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THE TAO OF MEKK

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1. ABSTRCT

Cloning and characterization of MEKK1 in 1993 revealed that in addition to Raf there were other pathways activated by extracellular stimuli that were responsible for ERK activation. Since then, three additional MEKK family members have been cloned adding even further diversity to the regulation of MAPK pathways. The MEKK family members are regulated by a diverse array of extracellular stimuli ranging from growth factors to DNA damaging stimuli and so are important for the cell to sense exposure to various environmental stimuli. One important aspect of MEKK biology is that they can potentially serve in more than one pathway. Regulation of MEKK family members often involves LMWG proteins, phosphorylation and subcellular localization. With regard to at least MEKK1, serine/threonine kinases such as NIK, GLK and HPK1 appear also to be important for regulation. Of the MEKK family members, the biological role of MEKK1 is best characterized and studies have shown that MEKK1 is important in mediating survival vs. apoptosis, possibly via its ability to regulate transcription factors, the expression of death receptors and their ligands. The biological roles of MEKK2, 3 and 4 are under investigation and undoubtedly homologous deletion of these MEKK family members will be invaluable at determining the biological functions of these MEKKs. At present, the MEKK family members are characterized as localized sensors that control cell responses at the level of gene expression, metabolism and the cytoskeleton

2. INTRODUCTION

Controlling the state of phosphorylation is an important mechanism by which signaling molecules regulate the activity of other proteins. A common theme in molecular signaling is the kinase cascade, in which a linear series of kinases is activated by phosphorylation by an upstream kinase. The mitogen-activated protein kinase (MAPK) pathway is a well characterized example of a sequential kinase cascade (figure 1). MAPKs are phosphorylated and activated by MAPK kinases (MKKs) which are dual specificity kinases that mediate phosphorylation of tyrosines and threonines. The MKKs are phosphorylated and activated by serine/threonine kinases that function as MKK kinases (MKKKs) which may be, in some pathways, phosphorylated and activated by MKKK kinases (MKKKKs). MKKKKs are either tyrosine or serine/threonine kinases, some of which are regulated by low molecular weight GTP binding (LMWG) proteins. The activity of several MKKKs are also regulated by LMWG proteins. Evolutionarily, many of the components of the MAPK pathways, as well as some aspects of their regulation, are conserved from yeast to man and establish a MKKKK-MKKK-MKK-MAPK sequential kinase pathway.

In 1990, mammalian p44 MAPK was cloned and referred to as extracellular signal-regulated kinase (ERK1). Since the initial discovery of ERK1, 12 MAPK genes encompassing five subfamilies have been identified in mammalian cells that are defined by sequence homology and functional similarity (table 1). The MAPK family members include ERK1/2, p38alpha, beta, gamma and delta, JNK1, 2, 3, ERK3, 4 and 5 (2). There are seven different MKKs that regulate the MAPKs with considerable specificity. MAPK/ERK (MEK) 1 and 2 regulate ERK1/2, whereas JNK kinase (JNKK; also known as SEK-1 or MKK4) and MAPK/ERK kinase 7 (MKK7) regulate JNK activity. MKK3 and MKK6 specifically phosphorylate and regulate p38 activity. MKK5 phosphorylates and regulates ERK5. There are currently 10 different groups of kinases encompassing over 22 different genes that act upstream and regulate the MKKs. One family, the MAPK/ERK kinase kinases (MEKKs), directly phosphorylate and activate specific MKKs and so are valid MKKKs (3). This review discusses what is currently known about the biological role and regulation of the MEKK family members, particularly with regard to regulation of MAPK pathways.

Table 1. Summary of MAPK pathway components in mammalian cells.

Acronym	Name
MKKKKs and MKKKs	
Raf1, A- and B-raf	
Mos	
MEKK1, 2, 3 and 4	MAPK/ERK kinase kinase 1-4
MAPKKK5/ASK-1	MAP kinase kinase kinase5/apoptosis-signal regulating kinase-1
MLK1, 2 and 3	Mixed lineage kinase 1-3
DLK	Dual leucine zipper bearing kinase
TAK	TGF-β-activated kinase
TPL2	Tumor progression locus 2
KSR	Kinase suppressor of ras
PAK1, 2 and 3	p21-activated kinase 1-3
GCK	Germinal center kinase
HPK1	Hematopoetic progenitor kinase 1
GLK	GCK-like kinase
KHS	Kinase homologous to Sps1/Ste20
NIK	Nck interacting kinase
MST1, 2 and 3	Mammalian sterile twenty-like kinase 1-3
SOK-1	Ste20/oxidant stress response kinase-1
MKKs	
MEK1 and 2	MAPK/ERK kinase
JNKK	JNK kinase (also known as MKK4 or SEK-1)
MKK3, 5, 6, 7	MAPK kinase
MAPKs	
ERK1, 2, 3, 4 and 5	Extracellular-signal regulated kinase
JNK1, 2, and 3	c-jun N-terminal kinase
p38alpha, beta, gamma, delta	

3. MEKKS: CLONING AND REGULATION

Four different MEKK genes have been cloned in mammalian cells based on homology to the yeast kinases Ste11 of S. *cerevisiae* and Byr2 of S. *pombe* (4-6). The sizes of the MEKK family members range from MEKK1 which is an 196 kDa protein and MEKK4 which is an 180 kDa protein, to MEKK2 and MEKK3 which are approximately 80 kDa proteins (figure 2). MEKK1 and MEKK4 are roughly 50% homologous to each other and to MEKK 2 and 3 in their C-terminal catalytic kinase domains. The N-terminal regulatory domains of MEKK 1-4 are quite different from each other (3). There are a number of interesting functional motifs found within the N-terminal regulatory domains of the MEKK1 and 4. MEKK1 and MEKK4 contain putative pleckstrin homology (PH) domains. PH domains associate with polyphosphoinositides and mediate localization to specific regions of the plasma membrane. As described below, this may explain why full length 196 kDa MEKK1 is associated with the membrane (7). MEKK1 and 4 also contain proline rich regions at the N-terminus which may be of functional significance. Proline rich regions have been shown to be important for binding to proteins that contain Src homology 3 (SH3) domains.

MEKK1 and 4, but not MEKK2 and 3 are regulated by LMWG proteins (8). MEKK4 contains a modified Cdc42/Rac interactive binding (CRIB) motif which is important for binding to Cdc42 and Rac (6). MEKK4 associates with Cdc42 and Rac in a nucleotide-independent manner. In contrast, MEKK1 associates with Rac and Cdc42 in a strongly GTP-dependent manner despite having no identifiable CRIB domain. In addition, MEKK1 also binds to Ras in a GTP dependent manner (9). Functional evidence indicates that MEKK1 and 4 are regulated by Cdc42 and Rac, as inactive forms of MEKK1 and 4 inhibit JNK activation by Cdc42 and Rac.

The MEKK family members also show binding specificity for 14-3-3 proteins. 14-3-3 proteins mediate protein-protein interactions and may serve as both chaperones and adaptor molecules (10). MEKK1, 2 and 3 but not MEKK4 selectively interact with 14-3-3 proteins (11). With regard to MEKK1, 14-3-3 proteins bind at the N-terminal regulatory domain. MEKK2 and 3 appear to contain 14-3-3 binding sites in both the N-terminal regulatory domains and with the C-terminal kinase domains. Although 14-3-3 association does not appear to dramatically affect MEKK activity, 14-3-3 proteins are probably important for MEKK regulation by mediating interactions with other regulatory proteins and for controlling subcellular localization of these kinases.

Several serine/threonine kinases have been shown to associate with and phosphorylate MEKK1 indicating that specific MKKKKs may regulate MEKK1 activity. Hematopoietic progenitor kinase 1 (HPK1), Nck interacting kinase (NIK) and GCK-like kinase (GLK) are serine/threonine kinases that resemble Ste20-like kinases in yeast. Ste20-like kinases are typically MKKKKs. HPK1, NIK and GLK associate with and phosphorylates MEKK1 suggesting that they may regulate MEKK1 activity (12-14). Since expression of kinase inactive MEKK1 inhibits JNK activation by HPK1, NIK and GLK, functional evidence supports the notion that these kinases reside upstream of

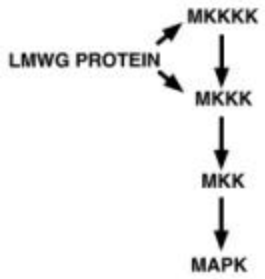

Figure 1. Sequential protein kinase pathway controlling the regulation of mitogen-activated protein kinase (MAPK) by extracellular stimuli. MAPK activity is controlled by a MAPK kinase (MKK) which is controlled by a MKK kinase (MKKK) which is controlled by a MKKK kinase (MKKKK). Low molecular weight GTP binding (LMWG) proteins regulate some MKKKKs and MKKKs.

Figure 2. Diagram illustrating MEKK family members and functional motifs encoded by these kinases that have been either demonstrated or hypothesized to be an important component of the regulation of these kinases. The hatched region indicates the kinase domain of each protein. One or more of the following motifs may be found in each kinase: proline rich region (PPP), pleckstrin homology domain (PH), Cdc42/Rac interactive binding motif (CRIB), Ras binding motif (RB).

MEKK1 in stress response pathways leading to JNK activation. Little is known about whether MEKK2, 3 and 4 are regulated by upstream kinases.

MEKKs are activated by a number of diverse extracellular stimuli, indicating that not only can these molecules affect a wide variety of downstream actions, they can also react to a diverse array of extracellular stimuli. For example, epidermal growth factor (EGF) receptor stimulation leads to an increase in MEKK1 activity in COS, T47D and PC12 cells (3,15). Other receptors including the tumor necrosis factor α (TNFα) receptor, FcεR1, and N-formyl methionyl leucine peptide receptor have been shown to activate MEKK1 (16,17). In addition, MEKK1 is activated in T cells by the T cell costimulatory receptor, CD28 (18). MEKK1 is also activated in response to DNA damaging agents such as irradiation, etoposide and cisplatin (19). Like MEKK1, MEKK2 is also activated in response to EGF stimulation of cells (G.R. Fanger and G.L. Johnson, unpublished observations). Little is presently known about what extracellular stimuli increase the activity of MEKK3 and 4.

4. MAPKS: REGULATION AND BIOLOGICAL ROLE

Based on similarity to yeast MKKKs, the MEKKs were first shown to be regulators of the MAPK pathways. When overexpressed in cells MEKK1, 2 and 3 but not MEKK4 are able to activate ERK1/2, whereas all of the MEKKs activate JNK. However, MEKK1-4 do not activate p38 when transiently expressed in HEK293 or COS cells. Although MEKK 2 and 3 are approximately 94% homologous in their C-terminal kinase domains, they are different with regard to their substrate specificities: MEKK2 preferentially activates the JNK cascade compared to the ERK cascade, while MEKK3 preferentially activates the ERK cascade in transient transfection experiments (5). There is evidence that JNK rather than ERK is preferentially activated by the kinase domain of MEKK1 (20-22). However, we have recently shown that MEKK1 contributes as a signaling intermediate in EGF stimulated ERK activity in COS cells (8). Furthermore, cells having the homologous disruption of MEKK1 expression have reduced ERK activation in response to LPA stimulation, as well as reduced JNK activation in response to stress, such as hyperosmolarity (23). Thus, like Ste11 in yeast, the MEKKs are involved in signaling more than one MAPK pathway.

MEKK1 activates ERK via the MKKs MEK1/2. ERK activation results in phosphorylation of cytoplasmic localized proteins and, following ERK translocation to the nucleus, activation of transcription factors. In the cytoplasm, ERK1/2 have also been shown to regulate a number of kinases including the EGF receptor, Raf1 and MEK, decreasing their catalytic activities in a possible feedback regulatory loop (24). Phosphorylation of S6 kinase $p90^{rsk}$ by ERK1/2 is associated with its activation (25). The microtubule associated proteins MAP-1, MAP-2, MAP-4 and Tau are phosphorylated by ERK1/2 (25). ERK1/2 also phosphorylate and activate cytoplasmic phospholipase A2 affecting the release of arachidonic acid (26). Upon translocation to the nucleus, ERK1/2 phosphorylate and regulate the activity of different transcription factors including Elk, Ets1, Sap1, c-Myc, Tal, STAT, Myb and c-Jun (27). Activation of ERK1 and ERK2 is often associated with proliferative signals (19,25,28), but has also been shown to inhibit proliferation in a few systems (29,30). Other biological outcomes have been associated with ERK 1 and ERK 2 activation, for example, protection from apoptotic stimuli (31,32) and terminal differentiation (33).

As mentioned, all four MEKKs are major regulators of the JNK pathway. MEKK-mediated JNK activation occurs via the MAPKK, JNKK, as well as perhaps MKK7. JNK activation has been correlated with widely different biological outcomes depending on the cell type and stimulus with which they are characterized. Several studies implicate JNK in apoptotic signaling, while other studies have suggested that JNK activity does not play a role in apoptosis (19,31,34-39). Still other studies have suggested that JNK activation is a protective response to apoptotic stresses or a proliferative signal (36,40,41). JNK1 and 2 have been shown to phosphorylate the transcription factors c-Jun, ATF-2, Elk-1, p53, DPC4 and NFAT4. The JNK family displays additional diversity in that each gene is alternatively spliced and the splice variants have been proposed to differentially influence the regulation of certain transcription factors (42).

5. ROLE OF MEKK1 IN APOPTOSIS AND CELL SURVIVAL

MEKK1 is important in regulating cell survival and apoptosis. PC12 cells transiently overexpressing the kinase domain of MEKK1 apoptosed in a JNK-dependent manner (31) In Swiss 3T3 cells and rat embryo fibroblasts, microinjection of the kinase domain of MEKK1 caused cells to undergo apoptosis independent of JNK activation (43). MEKK1 is required for apoptosis following DNA-damaging stresses such as UV irradiation, cisplatin, etoposide and mitomycin C, as well as following detachment from the extracellular matrix (anoikis) in MDCK cells (19,44). Consistent with the role of MEKK1 in apoptosis, MEKK1 is a substrate for caspases, a family of proteases required for apoptosis. The apoptotic signaling appears to be dependent on cleavage of full length MEKK1 and subsequent caspase activation, as cleavage resistant mutants do not induce apoptosis and can inhibit some apoptotic signals (19,44). Caspase-mediated cleavage of MEKK1 potentiates apoptosis by a currently ill-defined mechanism which may include upregulation of Fas ligand and activation of "death receptors" (39). Recently, our laboratory discovered that the full length 196 kDa form of MEKK1 may be involved in promoting cell survivial as embryonic stem cells with a homologous disruption of MEKK1 expression undergo apoptosis to stress stimuli at a significantly greater rate than wild type cells (23). Thus, MEKK1 plays a pivotal role in regulating cell survival and death, acting as a molecular switch when cleaved by caspases. MEKK1 changes from a survival promoting kinase to an effector of cell death when cleaved by caspases.

Subcellular localization appears to be a critical regulatory mechanism that controls the survival-promoting vs. apoptosis-inducing role of MEKK1. Full length 196 kDa MEKK1 is cleaved by caspases to form a 91 kDa fragment which contains the kinase domain and a 105 kDa N-terminal fragment (19,44). Only the 91 kDa form and not the 196 kDa form of MEKK1 is proapoptotic. The 196 kDa full length form of MEKK1 is membrane associated and following caspase activation, the 91 kDa cleavage product localizes to the soluble fraction of the cytoplasm and is no longer tethered to membranes (7). Tethering the 91 kDa form of MEKK1 to the cell membrane via a CAAX box modification prevents MEKK1-mediated apoptosis (45). It is possible that a putative pleckstrin homology (PH) domain found at the N-terminus of 196 kDa MEKK1 may mediate membrane tethering as it does for the guanine nucleotide exchange factor son of sevenless (SOS) (46). Another potential mechanism that may mediate membrane tethering is the association of 14-3-3 proteins which bind to phosphorylated serine motifs and associate with MEKK1 at the extreme N-terminus (11). Upon caspase cleavage, the kinase domain of MEKK1 no longer binds to 14-3-3 proteins nor contains the putative pleckstrin homology domain, thus relocalizes to the cytosol and so may interact with a different pool of effectors stimulating apoptotic pathways. With regard to regulation of the MAPK pathways, overexpression of the full length 196 kDa, but not the 91 kDa form of MEKK1 induces activation of ERK, a signal responsible for cell survival and proliferation (45). Thus, appropriate localization is critical for apoptosis and for determining the biological role of MEKK1.

6. REGULATION OF NFKB BY MEKK1

Independent of the MAPK activity, MEKK1 has been proposed to play an important role in regulating the transcription factor NFκB. NFκB is a dimer which is maintained in the cytoplasm via the inhibitory regulatory subunit IkB. Upon stimulation with specific cytokines or environmental stress, IkB is phosphorylated by IκB kinase which induces proteolytic degradation of IκB releasing NFκB to translocate to the nucleus effecting changes in transcription. Overexpression of MEKK1 potently activates NFκB activity. MEKK1 phosphorylates and activates both IκBα and β kinases (47). IκB kinase is required for MEKK1-mediated NFκB activation as an inhibitory form of IκB kinase blocks NFκB activation by MEKK1 expression. Additional support that MEKK regulates NFκB is provided by the properties of the HTLV-1 protein Tax (48). Tax is reported to bind to the N-terminus of MEKK1 and stimulate MEKK1 activity with subsequent NFκB activation and nuclear translocation. Inhibitory mutants of MEKK1 inhibit NFκB activation by Tax. NIK (NFκB interacting kinase) also appears to play an important role in NFκB activation as expression of NIK will induce NFκB activation and coexpression of NIK with MEKK1 strongly potentiates NFκB translocation and activation (49,50). The NFκB regulatory NIK unfortunately has the same abbreviation as the Nck-interacting kinase (NIK) that is proposed to regulate MEKK1 (see above); the two should not be confused because they are independent of one another. There is evidence that NIK phosphorylates IκB kinase and does not require MEKK1 for NFκB activation (49).

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