevance of MEKK4/CIN85 interaction. In support of CIN85's role in mediating the formation of signaling complexes, the MEKK-3R mutant was defective in activating the p38 pathway intermediate MKK6. The cells also showed a reduction in p38 activation in response to both oxidative stress and EGF stimulation. Thus, the CIN85 interaction might help MEKK4 activate the p38 pathway through MKK6 in response to specific stresses. Interestingly, the MEKK4-3R mutant was less prone to ubiquitination. It had not been previously shown that the kinase is ubiquitinated in vivo, but tagged ubiquitin was observed to precipitate with tagged MEKK4 [Aissouni et al., 2005].

A third type of regulation is the interaction of activator proteins with MEKK4. A recent study has shown that MEKK4 is tyrosine-phosphorylated in response to interferon-γ (IFN-γ) in human keratinocytes [Halfter et al., 2005]. The IFN-γ receptor, composed of two heterodimers, is expressed on almost all cell types [Boehm et al., 1997]. Upon ligand binding, the heterodimers associate and activate a signaling cascade. This signaling pathway involves activation of the calcium-dependent tyrosine kinase Pyk2 and the calcium-binding protein annexin II, both of which co-immunoprecipitate with MEKK4 in an agonist- and time-dependent manner [Halfter et al., 2005]. Upon IFN-γ receptor activation Pyk2 is activated and phosphorylates MEKK4 that in turn phosphorylates MKK6, leading to p38 activation. To keep the pathway in check the tyrosine phosphatase SHP2, which also co-immunoprecipitates with MEKK4 in an agonist-dependent manner, de-phosphorylates both MEKK4 and its upstream activator Pyk2 [Halfter et al., 2005]. This inhibitory interaction represents yet another level of MEKK4 regulation.

Interestingly, MEKK4 is also involved in the production of IFN-γ. IFN-γ is produced by differentiated T_H1 cells via two mechanisms; T cell receptor (TCR)-induced production in response to antigen receptor challenge, or TCR-independent production stimulated by the cytokines interleukin 12 (IL-12) and IL-18. Treatment of differentiated T_H1 cells with IL-12 and IL-18 upregulates GADD45β and γ production, and overexpression of GADD45β augments IFN-γ expression in response to those cytokines. This cytokine-induced IFN-γ production involves the GADD45-binding domain of MEKK4 since truncation mutants which contained the GADD45-binding domain but lacked the kinase domain selectively inhibited cytokine-induced IFN-γ production [Yang et al., 2001]. Indeed, differentiating T_H1 cells from MEKK4−/− mice exhibit a reduced level of IFN-γ production in response to cytokine treatment [Chi et al., 2004].

The role of MEKK4 in TCR-induced IFN-γ production is less clear. MEKK4 mutants lacking the kinase domain did not inhibit TCR-induced production [Yang et al., 2001], while differentiated T_H1 cells from MEKK4−/− mice show 70% less IFN-γ production upon TCR stimulation [Chi et al., 2004]. In support of the latter theory, T_H1 cells from GADD45β−/− and GADD45γ−/− mice have impaired p38 activation and produce significantly less IFN-γ in response to TCR signaling [Lu et al., 2001; Lu et al., 2004].

CONCLUSIONS AND FUTURE DIRECTIONS

Although conventional logic might predict that individual pathways should consist of proteins that function solely in that signaling cascade, this is certainly not the case in nature. Both MEKK4 and Ssk2p serve as one of a number of MAPKKK isoforms in a single pathway. Instead of merely increasing signal or providing redundancy, the multiple functions of these MEK kinases fine tune the cellular response to specific stressors. In addition to its role of transmission within the HOG MAPK pathway, Ssk2p is both regulated by and a regulator of the actin cytoskeleton. This regulation has been maintained throughout evolution, indicative of the importance of this kinase in cellular adaptation to stress. The spatial and temporal regulation of Ssk2p and MEKK4 by scaffolding has also been preserved, highlighting the importance of this aspect of function. Yeast has proven to be a powerful system for unraveling the fundamental aspects of MAP kinase pathway function and regulation. Moreover, investigators interested in studying the specifics of MEKK4 function have quite productively benefited from studies of the HOG pathway of yeast and also by studying the human kinase expressed in yeast. These studies have shown that even specialized and seemingly esoteric functions and interactions of this kinase have been conserved. The synergy derived from working in these seemingly disparate systems should encourage other investigators to avail themselves of the power of model systems to study their signaling pathways of choice. Concerning Ssk2p and MEKK4, clearly there is still much to learn about their regulation and mechanisms of action, a process that is likely to continue to benefit from cross-organism studies.

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