Abstract

The MAP kinases, discovered approximately 20 years ago, together with their immediate upstream regulators, are among the most highly studied signal transduction molecules. This body of work has shaped many aspects of our present views of signal transduction by protein kinases. The effort expended in this area reflects the extensive participation of these regulatory modules in the control of cell fate decisions, i.e., proliferation, differentiation and death, across all eukaryotic phyla and in all tissues of metazoans. The discovery of these kinases is reviewed, followed by a discussion of some of the features of this signaling module that account for its broad impact on cell function and its enormous interest to many investigators.

© 2006 Elsevier B.V. All rights reserved.

Keywords: MAP kinase; ERK; JNK; p38; SAPK; Protein phosphorylation; Protein kinase cascade; Signal transduction; Docking site; Scaffold protein; Transcriptional regulation; Cell fate

1. Discovery of the MAP kinase

Building on evidence that insulin and mitogens acted through novel, possibly convergent mechanisms to promote intracellular protein phosphorylation [1,2] through the activation of protein (Ser/Thr) kinases [3,4], Sturgill and Ray [5] detected an insulin-activated protein (Ser/Thr) kinase activity in extracts of 3T3-L1 adipocytes, capable of phosphorylating a contaminating high molecular weight polypeptide identified as microtubule-associated protein-2 (MAP-2). Partial purification indicated that the kinase, which behaved as a 35–40 kDa polypeptide, was stably activated by insulin treatment, and its activation was accompanied by an increase in 32P incorporation into 32P-Tyr and 32P-Thr residues on a copurifying 40 kDa polypeptide [6]. The kinase activity also could be adsorbed by anti-phosphotyrosine antibodies, confirming the occurrence of tyrosine phosphorylation of the kinase polypeptide concomitant with its activation [7]. In addition, the kinase could be deactivated by treatment in vitro with either a tyrosine-specific or a serine/threonine-specific protein phosphatase [8]. Few bona fide regulatory tyrosine phosphorylations had as yet been identified, apart from those on various receptor and nonreceptor tyrosine kinases themselves [9]. Moreover, even in cells expressing constitutively active tyrosine kinases such as vSrc, the absolute increase in Ser/Thr phosphorylation of cellular proteins exceeded the increase in Tyrosine phosphorylation by 100–1000 fold [10]. The possibility that this MAP-2 kinase might be a ubiquitous effector of mitogenic stimuli was reinforced by the finding that the MAP-2 kinase polypeptide was identical to the 41–43 kDa polypeptides [11] characterized previously whose tyrosine phosphorylation was stimulated by many polypeptide growth factors [12–15] and by active phorbol esters [15–17]. This realization prompted the redesignation of acronym “MAP” from “microtubule-associated protein” to “mitogen-activated protein”, and thus the MAP kinase as it is now known. These features created intense interest in the MAP kinase; a protein (Ser/Thr) kinase that was activated by insulin and growth factors through tyrosine-specific phosphorylation promised to be an important downstream effector, and perhaps even a direct substrate of the tyrosine kinases.
2. A highly conserved protein kinase cascade

The MAP kinase was not the first insulin-mitogen activated protein (Ser/Thr) kinase described. Work from several labs had shown earlier that the Ser/Thr phosphorylation of the ribosomal protein S6 that occurs in vivo [18–20] as a nearly universal response to insulin or mitogen stimulation is paralleled by the appearance of stably activated, 40S-S6 selective protein kinase activities in extracts prepared from the insulin/mitogen treated cells [3,4]. The first of these S6 kinases to be purified (now called Rsks) were from Xenopus oocytes [21,22], and much evidence indicated that these S6 kinases were activated downstream of tyrosine kinases, including by the direct microinjection into oocytes of tyrosine kinase polypeptides such as vSrc [23], vAbl [24], and the insulin receptor itself [25]. Nevertheless, the Xenopus S6 kinase was activated exclusively by Ser/Thr phosphorylation, as the insulin-activated S6 kinase lacked 32P-Tyrosine and its activity was abolished by (Ser/Thr)-specific phosphatases [26]. Remarkably, the partially purified, insulin-activated MAP kinase was shown to phosphorylate directly and activate the purified Xenopus S6 kinase [27]. Independent studies contemporaneously identified a set of mitogen activated S6 peptide kinases in extracts of EGF-treated NIH3T3 cells that were themselves activated by upstream, EGF-regulated kinases [28–30].

The ability of the MAP kinase to activate an S6 kinase identified what proved to be the first physiologic MAPK substrate, and represented an important milestone in growth factor signaling. The concept of a protein kinase cascade, however was not novel; the first example had been defined by Krebs and colleagues in the activation of phosphorylase b kinase by the cyclic AMP-dependent protein kinase [31]. Moreover it was already appreciated that the AMP-activated protein kinase required phosphorylation by an upstream kinase (s) [32] whose identities (LKB1, CAMKKβ, TAK1) have been uncovered only recently [33–35]. The most startling aspect of the MAPK cascade was revealed by the molecular cloning of the MAPK polypeptide [36]; the primary sequence of the p44 MAPK (called ERK1) was nearly 50% identical to the sequences of a pair of then recently described S. cerevisiae protein kinases KSS1 [37] and FUS3 [38], identified as participants in the yeast mating pathway. The remarkably high conservation of structure between these yeast and mammalian kinase polypeptides across a vast phylogenetic distance indicated that the role of this family of protein kinases as mediators of receptor-regulated cellular differentiation and proliferation was both ancient and highly conserved. Moreover, it was known by then that the S. cerevisiae mating pathway contained at least two other indispensable protein kinases, STE7 and STE11 [39–42] (STE20 was discovered somewhat later; [43]). Thus an intense effort followed to define the order and biochemical actions of each of these yeast kinases, as well as the identity of the upstream activators of the MAPKs evident in various vertebrate systems. Numerous reports described the partial purification of a MAPK activator as a cytoplasmic protein of 50–60 kDa capable of promoting the phosphorylation of the MAPKs ERK1 and ERK2 in vitro on both Thr and Tyr residues, accompanied by an increased MAPK catalytic activity (e.g., [44,45]; reviewed comprehensively in [46]); these findings eliminated the possibility that the MAPK was the direct substrate of a tyrosine-specific kinase. The MAPKs are able to autoactivate slowly in vitro by autophosphorylation; this property together with the inability of the MAPK activators to catalyze significant phosphorylation of other polypeptides created uncertainty as to whether the MAPK activators were protein kinases. Nevertheless, their ability to phosphorylate catalytically-inactive mutant MAPK polypeptides resolved this issue. Designated MAP kinase kinases [MKKs] or MAP and ERK kinases [MEKs], the primary sequences of MKKs from various sources, obtained initially as partial peptide sequences [47–50], and subsequently from cDNAs [51,52], disclosed that the vertebrate MKKs were 30–40% identical in overall primary sequence to STE7; two kinases, MKK1 and MKK2, each capable of activating ERK1 and ERK2, were identified. Hereafter, the MAP kinase kinases and their upstream kinase regulators will be referred to as MAP2Ks, MAP3Ks and MAP4Ks (Table 1).

Many studies demonstrated that the mammalian MAP2K activity was itself inactivated by treatment with protein (Ser/Thr) phosphatase, indicating that at least one additional protein (Ser/Thr) kinase lay upstream [46]. In S. cerevisiae, the ability of mating pheromone to induce the dual (Tyr/Thr) phosphorylation of FUS3 was shown to require both the STE7 and STE11 kinases [53]. STE11 was known from genetic analyses to be upstream of STE7 [53,54], and although evidence was then lacking that STE11 acted directly on STE7, the knowledge that MAP2K1 was itself regulated by Ser/Thr phosphorylation, together with the parallels between FUS3/KSS1 and ERK1/ERK2 and STE7 and MAP2K1, both in primary structure and in their regulatory relationship, led to an expectation that STE11 acted directly on STE7 and a mammalian STE11 homologue would prove to be the physiologic activator of MAP2K1/2 downstream of the RTKs. The yeast mating pathway however is regulated by a GPCR [55]; yeast do not contain receptor- or nonreceptor-tyrosine-specific protein kinases [56]. Yeast also lack orthologs of Raf kinases, and a role for Raf acting

<table>
<thead>
<tr>
<th>MAP4K</th>
<th>MAP3K</th>
<th>MAP2K</th>
<th>MAPK</th>
<th>MAPKAPKs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAK-3 (cRaf-1)</td>
<td>ARaf, BRaf cRaf-1, cMos, Tpl2/COT</td>
<td>MAP2K1/2</td>
<td>ERK1/2</td>
<td>Rsks (1–4) MNKs MSKs</td>
</tr>
<tr>
<td>GCK GCKR HPK1</td>
<td>MEKK1–4 ASK1, Tpl2/COT TAK1</td>
<td>MAP2K4/7</td>
<td>JNK1/SAPK JNK2/SAPKα</td>
<td></td>
</tr>
<tr>
<td>PKb1/2</td>
<td>MLK2/3, DLK</td>
<td></td>
<td>JNK3/SAPKβ</td>
<td></td>
</tr>
<tr>
<td>WNK1</td>
<td>MEKK3,4 ASK1 TAK2 TAO1/2</td>
<td>MAP2K3/6</td>
<td>P38a,β P38γ,δ</td>
<td>MAPKAPK2,3,5 MNKs MSKs</td>
</tr>
<tr>
<td></td>
<td>MEKK2,3</td>
<td>MPK5</td>
<td>ERK5</td>
<td></td>
</tr>
</tbody>
</table>
downstream of receptor tyrosine kinases had been established by genetic analyses of receptor tyrosine kinase signaling in the metazoans, *C. elegans* and *D. melanogaster* (reviewed in [57,58]). The finding that NIH3T3 cells transformed by a vRaf oncogene exhibited a constitutive activation of endogenous MAP2K raised the possibility that the vRaf (Ser/Thr) kinase itself might act directly on MAP2K; in fact the cRaf1 kinase catalyzed the in vitro phosphorylation and robust activation of MAP2K [59], a finding rapidly confirmed [60,61].

*Drosophila* and *C. elegans* have only one Raf ortholog, and genetic evidence ultimately established that the Raf kinase is the only MAP3K recruited by receptor tyrosine kinases for activation of the ERK pathway. Other essential components of RTK signaling revealed through fly and worm genetics included the Ras GTPase, its guanyl nucleotide exchange factor (SOS) and the SH2/SH3 domain adaptor protein Sem5/DRK, the worm/fly homolog of the mammalian Grb2 polypeptide [57,58]. The latter was found to be constitutively associated with SOS and the complex was recruited to the membrane by Grb2-mediated binding to the activated, tyrosine phosphorylated RTK, thereby promoting Ras GTP charging [62]. A variety of transfection and microinjection studies in mammalian and *Xenopus* cells had demonstrated that RTK activation of the MAPK required active Ras, and that Ras-GTP itself is able to activate the MAPK pathway (e.g., [63–69]). The subsequent finding that Raf binds selectively to the GTP loaded form of Ras ([70–75]; reviewed in [76]) thus uncovered the last of the central biochemical steps in RTK activation of the MAPK pathway. These discoveries provided the first understanding of the physiologic functions and biochemical mechanisms of cellular protooncogenes that, in mutant form contribute with high frequency to human cancers. In addition, the demonstration that Ras, through its switch1 loop, a segment whose configuration was shown previously to be GTP-dependent, binds in a GTP-dependent manner to a specific, structurally conserved domain in the cRaf-1 aminoterminal noncatalytic segment, thereby recruiting cRaf-1 to the membrane and initiating its activation, established a paradigm for the biochemical mechanism of action of the vast majority of all small GTPases.

3. A favored architecture for signal transduction

The discoveries that unveiled the MAPK pathway and its regulation by receptor tyrosine kinases (RTK) were exciting because of their rapid convergence from studies on evolutionarily distant systems, providing a forceful demonstration that evolution conserves not only critical housekeeping molecules, but useful regulatory modules and designs as well. Nevertheless, the description of RTK regulation of the MAPK pathway proved to be only the opening act in the MAPK story. Protein kinases other than Raf capable of activating MAP2K1 and 2 were soon identified; thus the cMos protein kinase was shown to be the physiologic MAP3K operating in the meiotic maturation of *Xenopus* oocytes [77–79], somewhat later, the COT/Tpl-2 protooncogene was shown to act as a physiologic activator of MAP2K1 or 2 in response to lipopolysaccharide acting through toll receptors [80]. The discovery in both *S. cerevisiae* and *S. pombe* of MAP3K/MAP2K/MAPK cassettes distinct from that of their mating pathways (reviewed in [81]) foretold the discovery of additional MAPK pathways in metazoans. Thus independently and contemporaneously, the SAPK/Jnk [82,83] and the p38 [84–87] subfamilies of mammalian MAPKs were discovered through a variety of independent approaches, and ERK5 slightly later [88]. Each of these newer MAPK subfamilies was shown to be regulated by distinct MAP2Ks; MAP2K4/7 for the SAPK/Jnks, MAP2K3 for p38α/β whereas MAP2K6 activates all four p38s (reviewed in [89]) and MAP2K5 for ERK5 [88]. MAPK activation in each of these cassettes is achieved by MAP2K-catalyzed phosphorylation of the ThrXaaTyr motifs in the MAPK activation loop; ERK3, which lacks this motif, remains somewhat an enigma [90,91]. Most unexpectedly, a plethora of MAP3Ks emerged [92–98], reflecting the exceptional variety of signals capable of recruiting these pathways, usually in combinatorial arrays. The model established by the Ras regulation of Raf was soon extended by the discovery that members of the Rho subfamily of GTPases, especially cdc42 and rac1 regulate the SAPK/Jnk and p38 pathways [99–103] acting at the level of the MAP3Ks of the MLK subfamily. Although the SAPK/Jnk and p38 pathways were initially characterized by their seemingly preferential activation by cellular stresses and inflammatory cytokines [104], this distinction from the insulin-mitogen activated MAPK pathway has become progressively blurred, as it is now evident that there is tremendous tissue- and developmentally-specific variation in the upstream input to each of these three MAPK subfamilies.

Once the basic architecture of these pathways and the identity of the core components had been accomplished, it became feasible to ask about the crucial determants of pathway activation and substrate selection. The dominant multifunctional effector of the MAP3K/MAP2K/MAPK cassette is the MAPK element. It became evident early that for the RTK input, the intensity and duration of the stimulatory input upstream of the MAP3K/MAP2K/MAPK cassette, as controlled e.g., by receptor density, exerted a profound impact on the cell fate chosen, by altering the duration of MAPK activation and thus the likelihood of nuclear entry and access to nuclear targets ([105]; reviewed in [106,107]).

4. MAPKs have a broad reach in cell regulation

The wide reach of these pathways in cell regulation emerged with the discovery of candidate MAPK substrates. Thus, prominent among MAPK substrates are other multi-target signal transduction proteins, most notably numerous transcriptional regulatory proteins and several other protein kinases. Notable among the early examples of transcriptional targets were a number of nuclear protooncogenes, e.g., cjun [108], cMyc [109] and p62TC/Ek1 [110] as well as ATF2 [111], MEF2C [112]. The protein Ser/Thr kinases that are regulated by the various MAPKs include the Rsk1–4 (by ERK1/2) [113,114], MAPKAP kinases (MK2,3: p38α/β;MK5:p38β/ERK3) [115,116], MSK1,2(by ERK1/2, p38α/β) [117–119].
and MNK1,2 (by ERK1/2, p38α/β) [120–122]. Each of these kinases, collectively referred to as MAPK-Activated Protein Kinases (MAPKAPKs), are themselves multifunctional regulators with a wide variety of targets, including many transcriptional regulatory proteins and a few translational regulators. Importantly however, the MAPKAPKs have a radically different substrate specificity than their upstream controlling MAPK, and this diversification of substrate specificity confers tremendous reach as to the targets whose phosphorylation is ultimately under the control of a MAPK. Early work established that the classic MAPKs (ERK1,2, SAPK/Jnks,p38s) are each, like the edks, “proline-directed” kinases [109,123,124]; the Ser/Thr residues they phosphorylate is always directly aminoterminal to a Pro. This Ser/ThrPro motif constitutes a negative determinant for kinases of the AGC and CAMK class [125]. The MK, MSK and MNK subfamilies are in the CAMK group of the human kinome and thus share general specificity of that class for substrates with an Arg situated at −3 [126]. The Rsk1s each encompass two catalytic domains, a carboxyterminal CAMK-related domain which is the primary target of the MAPK, but whose function is exclusively devoted to activation of aminoterminal AGC-related catalytic domain (acting in concert with PDK1); only the aminoterminal domain is active toward exogenous substrates [114], with a preference for Arg at −3 and −5. The ability of ERK1/2 and p38α/β to control AGC and CAM kinases allows these MAPKs to control the phosphorylation of many of the same substrates/sites that are regulated by the cyclic nucleotide regulated kinases (AGC), the Type 1 Ptd Ins lipid kinases (acting through the AGC subfamily of PKBs), and the Ca++/DAG regulated kinases (AGC subfamily of PKCs, CaMK1–4) [125]. A comprehensive catalog of MAPK and MAPKAPK substrates is not available, however a recent review [127] enumerated 160 well-documented substrates of ERK1/2 alone, suggesting that the total number of proteins whose function is regulated by these protein kinases is likely to exceed one thousand.

5. MAPK substrate selection

Early studies of MAPK specificity using synthetic peptide substrates based on the sequence of myelin basic protein as well as bona fide MAPK substrates clearly showed the requirement for Pro [109,123], with ERK1/2 preferring the motif of ProXaaSer/ThrPro surrounded by basic residues and SAPK/Jnk preferring Ser/ThrPro in a more acidic context [128]. Nevertheless, the peptide substrates exhibited very high Kms (e.g., 200–300 μM) as compared to the native polypeptide substrates, suggesting that the proline directed specificity of the MAPKs should be viewed as a qualifying feature of substrate selection, rather than as a determinant. These results were radically different than had been observed in the analysis of the specificity of PKA, wherein short synthetic peptides based on the amino acid sequence surrounding a substrate phosphorylation site e.g., of pyruvate kinase [129,130], or denatured pyruvate kinase [131] were phosphorylated with Kms (~10 μM) approaching those observed for the native protein, suggesting that the major determinants of PKA substrate selection were located very close in the primary sequence to the phosphorylation site. The low affinity of peptides based on MAPK phosphorylation sites, and the adverse effects of substrate denaturation strongly suggested that the major determinants of MAPK substrate selection were located in segments of the primary sequence that were remote from the phosphorylation site in primary sequence and perhaps conformationally determined. The validity of this inference was first established for the SAPK/Jnk catalyzed phosphorylation of cJun, where a segment in the cJun aminoterminus (the delta domain, AA31–47, deleted in vJun) acts as a high affinity binding site for the kinase, which phosphorylates SerPro sites at AA63 and 73 [82,132,133]. Subsequent studies revealed docking sites in many substrates of each of the three canonical MAPK families usually called the D domain, whose general features include a cluster of basic residues upstream of an LXX motif ([K/R]2−3−X1−6[L/I]−X−[L/I]), occasionally with an additional cluster of hydrophobic residues further carboxyterminal [134–136]. In a substrate such as SAP-1 that is shared by ERK1/2 and p38, systematic mutagenesis can identify residues that preferentially affect phosphorylation by either kinase [134]. Nevertheless, simple swapping of specificity by swapping D-domains is only occasionally successful, suggesting that multiple factors contribute to docking including weaker interactions near the phosphorylation site and elsewhere, the latter dependent on overall conformation. The D domain can be situated on either side of the Ser/ThrPro; in some substrates other than the downstream kinases, mostly transcription factors, a PhexaaPhe(Pro) (called “docking site for ERK, FXF” or DEF domain) is sometimes found on the carboxyterminal side of phosphorylation site, located much closer in primary sequence to the Ser/ThrPro than is the D domain (reviewed in [135,136]). Functional PhexaaPhe(Pro) docking motifs are sometimes found in the absence of the common, basic-hydrophobic motif, as in cFos [137]; when both a D domain and a DEF motif are present, they may direct the phosphorylation at the same or different sites. The ERK1 and 2/p38 downstream kinases MNK and MSK as well as several other substrates contain the docking motif LeuXaaXaaArgArg [136]. Docking site motifs have not been identified in all candidate MAPK substrates, so that the possibility remains that additional motifs remain to be discovered, or that intermediating proteins, i.e., “scaffolds”, some of which contain D domains, act to confer specificity, as occurs with PKA and the AKAPs [138,139]. Docking sites are well described for protein kinases other than the MAP kinases [140] and the relative contributions of docking versus phosphorylation site sequence to the determination of protein kinase specificity is under continued study [141].

6. Docking motifs critical to pathway regulation

6.1. Docking on the MAPKs

Docking motifs similar to the D domain on the MAPK substrates have also been identified in the majority of MAPK regulatory proteins, i.e., the MAP2Ks, the dual specificity MAPK phosphatases (MKPs) and certain scaffold proteins
(136]; see below); moreover, each of these regulators binds to a common region(s) on the MAPK.

The first such MAPK region (the “common docking” or CD domain) was that defined on the large lobe of the SAPK/Jnk catalytic domain (142). CD domains, now defined on all three major classes of MAPK, contain several acidic residues that presumably interact with the Arg/Lys of the substrate D domain. In addition, the LeuXaaLeu motif in the D domain binds to hydrophobic residues in the conserved kinase core of p38α located within a groove formed between β7−β8 and αD-αE loop regions [143]. The PheXaaPhePro/DEF motif binds to a pocket formed by the P+1 site, αF helix and the MAP kinase insert segment that is occluded by the unphosphorylated activation loop and thus available to substrates only after MAPK activation [144]. An additional region on the kinase catalytic domain conferring specificity, the ED site, was first detected as a pair of acidic residues in p38 that contribute to p38 binding to its specific substrates MK2 and MK5. The corresponding sequence in ERK2 is ThrThr; replacement by GhuAsp confers on ERK2 the ability to bind MK2 and enhances its ability to activate MK2 in vivo [145]. The CD domain and ED site are not close in primary sequence but are nearby on the surface and define a surface groove that mediates MAPK binding not only to substrates, but to all partners. Thus at least three separate regions on the MAPK catalytic domain surface, distinct from the catalytic site, have been identified as conferring specific interaction with MAPK partners. This number may well increase as more substrates are identified.

6.2. Docking on the MAP2Ks-

The MAP2Ks are the chokepoint in the pathway, inasmuch as they funnel the upstream input from numerous MAP3Ks into a very limited number of MAP2Ks. Thus MAP2K1/2 phosphorylates ERK1/2, MAP2K4/7 the SAPK/Jnks (although some input of MAP2K4 into p38 also occurs), MAP2K3 acts on p38αβ and MAP2K6 all p38 isoforms, and MAP2K5 on ERK5. MAP2K1,2,3,4,6,7 contain one or more (e.g., MAP2K7) D domains onto which the MAPKs dock [146]. The MAP2K D domains are uniformly situated aminoterminally to the MAP2K catalytic domain, although the MAP2K2 D domain has been shown to function effectively if moved to the carboxyterminal side of the catalytic domain [147]. MEK5 does not contain a D-site but instead contains a novel MAPK-docking site of acidic character [148]. The MAP2K D site is critical for efficient MAPK activation in mammalian cells however, the binding of MAP2Ks to MAPKs parallels the pattern of MAP2K-catalyzed phosphorylation only roughly. Thus, the D domains of MAP2K1/2 do not bind SAPKα/Jnk2 effectively, however replacement of the MAP2K2 D domain by that from the ERK1/2 substrate ets1, or by the D domain from STE7 enables effective MAP2K2 activation of ERK1/2 [147]. Thus the main functions of the D domain appear to be in providing a high local substrate concentration to the MAP2K and in cytoplasmic tethering of the inactive MAPK (the MAP2Ks are constitutively cytoplasmic). Importantly, MAPK activation is accompanied by dissociation from the MAP2K, freeing the MAPK to interact with its substrates, with the MKPs [149−151] and when activation is sustained, enabling the cytoplasmic MAPK to cycle rapidly through the nucleus and achieve a high activity in that compartment [105−107].

MAP2Ks can also interact stably with their upstream regulators, the MAP3Ks. The first example was provided by the Pbs2p kinase, the MAP2K in the S. cerevisiae osmosensing pathway [152]. Pbs2 binds the MAP3K, STE11 as well as the MAPK, Hog1; upon exposure to hyperosmolarity Pbs2p is recruited by its proline-rich motif to the cytoplasmic SH3 domain of the membrane osmosensor Sho1p, enabling STE11 to be phosphorylated and activated by the membrane associated STE20p kinase [153]. Pbs2p, through another segment aminoterminally to its catalytic domain also binds the MAP3Ks, Ssk2/22; the latter are activated by a two-component histidine kinase osmosensor, Sln1 (via the relays Ypd1/Ssk1), and activate Pbs2p entirely separate from STE11 [154]. Recently, a docking motif on the MAP2K1,3,4,6,7 utilized for many MAP3Ks has been identified, located at the carboxyterminal end of the catalytic domain extending beyond the catalytic core [155]. This MAP2K segment, called the DVD site (domain for versatile docking), contributes detectably to the specificity of individual MAP3Ks for the MAP2K, however DVD site swapping studies indicate that additional determinants on the MAP2Ks remain to be elucidated. The SAPK/Jnk and p38 pathways are usually activated in tandem, so it is not surprising that many of the MAP3Ks that activate MAP2K4/7 also activate MAP2K3/6; the overlapping specificity of the DVD sites on those MAP2Ks for various MAP3Ks probably accounts, at least in part, for this sharing; in contrast, the MAP2K1 DVD site binds Raf but not other classes of MAP3Ks. The DVD site appears to interact with the small lobe of the MAP3K catalytic domain, but as with the MAP2Ks, other MAP3K segments are also important for the MAP3K/MAP2K association. It may be at this level of the cassette that scaffold proteins make their greatest contribution.

7. An abundance of MAP3Ks and MAP4Ks

The MAP3Ks are the most numerous of the core kinase elements. The challenge is in defining the specific upstream inputs and cellular functions mediated by each. It appears likely, based on the phenotypes of several murine MAP3K gene deletion models, that whatever functional overlap exists, each MAP3K utilizes the same core MAP2K/MAPK module to execute at least some distinct signaling events. The three members of the Raf family only mediate the activation of MAP2K1/2 and underlie ERK1/2 activation in the vast majority of examples adduced thus far. Inactivation of the murine cRaf-1 [156] and Braf [157] genes each results in embryonic lethality; nevertheless, the phenotypes of the null embryos differ. Thus Rafs are not redundant and their biologic outputs differ, at least somewhat. Unexpectedly, a mutant form of cRaf-1 that lacks MAP2K catalytic activity can overcome the susceptibility of the murine cRaf-1(−/−) cells to apoptosis, demonstrating that ERK1/2 activation does not fully explain the biologic role of cRaf-1 [158]. Thus, although cRaf-1 is undeniably an active
protein kinase, it appears to have functions that are executed purely as a scaffold [159,160], independent of its catalytic activity. Interestingly, this is the dominant (probably sole) phenotype of the KSRs, the kinase subfamily most closely related in primary sequence to the Rafs.

The diversity of MAP3Ks that participate in regulation of the SAPK/Jnk and p38 pathways is especially striking, but it is clear that the MAP3Ks in this pathway are highly specialized; e.g., MEKK1, 2 and 3 each activate the SAPK/Jnk pathway, but inactivation of those genes results in entirely distinct patterns of gene expression [161]. This diversity of MAP3K function may in part be due to differing patterns of tissue-specific expression or to MAP3K targets other than the MAP2Ks, as well as to differences in the composition of the multicomponent MAP2K/MAPK assemblies that are organized by the diverse noncatalytic segments of the MAP3Ks or by the associated noncatalytic scaffolding proteins. These factors override the structural homology within the MAP3K subfamily catalytic domains to enable differing subcellular localization and downstream targets. In addition to the scaffolding functions of certain MAP3Ks, e.g., MEKK1 (and noncatalytic proteins, see next), the MAP4Ks may also play such a role. The vast majority of MAP4Ks are from the large class of Ste20 protein kinases; Dan et al. [162] have enumerated nearly 30 human Ste20-related kinases, subdivided into ten classes. It is most convenient to separate the PAKs, which have a carboxyterminal catalytic domain, from the more numerous GC kinase-related polypeptides, which have an aminoterminal catalytic domain; all Ste20 subfamilies exhibit highly diverse noncatalytic segments. Nearly all of the Ste20-related kinases tested can act as upstream regulators of the MAP3Ks, either serving in a noncatalytic mode as a scaffold to promote pathway assembly and MAP3K autoactivation, or additionally as a catalyst to phosphorylate and transactivate the MAP2K. Moreover, it is clear that the outputs of essentially all the Ste20/MAP4Ks are much broader than control of MAPK pathways [163].

8. Noncatalytic scaffolds for the MAPK pathway

The first noncatalytic MAPK pathway scaffold identified was the STE5p protein of the S. cerevisae pheromone mating pathway [164–166]. Ste5 binds independently and concomitantly STE11p, STE7p and the MAPKs, KSS1p and FUS3p and is recruited to the membrane by its ability to bind the free Gαγ subunits released upon engagement of the pheromone receptor; once there, STE20p, activated by Cdc42, phosphorylates and activates STE11p. Although docking sites are present in STE11 and STE7, inactivation of STE5 abolishes the mating response. Thus the noncatalytic scaffold STE5 is essential for signal transmission in the pheromone pathway. Although Ste5 may participate actively in signal transmission [167], its essential function appears to be as a tether, inasmuch as substitution of heterologous binding sites permits effective function [168]. Vertebrate homologs of STE5 do not exist, however a variety of noncatalytic polypeptides capable of binding two or more of the core kinases have been identified for each of the three major MAPK pathways (reviewed in [169–171]). The first identified was the Drosophila KSR recovered as a kinase-like polypeptide that acted as a suppressor of activated Ras. The KSR polypeptides exhibit alterations at several conserved residues in the kinase catalytic domain that are required for activity and most reports detect no KSR-specific catalytic activity [169]. Nevertheless, some reports describe KSR kinase activity [172], but inasmuch as KSR can bind constitutively with MAP2K1 and can interact, stably or transiently, with several other protein kinases (in addition to cRaf-1 and ERK1/2), the ability of KSR to catalyze phosphorylation remains unproven. KSR is one of the first and among the best studied of the “pseudokinases”, which constitute approximately 10% of the 518 members of the human kinome [173]. KSR is recruited to the membrane upon Ras activation, where it interacts with Raf, and Ras controls KSR turnover through the ubiquitin ligase, IMP [174]. KSR(−/−) mice are normal, but subtle abnormalities of ERK1/2 activation can be detected, e.g., in T cells, and these animals are relatively resistant to ras-induced tumorigenesis [175]. A reasonable conclusion is that KSR may be necessary for optimal Ras activation of ERK1/2 in specific settings, but unlike STE5, is not required for adequate pathway performance. A considerable number of other noncatalytic polypeptides have been described as stably interacting with one or more elements of the Raf/MAP2K1or2/ERK1/2 cassette, including CNK, MP1, MORG1, SUR-8, RKIP, sprouty/spred, and the beta arrestins (which couple the cassette to several classes of GPCR); depletion or overexpression of these elements results in phenotypes relating to altered MAPK signaling [169–171]. Several of these polypeptides exhibit multidomain structures and participate in signaling through other pathways, in addition to their MAPK-related outputs. Thus far, unlike STE5, few examples in mammals are available wherein depletion of these noncatalytic scaffolds in the ERK1/2 pathway results in dramatic inference with physiologic pathway function. Many of the “positively-acting” scaffolds may be dispensable for physiologic logic operation of the ERK1/2 cassette in the most settings, but essential in a few specific responses, or in the infrequent circumstances wherein maximal activity is required. Some of these scaffolds may in addition participate in maintenance of low basal pathway activity, yet their absence may be compensated by ambient protein phosphatase activities.

Among the various noncatalytic scaffolds identified for the SAPK/Jnk and p38 pathways [169], e.g., beta arrestin-2, ABP280/filamin, IKAP, POSH, JIP1-4, etc. the phenotypes seen with deletion of the murine JIP1 and 3 genes provide excellent illustrations of the narrow, but important role of scaffolds. JIP1-3 each interact with Jnk1-3, MAP2K7 and one or more MAP3Ks of the MLK family, as well as with the light chain of kinesin-1. The latter conveys JIP1 to synapses; JIP3 is localized to neuronal cell bodies Deletion of the Jip3 gene results in major defects in brain development culminating in death at birth due to failure to breathe [176]. JIP1 deficient mice have a much more subtle phenotype; they are viable and phenotypically normal under usual conditions. Nevertheless, if their CNS is subjected to excitotoxic or anoxic/ischemic stress, a reduced activation of SAPK/Jnk is observed as compared to
wildtype mice, associated with reduced size of ischemic infarcts [177]. JIP1, in addition to its expression in brain, is also expressed at lower levels in adipose tissue and skeletal muscle. JIP1 null mice exhibit diminished activation of Jnk1 in those tissues in response to a high fat diet, accompanied by diminished phosphorylation of IRS1 at ser307 and improved insulin responsiveness, as compared with wildtype mice fed such diets [178]. These findings indicate that, with some exceptions (e.g., JIP3), elucidation of the biologic roles of the noncatalytic scaffolds will require sophisticated examination of gene-deleted murine models stressed in a variety of ways.

9. Open questions

It has been known for some time that the frequency of activating ras mutations in human cancers approaches 30% [179]; this together with the essentiality of the ERK pathway for ras-directed proliferation identified this pathway as a prime target for anticancer therapies. The more recent discovery of activating mutations in Braf, especially in malignant melanoma [180] added further impetus to the search for clinically useful inhibitors of this pathway, and several agents directed toward Raf and MAP2Ks are now in clinical trials [181,182]. The important roles played by the SAPK/Jnk and p38 kinases in response to a variety of cell intrinsic and extrinsic stressors suggested strongly that these kinases are potentially important therapeutic targets in a variety of inflammatory and degenerative diseases. Several inhibitors of these MAPKs or upstream elements in these pathways have now entered clinical trials, especially in Rheumatoid arthritis [183,184], and others are planned for illnesses such as Crohn’s disease and psoriasis. Neurodegenerative conditions such as Parkinson’s disease, Alzheimer’s disease, hearing loss and others [185,186] are other areas of especial activity. Although specific pathway inhibitors of undeniable clinical efficacy have yet to make an appearance, optimism remains strong.

The development of specific inhibitors, along with gene modification will certainly advance our understanding of the biologic roles of the MAPK pathways in development, and in adult physiology. Biochemical problems that occupy considerable effort include the identification of additional substrates and the mechanisms of substrate selection; the identification of all components that regulate pathway activity, their mechanism and specific impact on the pathway output under physiologic conditions; the mechanisms that control the subcellular localization of pathway components and the significance for pathway regulation; the biochemical mechanisms that control pathway activation state and pathway cross-regulation. Work on the roles of the MAPKs in plants has accelerated in recent years [187–189]. Substantial gains remain to be made in these and other areas.

Acknowledgements

Work cited herein from the author’s lab was supported by awards from NIDDK, NCI, ACS and HHMI. I thank my collaborators, U.R. Rapp, J.R. Woodgett, J.M. Kyriakis and M. S. Marshall. Apologies to those whose significant contributions have not been cited directly. J. Prendable is acknowledged for assistance in preparation of the manuscript.

References


J. Han, Y. Jiang, Z. Li, V.V. Kravchenko, R.J. Ulevitch, Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation, Nature 386 (1997) 296–299.


G. Zhu, K. Fuji, N. Belkina, Y. Liu, M. James, J. Herrero, S. Shaw, Exceptional disfavor for proline at the P + 1 position among AGC and CAMK kinases establishes reciprocal specificity between them and the proline-directed kinases, J. Biol. Chem. 280 (2005) 10743–10748.


S. Grewal, D.M. Molina, L. Bardwell, Mitogen-activated protein kinase


