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Review

MAP kinase pathways: The first twenty years

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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 phylla 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.

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 covergent 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 ^{32}P incorporation into ^{32}P-Tyr and ^{32}P-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.

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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 ^{32}P-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 a cDNA encoding 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 phyllogenetic 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 ultimately 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 1

MAP4K	MAP3K	MAP2K	MAPK	MAPKAPKS
PAK-3 (cRaf-1)	ARaf, BRaf cRaf-1, cMos, Tpl2/COT	MAP2K1/2	ERK1/2	Rsks (1–4) MNKs MSKs
GCK GCKR HPK1	MEKK1–4 ASK1, Tpl2/COT TAK1	MAP2K4/7	JNK1/SAPKγ JNK2/SAPKα	
PAK1/2	MLK2/3, DLK		JNK3/SAPKβ	
	MEKK3,4 ASK1 TAK2 TAO1/2	MAP2K3/6	P38a,β P38γ,δ	MAPKAPK2,3,5 MNKs MSKs
WNK1	MEKK2,3	MP2K5	ERK5	