Promega Notes Magazine Number 59, 1996, p. 37

MAP Kinase Signaling Pathways

By Melanie H. Cobb, University of Texas Southwestern Medical Center*
and Erik M. Schaefer, Promega Corporation

**Department of Pharmacology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9041*

Mitogen-activated protein kinases (MAPKs) have recently been the focus of intensive study. Their involvement in a variety of signaling pathways, their modes of action, and mechanisms which contribute to their specificity, are the subject of this review.

Introduction

Over the last five years, the mitogen-activated protein kinases (MAPKs) have been at the forefront of a rapid advance in the understanding of cellular events in growth factor and cytokine receptor signaling (1-4). The MAP kinases (also referred to as extracellular signal-regulated protein kinases, or ERKs) are the terminal enzymes in a three-kinase cascade. The reiteration of three-kinase cascades for related but distinct signaling pathways gave rise to the concept of a MAPK pathway as a modular, multifunctional signaling element that acts sequentially within one pathway, where each enzyme phosphorylates and thereby activates the next member in the sequence (5; Figure 1 and Table 1). A canonical MAPK module thus consists of three protein kinases: a MAPK kinase kinase (or MEKK) that activates a MAPK kinase (or MEK) which, in turn, activates a MAPK/ERK enzyme. In the MAPK module of the yeast pheromone response pathway, a scaffold protein Ste5p binds the three analogous enzymes of its module, suggesting that the module may function as a physically stable unit (5).

Table 1. Enzymes Involved in MAP Kinase Signaling Pathways.

Generic Pathway	
MAPK	Mitogen-activated protein kinase (or ERK) superfamily, has TXY consensus sequence in the catalytic core. ERK1/2, p38/HOG and JNK/SAPK represent related yet distinct terminal enzymes in parallel pathways.
ERK	Extracellular signal-regulated protein kinase (or MAPK).
MEK	MAPK (ERK) kinase, Ser/Thr/Tyr-specific protein kinase that activates MAPKs by phosphorylating both Thr and Tyr within the TXY consensus sequence.
MEKK	MEK kinase or MAPK kinase kinase, Ser/Thr-specific protein kinase that dually phosphorylates — and thereby activates — one or more of the MEK enzymes on Ser or Thr residues (Ser-X-X-X-Ser/Thr) within the catalytic core.
ERK/MAPK Pathway	
MAPK	Mitogen-activated protein kinase subfamily, refers to ERK1 and ERK2, which have the TEY consensus sequence in the catalytic core.
ERK	Extracellular signal-regulated protein kinase (or MAPK). Examples are ERK1 (p44) and ERK2 (p42).
Raf	MEKK, known to activate the MAPK/ERK pathway. Raf has three isoforms (A-Raf, B-Raf and C-Raf-1). Raf is activated by several events, including phosphorylation at multiple residues and interaction with p21ras.
MOS	Another MEKK enzyme known to activate MAPK/ERKs.
p21ras	Guanine-nucleotide binding protein (binds GTP and hydrolyzes it to GDP). While GTP is bound, p21ras is in the active conformation. Becomes localized to the membrane as a result of being isoprenylated (attachment of a C15 or C20 lipid molecule) post-translationally.
GRB2	Adaptor proteins containing Src homology 2 and 3 (SH2 and SH3) domains that link protein tyrosine kinases (PTKs) to p21ras, thereby facilitating p21ras-mediated activation of Raf.
SOS	Ras guanine-nucleotide exchange factor that catalyzes the exchange of GDP for GTP on p21ras to activate it.
JNK/SAPK Pathway	
JNK	c-jun amino terminal protein kinase (or SAPK). MAPK superfamily member activated by stress, UV and inflammatory cytokines. Has TPY consensus sequence in catalytic core.
c-jun	Transcription factor regulated by protein phosphorylation on Ser residues. Forms homo- and heterodimers with jun and fos family members, which enables binding to promoter elements and activation of transcription.
SAPK	Stress-activated protein kinase (or JNK).
JNKK	Ser/Thr/Tyr specific protein kinase that activates the JNK/SAPK enzymes (or MEK4).
PAK	Protein Ser/Thr kinase activated by small GTP-binding proteins like RAC/Cdc42.
RAC	Small GTP binding protein that activates PAK and several other effectors. Becomes localized to the membrane as a result of being geranylgeranylated (attachment of a C20 lipid molecule).
p38/HOG Pathway	
p38	Mammalian MAPK superfamily member activated by stress, ultraviolet light and inflammatory cytokines. Has TGY consensus sequence in catalytic core.
HOG	Yeast homolog of mammalian p38 enzyme. Activated by osmotic stress.

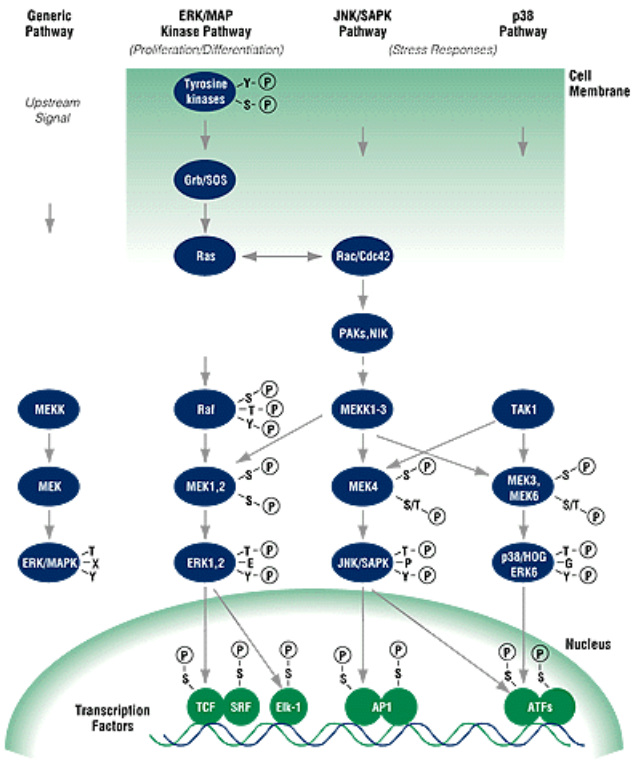

Figure 1. Parallel MAPK cascades involve specific MAPK enzyme modules. Each of the MAPK/ERK, JNK and p38 cascades consists of a three-enzyme module that includes MEKK, MEK and an ERK or MAPK superfamily member. A variety of extracellular signals triggers initial events upon association with their respective cell surface receptors and this signal is then transmitted to the interior of the cell where it activates the appropriate cascades. The shaded area indicates those signaling molecules that become associated with the intracellular surface of the plasma membrane upon activation.

The recent identification of distinct MAPK cascades that are conserved across all eukaryotes indicates that the MAPK module has been adapted for interpretation of a diverse array of extracellular signals. Although mitogen activation of the MAPK subfamily (e.g., ERK1 and ERK2) has dominated efforts to understand MAPK signaling, increasing appreciation of the role of the stress-activated kinases, JNK and p38, illustrates the diverse nature of the MAPK superfamily of enzymes (Figure 1). Although sequence similarities among components of the individual MAPK modules used for activation of ERK1/2, JNKs and p38 are considerable, the fidelity that is maintained in order to translate specific extracellular signals into discrete physiological responses illustrates the selective adaptation of each MAPK module. Understanding how such specificity is maintained, and the extent and significance of cross-talk between each signaling cascade, are fundamental issues that are actively being investigated by the research community.

Regulation and function of MAP Kinases

Multipurpose signal transducers

The MAPK superfamily of enzymes is a critical component of a central switchboard that coordinates incoming signals generated by a variety of extracellular and intracellular mediators. Specific phosphorylation and activation of enzymes in the MAPK module transmits the signal down the cascade, resulting in phosphorylation of many proteins with substantial regulatory functions throughout the cell, including other protein kinases, transcription factors, cytoskeletal proteins and other enzymes. The diversity of signals that culminates in MAPK activation indicates that these enzymes are not the dedicated hardware of any single growth factor, hormone or cytokine system. Instead, MAPKs -- like cAMP-dependent protein kinase (PKA) and Ca^{2+}- and phospholipid-dependent protein kinases (PKC) -- serve many signaling purposes. Because activation of the MAPK pathways are triggered to varying extents by a large number of receptor systems, temporal and spatial differences are critical to determining ligand- and cell-type-specific functions.

MAP kinase modules

Following activation of cells with the appropriate extracellular stimuli, the signal is transmitted to the canonical MAPK module comprising the three protein kinases (Figure 1). The progression of events for each enzyme cascade is the same, although specific isoforms of each enzyme confer the required specificity within each pathway. The first enzyme in the module is a MEKK enzyme, of which Raf and its isoforms are one example. The MEKK enzymes are Ser/Thr protein kinases that activate the MEK enzymes by phosphorylating two serine or threonine residues within a Ser-X-X-X-Ser/Thr motif. Once activated, the MEK enzymes, which are

mixed function Ser/Thr/Tyr protein kinases, phosphorylate the MAPK/ERK enzymes on Thr and Tyr residues within the Thr-X-Tyr (TXY) consensus sequence (Figure 2). A critical and common feature of the MAPK superfamily of enzymes is that they are activated upon dual phosphorylation within the TXY consensus sequence present in the activation loop of the catalytic domain (6-8). The central amino acid differs for each MAPK superfamily member, corresponding to Glu for ERK1/2, Gly for p38/HOG and Pro for JNK/SAPK, although MEK specificity is not limited to these residues (9). Phosphorylation at only one of the two positions does not activate the enzyme, although it may prime the kinase domain for receipt of the second phosphorylation event.

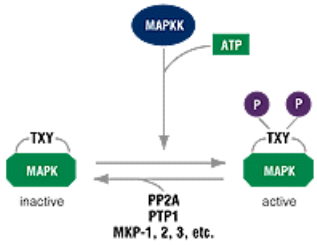

Figure 2. Dual phosphorylation activates the MAPK enzymes. A critical common feature of the MAPK superfamily of enzymes is that they are activated by MEK enzyme-mediated dual phosphorylation within the TXY consensus sequence in the activation loop of the catalytic domain. Phosphorylation of only one of the two positions does not activate the enzyme although it may serve to prime the kinase domain for receipt of the second phosphorylation event. A number of protein phosphatases (e.g., PP2A, PTP1 and MKPs) are involved in down-regulation of the signal, by dephosphorylating either one or both phosphorylated residues.

Regulation of ERK enzyme activation

ERK1 and ERK2 were the first members of the MAPK superfamily whose cDNAs were cloned (10,11) and the signaling cascades that lead to their activation are the best characterized to date. Potent activation of ERK1 and ERK2 can be initiated through activation of transmembrane receptors with intrinsic or associated protein tyrosine kinase (PTK) activity (reviewed in 12). In this scenario, binding of extracellular ligands to their respective cell surface receptors results in receptor autophosphorylation and enhanced PTK activity. The subsequent association of the Src homology 2 (SH2) domains of adaptor proteins such as Grb2 and Shc with the autophosphorylated receptors, or with additional "docking" proteins, provides the molecular interactions that bring the required signal transduction molecules into close proximity. Receptors without intrinsic PTK activity but which harbor sites for tyrosine phosphorylation may also activate the cascade via association of their phosphotyrosine residues with adaptor molecules. For example, the SH3 domain of Grb2 binds a proline-rich region of the guanine nucleotide-exchange protein SOS which, in turn, increases the association of Ras with GTP. The GTP-bound form of Ras binds to Raf (a MAPK kinase kinase) isoforms, including C-Raf-1, B-Raf and A-Raf. This action targets Raf to the membrane, where its protein kinase activity is increased by phosphorylation (12). MAPK kinases (MEK1 and MEK2) (2), are phosphorylated and activated by Raf. MEK1 and MEK2 are dual-specificity protein kinases that dually phosphorylate the ERK enzymes (corresponding to Thr^{183} and Tyr^{185} of p42ERK2), thereby increasing their enzymatic activity by approximately 1,000-fold over the activity found with the basal or monophosphorylated forms (13). Phosphorylation of these residues causes closure of the kinase active site and induces conformational changes necessary for high activity (7).

MAPK enzyme inhibition provides insight to cellular functions

MAPK mutants, lacking either a lysine required for catalytic activity or the prerequisite TXY phosphorylation sites, inhibit signaling by the native enzymes in cells. In the case of ERK1 and ERK2, these mutants have been used with repeated success. For example, mutant ERK2 completely blocks proliferation in response to epidermal growth factor (EGF) and v-Raf, and partially blocks induction by serum or small t antigen (14). ERK1 antisense mRNA and an ERK1 phosphorylation site mutant interfere with thrombin-induced transcription as well as serum-dependent proliferation (15). These findings suggest an essential role in proliferation and transformation for the ERK/MAPK pathway.

Regulation of JNK/SAPK and p38/hog

The JNK/SAPK and p38/HOG pathways are activated by ultraviolet light, cytokines, osmotic shock, inhibitors of DNA, RNA, and protein synthesis, and to a lesser extent by growth factors. This spectrum of regulators suggests that the enzymes are transducers of a variety of stress responses. In contrast to activation of ERK1 and ERK2, upstream signal transduction mechanisms for the JNK and p38 cascades are less well understood (Figure 1). When transfected into mammalian cells, a diverse group of protein kinases including the mixed lineage kinases (MLKs) and relatives of the yeast Ste20p -- e.g., the p21-activated kinases (PAKs) and germinal center kinase (GCK) -- cause activation of JNK/SAPK. Similarly, GTP-bound forms of the small GTP-binding proteins, Rac and Cdc42, activate the JNK/SAPK pathway and, to a lesser extent, the p38 pathway (16). Direct activation of both pathways by PAKs also has been demonstrated (17), suggesting that PAKs may be the relevant effectors for these small G proteins. The PAKs are homologs of the yeast kinases Ste20p and Shk1, enzymes upstream of the MAPK modules in yeast pheromone response pathways (5). Both yeast and mammalian protein kinases contain a binding site for Rac/Cdc42 and share the property of being activated *in vitro* through association with these small G proteins when in their GTP-bound states. In yeast, Ste20p is believed to phosphorylate and activate the MEKK

isoform Ste11p, suggesting that MEKKs may be PAK targets.

Determinants of MAPK Module Specificity

Because of the pleiotropic potential of MAPKs, their activities are tightly controlled by both positive and negative mechanisms. A variety of factors is known to modulate MAPK activity including substrate specificity, protein-protein interactions, subcellular localization, and dephosphorylation by protein phosphatases. These regulatory mechanisms are discussed below.

MEKS as determinants of MAPK signaling specificity

MEK1 and MEK2 are the only known activators of ERK1 and ERK2 and are believed to phosphorylate only these two substrates. Other MAPK family members retain the TXY phosphorylation sites but are poor substrates of MEK1 and MEK2 (9). Similarly, cloning efforts have uncovered MEK-like enzymes that show high selectivity in phosphorylating JNK/SAPKs and p38, further illustrating the important contribution of the MEK enzymes in determining MAPK signaling specificity.

Protein-protein interactions

As noted earlier, Ste5p promotes the formation of complexes among enzymes of the MAPK module that mediate pheromone-induced mating. Also, Ste5p is absolutely required for signaling through this module since deletion of this gene, or overexpression of a Ste5p mutant that cannot bind the yeast MAPK enzyme, blocks the pheromone response (5). Given the known conservation between yeast and mammals of components in MAPK signaling cascades, and the existence of scaffolding factors for a number of protein phosphatases and other protein kinases (18), scaffolding factors like Ste5p are likely to exist in metazoans. These proteins may restrict the function of potentially promiscuous enzymes, such as MEKK1, in order to maintain the fidelity of individual signaling pathways.

Intracellular distribution and pathway specificity

Subcellular localization undoubtedly plays an important part in directing ERK signaling and in limiting cross-activation between related modules. In quiescent cells, ERK1 and ERK2 are found in the cytoplasm and are associated with microtubules. Stimulation of cells with mitogens results in activation of the ERK enzymes within the cytoplasm, with some of the enzyme located in specialized membrane compartments. With mitogenic activation of certain cell lines, a fraction of the activated ERK enzymes is efficiently translocated to the nucleus (19). A similar story is unfolding for JNK/SAPKs and p38. The kinetics of ERK activation influence the efficiency of nuclear translocation and, thus, access to nuclear substrates. If the kinase is rapidly inactivated, as occurs following EGF stimulation of PC12 cells or by nonmitogenic stimulation of CCL39 cells (19), it may not enter or remain in the nucleus. In contrast, a more prolonged period of activation, as occurs with nerve growth factor (NGF)-stimulated PC12 cells (20) or thrombin-treated CCL39 cells, results in nuclear retention and this may be critical for establishing the differentiation signal (4).

Protein phosphatases

Because phosphorylation of both tyrosine and threonine is required to activate the MAPK enzymes, certain dual-specificity protein phosphatases, such as the immediate early gene MKP-1, appear well-suited to inactivate the MAPK family members by dephosphorylating one or both sites (21). However, individual Ser/Thr (e.g., PP2A) or Tyr (e.g., PTP1) protein phosphatases also can regulate ERK activity by dephosphorylating only one of the two phosphorylated residues and thereby inactivating the enzyme (12,21,22). The relatively high abundance and high specific activity of these Ser/Thr or Tyr protein phosphatases provides another important mechanism for regulating ERK enzyme activity. The final physiological outcome of these signal transduction pathways therefore is determined by the interplay between these protein kinases and protein phosphatases.

Summary

Much work remains to fully understand the properties and functions of MAPK pathways. Among significant challenges are the complete clarification of the regulatory steps and specificity determinants in these MAPK cascades. In addition, the identification and characterization of new regulatory signal transduction pathways for which the paradigm of the MAPK cascade module is conserved represents an exciting avenue to be explored.

References

1. Cobb, M.H. and Goldsmith, B.S. (1995) *J. Biol. Chem.* **270,** 14843.
2. Seger, R. and Krebs, E.G. (1995) *FASEB J.* **9,** 726.
3. Cano, E. and Mahadevan, L.C. (1995) *Trends Biochem. Sci.* **20,** 117.
4. Marshall, C.J. (1995) *Cell* **80,** 179.
5. Herskowitz, I. (1995) *Cell* **80,** 187.
6. Payne, D.M. *et al.* (1991) *EMBO J.* **10,** 885.
7. Zhang, J. *et al.* (1995) *Structure* **3,** 299.
8. Doza, Y.N. *et al.* (1995) *FEBS Letters* **364,** 223.

9. Robinson, M.J. *et al.* (1996) *J. Biol. Chem.,* in press.
10. Boulton, T.G. *et al.* (1990) *Science* **249,** 64.
11. Boulton, T.G. *et al.* (1991) *Cell* **65,** 663.
12. Hunter, T. (1995) *Cell* **80,** 225.
13. Robbins, D.J. *et al.* (1993) *J. Biol. Chem.* **268,** 5097.
14. Sontag, E. *et al.* (1993) *Cell* **75,** 887.
15. Pages, G. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8319.
16. Minden, A. *et al.* (1995) *Cell* **81,** 1147.
17. Zhang, S. *et al.* (1995) *J. Biol. Chem.* **270,** 23934.
18. Faux, M.C. and Scott, J.D. (1996) *Trends Biochem. Sci.* **21,** 312.
19. Lenormand, P. *et al.* (1993) *J. Cell. Biol.* **122,** 1089.
20. Traverse, S. *et al.* (1992) *Biochem. J.* **288,** 351.
21. Alessi, D.R. *et al.* (1995) *Current Biology* **5,** 283.
22. Keyse, S.M. (1995) *Biochim. Biophys. Acta.* **1265,** 152.
