MAP kinase pathways

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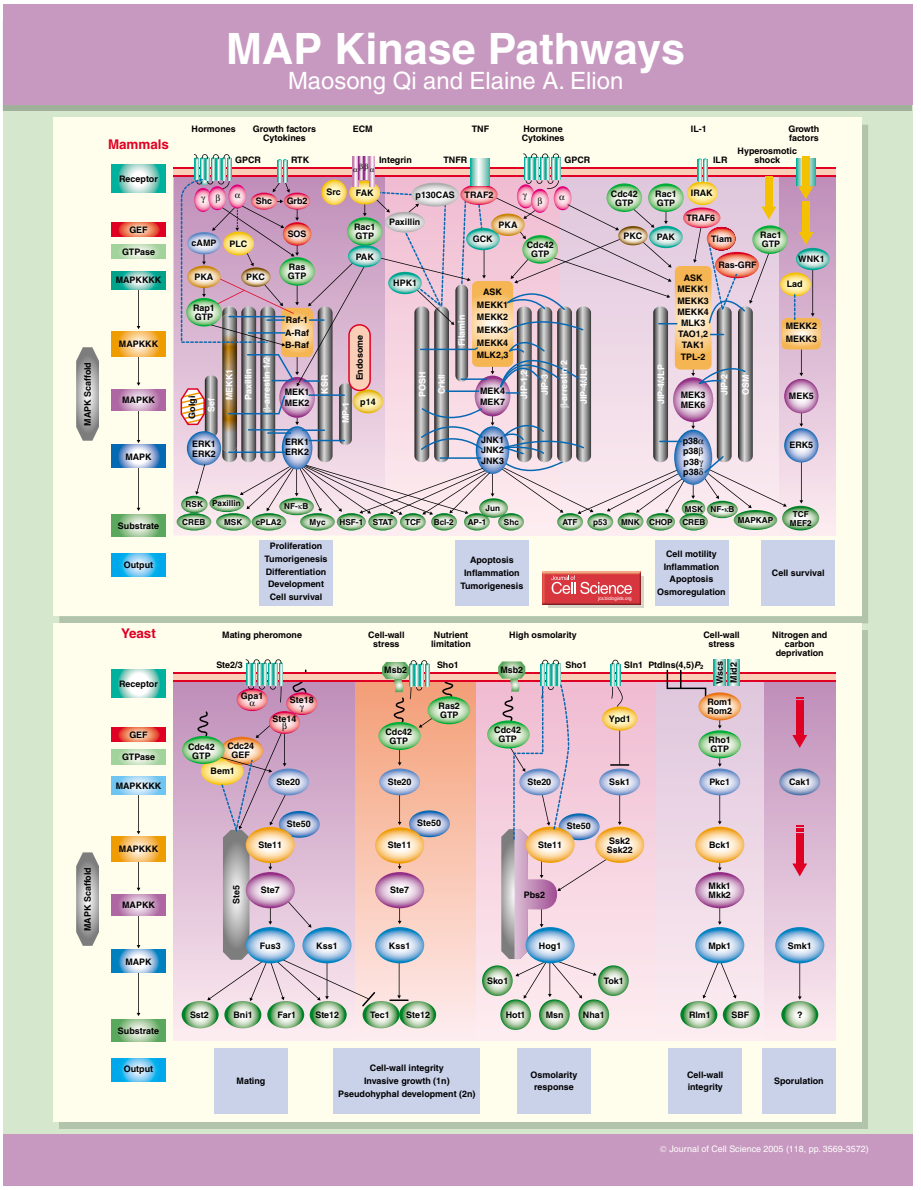
Journal of Cell Science 118, 3569-3572
Published by The Company of Biologists 2005
doi:10.1242/jcs.02470

Mitogen-activated protein kinase (MAPK) pathways regulate diverse processes ranging from proliferation and differentiation to apoptosis. Activated by an enormous array of stimuli, they phosphorylate numerous proteins, including transcription factors, cytoskeletal proteins, kinases and other enzymes, and greatly influence gene expression, metabolism, cell division, cell morphology and cell survival.

Each MAPK pathway contains a three-tiered kinase cascade comprising a MAP kinase kinase kinase (MAPKKK, MAP3K, MEKK or MKKK), a MAP kinase kinase (MAPKK, MAP2K, MEK or MKK) and the MAPK. This three-tier module mediates ultrasensitive switch-like responses to stimuli (Ferrell, 1996). Frequently, a MAPKKK kinase (MAPKKKK, MAP4K or MKKKK) activates the MAPKKK. The MAPKKKK or MAPKKK can be linked to the plasma membrane – for example, through association with a small GTPase or lipid (e.g. Ste20, PKC, PAK MAPKKKKs and Raf MAPKKKs).



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Numerous different MAPKKKs exist, including Raf isoforms, Ste11 relatives MEKK1-MEKK4, mixed lineage kinases (MLKs), Tao proteins and Mos. The MAPKKs and MAPKs are more highly related (Caffrey et al., 1999; Chen et al., 2001; Uhlik et al., 2005). The MAPKKKs can have large regulatory domains that interact with upstream regulators (e.g. Rho, Ras), have functions such as ubiquitylation and are activated by relief of autoinhibition and oligomerization. MAPKKs typically have smaller regulatory domains and are activated by dual phosphorylation of serine and threonine residues within the activation loop of the catalytic domain. They exhibit great specificity for their cognate MAPK but are regulated by many MAPKKKs.

MAPKs are activated by dual phosphorylation of conserved threonine and tyrosine residues within the activation loop (denoted T-X-Y) and phosphorylate targets on serine and threonine residues within a consensus PXT/SP motif (X can depend on the MAPK) (Songyang et al., 1996; Chen et al., 2001). Pathway specificity is regulated at several levels, including kinase-kinase and kinase-substrate interactions, colocalization of kinases by scaffold proteins, and inhibition of cross-talk/output by the MAPKs themselves. Scaffolds regulate MAPK signaling in multiple ways beyond simple tethering (Burack and Shaw, 2000; Elion, 2001).

MAPKs bind stably to substrates, MAPKKs and scaffold proteins through multiple docking domains (e.g. CD) distinct from their active sites that can recognize homologous sites on different targets (e.g. D and FXF domains), which raises the question of how this influences signaling (Chang et al., 2002; Tanoue and Nishida, 2003). MAPKs can also inhibit signaling by binding substrates when catalytically inactive (Madhani and Fink, 1998). MAPKs are attenuated by dual specificity MAPK phosphatases (MKPs), tyrosine phosphatases and serine/threonine phosphatases (Keyse, 2000). Continuous basal repression of

MAPKs by phosphatases occurs and may poise a pathway for activation above a particular threshold.

Signaling complexes of MAPK module components can vary with time and location. Active MAPKs frequently translocate from the cytoplasm to the nucleus to phosphorylate nuclear targets, and MAPKKs can shuttle in and out of the nucleus carrying the MAPK as a passenger and anchor them in the cytoplasm. MAPK scaffold proteins may function similarly (Elion, 2001). MAPKs localize to numerous subcellular structures, including microtubules, endosomes, the ER and the actin cytoskeleton. MAPK phosphatases may attenuate MAPKs in a site-specific manner.

Yeast MAPK pathways

All *Saccharomyces cerevisiae* MAPK pathways were defined genetically. Although components are shared between different pathways, erroneous crosstalk does not occur. This is because of insulation mechanisms that result in exquisite specificity. The mating pathway was the first MAPK module to be defined, sequence similarity between the Ste11 MAPKKK and Ste7 MAPKK and their mammalian equivalents aiding cloning of the latter.

Five MAPK pathways, utilizing six MAPKs have been defined: the Fus3 and Kss1 MAPKs are most similar to mammalian ERK1/2; the Hog1 MAPK is most similar to mammalian p38; and the Mpk1 (also known as Slt2) Mpl1 and Smk1 MAPKs, which are distinct (Caffrey et al., 1999). Fus3 and Kss1 regulate mating in response to peptide mating pheromones and have shared and unique substrates but only Fus3 is essential for mating, Kss1 functions in additional pathways that regulate invasive growth/pseudohyphal development and cell wall integrity (Wang and Dohlman, 2004; Bardwell, 2005; Elion et al., 2005). Hog1 regulates intracellular osmolarity in response to extracellular osmolarity and citric acid stress, Mpk1 regulates cell integrity and budding in response to mechanical changes at the cell wall/plasma membrane (with undefined input by Mpl1) and Smk1 regulates sporulation, being expressed only after meiosis has been initiated in response to carbon and nitrogen deprivation. Fus3 is activated by the MAPKK Ste7 on the Ste5 scaffold through localizing interactions involving a heterotrimeric G protein and Cdc42 GTPase, which guide the MAPKKK Ste11 to the MAPKKKK Ste20. Kss1 can be activated by Ste7 that is not bound to Ste5. Hog1 can be activated by Ste11 through linkage of the Pbs2 scaffold to a plasma membrane sensor, Sho1. It is also activated by a two-component relay system involving the sensor Sln1 (an autophosphorylating protein-histidine kinase), a phosphotransfer protein, Ypd1, and a receiver, Ssk1, that activates two MAPKKKs Ssk2 and Ssk22 that activate Pbs2 MAPKK (Chellapan, 2000; Westfall et al., 2004). Mpk1 (and possibly Mpl2) regulates cell integrity and is activated by the MAPKKK Bck1, and the MAPKKs Mkk1 and Mkk2 in response to stimulation of integrin-like proteins (i.e. Wsc1-4, Mid2) linked to guanine nucleotide exchange factors (Rom1 and Rom2) that activate the Rho1 GTPase, which activates protein kinase C (PKC) 1, a MAPKKKK for the pathway (Hohmann, 2002).

Scaffold proteins are essential for mating and high-osmolarity signaling. Ste5 and Pbs2 provide specificity by segregating shared kinases with pathway-specific kinases and receptors that sense stimuli (Elion, 1998). Ste5 provides separate binding sites for Ste11, Ste7 and Fus3 and stimulates phospho-relay by proximity effects, oligomerization, and conformational changes (Elion, 2001; Ferrell and Cimprich, 2003). The Pbs2 scaffold joins Ste11 with Hog1 and also links Ste11 to Cdc42-GTP-bound Ste20, through binding of its proline-rich domain to an SH3 domain of Sho1, which also binds to Ste11.

The yeast MAPKs play unportant roles in cell physiology although they are nonessential – *hog1Δ* and *mpk1Δ* single mutants grow poorly, strains lacking all of the MAPKs are very sick, and sexual reproduction and sporulation are blocked in *fus3Δ kss1Δ* and *smk1Δ* mutants, respectively. The MAPKs are inhibited to varying degrees by MAPK phosphatases, Msg5 and Sdp1, tyrosine phosphatases, Ptp2 and Ptp3, and Ptc1 – a type 2C Ser/Thr phosphatase (PP2C).

Yeast MAPKs help maintain signaling specificity: Hog1 prevents misactivation of Fus3 and Kss1 by Ste11 during high osmolarity stress (Sprague, 1998) and Fus3 induces degradation of invasive growth regulator Tec1 and attenuates Kss1 (Elion et al., 2005). Additional factors may prevent misactivation of the HOG pathway by activated Ste11 during mating and filamentous growth and enhance activation of Fus3 over that of Kss1 during mating.

Mammalian MAPK pathways

Mammalian MAPK pathways are difficult to assign because of the many kinases, cell lines, tissue types, experimental conditions (which can yield conflicting results) and functional redundancy. Genetic analysis in *Drosophila melanogaster* and *Caenorhabditis elegans* has greatly helped, however (Chang and Karin, 2001). Five families of MAPKs have been defined in mammalian cells: extracellular signal-regulated kinases (ERK1 and ERK2), Jun N-terminal kinases (JNK1, JNK2 and JNK3); p38 kinase isozymes (p38α, p38β, p38γ and p38δ); ERK3/ERK4; and ERK5 (Davis, 2000; Chen et al., 2001; Chang and Karin, 2001; Johnson and Lapadat, 2002; Roux and Blenis, 2004). The first three, and their activators, are implicated in human diseases and are targets for drug development (Manning and Davis, 2003; English and Cobb, 2002). Mammalian MAPK modules associate with numerous scaffold proteins that regulate their activity and localization in various cells (Morrison and Davis, 2003). The scaffold proteins can bind to other proteins, including phosphatases and cytoskeletal proteins. While the significance of scaffolding is not yet fully defined in most instances, it appears to be a method generally used to segregate individual MAPK modules and may hep define specific MAPK pathways.

ERK1 and ERK2 regulate proliferation, differentiation and meiosis, and learning and memory in nerve cells. They are activated by mitogenic stimuli such as growth factors, cytokines and phorbol

esters, which activate a variety of receptors and G proteins. A complete pathway from receptor to MAPK has only been fully defined for the ERK1 and ERK2 kinases, which act downstream of the Ras proto-oncoprotein. The ERKs are expressed in many tissues and form part of a MAPK module that includes Raf MAPKKKs (A-Raf, B-Raf, C-Raf/Raf-1) and theMEK1/MEK2 MAPKK. Ligands bind to cell-surface-receptor tyrosine kinases or G-protein-coupled receptors. This leads to activation of Ras by associated SOS (son of sevenless). Ras-GTP then triggers activation of Raf isoforms and recruitment of Raf to the plasma membrane (Wellbrock et al., 2004), followed by phosphorylation of MEK1/MEK2 and then ERK1, ERK2. This is facilitated by the scaffold protein KSR, which links the three tiers of kinases to Ras. Other scaffold proteins can also tether the ERK module, including β-arrestin1/2 and MP-1, which links MEK1 and ERK1 to p14 and endosomes. ERK1/ERK2 have many known targets, including key transcription factors, such as AP-1, NF-κB, Myc, kinases, such as Rsk, the cell survival regulator Bcl-2, cPL2 and the cytoskeletal scaffold paxillin. Depending on the strength and duration of stimulation, activation of the ERK1/ERK2 MAPKs can lead to either proliferation or differentiation (Marshall, 1995). Ras is frequently mutated in many tumors, and associated constitutive activation of ERK1/ERK2 promotes tumor cell proliferation. Gene targeting experiments indicate ERK1 is important for T cell responses whereas ERK2 plays a role in mesoderm differentation and placenta formation. More recent work suggests roles for ERK1 and ERK2 in neuronal responses including memory and apoptosis.

Members of the JNK family play crucial roles in regulating responses to various stresses, and in neural development, inflammation, and apoptosis. They are activated by radiation and other environmental stresses and by growth factors. JNK1 and JNK2 are the products of alternative splicing of a single gene and are expressed in many tissues, but JNK3 is specifically expressed in brain. JNK activation is much more complex than that of ERK1/Erk2 owing to inputs by a greater number of MAPKKKs (at least 13, including MEKK1-MEKK4, ASK and MLK, which are activated by upstream Rho-family GTPases). These activate JNK MAPKKs MEK4 and MEK7. The JNK MAPK modules are regulated by a number of different scaffold proteins, including JIP1, JIP2, JIP3/JSAP1, JIP4, β-arrestin 2, filamin and CrkII. The scaffold proteins presumably target the MAPK modules to different sites in the cell and play roles in kinase activation and/or substrate selection. It is currently not well understood whether these scaffolds are linked to upstream elements in a manner similar to Ste5, Pbs2, and KSR, although several possible linkages have been described. *JIP1*$^{-/-}$ mice have early embryonic defects, defects in primary neurons in adult mice and defects in excitotoxic stress-induced neuronal apoptosis. *JSAP1*$^{-/-}$ mice have neuronal defects in telencephalon morphogenesis. Gene targeting experiments indicate that JNK1 regulates differentiation of the Th2 subset of T cells, T-cell activation, apoptosis of thymocytes and the insulin response, whereas JNK1 and JNK2 are required for neural tube development, control of IL-2 production in T cells and responses to UV.

The p38 MAPKs play an important role in asthma and autoimmunity in humans and are activated by numerous physical and chemical stresses, including hormones, UV irradiation, ischemia, cytokines including interleukin-1 and tumor necrosis factor, osmotic shock and heat shock. Numerous MAPKKKs participate in p38 modules, including ASK1, MEKK1-MEKK 4, MLK2 and 3, DLK, ASK1, Tpl2, Tak1 and Tao1/Tao2. By contrast, only two MAPKKs have been identified, MEK3 and MEK6. All four isoforms of p38 (α, β, γ and δ) can be activated by MEK6; p38α and p38β are also activated by MEK3. Among the targets of p38 MAPKs are several important transcription factors, including NF-κβ, p53 and ATF, and those modulate the expression of genes encoding inflammatory cytokines, which induce inflammation. The p38 MAPK modules are also linked to scaffold proteins including JIP2, JIP4 and the recently described OSM protein, which interacts with actin cytoskeleton. Gene targeting experiments reveal that p38α is required for angiogenesis and Epo production.

The least is known about the ERK3 and ERK5 families. ERK3 is called an atypical MAPK kinase because it lacks the conserved threonine and tyrosine residues in the activation loop and is mainly regulated by its own autophosphorylation and control of protein stability. ERK5 [also called Big MAPK1 (BMK1) owing to its large C-terminal tail] functions in a MAPK module that includes MEK5 and MAPKKKs MEKK2 and MEKK3 and is activated by growth factors. It is required for angiogenesis and cardiovascular development.

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