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EDITORIAL REVIEW

Multiple intracellular MAP kinase signaling cascades

Mitogen-activated protein (MAP) kinases are important mediators involved in the intracellular network of interacting proteins that transduce extracellular cues to intracellular responses. Intracellular signaling pathways display a high level of evolutionary conservation. Recent developments have extensively characterized the extracellular signal-regulated kinase (ERK) cascade. The ERK cascade remains the best-studied MAP kinase signaling cascade and may be considered as the archetypal MAP kinase cascade. The utilization of powerful molecular genetic tools helped to elucidate the existence and the physiological role of independent MAP kinase signaling cascades in yeast. Based on this knowledge, new MAP kinase isoforms (SAPK, p38 HOG1 kinase and ERK5) have recently been described in mammalian cells. These MAP kinases respond to distinct extracellular stimuli and have different intracellular substrates. A common feature of all MAP kinase isoforms is the requirement for phosphorylation of both threonine and tyrosine regulatory sites by a specific upstream protein kinase for activation. Thus, not only protein kinases that catalyze phosphorylation, but also protein phosphatases that are capable of dephosphorylation and thereby inactivation of MAP kinases are of interest in the regulation of these intracellular signaling pathways. A diverse array of extracellular signals utilize MAP kinase signaling cascades to initiate a variety of cell signaling outcomes. The pleiotropic potential of MAP kinases emphasizes the importance of a tight control of their activation. In response to extracellular stimuli MAP kinases regulate the transcriptional activity of several transcription factors via phosphorylation of either stimulatory or inhibitory regulatory sites, thereby initiating the expression of a variety of immediate and delayed early response genes. This regulation of gene expression and the phosphorylation and regulation of cytosolic as well as nuclear targets by MAP kinases is critical for cell signaling outcomes. MAP kinases have not only been suggested to play a pivotal role in fundamental cellular processes like DNA synthesis, progression through the cell cycle and cellular proliferation, but have also been implicated in G1 phase arrest and cellular differentiation [1, 2]. However, we would like to emphasize that this review by no means suggests that MAP kinase cascades are an exclusive system to regulate fundamental cellular processes. For more detailed information on other signaling systems, for instance the JAK/STAT pathway or PI 3-kinase, please refer to related reviews [3, 4].

In this review we will discuss the mechanisms of stimulation of parallel MAP kinase cascades. The ERK cascade is described in more detail, since it is the best studied MAP kinase cascade and most of its stimulating mechanisms appear to be representative of other MAP kinase cascades. Furthermore, we will discuss mechanisms of down-regulation of MAP kinase signaling cascades and emphasize the physiological relevance of these MAP kinase cascades in renal and cardiovascular regulation.

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The ERK cascade

The activation of ERK in response to extracellular stimulation can be schematically devided into membranous and cytoplasmic phases [1] (Fig. 1). The pattern of activation during the membranous phase is mostly determined by the receptor sensing the extracellular signal, whereas the cytoplasmic phase shows high homology between the MAP kinase isoforms. Protein-tyrosine kinase receptors [3, 5], G-protein coupled receptors [6–11] and cytokine receptors [12, 13] were shown to be capable of activating the ERK cascade. Many studies focus on the role of ERK in response to growth factors, since alteration of growth factor regulated cellular processes is frequently accompanied by altered cellular proliferation [1].

Growth factors like epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) bind to receptors with intrinsic protein-tyrosine kinase activity. Ligand-induced dimerization of the receptor in the plane of plasma membrane is a property common to the signaling mechanisms of these growth factor receptors [14]. Dimerization leads to activation and to autophosphorylation of tyrosine residues in the intracellular domains of growth factor receptors [15–17]. The breakthrough in the coupling of growth factor receptors to activation of Ras signaling pathway came with the understanding of the role played by recently discovered proteins termed adaptor proteins.

Adaptor proteins mediate protein-protein interactions in signal transduction pathways activated by protein tyrosine kinases. Adaptor proteins do not possess any intrinsic enzymatic activity and consist only of modular binding domains [18–20]. Src homology two (SH2) domains bind to short phosphotyrosine containing sequences in growth factor receptors and other phosphoproteins. Together with Src homology three (SH3) domains, which bind to target proteins through sequences containing proline and hydrophobic amino acids, SH2 domains determine the selectivity of signaling pathways. The specificity in binding of SH2 domains to phosphotyrosine residues has been investigated using a library of synthetic peptides containing phosphotyrosine. The preferential selection of defined amino acids at one or more positions relative to the phosphotyrosine by different SH2 domains has been demonstrated [21]. These data and the identification of physiological binding sites of these SH2 domains on growth factor receptors provided the consensus binding sites for a number of SH2 domain containing signaling molecules [19, 21]. With the SH2 domain of adaptor protein bound to specific tyrosines in phosphorylated proteins, the SH3 domains are free to effect the downstream signal, resulting in the formation of multisubunit signaling complexes.

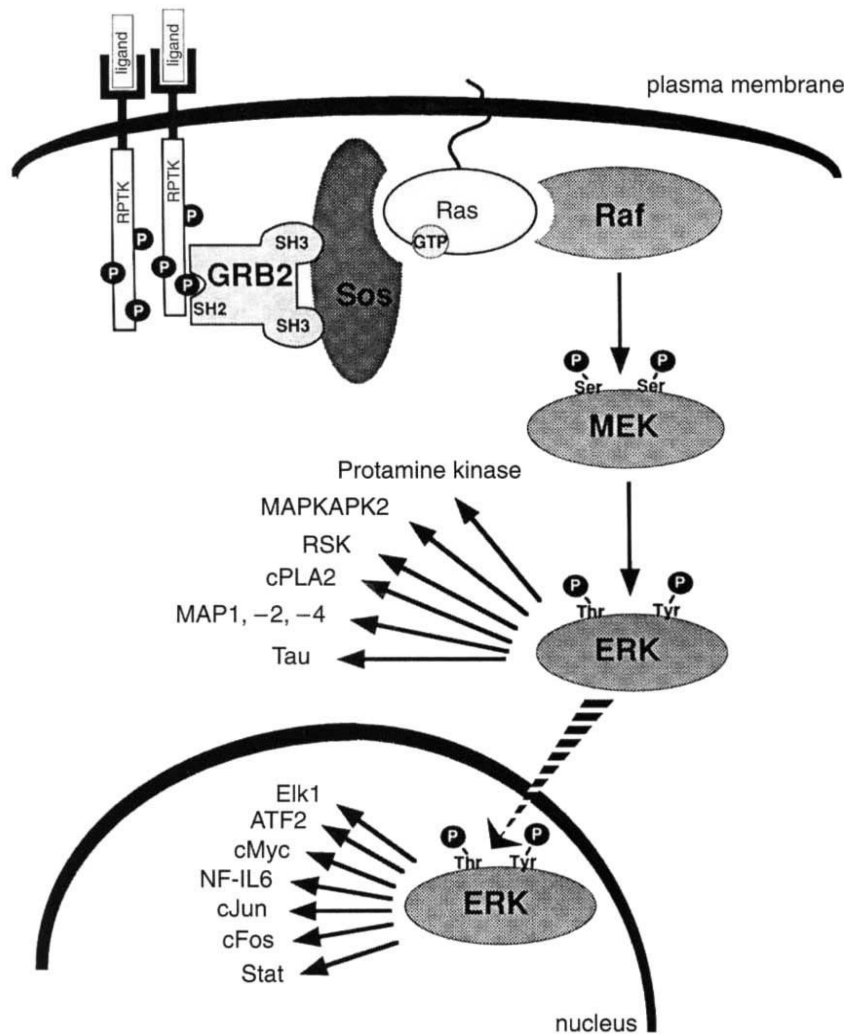

Fig. 1. *Stimulation of the ERK cascade after binding of a growth factor to its receptor protein tyrosin kinase (RPTK) and downstream targets of ERK.* ERK is a proline-directed kinase that phosphorylates serine or threonine residues on its substrates.

An extensive body of data indicates that function of the mammalian adaptor protein Grb2 (growth factor receptor-bound protein 2) is the linkage of receptor tyrosine kinases to Ras signaling pathways. The SH3 domain of Grb2 was shown to recruit guanine-nucleotide exchange factor Sos (son of sevenless, gene product in Drosophila) and to enforce its translocation to the plasma membrane [22]. This translocation is thought to bring Sos in close proximity with Ras, a small GTP-binding protein located at the cytoplasmic surface of the plasma membrane [23]. Sos induces the dissociation of GDP from Ras, allowing the formation of an activated GTP-Ras complex (Fig. 1) [24].

Thereafter several cytoplasmic protein kinases are sequentially stimulated, collectively known as the MAP kinase signaling cascade. The cytoplasmic phase of ERK activation is thought to be critical for rapid signal amplification. Activated Ras binds to the NH_2-terminal portion of the serine-threonine protein kinase Raf-1 thereby recruiting Raf-1 to the plasma membrane. Once at the membrane Raf-1 is activated by an unknown mechanism [25–27]. Raf-1 is a member of a family of related kinases which also includes B-Raf [26]. In contrast to the ubiquitous Raf-1, B-Raf is detectable in only a few tissues, such as neuronal cells, and seems to be responsible for the activation of ERK in PC12 cells [28, 29]. Raf activity may be modulated by upstream kinases like protein kinase C (PKC) [29, 30], protein kinase A (PKA) [28] or, since phosphorylation on tyrosine is important for Raf-1 activation [31], by an unidentified protein tyrosine kinase. Raf-1 exhibits high substrate specificity towards MEK (MAP kinase/ERK kinase) [32]. However, Raf-1 is also suggested to phosphorylate I kappa B thereby releasing the active transcription factor NF-kappa B [33] and to activate the cell cycle by activation of Cdc25 [34]. Raf-1 activates the MAP kinase kinase isoforms MEK1 and MEK2 by phosphorylation of two regulatory serine residues [35–37]. In addition the activity of MEKs may be modulated by phosphorylation on threonine residues [38, 39]. MEKs belong to the small group of dual specificity kinases that catalyze both serine/threonine as well as tyrosine phosphorylation [40]. Despite findings that MEKs neither translocate to the nucleus nor localize in the nucleus of several cell types [41, 42], recent data suggest that the nuclear localization of MEKs is enhanced by down regulation of protein kinase C [43]. Furthermore, MEK may be important for the long-term regulation of the ERK cascade since MEK protein levels are up-regulated in response to chronic mitogenic cellular stimulation [44]. MEK1 and MEK2 are highly selective activators of the MAP kinases

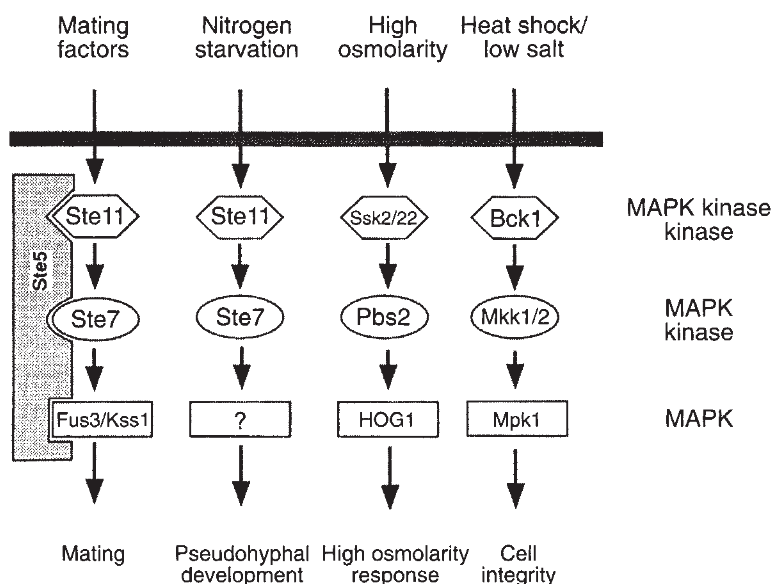

Fig. 2. *Multiple MAP kinase signaling pathways in S. cerevisiae.*

ERK1 and ERK2 (also refered to as p44 MAP kinase and p42 MAP kinase, respectively) by phosphorylation of both threonine and tyrosine regulatory sites [45, 46] (Fig. 1).

ERK was the first cloned MAP kinase in mammalian cells [47]. ERK1 and ERK2 are usually considered to be functionally redundant [1]. The regulatory tripeptide motif -Thr-X-Tyr- (X can be Glu, Gly or Pro) is a common feature of all MAP kinases. The ERK subfamily of MAP kinases is more exactly defined by a -Thr-Glu-Tyr- regulatory motif [48]. The phosphorylation sites have been identified as Thr183 and Tyr185 in mammalian ERK2 [46] and phosphorylation of both residues is required for full activation [1]. The three-dimensional structure of ERK2 [49] suggests that conformational changes are responsible for its activation in response to phosphorylation of the regulatory motif [49, 50].

ERK belongs to the group of serine/threonine kinases and analysis of its substrate specificity demonstrated that ERK is a proline-directed protein kinase that phosphorylates -Ser/Thr-Pro- motifs [51]. After mitogenic stimulation ERK is capable of translocation to the nucleus [41, 52]. Therefore, not only cytoplasmic but also nuclear proteins can be phosphorylated by ERK. Putative nuclear targets are several transcription factors indicating the importance of ERK in the regulation of transcriptional activity [53]. The transcription factor Elk-1 is one of the best characterized substrates of ERK. Elk-1 binds together with the serum response factor to the serum response element in the promoter region of many genes. The serum response element plays an especially significant role in the promoter of the transcription factor c-*fos* [54]. Phosphorylation of Elk-1 by ERK increases its transcriptional activity [55–57]. Other transcription factors implicated as substrates of ERK are c-*Myc* [58], ATF-2 [59] and NF-IL6 [60]. Furthermore, ERK was shown to phosphorylate c-*Jun* but the significance of this finding *in vivo* remains to be determined, since recently identified MAP kinases distinct from ERK were shown to regulate c-*Jun* (see next section). Recent studies suggest the ERK cascade to be involved in cytokine-activated signaling cascades that are mediated by the Janus family of tyrosine kinases and the STAT proteins, since the DNA binding capacity of the STAT transtriction factors is increased by a ERK-dependent phosphorylation of serine residues of STATs [61, 62]. The STAT proteins are suggested to be the direct substrate of ERK [61, 62].

Protein kinases are another major group of substrates for ERK. $p90^{RSK}$ (RSK) was first identified as a substrate of ERK [63]. RSK is activated by phosphorylation on threonine [64]. The ribosomal protein S6 was the first described substrate of this serine/threonine kinase. However, recent reports indicate that the kinase $p70^{S6K}$ is responsible for S6 phosphorylation *in vivo*. Another substrate of RSK is the transcription factor c-*fos* [65]. This phosphorylation may be important in the regulation of the transcriptional activity of the AP-1 complex. Other downstream kinases serving as substrates for ERK are MAP kinase-activated protein kinase 2 (MAPKAP kinase 2) [66] and protamine kinase [67]. The ability of ERK to phosphorylate several upstream proteins of the ERK cascade including the NGF receptor, Sos, Raf-1 and MEK might serve as a mechanism of negative feedback regulation [1, 68, 69].

Phosphorylation of cytoskeletal elements like microtuble-associated proteins (MAP)-1, MAP-2, MAP-4 and Tau by ERK appears to important for the regulation of cellular morphology and cytoskeletal rearrangements [70] and activation of phospholipase A2 by ERK ties the ERK signaling cascade to arachidonate metabolism [71].

Multiple MAP kinase signaling cascades

Data about signaling pathways in the yeast *Saccharomyces cerevisiae* were useful in the identification of the ERK- and other MAP kinase-cascades in mammalian cells. Several independent signaling pathways employing MAP kinase homologues have been described in *S. cerevisae* (Fig. 2). A common theme among these pathways is the requirement of a sequential protein kinase reaction to phosphorylate and activate the next kinase in the pathway [72]. In general, the MAP kinase isoforms are activated by phosphorylation on the regulatory threonine and tyrosine

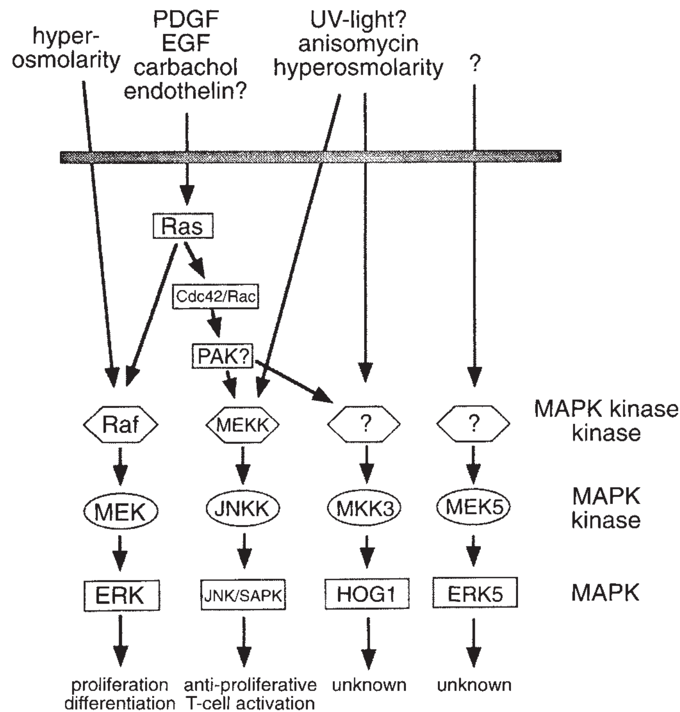

Fig. 3. *Multiple MAP kinase signaling pathways in mammalian cells.*

residues by dual specificity protein kinases, also referred to as MAP kinase kinases. The MAP kinase kinase isoforms are activated by an upstream kinase, also referred to as MAP kinase kinase kinase (Fig. 2).

The MAP kinase signaling pathways in *S. cerevisae* function independently of one another in the regulation of osmotic stress response, mating, pseudohyphal development and cell-wall biosynthesis [73–75]. The conformational requirements of the substrates recognized by MAP kinase kinases and MAP kinasae kinase kinases may be a mechanism to maintain the signaling specificity of each MAP kinase pathway [72]. The mammalian kinases RAF-1 and MEK, for example, recognize only their native substrates MEK and ERK, respectively. Denatured substrates or peptides encoding the regulatory sequence of ERK are not recognized [72]. Another mechanism of signaling specificity may be brought about by formation of complexes involving members of a MAP kinase cascade. The protein binding protein STE5, identified in *S. cerevisiae*, was shown to bind to the members of the mating pathway, STE11, STE7, KSS1 and FUS3, rather than kinases of the osmosensing or cell-wall biosynthesis pathway [72, 76–78] (Fig. 2). These STE5 containing protein complexes would allow rapid and selective regulation of the pheromone-responsive mating pathway in *S. cerevisiae*. However, so far mammalian equivalents of STE5 have not been described.

Until recently, ERK1 and ERK2 were the only well-characterized mammalian MAP kinases (as described above). The discovery of other MAP kinases introduced further tiers to the complex system of intracellular signal transduction (Fig. 3). The c-*Jun* N-terminal kinase (JNK), also referred to as stress-activated protein kinase (SAPK), was recently described as a new subgroup of MAP kinases in mammalian cells [79–81]. Two isoforms of 46 kDa and 55 kDa were named JNK1 and JNK2, respectively, and seem to be functionally redundant [79, 82]. JNK/SAPK regulates c-*Jun* transcriptional activity by phosphorylation of the N-terminal activating domain, Ser-63 and -73 [83], whereas ERK phosphorylates the inhibitory C-terminal site, Ser-243 [83, 84]. N-terminal phosphorylation of c-*Jun* induces the formation of c-*Jun*/c-*Fos* heterodimers and c-*Jun* homodimers that increase the transcriptional activity of many genes by binding to the AP-1 sites in their promoter region [85]. Other nuclear targets of JNK/SAPK are the transcription factors ATF2 and Elk-1 that are phosphorylated and activated by JNK/SAPK [86–88]. JNK/SAPK is activated by extracellular stress (UV-light, heat shock, osmolarity), cytokines (TNF-α, IL-1) or growth factors (EGF) [48].

Recently, JNK kinase (JNKK), also referred to as MKK4 or SEK1, the upstream dual specificity protein kinase of JNK/SAPK, has been described by different groups [89–91]. The upstream kinase of JNKK is MEKK (Fig. 3) [92, 93]. MEKK was originally described as a MEK activating kinase based on its ability to phosphorylate MEK *in vitro* or during overexpression [94]. But recent studies showed *in vivo* that Raf-1 acts upstream of MEK in the ERK signaling cascade whereas MEKK selectively phosphorylates and activates JNKK in the JNK/SAPK signaling cascade, thereby describing two independent MAP kinase cascades in mammalian cells [89, 92, 93]. Like ERK, JNK/SAPK is activated in response to extracellular stimuli binding to both tyrosine kinase receptors [93, 95] or G protein-coupled receptors [96, 97]. Other potent agonists of JNK/SAPK are intra- and extracellular stress stimuli like hyperosmolarity, UV-light or protein synthesis inhibitors [48]. Growth factors like epidermal growth factor (EGF) induced stimulation of JNK/SAPK is Ras-dependent [93] whereas activation by cytokines like tumor necrosis factor α (TNF-α) is Ras-independent [93]. Recently members of the Rho family, a subgroup of the Ras superfamily of small GTP-binding proteins, have been reported to be important for activation of the JNK/SAPK cascade [95, 98]. The Rho-like proteins Rac1 and CDC42 were shown to be essential for the Ras-dependent activation of the MEKK-JNKK-JNK/SAPK cascade by EGF and the Ras-independent activation by TNF-α [95, 98]. However, JNK/SAPK activation by protein synthesis inhibitors like anisomycin is independent of the small GTP binding proteins Rac and Cdc42 [95]. Rac and Cdc42 can bind to the p21-activated serine/threonine kinase PAK and stimulate its autophosphorylation activity [99, 100]. PAK may mediate the effect of Rac and Cdc42 to the JNK/SAPK cascade [98] (Fig. 3).

A third isoform of mammalian MAP kinases, p38 HOG1 kinase, has been cloned and shown to be similar to the yeast high-osmolarity glycerol response 1 (HOG1) kinase [101–103]. The physiological substrate of p38 HOG1 kinase remains to be determined in mammalian cells. p38 HOG1 kinase is defined by the regulatory tripeptide dual phosphorylation motif -Thr-Gly-Tyr- in place of -Thr-Glu-Tyr- in ERKs and -Thr-Pro-Tyr- in JNK/SAPKs. Recently MKK3 (MAP kinase kinase 3), the upstream dual specificity kinase of p38 HOG1 kinase has been identified [90], whereas the upstream kinase of MKK3 in the p38 HOG1 kinase cascade is unknown (Fig. 3). Interestingly, JNKK (also referred to as MKK4 or SEK1) was also shown to phosphorylate and activate p38 HOG1 kinase, but MEKK dependent activation of JNKK *in vivo* induced JNK/SAPK activation but had only weak effect on p38 HOG1 kinase activity [89, 90]. In

correlation with the adaptor protein STE5 in yeast, as described above, an adaptor protein in mammalian cells that formats a complex with MEKK, JNKK and JNK/SAPK may explain this finding. This complex could be responsible for the MEKK-induced activity of JNKK towards JNK/SAPK rather than p38 HOG1 kinase. However, since p38 HOG1 kinase, like JNK/SAPK, is inducible by the GTP binding proteins Rac and CDC42 and responds to the same extracellular stimuli as the JNK/SAPK cascade, its unique physiological function remains to be established.

Recently, another mammalian MAP kinase, ERK5, and its dual specificity kinase MEK5 have been cloned [104]. The upstream kinase or downstream substrates are so far unknown.

Phosphatases regulating MAP kinase cascades

Recently, it was shown, in PC12 cells, that the duration of ERK activation by extracellular stimuli is critical for cell signaling outcomes, since transient activation of MAP kinase induced mitogenesis whereas sustained activation of MAP kinase induced cell differentiation [2]. These data emphasize the importance of mechanisms to terminate the activity of ERK. Protein phosphorylation is a reversible and dynamic process and is balanced by the antagonism of kinases that catalyze phosphorylation and phosphatases that catalyze dephosphorylation. In analogy to kinases, phosphatases are divided into two major groups: protein serine/threonine phosphatases (PSP) and protein tyrosine phosphatases (PTP) [105, 106].

As described above, MAP kinases are activated by phosphorylation on both threonine and tyrosine regulatory sites. Therefore, recently cloned dual specificity PTPs that exhibit dual catalytic activity toward phosphotyrosine and phosphothreonine in substrate proteins are of special interest in the regulation of the MAP kinase signaling pathways. The vaccina H-1 gene product (VH-1) was the first phosphatase shown to effectively hydrolyze both phosphotyrosine and phosphoserine/phosphothreonine [107]. Recently isolated mammalian VH-1-like dual specificity PTPs with significant structural similarities over a stretch of 50 amino acid residues within the catalytic domain, exhibit catalytic activity towards both regulatory sites in ERK [108, 109]. CL100 and B23 are widely expressed human dual specificity PTPs [110–112], whereas PAC1 is expressed in T-lymphocytes, human mesangial cells and human umbilical vein endothelial cells [112, 113]. VHR, the smallest member of mammalian VH-1-like PTPs, dephosphorylates ERK *in vitro* but failed to exhibit a substrate specificity towards ERK [110, 114]. MKP-1 (MAP kinase phosphatase 1, also refered to as 3CH134 [115]), the mouse homologue of CL100 [116] (97% identity), and PAC1 inactivate ERK *in vivo*.

Expression of MKP-1 in COS cells [117] or rat embryonic fibroblasts REF-52 cells [118] prevents the activation of ERK not only by serum or tetradecanoyl phorbol (TPA), but also by oncogenic v-Ras and activated Raf. Expression of PAC1 in COS cells and T-lymphocytes leads to inhibition of ERK activity normally stimulated by EGF, TPA or T-cell activation [119]. MKP-1 and PAC1 are immediate early response genes and both are expressed transiently in response to mitogenic stimulation. ERK is also transiently activated and the kinetics of ERK down-regulation coincides with the appearance of newly synthesized MKP-1 protein in NIH3T3 fibroblasts [117]. In addition, inhibition of protein synthesis blocks the down-regulation of ERK in NIH3T3 cells [117] and vascular smooth muscle cells [120], suggesting the synthesis of MKP-1 being required for ERK inactivation. Furthermore, a physical association between ERK and a catalytically inactive mutant of MKP-1 has been demonstrated [117]. Recently it was shown that MKP-1 antisense oligonucleotides prolonged the activation of ERK in vascular smooth muscle cells but did not affect the down-regulation of MEK, the upstream kinase of ERK [120]. Taken together, the above results strongly suggest that ERK is a physiological substrate of the dual specificity PTPs MKP-1 and PAC1 (Fig. 4).

However, this may not be the case in all cell systems. The inactivation of ERK following mitogenic stimulation of chromaffin cells (PC12), adipose cells (3T3-L1) or endothelial cells (PAE) occurs normally when protein synthesis is inhibited [121, 122]. In PC12 cells the protein serine/threonine phosphatase PP2A and an unidentified protein tyrosine phosphatase are suggested to be responsible for down-regulation of stimulated ERK [122]. Specific inhibition of PP2A in CV1 cells results in up-regulation of MEK and ERK *in vivo* [123]. Since PP2A is largely located in the cytoplasm [108, 124] it may be responsible for inactivation of MEK and cytosolic ERK. In contrast, dual specificity PTPs are located in the nucleus [113, 125, 126] and may therefore be responsible for dephosphorylation of ERK in the nucleus in several cell systems.

Little is known whether distinct dual specificity PTPs serve specific functions in the control of intracellular signaling or are functionally redundant. Dual specificity phosphatases are regulated on the transcriptional level [127]. Recent data demonstrating a differential regulation of VHR and B23 in liver cell lines and CL100, PAC1 and B23 in human mesangial cells [112] suggest unique roles of distinct dual specificity PTPs in intracellular signaling. Furthermore, the substrate specificity of distinct dual specificity PTPs towards other MAP kinases like JNK/SAPK or p38 HOG1 kinase remains to be determined. JNK/SAPK has been tested as a substrate of MKP-1 by two independent groups with conflicting results. Transfection of HeLa cells with MKP-1 inhibited the activation of JNK/SAPK *in vivo* [128], whereas *in vitro* and *in vivo* data in rat fibroblasts suggest a relative selectivity of MPK-1 for ERK compared to JNK/SAPK [118]. Recently two new members of the group of dual specificity PTPs have been described [129, 130]. Since it has been demonstrated that dual specificity kinases exhibit high substrate selectivity towards distinct MAP kinases (see above), it might be expected that dual specificity PTPs will exhibit a similar selectively, each targeting a distinct MAP kinase. If this proves to be the case, dual specificity PTPs would introduce further tiers of control into the regulatory network. Our recent data indicate that MKP-1 transcription is mediated by the MEKK-JNKK-JNK/SAPK pathway rather than the Raf-MEK-ERK pathway [131], thereby suggesting a crosstalk between both pathways since MKP-1 is capable of inactivating ERK. This may be an important mechanism to maintain signaling specificity.

Several other components of the ERK cascade are regulated by protein phosphorylation and are therefore potential substrates of protein phosphatases. As described above, the regulatory phosphoserines in MEK can be hydrolyzed by PP2A *in vitro* [132] and *in vivo* [123]. Recently, thus far unidentified membrane-associated protein phosphatases were shown to be responsible for Raf-1 dephosphorylation and inactivation (Fig. 4) [133].

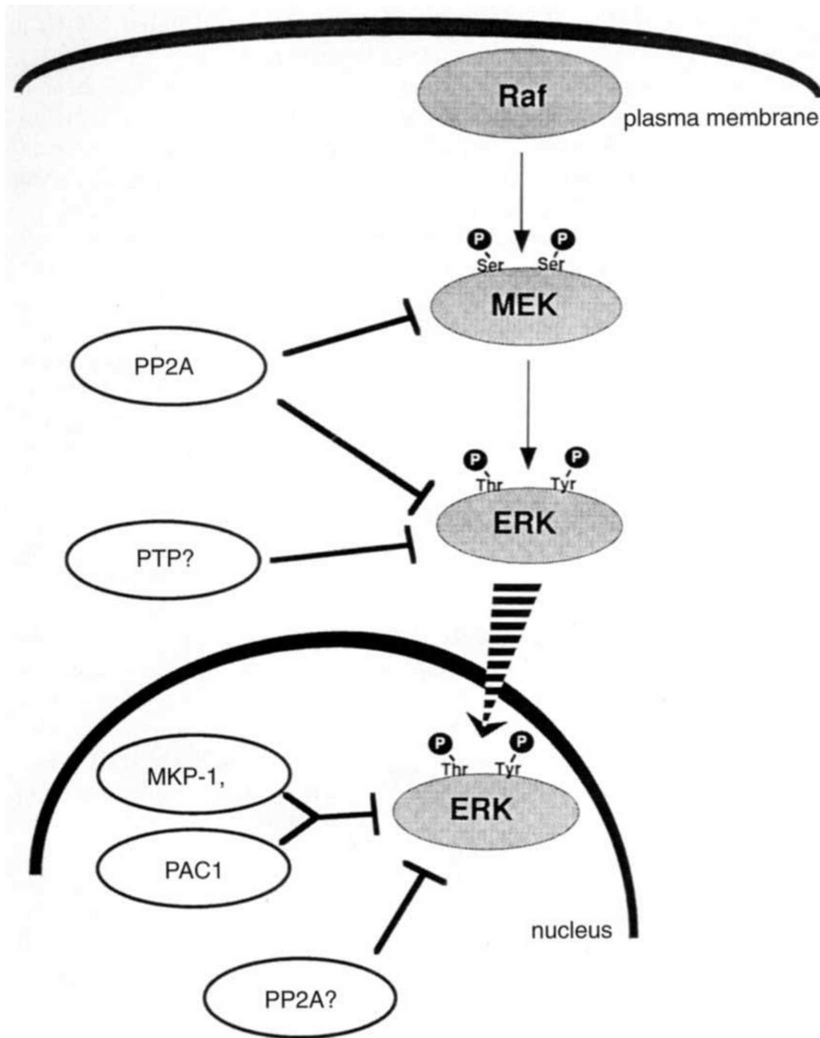

Fig. 4. *Phosphatases involved in the down-regulation of the ERK cascade.*

Physiological relevance of MAP kinase signaling cascades

Proliferation and differentiation

The ERK-signaling cascade plays a pivotal role in growth factor-induced cell proliferation. In most cells mitogenic stimulation by various extracellular agonists correlates with activation of ERK. More important are data from studies using antisense approaches and inactive or constitutively active mutants of components of the ERK cascade. Dominant negative interfering mutants of Ras or Raf-1 were shown to inhibit growth factor induced cell proliferation [134, 135], whereas constitutively activated Raf-1 induced cell proliferation [134]. Furthermore, dominant negative or constitutively active mutants of MEK inhibit or accelerate cell proliferation of NIH3T3 cells respectively [136, 137]. Finally mutants of ERK and its antisense cDNA caused an inhibition of proliferation [138]. Moreover, at least a third of tumors contain mutated Ras genes [139], indicating the importance of Ras and Ras-dependent signaling in oncogenesis. Like Ras, Raf kinases were first described as constitutively active mutants with the ability to transform cells oncogenically [140]. Recently, activated MEK has been shown to induce cellular transformation [141]. These data point to an important role of the ERK cascade in the control of cell proliferation and oncogenesis. However, in some cases cellular proliferation may occur independent of ERK activation [142, 143].

In contrast to the ERK cascade, the JNK/SAPK intracellular signaling pathway is capable of mediating inhibition of cell growth. As described above, JNK/SAPK is strongly inducible by extra- and intracellular stress stimuli that induce cell death. Furthermore, expression of a constitutively active mutant of MEKK, an upstream kinase of JNK/SAPK, inhibits cell growth [92]. Recent data suggest that activation of JNK/SAPK and of p38 HOG1 kinase induce apoptosis, while activation of the ERK cascade prevents apoptosis in PC12 cells after withdrawal of neural growth factor [144].

Cellular differentiation appears to be another physiological response linked to the ERK signaling pathway. Differentiation of PC12 cells [145, 146], monocytes [147], T-cells [148] and mast cells [149] is mediated by the ERK cascade. Differentiation of PC12 cells, characterized by neurite outgrowth, is induced by extracellular stimulation with neural growth factor (NGF) [145]. A sustained activation of the ERK cascade by NGF seems to cause the cellular differentiation [146, 150]. However, other intracellular events may require integration with the ERK cascade to induce differentiation in PC12 cells [151]. In contrast, epidermal growth

factor (EGF) induces proliferation of PC12 cells [2]. The prolonged activation of ERK by NGF versus the transient activation of ERK by EGF is suggested to be responsible for the cell signaling decision [2]. This idea is supported by several studies [reviewed in 2]. For instance, transfection of PC12 cells with oncogenic Ras or Raf induces sustained activation of ERK and cell differentiation [151, 152].

Based on the data described above, both cellular proliferation and differentiation are closely related to the ERK signaling pathway and the duration of activation may be critical for cell signaling outcomes.

MAP kinase cascades in the cardiovascular system

Cardiac hypertrophy causes impaired systolic and diastolic function and reduces the coronary reserve, therefore leading to higher mortality in heart failure and ischemic heart desease. Left ventricular hypertrophy occurs as an adaptive process to increased workload. *In vitro* mechanical stress induces hypertrophic responses [153] and stimulates the activation of all components of the Raf-MEK-ERK signaling cascade in neonatal cardiac myocytes [153, 154]. However, transfection studies investigating the effect of constitutively active Raf-1 in cardiac myocytes suggested that the ERK signaling pathway is critical to induce gene expression associated with hypertrophy, but is not sufficient to induce cardiac myocyte hypertrophy [155]. In accordance with an important role of the renin-angiotensin system in stretch-induced cardiac hypertrophy [154, 156], several studies demonstrated a reduced ERK activation by mechanical stress in the presence of an angiotensin II antagonist [154, 157]. Angiotensin II itself was shown to be a potent stimulator of ERK in cardiac myocytes [158]. The MEKK-JNKK-JNK/SAPK pathway may also be involved in intracellular signaling in stretch-induced hypertrophy, since MEKK is activated in response to mechanical stress in cardiocytes [154]. However, the physiological relevance of this finding remains to be determined.

Endothelin-1 (ET-1) and fibroblast growth factor (FGF) are other agonists that induce a response of cardiac myocytes *in vitro* akin to the hypertrophic response *in vivo*. Both ET-1 and FGF stimulation of cardiocytes induce activation of ERK [158–160], reinforcing the hypothesis that the ERK cascade is relevant to the hypertrophic response of the heart. Moreover, ERK has been suggested to play a role in the recovery from ischemia, since ERK is activated after metabolic inhibition of cardiac myocytes and its activation parallels the induction of immediate early response genes like c-*Jun* and c-*Myc* [161].

Proliferation of vascular smooth muscle cells (VSMC) is an important pathophysiological mechanism in hypertension and atherosclerosis. Vasoconstrictors like angiotensin II, ET-1 or vasopressin and growth factors like PDGF or EGF were shown to induce proliferation of VSMC. These mitogens activate ERK in VSMC [162–165] whereas substances like heparin [166] or elevated levels of cAMP [167], that were shown to be antiproliferative in VSMC, inhibit the activation of ERK in this cell system. This correlation strongly suggests an important role of the ERK cascade in the control of cellular proliferation in VSMC. Recently it was shown that mechanical load of carotid arteries induces ERK activation [168]. Therefore, ERK may also be involved in vascular remodeling in response to high intravascular pressure.

MAP kinase cascades in the kidney

Growth factors play a pivotal role in renal physiology and pathophysiology. For instance, mesangial cell proliferation and expansion that accompanies several forms of glomerulonephritis are suggested to be related to PDGF [169]. Furthermore, strong evidence points to ET-1 and angiotensin II as important mediators of cyclosporine A side-effects including glomerulosclerosis [170, 171], which may be due to cellular proliferation. The mitogens PDGF, ET-1 and angiotensin II were shown to induce ERK activation in mesangial cells [4, 172, 173]. Based on the data in fibroblasts, describing the pivotal of ERK in proliferation (see above), it can be assumed that the activation of ERK in mesangial cells also contributes to the proliferation of mesangial cells. In addition ET and PDGF stimulate *de novo* synthesis of MEK and ERK in mesangial cells [44, 174, 175], thereby contributing to a sustained activation of the ERK cascade. Therefore, the ERK cascade may be an crucial mediator of mesangial cell proliferation in renal deseases mediated by growth factors like ET-1 or PDGF. Interestingly, mesangial cell proliferation is not only induced by growth factor dependent activation of kinase cascades but also by vanadate, an inhibitor of protein tyrosine phosphatases [176]. This finding points to the antagonism of kinases and phosphatases as an important regulator of cellular activation. Heparin is a potent inhibitor of mesangial cell proliferation [177] and exhibits beneficial effects in renal injury in some animal models that are accompanied by cellular proliferation of the mesangium [178]. These effects of heparin may be due to its ERK inactivating capacity in mesangial cells [179] or due to its antiinflammatory effect by inhibition of the expression of prostaglandin endoperoxide synthase-2 (PGHS-2) [180]. Recent data suggest the ERK and the JNK/SAPK signaling pathways to induce the expression of PGHS-2 [181, 182], and therefore the antiinflammatory effect of heparin may also be due to inhibition of ERK.

Sphingolipid metabolites have been implicated in cellular proliferation. The catalysis of sphingomyelin, the major membrane sphingolipid, to the cell growth regulatory lipids ceramide and sphingosine is regulated in response to growth factors and cytokines in rat mesangial cells. Recently, the mitogenic metabolite sphingosine was shown to stimulate ERK with no effect on JNK/SAPK whereas ceramide, an antiproliferative lipid, selectively stimulates JNK/SAPK in rat mesangial cells [183]. These findings suggest that the growth regulatory effect of sphingolipids is mediated through activation of separate MAP kinase cascades reinforcing the pivotal role of MAP kinases in the control of cell growth in mesangial cells.

Furthermore, phospholipase A_2 activity regulates the release of arachidonate metabolites, which modulate renal blood flow and glomerular filtration [184]. Phospholipase A_2 is known to be regulated through phosphorylation by the ERK cascade in response to growth factors [71], and it is tempting to speculate that the ERK signaling pathway is critical in the regulation of phospholipase A_2 in renal cells.

MAP kinase signaling pathways have also been implicated in the renal response to hyperosmolarity. The p38 HOG1 kinase pathway has been reported to be involved in the osmosensing signal transduction in yeast, since organisms with inactive mutants of p38 HOG1 kinase failed to grow normally in a hyperosmolar environment [74]. In mammalian distal tubular cells not only ERK but also JNK/SAPK and p38 HOG1 kinase are stimulated by

Table 1. Summary of the physiological relevance of MAP kinase cascades in the heart, vasculature and kidney

Organ	Physiological status	Critical MAP kinase cascade
Heart	-cellular hypertrophy of cardiac myocytes	-ERK and JNK/SAPK cascades
	-recovery from ischemia	-ERK cascade
Vasculature	-proliferation of smooth muscle cells	-ERK cascade (in response to growth factors and mechanical stress)
Kidney	-mesangial cell proliferation	-ERK cascade (in response to growth factors and sphingolipids)
	-inflammatory response	-ERK cascade (induces PGHS-2 expression in mesangial cells)
	-response to hyperosmolarity	-ERK, JNK/SAPK and p38 HOG1 kinase (in tubular epithelial cells)

hyperosmolarity [185–188]. The physiological relevance of each MAP kinase cascade in the cellular response to osmotic stress remains to be determined. However, based on the well examined function of these pathways in yeast it is tempting to speculate that the p38 HOG1 kinase pathway may also play a pivotal role in the cellular osmotic stress response and regulation of osmolyte transporter genes. This hypothesis is supported by the finding that arginine vasopressin (AVP), a hormone responsible for water and electrolyte transport in the distal tubule under hyperosmolar conditions [189, 190], inhibits epidermal growth factor (EGF) activation of the ERK cascade in Madin-Darby canine kidney (MDCK) epithelial cells [185, 191]. The elevation of intracellular cAMP in response to AVP has been suggested to mediate this inhibition [191]. Furthermore, growth factors like EGF were reported to induce ERK activation and DNA synthesis in distal tubule cells, whereas the ERK activation by hyperosmotic stress is accompanied with reduced DNA synthesis [187]. Thus, additional intracellular pathways, like the JNK/SAPK or p38 HOG1 kinase pathway, are almost certainly involved in the cellular response to hyperosmolarity.

Conclusion

Based on the data presented in this review the regulatory network of intracellular signaling cascades is not only an important factor in cellular physiology, but may also be critical in cardiovascular and renal physiology and pathophysiology (Table 1). A better understanding of the intracellular mechanisms that regulate fundamental cellular responses like cellular proliferation may provide new insights for renal desease *in vivo*. For instance, the development of proliferative glomerulonephritis appears to be dependent on the combined effects of a variety of extracellular mediators that might converge at critical intracellular signaling modules, like MAP kinases, to mediate their pathophysiological effects. Therefore, in future therapeutic strategies in complex renal diseases it might be more promising to target these essential intracellular signaling modules than blocking any single extracellular mediator.

However, there are still several questions about the regulation and function of MAP kinase cascades that remain unanswered. For example, the substrates of the recently described MAP kinases, p38 HOG1 kinase and ERK5, are still unknown. Furthermore, the role of distinct dual specificity PTPs in the control of intracellular signaling is unclear. The specificity of distinct dual specificity kinases (MEK-ERK, JNKK-JNK/SAPK, MKK3-p38 HOG1, MEK5-ERK5) leads one to speculate that, working in parallel, distinct members of the group of dual specificity PTPs will be shown to exhibit selective catalytic activity towards distinct MAP kinases, thereby introducing further tiers to the regulatory network. In addition, one can expect to get important information about the developmental and physiological relevance of intracellular MAP kinase signaling pathways from experiments inactivating genes encoding MAP kinases utilizing dominant negative mutants or antisense strategies in cell culture and gene knock out strategies in mice. Nonetheless, the description of multiple MAP kinase cascades and of the mechanisms involved in their regulation is a major discovery in intracellular signal transduction.

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