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Mitogen-Activated Protein (MAP) Kinase/MAP Kinase Phosphatase Regulation: Roles in Cell Growth, Death, and Cancer

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***Abstract*——Mitogen-activated protein kinase dual-specificity phosphatase-1 (also called MKP-1, DUSP1, ERP, CL100, HVH1, PTPN10, and 3CH134) is a member of the threonine-tyrosine dual-specificity phosphatases, one of more than 100 protein tyrosine phosphatases. It was first identified approximately 20 years ago, and since that time extensive investigations into both mkp-1 mRNA and protein regulation and function in different cells, tissues, and organs have been conducted. However, no general review on the topic of MKP-1 exists. As the subject matter pertaining to MKP-1 encompasses many branches of the biomedical field, we focus on the role of this protein in cancer development and progression, highlighting the potential role of the mitogen-activated protein kinase (MAPK) family. Section II of this article elucidates the MAPK family cross-talk. Section III reviews the structure of the *mkp-1* encoding gene, and the known mechanisms regulating the expression and activity of the protein. Section IV is an overview of the MAPK-specific dual-specificity phosphatases and their role in cancer. In sections V and VI, mkp-1 mRNA and protein are examined in relation to cancer biology, therapeutics, and clinical studies, including a discussion of the potential role of the MAPK family. We conclude by proposing an integrated scheme for MKP-1 and MAPK in cancer.**

I. Introduction

MAP[1] kinase phosphatase-1 (MKP-1) is one of many phosphatases coded by the mammalian genome (Keyse, 2000; Alonso et al., 2004c; Arena et al., 2005; Tonks, 2005) and is a member of a subfamily of phosphatases

[1] Abbreviations: MAP, mitogen-activated protein; MKP-1, MAP kinase phosphatase-1; ERK, extracellular regulated kinase; MAPK, mitogen activated protein kinase; JNK, Jun N-terminal kinase; MEK, mitogen activated protein kinase kinase (MAPK)/extracellular regulated kinase (ERK) kinase; H-Ras, Harvey-rat sarcoma virus oncogene; K-Ras, Kristen-rat sarcoma viral oncogene; N-Ras, neuroblastoma ras oncogene; Raf-1, v-raf-1 murine leukemia viral oncogene homologue 1; c-Myc, cellular-myelocytomatosis oncogene; CDK, cyclin-dependent kinase; AP, activator protein; PAK, p21-activated protein kinase; FAK, focal adhesion kinase; Src, rous sarcoma oncogene; MAPKKK, mitogen activated protein kinase kinase kinase; MKK, mitogen-activated protein kinase kinase; MEKK, mitogen activated protein kinase kinase kinase (MAPK)/extracellular regulated kinase (ERK) kinase kinase; MLK, mixed-lineage kinase; TAK-1, transforming growth factor-β-activated kinase-1; ASK, apoptosis signal-regulating kinase; JIP, JNK-interacting proteins; IκBα, inhibitor of nuclear factor-κBα; KO, knockout; TAB, TAK-1 binding protein, transforming growth factor-β-activated protein kinase binding protein; MAPKAP-K, MAPK-activated protein kinase; TTP, tristetraprolin; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; HSP, heat shock protein; MAPKK, mitogen activated protein kinase kinase; PI3K, phosphatidylinositol 3-kinase; SOS, son of sevenless; HUVEC, human umbilical vein endothelial cell; ER, estrogen receptor; HSF-1, heat shock factor-1; WT, wild-type; TNF-α, tumor necrosis factor-α; miRNA, microRNA; EGF, epidermal growth factor; DSIF, 6-chloro-1-β-D-ribofuranosylbenzimidazole sensitivity-inducing factor; ELK-1, Ets-like protein-1; Skp2, S-phase kinase-associated protein-2; PK, protein kinase; SCF, Skp1/Cul1/F-box; SB203580, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole; cisplatin, (*cis*-diaminedichloroplatinum II); LPS, lipopolysaccharide; Ro 31-8220, 3-[1-3-(amidinothio)propyl-1*H*-indol-3-yl]-3-(1-methyl-1*H*-indol-3-yl) maleimide (bisindolylmaleimide IX); VHR, vaccinia virus

known as the dual-specificity phosphatases (Camps et al., 2000; Theodosiou and Ashworth, 2002; Farooq and Zhou, 2004; Dickinson and Keyse, 2006). Initially identified as one of a set of genes that are expressed in cultured murine cells during the G_0/G_1 transition (Lau and Nathans, 1985), it is part of a family, most of which demonstrate a wide tissue distribution (Camps et al., 2000). As their name implies, the dual-specificity phosphatases can dephosphorylate two types of residues, threonine and tyrosine. The function of these phosphatases is to dephosphorylate and therefore inactivate the MAP kinases (Slack et al., 2001; Liu et al., 2005a), ERKs (Sun et al., 1993; Duff et al., 1995; Sarkozi et al., 2007), p38MAPKs (Kaiser et al., 2004), and JNKs (Sánchez-Pérez et al., 1998). This dephosphorylation activity has been shown to be context-dependent; not all three types of MAP kinases are targeted for dephosphorylation in a given situation, at least in the case of MKP-1 (Wu and Bennett, 2005; Wu et al., 2005b).

As the archetypal member of its family, MKP-1 continues to be the most exhaustively studied. Thus, much of the function and domains of MKP-1 is known; unfortunately, the three-dimensional structural analysis remains to be determined. Although the MKP-1 protein function is characterized in many cells and tissues in both animal models and humans, in this review we will focus on the relationship between MKP-1 and the axes of the MAPK family, emphasizing the involvement of both MKP-1 and the family of MAPK in cancer. As MKP-1 is one of the MAPK-specific dual-specificity phosphatases, which include other MKPs and atypical dual-specificity phosphatases, we also summarize the substrate specificity, the expression pattern, and the involvement of these phosphatases in cancer. Finally, we also review the expression of MKP-1 and chemotherapeutic agents, their mechanism of action, and relevant clinical trials.

homolog 1-related; DUSP1, dual specificity phosphatase-1; HVH, human vaccinia virus homolog; PAC-1, phosphatase in activated T-cells; Pyst, phosphorylates tyrosine serine threonine; JKAP, JNK pathway-associated phosphatase; JSP, JNK stimulatory phosphatase; LMW-DSP, low-molecular-weight dual-specificity phosphatase; AKT, v-akt murine thymoma viral oncogene homolog 1; STYX, phospho-serine/threonine/tyrosine interacting like-1; MDSP, muscle-specific dual specificity phosphatase; TMDP, testis and skeletal muscle dual-specificity phosphatase; IL-6, interleukin-6; IL-10, interleukin-10; IL-12p70, interleukin-12 protein 70 kDa; GSK glycogen synthase kinase; MEFs, mouse embryonic fibroblasts; shRNA, short hairpin RNA; PDK, phosphoinositide-dependent kinase-1; γ2A/AT_1, angiotensin type 1 receptor only; Jak, Janus tyrosine kinase; siRNA, short inhibitory RNA; transplatin, *trans*-diaminedichloroplatinum II; SP600125, anthra[1,9-*cd*]pyrazol-6 (2*H*)-one; Fas, apoptosis-mediating surface antigen; FasL, Fas ligand; BBI, Bowman-Birk inhibitor; MG132, *N*-benzoyloxycarbonyl (*Z*)-Leu-Leu-leucinal; DETA-NONOate, (*Z*)-1-[*N*-(2-aminoethyl)-*N*-(2-ammonioethyl) amino]diazen-1-ium-1,2-diolate; NO, nitric oxide; PARP, poly(ADP-ribose) polymerase; NF-κB, nuclear factor κB; HIF-1, hypoxia inducible factor-1; CBP, cyclic AMP response element-binding protein; APC, adenomatous polyposis coli; Wnt, wingless; NSCLC, non–small-cell lung cancer.

II. Mitogen-Activated Protein Kinase Family: An Overview

To appreciate the possible consequences of phosphatase function, a thorough overview of the axes of MAPK will be undertaken. Although for simplicity, a linear presentation is given for each of the three branches of the MAPK cascade (Cuevas et al., 2007) (Fig. 1), the MAPKs interact with one another, either directly or indirectly, which will be addressed in section II.E (Fig. 2). An overview of the contribution of MAPK modules to the tumorigenic phenotype will be presented in sections V.C on MAPK and cancer and also in sections V.D and VI.

A. Ras-Raf-Mitogen-Activated Protein Kinase Kinase/Extracellular-Regulated Kinase Kinase Kinase-Extracellular-Regulated Kinase Interactions

A thorough review of this branch of MAPK signal transduction must start with the monomeric GTPase Ras, which is associated with this archetype-signaling module Raf-MEK-ERK. Ras has three isoforms: H-Ras, N-Ras, and K-Ras; the latter isoform has two splice forms that result from the use of an alternative C terminus, leading to the products K-Ras4A and K-Ras4B (Schubbert et al., 2007). Furthermore, the signaling of these isoforms is partially controlled on the basis of Ras subcellular distribution, in addition to regional distribution across the plasma membrane (Hancock, 2003; Mor and Philips, 2006) that results from post-translational modifications (Konstantinopoulos et al., 2007). Adding to this, there are three Raf isoforms: Raf1 (also called C-Raf), A-Raf, and B-Raf (Wellbrock et al., 2004) that serve nonredundant functions based on the phenotypes of knockout mice (Gerits et al., 2007). The Ras isoforms have different binding affinities to the Raf proteins (Wellbrock et al., 2004). In the context of cells, B-Raf seems to be the dominant form that activates MEK1/2, whereas the isoforms Raf1 and A-Raf may regulate duration of signaling of MEK-ERK among other functions (Wellbrock et al., 2004).

Another means of defining signal specificity is by controlling subcellular localization, a key element of signal transduction, which can be mediated via scaffolding protein. Scaffolds such as MORG1, KSR, and paxillin, in conjunction with other proteins (MP1, p14, and 14-3-3) that act as scaffolds in their own right, target the Raf1-MEK-ERK module to late endosome, plasma membrane, and focal adhesions, respectively (Kolch, 2005). Although MEK can interact with ERK in their unphosphorylated forms with or without scaffolding proteins (Yoon and Seger, 2006), the activation of ERK by MEK forces them apart because of structural changes in phosphorylated ERK. Once activated, ERK1 and ERK2 catalytic activities increase (170,000–250,000 times over basal activity) and have at least 160 potential effectors in the cytosol and nucleus (Yoon and Seger, 2006).

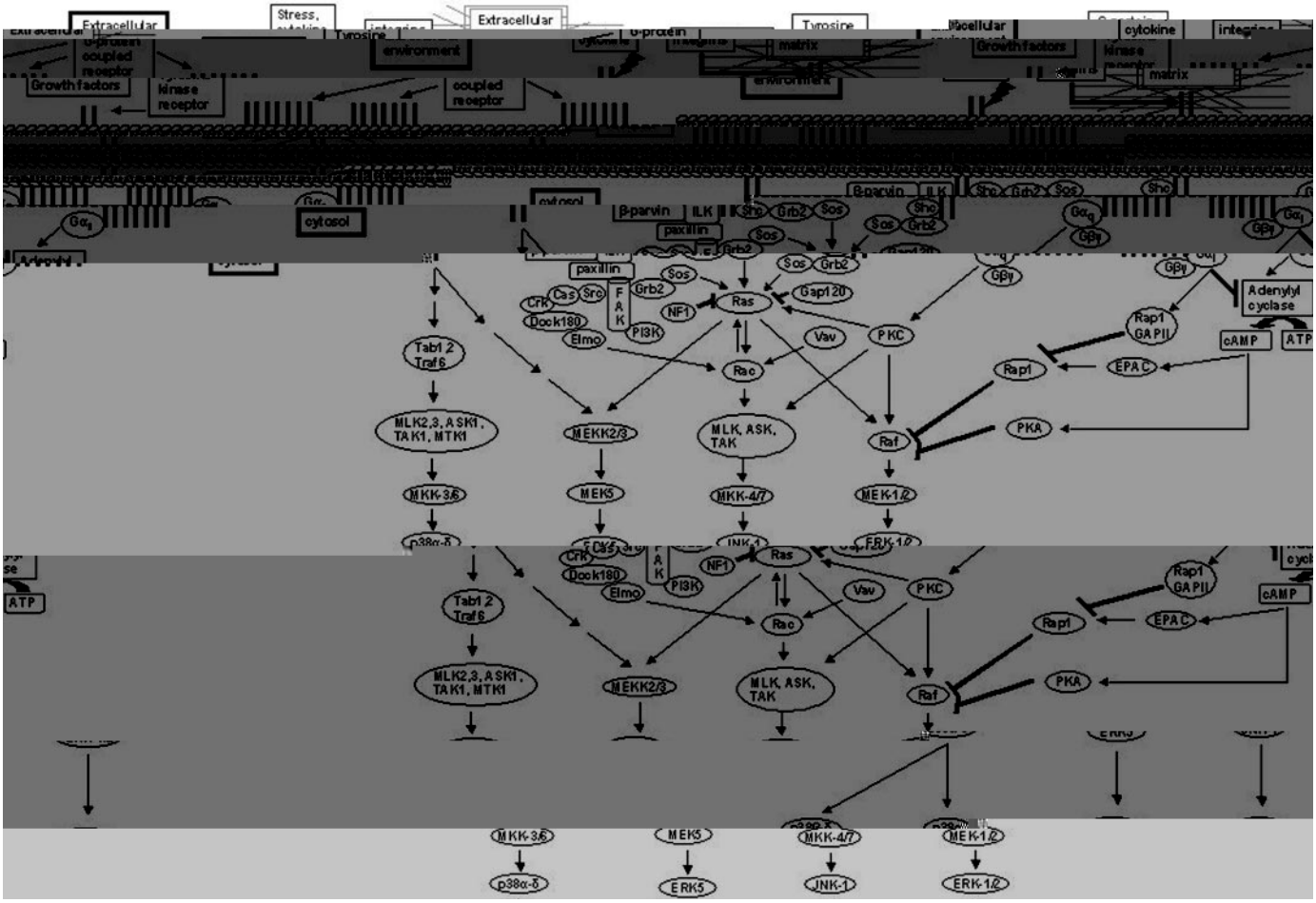

FIG. 1. Linear aspect of MAPK signal transduction. This figure summarizes some of the components in the MAPK signal transduction pathway from the extracellular milieu to the phenotypes associated with the more "classic" MAPK family members, with an emphasis on a linear interaction. SOS, Vav, and exchange protein directly activated by cAMP (EPAC) are guanine nucleotide exchange factors that activate GTPases (Ras, Rac, and Rap1) and neurofibromin 1 (NF1). Gap120 and Rap1 GapII are GTPase-activating proteins (GAPs) that increase catalytic activity of GTPases and inactivate them. For each of the MAPK members represented, the more important functions have been enumerated. See sections II.A–D and V.D. ILK, integrin-linked kinase; Cas, CRK-associated substrate; Elmo, engulfment and cell motility protein.

Among these are transcription factors, kinases, phosphatases, cytoskeletal proteins, and apoptosis-related proteins (Yoon and Seger, 2006).

B. Extracellular-Regulated Kinases

The oldest known and best characterized members of the MAPK family, the ERKs boast at last count six isoform family members (ERK1–5 and ERK7/8) (Bogoyevitch and Court, 2004). By far the most studied are ERK1/2 and ERK-5. ERK1/2 were the first members to be characterized (Bogoyevitch and Court, 2004). The signaling events activating ERK1/2 are usually initiated at the plasma membrane via receptor tyrosine kinases (McKay and Morrison, 2007) and funnel, in part, through the Raf-MEK1/2-ERK module, the outcome of which depends on cell surface receptor density, the amount of ligand, the duration of signaling dictated in part by the rate of internalization and inactivation of receptor, and the cell type under consideration (Marshall, 1995; Murphy and Blenis, 2006). More importantly, duration and strength of signaling can be uncoupled. Two signals may last for an equal amount of time but have different strengths and, conversely, signaling strength can be equal but last for various lengths of time. A combination of strength and duration dictates different outcomes for a given cell-type in response to stimuli (Murphy and Blenis, 2006), with possible outcomes ranging from sustained high activation corresponding to senescence, apoptosis, and differentiation, whereas sustained lower levels of activation correlate with cell proliferation (Agell et al., 2002). The opposite situations in which sustained or transient ERK activation leading to cell proliferation can also be found, depending on cell type, receptor number, and other factors (Marshall, 1995). Tyrosine kinase receptor-mediated activation of ERK1/2 proceeds via a Raf-MEK-ERK signaling module and the activation of ERK1/2 requires threonine and tyrosine dual phosphorylation in a threonine-glutamic acid-tyrosine (T-E-Y) motif (Turjanski et al., 2007). Other cell surface receptors, such as G protein-coupled receptors, e.g., the integrins, among others, can also transmit their signal via MAPK modules or modu-

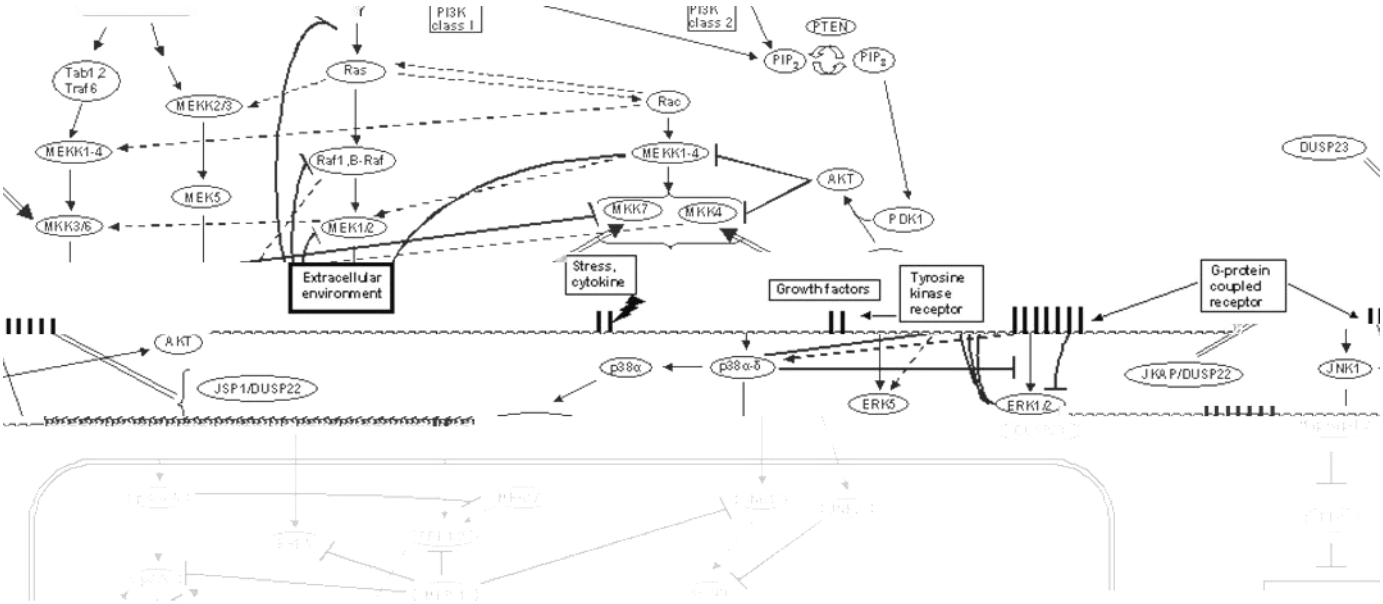

FIG. 2. Cross-talk of the MAPK signal transduction pathway. Summary of the cross-talk and feedback inhibition implicating the MAPK signal transduction pathway. Solid single arrows indicate activating signal transduction within a module (except JNK → Akt which is a priming phosphorylation). Hatched arrows indicate activating cross-talk between signaling modules. Solid double arrows indicate activating dephosphorylation by phosphatases. Solid lines-bars are inhibitory phosphorylation or dephosphorylation. Open arrows indicate cycling between two forms of phosphatidylinositol phosphate (PIP). For more details about these interactions, see section II.E. on MAPK cross-talk and section IV, an overview of the dual-specificity phosphatase family. PTEN, phosphatase with tensin homology.

late the tyrosine kinase receptor signaling (Martin and Vuori, 2004; Olson and Hallahan, 2004; Hannigan et al., 2005; McLean et al., 2005; Cully et al., 2006; Engelman et al., 2006; Holz et al., 2006; Kohno and Pouyssegur, 2006; Goldsmith and Dhanasekaran, 2007; Hehlgans et al., 2007; Mayor et al., 2007; Schubbert et al., 2007).

ERK1/2 activity or a lack thereof has many consequences that will be considered in the context of their functions. Much is known about the involvement of these kinases in various biological processes; however, as ERK1 (p44MAPK) and ERK2 (p42MAPK) share 83% identity and are regulated by similar factors and conditions, distinguishing between the contribution of either kinase to ERK signaling has been challenging. As a detailed review of ERK1/2 in cell cycle progression has been undertaken elsewhere (Chambard et al., 2007; Meloche and Pouyssegur, 2007), we will provide an overview of some of the functions reported for ERK1/2 at different phases of the cell cycle as follows:

- **Preparation for the cell cycle:** ERK1/2 prepare the cell for the cell cycle by 1) synthesis of ribosomal RNA, 2) contribution to chromatin remodeling, 3) increased pyrimidine nucleotide synthesis (Chambard et al., 2007), 4) increased protein synthesis (Meloche and Pouyssegur, 2007), and 5) participation in protein translation (Meloche and Pouyssegur, 2007).
- **G_0-G_1 transition:** ERK1/2 are also implicated in the stabilization of c-Myc, which together with Max forms a heterodimeric transcription factor, one of the essential steps for cells to proceed from G_0 to late G_1 (Jones and Kazlauskas, 2001).
- **G_1-S transition:** 1) Both transient and sustained activation of ERK1/2 can phosphorylate Elk and induce transcription of the c-*fos* gene; however, only sustained ERK1/2 nuclear activity can stabilize c-Fos protein within the nucleus, which leads to increased fra-1 transcription and decreased cyclin D1 transcription, among other transcriptional activities. The sustained ERK1/2 nuclear activity and c-Fos stability dwindle, and Fra-1 and other transcription factors initiate transcription of cyclin D1. 2) ERK1/2 can also up-regulate cyclin D1 via increased stability of c-Myc transcription factor

(Chambard et al., 2007). 3) ERK1/2 phosphorylation of CDK family members, which in turn phosphorylate the retinoblastoma family, is a necessary step for retinoblastoma family members to dissociate from the E2F family members and initiate transcription of genes coding for proteins involved in DNA replication (Meloche and Pouyssegur, 2007). 4) ERK1/2 mediate inhibition of antiproliferative gene transcription throughout the G_1 phase by an AP-1-dependent mechanism (Yamamoto et al., 2006).

- **G_2-M transition:** ERK1/2 activity is required during the early G_2 phase and DNA damage checkpoints during G_2 (Meloche and Pouyssegur, 2007) but is dispensable from this point on for the rest of mitosis (Shinohara et al., 2006). ERK1/2 are also involved in cell cycle arrest, a topic that will be discussed in section V.C on MAPK and cancer.

Although the facet of ERK1/2 function in the control of cytoskeletal remodeling is reviewed in Pullikuth and Catling (2007), we summarize some of the more important points for this review. MP1-p14-PAK signaling has been implicated in PAK-mediated MEK1-ERK activation involving the remodeling of focal adhesion and actin filaments during cell spreading (Pullikuth and Catling, 2007). ERK1/2 also increase tubulin polymerization via inhibitory phosphorylation of a tubulin-sequestering protein, stathmin/Op18, which is also targeted by PAK and CDK5 for inactivating phosphorylation. Although ERK1/2 phosphorylate and activate the calcium-dependent protease calpain, which is implicated in focal adhesion assembly and disassembly, the relationship of calcium, calmodulin, and MAPK signal transduction is a complex network of interactions that goes beyond the scope of this review (Agell et al., 2002; Cook and Lockyer, 2006). However, we will briefly summarize the interaction of some of the previously mentioned players. FAK directs calpain to focal adhesions and is itself degraded by calpain during focal adhesion turnover. Furthermore, FAK may serve as scaffold for ERK1/2 and calpain, allowing these two proteins to interact. These findings are further supported by evidence that *Fak*-null cells have decreased ERK1/2 signaling, decreased calpain activity, and decreased microtubule stability, leading to a reduction in focal adhesion disassembly and reduced cell migration. Such results are explained in light of other data implicating FAK as a necessary component of focal adhesion disassembly but not in focal adhesion formation. Moreover, growing microtubules can target focal adhesions for disassembly. In this way, ERK-mediated tubulin polymerization targets focal adhesion disassembly and participates in the remodeling of the cytoskeleton, focal adhesion turnover, and cell shape and motility (Pullikuth and Catling, 2007).

ERK1/2 also participate in cell differentiation by modulating the stability, activity, and protein-protein interaction of various proteins in a cell type-specific manner (Yoon and Seger, 2006). ERK1/2-mediated degradation of BCL6 allows B-cell differentiation and participates in antibody production. ERK1/2 phosphorylation of the Ets transcription factor ERF, results in a decrease in its repression of gene transcription. Another ERK effector, GATA1, increases its protein-protein interaction after phosphorylation. This modification is implicated in both the development and differentiation of erythroid cells. Stathmin-related functions in the cell cycle and differentiation are also regulated by ERK-mediated phosphorylation (Yoon and Seger, 2006).

The previously mentioned studies were complemented by work on ERK1 and ERK2 knockout mice in an attempt to distinguish between the functions of these isoforms. Some *Erk1*-null mice have no overt phenotype, develop normally, and are fertile, whereas others reveal increased locomotor activity (Gerits et al., 2007) and decreased adiposity due to impaired adipocyte differentiation (Aouadi et al., 2006), among other phenotypes (Gerits et al., 2007). *Erk2*-null mice die in utero from embryonic day 6.5 from lack of mesoderm differentiation to embryonic day 8.5 from lack of proper placental development, depending on the murine background (Gerits et al., 2007). Although ERK1 and ERK2 are frequently thought of as being simultaneously regulated, these results demonstrate that they have nonoverlapping functions under certain conditions. Systematically identifying effectors that recognize one or the other kinase would go a long way to determine the nonoverlapping functions of each kinase.

ERK5, the next best characterized member of the family was cloned in 1995 (Wang and Tournier, 2006) and is also called big MAPK1 (BMK1) owing to its molecular mass of 98 kDa. In mice, this MAPK has three splice forms: ERK5a, ERK5b, and ERK5c. ERK5a is a catalytically active kinase, whereas the shorter N-terminal-truncated forms, ERK5b and ERK5c, are kinase-dead (Yan et al., 2001). The latter two kinases can interfere with ERK5a binding to its upstream kinase MEK5. The N-terminal domain of ERK5a contains three domains: 1) cytoplasmic targeting, 2) MEK5-binding domain, and 3) oligomerization domain (Yan et al., 2001). MEK5 is expressed as two splice forms, MEK5α and MEKβ. The former activates ERK5, whereas the latter acts as a dominant-negative regulator of ERK5 activation (Cameron et al., 2004). In addition, The ERK5 C-terminal kinase contains both a nuclear localization signal and nuclear export signal and an autoinhibitory domain. Upon activation, ERK5 can phosphorylate its C terminus, thereby exposing its nuclear localization sequence (Buschbeck and Ullrich, 2005). A C-terminal truncated splice form, termed ERK5-T, is unable to translocate to the nucleus although it can bind to and sequester ERK5 in the cytosol (McCaw et al., 2005). In nonstimulated cells, ERK5 is either nuclear or diffuse throughout the cell, depending on cell type. Activation of

ERK5 requires a T-E-Y motif (Mody et al., 2003) and can result from mitogenic factors or chemical or physical stresses.

ERK3 and ERK4 are from the same subfamily and are covered together here because there is very little published information on ERK4. ERK3, first identified in 1991 (Boulton et al., 1991), is believed to be a protein of approximately 100 kDa. A 63-kDa form, once believed to be a form of ERK3, has been renamed ERK4 (Coulombe and Meloche, 2007). This ERK4 is not to be confused with the presumed 45-kDa ERK1b splice form, which shares immunoreactivity with ERK1 and is also named ERK4. The mRNA of both *erk3* and *erk4* can be detected in multiple tissues although ERK4 has a more restricted expression pattern (Coulombe and Meloche, 2007). The common ERK1/2 and ERK5 activation motif T-E-Y is replaced by S-E-G in ERK3 and ERK4. Thus, on the basis of current evidence, it is believed that neither of these kinases is dually phosphorylated. However, B-Raf and MEK1/2 have been implicated in the increase of ERK3 protein levels (Hoeflich et al., 2006). ERK4 has thus far not been characterized. Although the function of ERK3 remains unknown, the subcellular distribution of ERK3 is both cytosolic and nuclear and is not affected by mitogens or chemical stress; it is targeted to the nucleus in response to thermal stress. Furthermore, the change in distribution does not require ERK3 to be enzymatically active or its activation motif to be phosphorylated (Coulombe and Meloche, 2007).

As ERK6 is also known as p38γMAPK, this protein is discussed in section II.D on p38MAPKs.

ERK7 was identified in 1999 (Abe et al., 1999), and ERK8 was initially identified as a novel MAPK (Abe et al., 2002). ERK8 is now considered to be the human ortholog of the rodent ERK7; thus, the ERK7 designation will be used for ERK7 and ERK8 (Saelzler et al., 2006; Coulombe and Meloche, 2007). ERK7 mRNA is ubiquitously expressed in human adult tissues. Although the protein has an activation loop with a T-E-Y motif similar to that of ERK1/2 and ERK5, it is constitutively phosphorylated, via autophosphorylation, whereas its half-life is determined by polyubiquitination and proteasomal degradation, similar to ERK3 (Coulombe and Meloche, 2007). The C-terminal portion of ERK7 also determines full kinase activity. Stress, mitogens, and kinases, such as Src and Ret, can induce phosphorylation and activation of ERK7 (Coulombe and Meloche, 2007).

C. c-Jun NH_2-Terminal Kinases

This kinase family is stimulated by stress, cytokines, and growth factors (Roberts and Der, 2007). These stimuli activate the signaling module comprising of MAPKKK-MKK4/7-JNK (Bogoyevitch and Kobe, 2006; Raman et al., 2007; Roberts and Der, 2007). More specifically, JNK-related MAPKKKs are composed of MEKK1, MEKK4, dual leucine zipper-bearing kinase, MLK1–4, leucine zipper-bearing kinase, TAK-1, ASK1, and zipper sterile-α-motif kinase. In turn, these funnel through MKK4 and MKK7, activating the JNKs (Raman et al., 2007; Roberts and Der, 2007). The c-Jun NH_2-terminal kinase family is composed of three isoforms, JNK1, JNK2, and JNK3, which are divided into splice forms. The four splice forms of JNK1 are arranged as follows: α1 and β1 splice forms (p46) and α2 and β2-splice forms (p54). The α1 and β1 splice forms (p46) differ from each other by alternative exon usage, leading to substitution between kinase domains IX and X, which determine substrate specificity. The same exon usage characterizes the difference between the α2 and β2 splice forms. The p46 differ from the p54 splice forms by the C-terminal region that is alternatively spliced. The function associated with the longer C-terminal region found in p54 and lacking in p46 remains unclear. JNK2 also has splice forms (α1, α2, β1, and β2) and molecular masses analogous to that of JNK1, whereas JNK3 has only the α1 (p46) and α2 (p54) splice forms (Waetzig and Herdegen, 2005). There are very few data comparing the effects of all 10 splice forms in a single biological system (Gupta et al., 1996; Yang et al., 1998; Tsuiki et al., 2003). Both JNK1 and JNK2 are ubiquitously expressed, and JNK3 is more specific to the brain and heart and testis. The activation of JNKs requires threonine and tyrosine dual phosphorylation in a threonine-proline-tyrosine (T-P-Y) motif. MKK7 preferentially targets threonine 183 and MKK4 phosphorylates tyrosine 185 (Bode and Dong, 2007).

Underlying all these splice forms are differently localized effectors, cytosol versus nucleus (Bogoyevitch and Kobe, 2006), scaffolding proteins such as JNK-interacting proteins (JIPs), among others, that sequester and determine subcellular localization, and functions of JNKs (Raman et al., 2007), which include cell migration activity (Huang et al., 2003., 2004; Bogoyevitch and Kobe, 2006) and proapoptotic and antiapoptotic activities (Liu and Lin, 2005) relating to the duration of JNK signaling (Ventura et al., 2006), among others (Bogoyevitch and Kobe, 2006). More than 1 h of signaling by JNK is associated with proapoptotic activity, whereas shorter durations are linked to antiapoptotic activities (Ventura et al., 2006). Adding to these observations is the seemingly contradictory JNK-mediated inhibition of the apoptotic pathway via phosphorylation (Yu et al., 2004) and stimulation of the prodeath pathway also via JNK activity (Maundrell et al., 1997; Fuchs et al., 1998b). JNK also participates in cell cycle progression via the c-Jun component of the AP-1 transcription factor (Mikhailov et al., 2005; Bogoyevitch and Kobe, 2006; Heasley and Han, 2006; Perdiguero et al., 2007).

JNK is also implicated in cell migration, as the chemical inhibition or the dominant-negative form of JNK can impair cell migration of human, rodent, and bovine cells (Huang et al., 2004). JNK phosphorylates paxillin on Ser-178, and a point mutation Ser-178→Ala preventing

TABLE 1

Summary of the MAP kinase dual-specificity phosphatases and their involvement in cancer

This table summarizes the findings concerning the MKPs that target MAP kinases as substrates. For the purposes of clarity, the first name of each phosphatase corresponds to the official symbol from the National Center for Biotechnology Information GENE database (http://www.ncbi.nlm.nih.gov/sites/entrez). Other names that vary with species are also given and can be found on other databases (PubMed, part of NCBI, or at http://harvester.fzk.de/harvester) or other review articles (Alonso et al., 2004). The expression level and function of each phosphatase is given in relation to the process of tumorigenesis, when available. The occasional contradiction in tissue distribution is caused by differences in detection methods (Northern blot versus reverse transcriptase-polymerase chain reaction). Finally, although for each phosphatase MAPK substrate specificity is given, depending on cell type and paradigm, the reported specificity can vary.

MKP Family	Primary Subcellular Localization	MAPK Substrate Specificity	Normal Tissue Expression and Distribution of the mRNA or Protein	Expression in Tumors relative to Normal Tissue, Consequences of Atypical Expression to Tumor Cell Lines and in Vivo Tumors	Knockout Mice Phenotype	References
DUSP1, *MKP-1*, *erp*, *CL100*, *HVH1*, *PTPN10*, *Ptpn16*, *3CH134*	Nuclear	p38 ~ JNK ≫ ERK	Inducible, ubiquitous	Increase or decrease, depending on tumor (see text)	Develop normally: immune- and diet-related phenotype revealed (see text)	See text
DUSP2, *PAC-1*	Nuclear	ERK ≫ p38 ~ JNK Pac-1 variant without intrinsic phosphatase activity increases MKP-1 phosphatase activity	Inducible in hematopoietic tissue Some expression in thymus, spleen, kidney. and lung Pac-1 variant has no phosphatase activity; found in large granular lymphocyte leukemia and pancreatic islet cells	Decreased in acute leukemia Increased in ovarian carcinoma Increased in breast cancer cell line: increased apoptosis Overexpressed in large granular lymphocyte leukemia No direct correlation with non-Hodgkin's lymphoma subtype, may be involved in lymphomagenesis	Develop normally: *Dusp2*$^{-/-}$ mice protected from arthritis; increased JNK1 activity with decreased ERK and p38 activities	Rohan et al., 1993; Ward et al., 1994; Chu et al., 1996; Kim et al., 1999; Kothapalli et al., 2003; Givant-Horwitz et al., 2004; Jeffrey et al., 2006; Cerhan et al., 2007; Wu et al., 2007
DUSP4, *MKP-2*, *HVH2*, *TYP*	Nuclear	ERK ~ JNK > p38 C-terminal domain of MKP-2 reduces its phosphatase activity	Ubiquitous, inducible	Increased MKP-2 protein if Raf-1/MEK is active Exocrine pancreatic tumor cell lines inverse correlation between survival of rats and *mkp-2* mRNA expression in ascites hepatomas Overexpressed in human breast cancers Early onset of high-grade breast cancer associated with loss of chromosome arm 8p, loss of genes including MKP-2 6 weeks after s.c. injection, in athymic female nude mice mammary pads, fewer mice had tumors when MKP-2-transfected MCF-7 cells were injected compared with MCF-7 containing empty vector or MCF-7 parental lines Ovarian serous borderline tumors express higher levels of MKP-4 than serous carcinomas Difference in phenotype may be due in part to MKP-4 expression MKP-2 siRNA protects MEFs from hydrogen peroxide-induced apoptosis MKP-2 can induce apoptosis in hydrogen peroxide-treated-*p53*$^{-/-}$ MEFs		Guan and Butch, 1995; King et al., 1995; Chu et al., 1996; Yokoyama et al., 1997; Yip-Schneider et al., 2001; Hutter et al., 2002; Wang et al., 2003, 2007a; Armes et al., 2004; Sieben et al., 2005; Shen et al., 2006
DUSP5, *HVH3*, *Cpg21*, *Gm337*	Nuclear	ERK ≫ JNK ~ p38 ERK-2 ~ ERK1 ≫ ERK3 ~ ERK5 ~ ERK7 DUSP5 transports and sequesters unphosphorylated ERK2 to the nucleus Phosphatase-dead DUSP5 translocates ERK2 to nucleus and MEK can activate ERK2 in nucleus Crystal structure of DUSP5 catalytic domain revels head-to-tail dimers	Inducible Bone marrow, tonsillar B-cells, brain, lung, and eosinophile	Overexpression in lung and colon cancer cell lines, causes decreased colony formation Granta 519 cells (used as an in-vitro model for mantle cell lymphoma) reduce their proliferation by ~50%, via an unknown mechanism, when DUSP5 siRNA is ectopically expressed		Temple et al., 2001; Ueda et al., 2003; Mandl et al., 2005; Jeong et al., 2007; Ortega-Paino et al., 2008

TABLE 1 *Continued.*

MKP Family	Primary Subcellular Localization	MAPK Substrate Specificity	Normal Tissue Expression and Distribution of the mRNA or Protein	Expression in Tumors relative to Normal Tissue, Consequences of Atypical Expression to Tumor Cell Lines and in Vivo Tumors	Knockout Mice Phenotype	References
DUSP6, *MKP-3*, *PYST1*, *rVH6*	Cytosolic	ERK ≫ JNK ~ p38 ERK1/2 ≫ ERK5 Monomer ERK binds to monomer MKP-3 ERK2 binding to MKP-3 → rearrangement MKP-3 catalytic site MKP-3 (C^{293}→S) causes cytoplasmic retention of ERK; this retention allows MEK to phosphorylate ERK ERK1/2 phosphorylates MKP-3 serine 159 and/or serine 197 and mTOR can phosphorylate MKP-3 at serine 159, leading to MKP-3 proteasomal degradation ERK5, p38, and JNK do not phosphorylate MKP-3	Constitutive Mkp-3 mRNA expression in mouse embryo in centers associated with cell proliferation and patterning: presegmental paraxial mesoderm, limb bud, brachial arch mesenchyme, midbrain/hindbrain isthmus, nasal, dental, hair, and mammary placodes	Expression of DUSP6 gene product is reduced by hypermethylation in human pancreatic cell lines and pancreatic tumors Decrease associated with invasive carcinoma phenotype of pancreatic cancer Tamoxifen treatment in conjunction with MKP-3 overexpression in MCF-7 cells increases 1) MCF-7 colony formation in soft agar, 2) growth rate in athymic ovariectomized nude mice, and 3) levels of phospho-ERK and reduces 4) MKP-3 phosphatase activity Inactivation of MKP-3 protein was associated with progression from high-grade dysplasia/pancreatic intraepithelial neoplasia (grade 3) to invasive carcinoma → lack of DUSP6 may be associated with invasive phenotype Ovarian cancer cell lines OVCAR3, OVCA420, OVCA429, OVCA433, DOV13–5, SKOV3, and A2780cp, reveal lower MKP-3 protein expression, compared with immortalized human ovarian surface epithelium; the lower expression was accounted for by inhibiting proteasomal degradation of MKP-2 40% of human ovarian cancer samples revealed reduced MKP-3 protein expression compared with normal ovarian samples Ectopic expression of MKP-3 in A2780cp cells 1) reduces cell proliferation and anchorage-independent growth in vitro, 2) reduces growth rate in vivo when injected into nude mice, and 3) increases cell sensitivity to cisplatin-mediated death in vitro and in nude mice (reduced tumor size)	1) At embryonic days 8.5–17.5 F_1 heterozygous offspring of germline chimeras: *DUSP6* (*MKP-3*)-null mice have normal mendelian frequency with no obvious phenotype 2) Postnatal development: *DUSP6* (*MKP-3*)-null mice reveal postnatal lethality depending on genetic background 3) Null mice that survived to weaning: can have skeletal dwarfism, craniosynostosis, developmental delays, and/or malformations of the middle ear component caused by negative regulation of FGF signaling	Groom et al., 1996; Muda et al., 1996; Wiland et al., 1996; Camps et al., 1998; Brunet et al., 1999; Stewart et al., 1999; Dickinson et al., 2002a; Furukawa et al., 2003, 2005; Kim et al., 2003b; Karlsson et al., 2004; Marchetti et al., 2005; Xu et al., 2005; Cui et al., 2006; Li et al., 2007; Arkell et al., 2008; Bermudez et al., 2008; Chan et al., 2008
DUSP7, *PYST2*, *MKPX*, *B59*	Cytosolic	ERK > p38 ≫ JNK PYST2-L (long) has phosphatase activity and PYST2-S (short) has no catalytic activity.	Constitutive Very little expression in peripheral blood mononuclear cells Humans: skeletal muscle, brain, heart, kidney, pancreas, placenta, little expression in lung and liver	Ectopic B59 expression reduces the number of foci per plate when v-raf or H-ras-transformed NIH 3T3 are cultured PYST2-S and PYST2-L are coded by different open reading frames of the same gene PYST2-L is coded by exons 1, 2, and 3b and PYST2-S is coded by exons 3a and 3b; the catalytic domain is coded by exon 2; PYST2-S may compete with PYST2-L for common factors at the transcription, translation, or regulatory levels Increased PYST2-L mRNA in leukocytes of patients with acute myelogenous leukemia than in with normal leukocytes; leukemic cell lines also reveal an increase that is not related to an amplification of the gene PYST2-L mRNA is also increased in solid tumors derived from different tissues PYST-2 is expressed 5-fold higher in leukemic leukocytes (before chemotherapy) when compared with leukocytes from the same patient after remission		Shin et al., 1997; Dowd et al., 1998; Levy-Nissenbaum et al., 2003a,b, 2004
DUSP8, *HVH-5*, *HVH8 Nttp1*, *M3/6*, *HB5*	Nuclear and cytosolic	JNK ~ p38 ≫ ERK M3/6 binds to JIP1 to dephosphorylate JNK M3/6 can also bind to JIP2 JNK phosphorylates M3/6 → does not affect stability	Constitutive Adult mouse tissue: eye, brain, lung, and heart Fetal human tissue: brain and lung Human: skeletal muscle, heart, and different structures of the brain may be involved in olfactory processing			Martell et al., 1995; Muda et al., 1996; Theodosiou et al., 1996; Nesbit et al., 1997; Bernabeu et al., 2000; Johnson et al., 2000; Willoughby et al., 2003

TABLE 1 *Continued.*

MKP Family	Primary Subcellular Localization	MAPK Substrate Specificity	Normal Tissue Expression and Distribution of the mRNA or Protein	Expression in Tumors relative to Normal Tissue, Consequences of Atypical Expression to Tumor Cell Lines and in Vivo Tumors	Knockout Mice Phenotype	References
DUSP9, MKP-4, Pyst3	Cytosolic Dependent on nuclear export signal and nuclear export protein CRM1	ERK ≫ p38 > JNK1 ERK2 > ERK1 ≫ ERK3, ERK5, ERK7 p38α ≫ p38γ, p38δ	Human placenta, kidney Mice: placenta, kidney, testis (protein)	Decrease of MKP-4 in squamous cell carcinoma leads to increased invasion and metastasis In developing tumors: squamous cell carcinoma-producing lineages coexpressing MKP-4 and GFP injected into neonatal BALB/C had smaller tumors that did not express GFP → conclusion: tumors derived from nontransduced cell population; in the same experiment, control cells transduced with GFP only developed into a tumor that was GFP-positive In established tumors: 35 days after inoculation of H1299 cells, with either MKP-4-Tet or control vector, into nude female mice, tumors formed and mice were fed tetracycline Tetracycline-responsive MKP-4-expressing H1299 cells formed smaller tumors than H1299 control cells; cells in MKP-4-expressing tumors were larger and had decondensed nuclei compared with control cells In vitro: MKP-4 expression caused cell enlargement and cell death in tumorigenic cells but not in the nontumorigenic parental cell line In vitro: overexpression of MKP-4 leads to cell death caused by cell cycle arrest at G_2-M and microtubule disruption	Embryonically lethal owing to lack of labyrinth development (placental defect) When placental defect rescued: not required for embryonic development Males$^{-/y}$ are born healthy and fertile males$^{-/y}$ derive from females$^{-/+}$ × males$^{-/y}$	Muda et al., 1997; Dickinson et al., 2002b; Christie et al., 2005; Liu et al., 2007
DUSP10, MKP-5	Nuclear and cytosolic	p38 > JNK ≫ ERK p38α,β ≫ p38γ, δ	Inducible Some expression in human liver and skeletal muscle; mouse heart, lung, liver, skeletal muscle, and kidney.	Decreased up-regulation of *mkp-5* mRNA in response to 1,25-D in cell lines from human prostate cancer and squamous cell carcinoma; low 1,25-D is associated with increased risk of prostate cancer in older men Overexpression of *mkp-5* reduces apoptosis in nonstressed *p53*-null cells expressing WT p53, constitutively active JNKK, and *mkp-5* compared with cells only expressing WT p53 and constitutively active JNKK	Develop normally: Protects against excessive T- cell cytokine production in response to viral infection.	Tanoue et al., 1999, 2001; Theodosiou et al., 1999; Buschmann et al., 2001; Zhang et al., 2004; Nonn et al., 2006
DUSP16, MKP-7, Mkpm	Cytosolic	JNK ~ p38 ≫ ERK p38α,β ≫ p38γ,δ MKP-7 binds to JIP1 to dephosphorylate JNK2 MKP-7 can also bind to JIP2 MKP-7 binds to β- arrestin 2 to dephosphorylate JNK3 MKP-7 binds to JNK via C-terminal (371–665) and Leu-166, Leu-168 in catalytic domain of MKP-7 MKP-7 binds to p38 via: Arg-56, Arg-57 in rhodanese domain and Leu-166, Leu-168 of MKP-7 MKP-7 binds to ERK via C-terminal (371–665) and Arg-56, Arg-57 of MKP-7 Dephosphorylation: Leu-166, Leu-168 of MKP-7 are important in MAPK inactivation Residues 569–604 of MKP-7 are involved in its degradation ERK phosphorylates MKP-7 (Ser-446), increasing its stability	Inducible Some expression in mouse heart, testis, and kidney and to lesser extent in brain and liver	Hemizygosity has no direct linkage to leukemia Overexpression reduces BCR-ABL-induced transformation in rat-1 fibroblasts in unstimulated cells MKP-7 binds to β-arrestin 2 in the cytoplasm Upon stimulation, β-arrestin 2 translocates to the receptor (angiotensin type 1a receptor) where MKP-7 dissociates from it; β-arrestin 2 can bind to ASK1 and indirectly to MKK4 to stimulate JNK3 activation; active JNK3 can cause MKP-7 to dissociate from β-arrestin 2; 30–60 min after stimulation, the MKP-7 reassociates with β-arrestin 2 to inactivate JNK3		McDonald et al., 2000; Matsuguchi et al., 2001; Tanoue et al., 2001; Hoornaert et al., 2003; Masuda et al., 2003; Willoughby et al., 2003; Katagiri et al., 2005; Willoughby and Collins, 2005
STYXL1, DUSP24, MK-STYX		Presumed catalytically inactive Cys→Ser substitution at catalytic site		Expression is increased in Ewing's sarcoma family of tumors		Wishart and Dixon, 1998; Siligan et al., 2005

mTOR, mammalian target of rapamycin; FGF, fibroblast growth factor; 1,25-D, 1,25-dihydroxyvitamin-D_3; JNKK, Jun N-terminal kinase kinase.

this phosphorylation causes a slowing of cell movement in a variety of cell types (Bogoyevitch and Kobe, 2006). JNK associates with focal adhesions, in a JIP-dependent manner, and MEKK1 localized with FAK and α-actinin (Huang et al., 2004; Bogoyevitch and Kobe, 2006). JNK has also been found to localize to membrane ruffles and along microtubules. JNK phosphorylates Tau, preventing it from promoting microtubule assembly or binding to microtubules if hyperphosphorylated (Goedert et al., 1997; Bogoyevitch and Kobe, 2006).

Active JNK mediates its function by regulating the activity and stability of some effectors via phosphorylation. However, active JNK can also mediate degradation of its effectors, such as c-Jun (Gao et al., 2004) and inhibitory molecules such as IκBα (Ki et al., 2007) by phosphorylating and targeting them for polyubiquitination and proteasomal degradation and also by increasing E3 ligase activity (Gao et al., 2004; Chang et al., 2006). Conversely, nonphosphorylated JNK can also bind to these same effectors, such as c-Jun, p53, activating transcription factor-2, and JUNB, among others, in nonstimulated cells, and target them for degradation via polyubiquitination and the proteasomal pathway, possibly by acting as an adaptor protein. In addition, decreasing the levels of JNK can increase the protein levels of JNK effectors (Fuchs et al., 1996, 1997, 1998a; Bode and Dong, 2007). Although the exact mechanisms governing these observations remain to be determined, overall it seems that JNK controls the levels of its effector proteins in both stimulated and nonstimulated conditions. Furthermore, as dephosphorylated JNK, via MKP-1 or other phosphatase (Tables 1 and 2), can target JNK effectors for degradation, inactivation of JNK would require monitoring for possible consequences, as this protein is not simply "turned off."

An array of JNK knockout mouse work has been done for JNK1, JNK2, and JNK3. JNK1 KO phenotypes vary from the normal phenotype to one with increased Th2 cytokine production and improved cardiac function under acute pressure overload. Similar variations can be seen for JNK2 KO mice, with phenotypes ranging from normal, prone to obesity, increased Th2 cytokine production, and so on. There are fewer results for JNK3 (fewer KO mice generated), which reveal either a normal phenotype or resistance to kainic acid treatment (Gerits et al., 2007). Furthermore, whereas JNK1/JNK3 and JNK2/JNK3 double knockouts develop normally, JNK1/JNK2 double knockouts die because of a lack of developmentally controlled apoptosis (Kuan et al., 1999; Gerits et al., 2007). Taken together, the JNK1/JNK2 double KO suggests that JNK1 and JNK2 can compensate for each other during development. In addition, the different phenotypes associated with JNK1 and JNK2 single KO mice may be associated with the multiplicity of the JNK1 and JNK2 splice forms or genetic background, as the severity of the phenotype associated with knockouts can be mouse strain-specific (Linder, 2006; Yoshiki and Moriwaki, 2006). Although the background of the mice was not always mentioned in the work (Kuan et al., 1999; Gerits et al., 2007), knocking in one splice form at a time, across genetic backgrounds, would uncover the contribution of the murine strain to the differences in phenotypes observed, whereas knocking them in, within the same genetic background, would reveal the functional differences between the various splice forms.

D. p38 Mitogen-Activated Protein Kinases

This signal transduction pathway is also stimulated by stress, cytokines, and growth factors (Roberts and Der, 2007). These stimuli mediate their effect by activating a signaling module that is composed of MAPKKK-MKK3/4/6-p38MAPK (Han and Sun, 2007). The MAPKKKs are composed of MLK2 and MLK3, dual leucine zipper-bearing kinase, ASK1, map three kinase-1, and TAK-1. These kinases, in turn, activate MKK3/4/6, which activate p38MAPK (Brancho et al., 2003; Han and Sun, 2007; Raman et al., 2007; Roberts and Der, 2007). Of note, the MKK-independent means of p38αMAPK activation/repression rely on TAK-1 binding protein (TAB1, TAB2, and TAB3 or T-cell receptor). These activators are more cell type and stimulus-restricted. How they differ in terms of the consequences of downstream signaling, in relation to the usual MKK-dependent activation or phosphatase-mediated repression is still being determined (Cuenda and Rousseau, 2007). The JNK scaffolding proteins JIPs, among other scaffolding proteins, can also bind to p38MAPKs and affect their subcellular localization (Raman et al., 2007).

The p38MAPK family, which is also part of the stress-activated MAPKs (Han and Sun, 2007), has four isoforms (α, β, γ, and δ) (Han and Sun, 2007; Raman et al., 2007). Expression patterns vary according to the isoform. p38αMAPK, which is the most extensively characterized of its family members, has a wide expression pattern and so does p38βMAPK (Cuenda and Rousseau, 2007; Mayor et al., 2007). p38γMAPK is expressed in skeletal muscle, whereas p38δMAPK is detected in small intestine, pancreas, testis, and kidney. Based on sequence identity and substrate specificity, these isoforms can be subdivided as α, β and γ, δ. A potential means of distinguishing these isoforms relies on the use of ATP competitive inhibitors as the ATP-binding pocket for the α, β isoforms is different in amino acid composition from the corresponding ATP-binding pocket in γ, δ. Furthermore, the level of expression of these isoforms as well as their upstream activators, MKK3 and MKK6, vary across stimuli and cell types. The latter kinases target a threonine-glycine-tyrosine (T-G-Y) motif on their p38MAPK substrates (Cuenda and Rousseau, 2007).

Some of the functions of the p38αMAPK isoform involve increasing mRNA stability (Dean et al., 2004) via MAPKAP-K2, which phosphorylates the RNA-binding

TABLE 2

Summary of the MAP kinase atypical dual-specificity phosphatases and their involvement in cancer

This table summarizes the findings concerning MKPs and atypical dual-specificity phosphatases that target MAP kinases as substrates. For the purposes of clarity, the first name of each phosphatase corresponds to the official

TABLE 2 *Continued.*

Atypical Phosphatase	Primary Subcellular Localization	MAPK Substrate Specificity	Normal Tissue Expression and Distribution of the mRNA or Protein	Expression in Tumors Relative to Normal Tissue, Consequences of Atypical Expression to Tumor Cell Lines and in Vivo Tumors	Knockout Mice Phenotype	References
DUSP19, *LDP-2*, *SKRP1*, *TS-DSP1*, *DUSP17*, *LMW-DSP3*	Cytosolic	JNK ≫ ERK ~ p38 SKRP1 also interacts directly with MKK7 and ASK1	Mouse: ubiquitous Human adult: heart, lung, liver, pancreas			Nakamura et al., 2002; Zama et al., 2002a,b; Cheng et al., 2003
DUSP21, *LMWDSP21*	Cytosolic and nuclear		Testis			Hood et al., 2002
DUSP22, *LMW-DSP2*, *TS-DSP-2*, *VHX*, *JSP1*, *JKAP*, *MKPX*	Cytosolic and nuclear (myristolated)	p38 ≫ JNK > ERK VHX inactivates ERK-2 JKAP associates with MKK7 *JKAP*-null cells have reduced JNK activation in response to cytokines JSP1 activates MKK4 LMW-DSP2 dephosphorylates STAT3 DUSP22 dephosphorylates ERα at Ser-118	Inducible Lymphoid cells adult murine tissue: heart, brain, liver, kidney and testis	LMW-DSP2 interacts directly and dephosphorylates STAT3 in murine testicular GC-1 cells; LMW-DSP2 also prevents STAT3 translocation to the nucleus DUSP22 mRNA increases in response to estrogen in ERα-positive human breast cancer cell lines MCF-7 and T47D Luciferase reporter assay in MCF-7 or HeLa cells: 1) DUSP22 siRNA enhanced estrogen/ERα-induced luciferase activation (HeLa cells were ERα-transfected, MCF-7 endogenous ERα); 2) DUSP22 siRNA reduced dihydrotestosterone/androgen receptor-induced luciferase activation (in androgen receptor HeLa cells); 3) DUSP22 siRNA had no effect on dexamethasone-induced luciferase activation in HeLa cells Pulldown assays indicate that DUSP22 interacts directly with ERα	JKAP-null mice are borne with predicted mendelian ratios and are healthy throughout adult life	Aoyama et al., 2001; Shen et al., 2001; Alonso et al., 2002; Chen et al., 2002; Sekine et al., 2006, 2007
DUSP23, *MOSP*, *VHZ*, *LDP-3*, *DUSP25*, *FLJ20442*	Cytosolic: apical submembrane area and nuclear: nucleolus	ERK ≫ p38 ~ JNK Enhances activation of p38 and JNK during osmotic stress via MKK4 and MKK6 activation	Human fetal tissue: ubiquitous except spleen; human adult: colon, testis, pancreas, liver heart, lung Mouse adult: ubiquitously expressed LDP-3			Alonso et al., 2004a; Takagaki et al., 2004, 2007; Wu et al., 2004a; Agarwal et al., 2008
DUSP26, *MKP8*, *NATA1*, *SKRP3*, *LDP-4*	Nuclear and Golgi apparatus	p38 ≫ JNK ~ ERK	Mouse: skeletal muscle and brain, cerebellum Mouse LDP-4 expressed in brain except hippocampus	Increased copy number of gene in anaplastic thyroid tumors and cell lines; DUSP26 inhibits caspase-3 activity in anaplastic thyroid cell lines Expression is also increased in retinoblastoma, neuroblastoma, and epithelioblastoma		Vasudevan et al., 2005; Hu and Mivechi, 2006; Takagaki et al., 2007; Yu et al., 2007
DUPD1, *DUSP27*, *FMDSP*	Cytosolic		Skeletal muscle, liver and adipose tissue			Friedberg et al., 2007
DUSP28, *VHP*						Strausberg et al., 2002
PTPMT1, *MOSP*, *PLIP*, *PNAS-129*	Mitochondrial: anchored to the matrix face of the inner mitochondrial membrane	Currently unknown Knockdown of *PTPMT1* changes P-Ser/Thr and P-Tyr profiles in mitochondrial proteins	Rat: testes, liver, kidney, and endocrine cells of the pancreas Rat islets and insulinoma cell line INS-1 832/13 express PTPMT1 but not the exocrine-derived cell line PANC-1	Knockdown of *PTPMT1* expression in the pancreatic insulinoma cell line INS-1 832/13 increases 1) ATP production by ~80%, 2) ATP/ADP ratio as ADP levels remain unchanged, and 3) glucose-stimulated insulin secretion		Pagliarini et al., 2005
STYX, *hStyxb*		Inactive phosphatase owing to Cys→Gly substitution in catalytic site	Ubiquitous		Null males are infertile because of abnormal sperm production. Female offspring of null animals are fertile	Wishart et al., 1995; Wishart and Dixon, 2002

STAT, signal transduction and activator of transcription; pNPP, *p*-nitrophenyl phosphate; P, phospho; MBP, myelin basic protein; ERα, estrogen receptor-α; VHP, VH1-like phosphatase1.

effectors, such as tristetraprolin (TTP) (Dean et al., 2004), heterogeneous nuclear ribonucleoprotein A0 (Rousseau et al., 2002), and poly(A)-binding protein (Bollig et al., 2003), thereby modulating their mRNA binding capacity. TTP and other AU-rich element-binding proteins are involved in reducing the stability of mkp-1 mRNA (Lin et al., 2008), VEGF mRNA (Claffey et al., 1998), and MMP mRNA (Akool et al., 2003; Huwiler et al., 2003), among other transcripts (Eberhardt et al., 2007). In addition, p38αMAPK and p38βMAPK phosphorylate KH-type splicing regulatory protein, an mRNA-binding protein, and inhibit its binding and destabilization of mRNAs coding for proteins involved in the differentiation of myoblasts into myotubes (Briata et al., 2005). In addition, p38δ is associated with keratinocyte maturation (Efimova et al., 2003; Cuenda and Rousseau, 2007). In the same line of thought, p38αMAPK also participates in inhibition of cellular proliferation (Mikhailov et al., 2005; Heasley and Han, 2006; Perdiguero et al., 2007).

p38αMAPK can modulate the actin cytoskeleton via MAPKAP-K2 and HSP27, a required step for migration (Rousseau et al., 2000, 2006). The unphosphorylated form of HSP27 binds to actin, capping it and blocking polymerization. Other proteins downstream of MAPKAP-K2, such as LIM kinase 1, induce actin polymerization via inhibitory phosphorylation of cofilin, an actin-depolymerizing protein and CapZ-interacting protein-CapZ also affect actin polymerization (Cuenda and Rousseau, 2007). These are only some of the proteins involved in actin polymerization, one of the actin remodeling events (Disanza et al., 2005). A complete picture of how p38MAPK modulates cell migration is still wanting.

Knockout mice for each of the p38MAPKs have been generated. Overall, mice null for the isoforms p38β, p38γ, or p38δMAPK reveal no overt phenotype and develop normally. Null mice for p38αMAPK are embryonically lethal because of either placental defects or erythroid differentiation, depending on the genetic background of mice (Gerits et al., 2007). None of the p38MAPK isoforms is able to substitute for the lack of p38αMAPK. Results from a recent study revealed that p38α embryo-specific knockout mice [placentas are *p38α*(+/+)] develop into pups; however, most die at the 4-day postnatal time point (Hui et al., 2007), whereas another study used mice with an inducible deletion of the *p38α* gene, which allows the mice to develop to adulthood (Ventura et al., 2007). The phenotypes of these mice will be discussed in light of MAPK involvement in cancer (see section V.C on MAPK and cancer).

E. Mitogen-Activated Protein Kinase Cross-Talk

Until now, for reasons of simplicity and clarity, the axes of the MAPKs (Raf-MEK1/2-ERK, MEKK-MKK4/7-JNK, and MEKK-MKK3/4/6-p38MAPK) have been discussed independently of one another regarding their families, functions, and signal transduction pathways as a MAPKKK → MAPKK → MAPK model. However, evidence reveals this is not the case in cell lines or primary cells. Relationships between the members of a given module are not always linear, and both stimulatory and inhibitory interactions exist within and across MAPK modules (Fig. 2).

The cross-talk between the modules occurs at every level. For example, Ras activates Rac independently of PI3K (Lambert et al., 2002) or via PI3K (Bar-Sagi and Hall, 2000), leading to cytoskeletal remodeling (Innocenti et al., 2003), cell migration (Holly et al., 2005; Shin et al., 2005), and directional movement (Sasaki et al., 2004; Sasaki and Firtel, 2006). However, the Ras-PI3K interaction is not always required (Lim and Counter, 2005) and can be inhibited when the MAPK pathway is constitutively active, leading to cell cycle arrest (Menges and McCance, 2008). Less is known about Rac-mediated activation of Ras (Zugaza et al., 2004). Other members of the MAPK signal transduction pathway also interact with both stimulatory and inhibitory consequences. Some of the functional consequences of p38α- and p38βMAPK-mediated inhibition of JNK signal transduction include decreased in vitro cell transformation and cell proliferation (Wada et al., 2008). In addition, the JNK effector c-Jun is also implicated in reduced liver regeneration, after partial hepatectomy, as seen in c-*jun*-null mice (Hilberg et al., 1993; Eferl et al., 1999) or conditional c-*jun*-null mice (Behrens et al., 2002). This phenotype was rescued in mice carrying a conditional deletion of c-*jun* and a complete *p53*-null mutation [c-*jun*(−/−)*p53*(−/−)] or a conditional c-*jun* and complete *p21*-null mutation [c-*jun*(−/−)*p21*(−/−)] (Stepniak et al., 2006). Interestingly, there is no overt effect of the *p53*-null mutation on the capacity of the liver to regenerate after partial hepatectomy (Stepniak et al., 2006). Adding to the previous observations, mice carrying c-*jun*(−/−)*p53*(−/−) or c-*jun*(−/−)*p21*(−/−) reveal lower levels of phosphorylated p38MAPK than conditional c-*jun*(−/−) mice do (Stepniak et al., 2006). Mice bearing both conditional null mutations for c-*jun* and *p38αMAPK* genes revealed normal liver regeneration (Stepniak et al., 2006; Wada et al., 2008). Together p53 and p21 increase phosphorylation levels of p38α, and all three inhibit cell cycle progression, whereas c-Jun, the downstream effector of JNK, inhibits p38α, p53, and p21. In the absence of c-Jun, all three are active and the cell cycle is inhibited, unless one of the three proteins is inhibited, allowing the cell cycle to progress. Although this relationship is seen in the regenerating liver (Stepniak et al., 2006), such interactions need to be established for other systems, especially when normal regulatory systems are overridden. Another study showed that p38α antagonizes the JNK-c-Jun pathway via inhibition of MKK7 (Hui et al., 2007). The regulatory loop between p38MAPK and JNK can be extended, as both p38α and the MKK4/7-JNK-c-Jun pathway inhibit ERK1/2 activation and function in cell lines (Zhang et al., 2001; Shen et al., 2003). More specifically, c-Jun

transcriptional activity is required for ERK inhibition (Shen et al., 2003), whereas JNK inhibits ERK1/2 activation in primary cells and possibly p38MAPK (Jeffrey et al., 2006) although the mechanism was not known for either study (Shen et al., 2003; Jeffrey et al., 2006).

MAPK family members can also modulate their own signal transduction pathway. This cross-talk occurs at many levels (Cuevas et al., 2007; Han and Sun, 2007; Schwacke and Voit, 2007); there is also feedback inhibition at the mRNA (Ambrosino et al., 2003) and protein levels (Dhillon et al., 2007). For example, 1) p38α reduces the stability of *mkk6* mRNA (Ambrosino et al., 2003), which is the transcript for one of the p38MAPK kinases, 2) ERK can directly phosphorylate and inhibit Raf1 and son of sevenless (SOS) (a RasGEF and activator of Ras) or indirectly phosphorylate and inhibit SOS via activation of ribosomal s6 kinase 2 (Dhillon et al., 2007), 3) ERK can also inhibit B-Raf and MEK via phosphorylation (Yoon and Seger, 2006), and 4) JNK2 reduces JNK1-mediated phosphorylation of c-Jun (Hochedlinger et al., 2002). Other regulatory mechanisms exist in the form of the plant homeodomain of MEKK1, one of the MKK4 kinases, ubiquitinating ERK1/2 and targeting them for proteasomal degradation (Lu et al., 2002). In addition, Raf1 can bind to ERK5 and enhance its phosphorylation in a MEK5-independent, Ras-mediated mechanism (English et al., 1999). Adding to this complex scenario, members of a signaling module, such as the Raf kinases, may have functions outside these modules (Hindley and Kolch, 2002), although the physiological relevance of Raf function outside of the MAPK module is still in debate.

Even as the consequences of the interactions between members of the MAPK family are increasingly being recognized (Hui et al., 2007; Ventura et al., 2007), we have a long way to go before we understand how, when, where, and what happens as a consequence of the interactions between the various isoforms and splice forms of the MAPK family, in addition to the contribution of their and other signaling modules. Knockout mice are available for the various members of the MAPK modules (Gerits et al., 2007), MEKK1–4 connection maps are available (Cuevas et al., 2007), and models of MAPK, MAPKK, and MAPKKK interactions have been designed, revealing the possible level of complexity (Schwacke and Voit, 2007). What we lack is detailed information, a connection map based on subcellular localization, MAPK splice form, cell, development, and stimulus-specific identification of the players involved in signal transduction. However, other variables also come into play. Among these are the consequences of phosphorylation, such as priming, inhibition, stabilizing, activating, and targeting of proteins for polyubiquitination and degradation. The consequences attributed to phosphorylation is a topic that is covered throughout the review. Furthermore, the function associated with nonphosphorylated JNK and possibly other MAPK members also needs to be considered. The influence of scaffolding proteins, which group different players and determine their localization and availability for interaction, also influence the outcome of signal transduction. When taken into account, all of these factors will modify the interaction maps and models. However, there is one facet that remains to be explored: the time line. Chronology is a dimension that can be used to hone in these models (Kholodenko, 2006) and determine when a specific interaction is occurring. All of these potential interactions need to be verified experimentally to distinguish factual from potential interactions.

The preceding sections have dealt with activation and inactivation of kinases by other kinases. However, cells have access to a host of phosphatases that modulate MAPK activity directly or by modulating activity of upstream kinases. Indeed, although our main focus is MKP-1, adding to all this complexity is an appreciable list of dual-specificity phosphatases, the MKPs and the atypical phosphatases that target MAPKs and their kinases with varying degrees of specificity in the cytosol or the nucleus (Tables 1 and 2; Fig. 2) (see also section IV on the dual-specificity phosphatase family). As MKP-1 is one of many phosphatases located at the MAPK nexus, understanding how all the players interact to modulate signal transduction pathways is a necessary task if we are to complete the picture vis-à-vis cell biology. To this end, we review what is known about MKP-1 regulation at the mRNA and protein levels, and we endeavor to determine how this phosphatase interacts with the MAPKs in the normal and pathological state.

III. Regulation of Mitogen-Activated Protein Kinase Phosphatase-1 Expression and Activity

A. mkp-1 *Gene Structure, Promoter, and Enhancer*

The human *mkp-1* gene contains four exons and three introns coding for an inducible mRNA that is approximately 2.4 kilobases long (Kwak et al., 1994). The promoter/enhancer region of this gene contains multiple AP-2, *trans*-acting transcription factor 1, and cAMP-responsive element sites but only one site for AP-1, neurofibromin 1, and TATA box (Kwak et al., 1994; Pursiheimo et al., 2002) (Fig. 3). Other binding motifs, such as an E box and three GC boxes are localized between positions –110 and –30 (Ryser et al., 2004). Finally, a possible binding site for p53 protein is found in the second intron (Li et al., 2003). These binding sites may explain the numerous factors that can transactivate the *mkp-1* gene (see section III.B). Interestingly, although there is an E box consensus sequence, as previously mentioned, in both the murine and human *mkp-1* promoter, the transcription factor dimer c-Myc/Max is unable to regulate *mkp-1* expression because of unfavorable flanking regions (Sommer et al., 2000). Thus, the presence of the consensus sequence of a transcription factor does not predict modulation via its sequence, and the presence of appropriate flanking regions

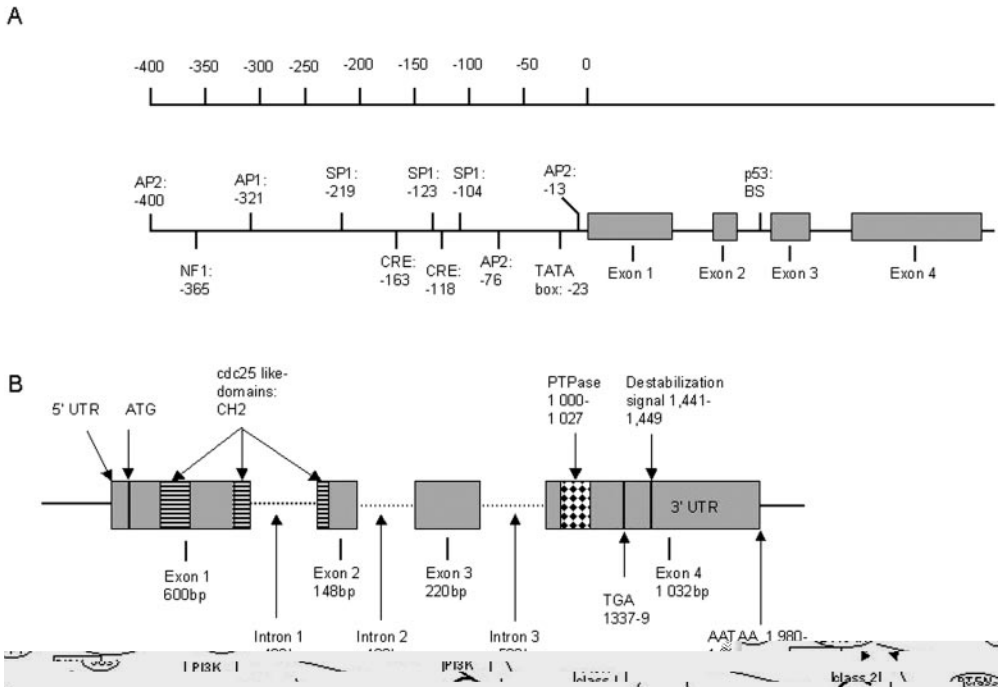

FIG. 3. MKP-1 promoter and gene. A, although the MKP-1 promoter demonstrates the potential for binding many transcription factors, caution should be used when inferring the binding of a transcription factor even when its consensus sequence is present in the promoter region, because flanking regions may influence binding (see text). B, a more detailed look at the *mkp-1* gene reveals that exons 1 and 4 bear the crux of the important domains of gene product, whereas the CH2B domain is coded by exons 1 and 2. Interestingly, at this time no known domain coded by exon 3 has been discovered. NF1, neurofibromin 1; Sp1, *trans*-acting transcription factor 1; CRE, cAMP-responsive element; UTR, untranslated region; PTPase, phosphoprotein tyrosine phosphatase; bp, base pairs.

can determine the final outcome as to gene transcription. Finally, the transcription factor E2F1 is necessary to ensure responsiveness of the *mkp-1* gene to hydrogen peroxide (Wang et al., 2007b).

Despite its high degree of conservation, there is a single nucleotide polymorphism reported in the *mkp-1* gene, although it occurs in the first intron and is not believed to influence transcription or translation efficiency. However the latter conclusion awaits formal experimental validation (Suzuki et al., 2001).

B. mkp-1 mRNA

1. Regulation of mkp-1 mRNA Expression Levels. As with many immediate-early genes coding for regulatory proteins, an abundance of factors up-regulate mkp-1 mRNA levels in different cell types, for example, serum in mouse and rat fibroblasts (Bokemeyer et al., 1996), dexamethasone, a glucocorticoid analog in human mammary epithelial cells MCF10A-Myc (Wu et al., 2004b, 2005b), glucagon in rat hepatocytes (Schliess et al., 2000), insulin in rat hepatoma cell line H4IIE-C3 (Lornejad-Schafer et al., 2003), and atrial natriuretic peptide in human umbilical-vein endothelial cells (HUVECs) (Furst et al., 2005). Other factors such as arachidonic acid increase mkp-1 mRNA expression in cultures of rat aortic vascular smooth muscle cells (Metzler et al., 1998). Stressful conditions, such as heat shock in human skin fibroblasts (Keyse and Emslie, 1992) and in murine macrophages (Wong et al., 2005), osmotic shock in rat hepatoma cell line H4IIE-C3 (Schliess et al., 1998; Lornejad-Schafer et al., 2003), hypoxia in neonatal rat tissues (Bernaudin et al., 2002), hypoxia in PC12, Hep3B (Seta et al., 2001), and HepG2 cells (Seta et al., 2001; Liu et al., 2003, 2005a,b), cobalt chloride, a hypoxia mimic, in HepG2 cells (Liu et al., 2003), and ischemia in rat forebrain (Takano et al., 1995; Wiessner et al., 1995) also modulate the *mkp-1* transcript. In addition, DNA-damaging agents such as hydrogen peroxide in human skin fibroblasts (Keyse and Emslie, 1992) and vascular smooth muscle cells (Metzler et al., 1998) and other DNA-damaging agents (Keyse and Emslie, 1992) produce similar effects. In contrast, no *mkp-1* transcript was detected in four different unstimulated hepatoma cell lines (Kwak and Dixon, 1995). It is not known whether the reported increases in mRNA correspond to increased stability or de novo synthesis of the mRNA.

There is no shortage of factors and conditions that regulate mkp-1 mRNA. These represent a potential arsenal from which to choose, corresponding to nearly every contingency that could arise should increased levels of mkp-1 mRNA be required. However, at what point, if any, do these factors use the same signal transduction mechanisms to generate their effect? Heat shock and hypoxia/ischemia-reperfusion, for example, generate reactive oxygen species and protein misfolding affecting the endoplasmic reticulum (ER) (Gorlach et al., 2006), the mitochondria (Benedetti et al., 2006), the cytosol (Hartl and Hayer-Hartl, 2002), and the nucleus (Ham-

mond et al., 2007). In turn, these compartments communicate with each other. The ER and mitochondria interact with each other via calcium and reactive oxygen species (Gorlach et al., 2006), and, for example, oxidative stress can cause calcium influx from both the extracellular milieu and ER or sarcoplasmic reticulum (Ermak and Davies, 2002). Furthermore, the ER-unfolded protein response, calcium flux, and reactive oxygen species also affect the nuclear compartment via transcriptional changes (Alonso et al., 2006; Gorlach et al., 2006) or DNA damage (Hammond et al., 2007). Although this is a simplified overview, it reveals that using some of the previously mentioned stimuli for the purposes of up-regulating mkp-1 mRNA may not be straightforward. As the responses to different stresses are adaptations that allow the cell to react to its internal and external environment, these stresses target a host of functions within the cell. Thus, although there are many factors that can up-regulate mkp-1 mRNA, finding a treatment that specifically targets *mkp-1* would be a first step.

2. mRNA Stability. The mkp-1 mRNA has a half-life of 1 to 2 h, which varies according to the stimulant (Lau and Nathans, 1985). This variation in half-life may stem from the different mechanisms that determine the amount of mkp-1 mRNA that accumulates. In addition, a given stimulus may lead to the activation of multiple effectors, only some of which would be relevant to the query of interest. For example, heat shock up-regulates p38MAPK activity, and inhibition of this MAPK reduces the heat shock-mediated increase in mkp-1 mRNA levels (Wong et al., 2005). Conversely, stabilization of the *mkp-1* transcript via heat shock in RAW 264.7 murine macrophages may not depend on HSF-1. A luciferase assay, using a WT *mkp-1* murine promoter upstream of the luciferase gene, demonstrated luciferase activity in *HSF-1*(−/−) fibroblasts (Wong et al., 2005).

Other mechanisms exist to modulate mRNA stability. TTP binds to AU-rich elements on the 3′-untranslated region of mRNAs and destabilizes them (Dean et al., 2004). TTP binds to and destabilizes mkp-1 mRNA via an unknown mechanism (Lin et al., 2008), although TTP, which also destabilizes TNF-α

calcium-mediated up-regulation of *mkp-1* is observed, in contrast to the calcium-independent up-regulation of the same gene via EGF (Ryser et al., 2001). In the former case, a calcium-sensitive block in elongation located within the first exon, 300 base pairs downstream of the transcriptional initiation start site, of the rat *mkp-1* gene was thought to be the cause. In the latter case, EGF-mediated enhanced initiation and elongation were calcium-independent (Ryser et al., 2001). Recently, this group revisited the mechanism of inhibition and reported that under basal conditions in the GH4C1 cell line, a complex made from 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole sensitivity-inducing factor (DSIF) and negative elongation factor-inhibited RNA polymerase II at the promoter proximal region of the *mkp-1* gene and not within the first exon (Fujita et al., 2007). Upon stimulation with thyrotropin-releasing hormone, the positive transcription elongation factor b was recruited and phosphorylated both the C-terminal repeats of Spt5, one of the subunits of DSIF (Yamaguchi et al., 1998), and Ser-2 within the C-terminal domain of polymerase II. These modifications allowed negative elongation factor to detach from polymerase II, which continued elongation with positive transcription elongation factor and DSIF complexed to it (Fujita et al., 2007).

This mechanism raises some questions. Is this calcium-sensitive block in elongation strictly part of "normal" calcium-mediated signaling or can it be the result of stress-related mechanisms? Many proteins are sensitive to intracellular calcium proteins, acting as sensors and calcium-binding proteins (Agell et al., 2002; Haeseleer et al., 2002; Cook and Lockyer, 2006). Determining to what extent this mechanism applies to other mRNAs and cell types would be the next step. As the main cellular calcium storehouse is the endoplasmic reticulum (Gorlach et al., 2006; H, yer-Hansen and Jaattela, 2007), could the calcium-sensitive block in elongation also be part of an ER stress response (Gorlach et al., 2006)? If not, could the calcium be derived from perinuclear or nuclear stores (Alonso et al., 2006)? The provenance of calcium would be a clue as to the mechanisms that govern this block.

As we have seen in this section, the many factors and mechanisms regulating the level of mkp-1 mRNA underscore the importance of the function of MKP-1. These multiple levels of control of the mkp-1 mRNA speak of an expression that is precisely and exquisitely controlled by stimulants in space and time. Furthermore, each one of the mechanisms represents a level of control with a potential for therapeutic intervention. However, this is only the control for mkp-1 mRNA expression levels. The next section will reveal that the MKP-1 protein also boasts its modulators.

C. Mitogen-Activated Protein Kinase Phosphatase-1 Protein Function and Affinity

This section presents an overview of MKP-1 affinity and function and the mechanisms that allow cells to modulate the function of this protein. The consequences of this modulation will be addressed in light of chemotherapeutic agents and cellular and animal models in sections V and VI.

MKP-1 is a nuclear phosphatase. This phosphatase dephosphorylates proteins of the MAPK family, in the following order of affinity: p38MAPK $\geq$ JNK $\gg$ ERK1/2 (Franklin and Kraft, 1997; Camps et al., 2000; Farooq and Zhou, 2004) and within these ERK2 $>$ ERK1 (Slack et al., 2001). MKP-1 also dephosphorylates ERK5 although it can bind to ERK5 in both stimulated and unstimulated cells (Sarkozi et al., 2007). However, MAPK binds to different sites within MKP-1; indeed, whereas MKP-1 binding of ERK2, ERK1, and p38αMAPK depends on the same arginine residues (arginine 53–55) (Fig. 4), that of JNK1 depends on as yet unidentified residues, although within the first N-terminal 188 residues of MKP-1 (Slack et al., 2001). Although ERK5 binding to MKP-1 may be similar to that of ERK1/2, this theory awaits experimental verification. When the arginine 53 to 55 residues are mutated, MKP-1 retains its phosphatase activity toward JNK1 but not toward ERK2 or p38α (Slack et al., 2001). Identification of the MKP-1 residues that bind JNK1 will allow more precise control for eventual therapeutic purposes. The ability either to modify the binding domains of MKP-1 (ERK/p38MAPK versus JNK1) independently of one another or to hinder the binding of the MKP-1 domains to effector molecules, via small competitive inhibitors, represents a promising avenue of endeavor. Determining the three-dimensional structure of MKP-1 would be necessary to distinguish between ERK and p38MAPK, which bind to the same site. More subtle target mutations within MKP-1 itself may be needed for the purposes of limiting MKP-1 interaction with either ERK or p38MAPK, while allowing binding to the other. With the advent of high-throughput assays for dual-specificity phosphatases (Tierno et al., 2007), inhibitors that distinguish between MKP-1 binding to ERK1/2, JNK1, and p38αMAPK may be identified soon.

Although the order of MAPK dephosphorylation is commonly accepted (Camps et al., 2000; Farooq and Zhou, 2004), with an occasional exception as seen in rat pinealocytes (Price et al., 2007), that of ERK1/2 has been debated. Indeed, MKP-1 does not seem to dephosphorylate ERK1/2 but competes with ERK effectors and prevents their binding and phosphorylation-mediated activation (Wu et al., 2005a). Based on this model, it is suggested that the binding of MKP-1 to ERK1/2 prevents the ERK1/2-ELK-1 interaction and therefore phosphorylation and activation of ELK-1 in the nucleus. In turn, this would prevent the activation of the serum response element by ELK-1 (Wu et al., 2005a). Elucidating the manner in which MKP-1 binds to the various MAPKs would be a first step in determining why a difference exists in the affinity between MKP-1 and the different MAPKs. Another possibility lies in the obser-

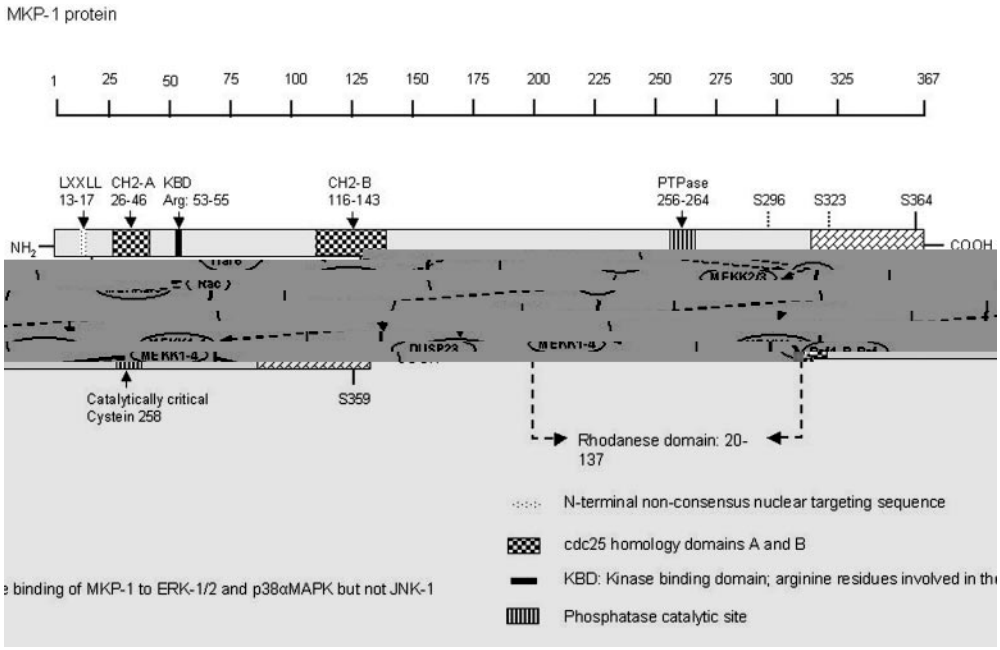

FIG. 4. MKP-1 protein. Although the N-terminal aspect of the protein is responsible for the nuclear localization and the binding of the MAPK (ERK1/2 and p38αMAPK, the residues responsible for binding JNK-1 await identification), the C-terminal part contains the catalytic and stabilization/destabilization domains of the protein. PTPase, phosphoprotein tyrosine phosphatase.

vation that phosphorylated ERK2 can homodimerize (Khokhlatchev et al., 1998) and thereafter actively translocate to the nucleus, whereas monomeric phospho-ERK can enter the nucleus by passive diffusion (Adachi et al., 1999). ERK dimers may simply be unable to interact with MKP-1.

The dephosphorylating activity of MKP-1 is restricted to the nucleus (Camps et al., 2000; Wu et al., 2005a). The targeting of MKP-1 to the nucleus is ascribed to a LXXLL motif located in the $NH_{2\ terminus}$ proximal to the cdc25 homology domains A (Wu et al., 2005a) (Fig. 4). Although this sequence is not considered to be a consensus nuclear targeting sequence (Christophe et al., 2000; Cartier and Reszka, 2002), it acts as a nuclear targeting sequence for MKP-1 (Wu et al., 2005a). MKP-1 also contains a rhodanese domain located within residues 20 to 137 (UniProtKB/Swiss-Prot), known to catalyze a sulfur transfer reaction. Interestingly, MKP-1 lacks a critical cysteine residue within the rhodanese domain that would allow it to be enzymatically active. The function of this catalytically inactive rhodanese domain is currently unknown (Bordo and Bork, 2002). Understanding how MKP-1 is targeted to the nucleus, on its own or via binding to another molecule, can add another level of control over this phosphatase. The possibility of sequestering MKP-1 in the cytosol, as a means of limiting its phosphatase activity in the nucleus, carries the underlying assumption that MKP-1 could not dephosphorylate its effector MAPKs while sequestered in the cytosol; otherwise one set of variables would be exchanged for another.

To summarize, MKP-1 is currently known as a nuclear phosphatase. Despite this, there is a report of a non-nuclear localization for this protein. Treating a human lymphoblastic cell line with nerve growth factor increases mkp-1 mRNA synthesis and protein stability (up to 6 h) with a corresponding increase in translocation of the MKP-1 protein to the mitochondrial compartment (Rosini et al., 2004). The significance of this finding awaits further validation.

1. Regulation of Mitogen-Activated Protein Kinase Phosphatase-1 Protein Expression Levels. In addition to the transcriptional regulation, MKP-1 protein expression is increased in various cell types by different factors as exemplified by the following: insulin-treated rat smooth muscle cells (Takehara et al., 2000) and rat hepatoma cells (Lornejad-Schafer et al., 2003), glucagon-treated rat hepatocytes (Schliess et al., 2000), dexamethasone-treated human breast epithelial cell lines MCF-7 and MDA-MB-231 (Wu et al., 2004b, 2005b), epidermal growth factor-treated mouse embryonic fibroblasts (Wu and Bennett, 2005), atrial natriuretic peptide-treated HUVECs and rat lung cells (Furst et al., 2005), overexpression of cytochrome P450 2C9 on treatment with 11,12-epoxyeicosatrienoic acid in HUVECs (Potente et al., 2002), arachidonic acid-treated rat aortic vascular smooth muscle cell cultures (Metzler et al., 1998), hypoxia-treated neurons of newborn piglets (Mishra and Delivoria-Papadopoulos, 2004), hypoxia-treated PC12 (Seta et al., 2001) and HepG2 cells (Liu et al., 2005a, 2005b), serum-treated mouse fibroblasts (Sun et al., 1993), and peroxide-treated vascular smooth muscle cells (Metzler et al., 1998) and HUVECs (Furst et al., 2005). As noted for mRNA levels, 1) it is unknown whether the increase in protein levels is due to de novo translation or increased stabilization, and 2) none of

these factors or treatments are specific for MKP-1 protein regulation as they target many effectors, whereas others are adaptations to changes in the environment. Although at first glance there seem to be fewer agents and conditions that up-regulate MKP-1 protein compared with the litany of agents and conditions that control its mRNA, this situation is due to the lack of investigation of MKP-1 protein regulation when its mRNA was studied.

2. Protein Stability and De Novo Protein Synthesis. The MKP-1 protein half-life varies between 40 min (Charles et al., 1992) and 2 h (Noguchi et al., 1993). This difference in half-life can be accounted for by the various mechanisms available to the cell for the modulation of MKP-1 protein stability, as will be seen presently. A single heat shock treatment of IMR-90 human lung fibroblasts causes aggregation of MKP-1 protein in an HSP72-dependent manner, preventing MKP-1 from denaturation and preserving its function; this aggregation is partially reversed in the recovery phase from the heat shock treatment (Yaglom et al., 2003). Moreover, ERK1/2 reduce MKP-1 protein degradation by phosphorylating the ^{359}Ser and ^{364}Ser residues (Brondello et al., 1999), whereas glucocorticoids also decrease MKP-1 degradation, albeit by an unknown mechanism (Kassel et al., 2001). On the other hand, ERK1/2 are also responsible for degradation of MKP-1 (Lin et al., 2003) via phosphorylation of ^{296}Ser and ^{323}Ser residues (Lin and Yang, 2006) (Fig. 4). Once phosphorylated, Skp2 (also called SCF^{Skp2} of Skp1/Cul1/F-box protein Skp2), targets MKP-1 for degradation via the ubiquitin proteasomal pathway (Lin et al., 2003). This degradation also involves PKCδ through an unknown mechanism (Choi et al., 2006). Although ERK-2 is a better substrate for MKP-1 than is ERK1, as determined by the yeast two-hybrid system (Slack et al., 2001), ERK1 can phosphorylate MKP-1 in vitro (Brondello et al., 1999). Furthermore, as ERK1 and ERK2 share 83% identity in protein sequence (Boulton et al., 1990), it remains possible that one is responsible for phosphorylation-mediated stabilization of MKP-1, whereas the other targets this phosphatase for proteasomal degradation via phosphorylation on other residues. Otherwise, ERK1 and ERK2 may serve redundant functions. Finally, the p38MAPK may also be a stabilizing agent or have a role in translational events, because SB203580, an inhibitor that preferentially targets p38α/p38βMAPK, partially blocks ERK-dependent *cis*-diaminedichloroplatinum II (cisplatin)-mediated accumulation of MKP-1 protein without affecting mRNA levels (Wang et al., 2006). Finally, in contrast to what happens in IMR-90 cells, which constitutively express MKP-1, COS-7 cells induced de novo MKP-1 synthesis during the recovery phase after heat shock (Yaglom et al., 2003), whereas hyperosmolarity delayed insulin-induced MKP-1 protein expression (Lornejad-Schafer et al., 2003).

3. Protein Activity. Dipyridamole, an inhibitor of phosphodiesterases and nucleoside transport, stimulates tyrosine phosphorylation of MKP-1 in the murine macrophage cell-line RAW 264.7 by as yet an unknown mechanism. Dipyridamole also blocks LPS-mediated up-regulation of cyclooxygenase-2 protein levels via inhibition of p38MAPK phosphorylation (Chen et al., 2006). In addition, a double heat shock treatment increases MKP-1 phosphorylation and activity via interaction with HSP70 in human bronchial epithelial cells (Lee et al., 2005). The chaperone functions of the HSP family, which include the control of activities of other proteins and their refolding, among other functions, are well documented (Bukau et al., 2006). In consideration of the fact that MKP-1 is being stabilized in its phosphorylated state, this stabilization may be the consequence of general chaperone functions, e.g., preventing MKP-1 from unfolding or part of a mechanism designed to limit the deleterious effects of fever. How MKP-1, fever, and cancer are related to each other is a topic that will be discussed in more detail in section V.

Another mechanism that limits MKP-1 protein activity lies within the C-terminal region of MKP-1 (Fig. 4), which autoinhibits the phosphatase activity. In the same line of thought, the C-terminal truncated form of MKP-1 has higher phosphatase activity without modifying its substrate specificity (Hutter et al., 2002). Thus far, there is overall consensus on the increased phosphatase activity of MKP-1 upon binding to one of a number of active MAPKs such as ERK1/2, JNK1, or p38MAPK (Slack et al., 2001), and, in turn, MKP-1 dephosphorylates the MAPK it was bound to (Hutter et al., 2000; Farooq and Zhou, 2004). However, if we consider that the C-terminal portion of MKP-1 can inhibit its phosphatase activity, determining whether the C-terminal fragment on its own can bind to other proteins may reveal another level of control for this phosphatase. Although previous binding assays failed to reveal interactions of MKP-1 with other proteins, other than the usual MAPKs, these assays relied on the phosphatase activity of MKP-1 for a readout.

Unfortunately, the three-dimensional structure for the MKP-1 protein is still unknown and thus "tailor-made" inhibitors are not available. However, compounds

The preceding sections have revealed a level of control over the MKP-1 protein that mirrors the many facets of control over its mRNA. Clearly the amount of MKP-1 protein is under strict censure in space and time. As is the case with the mRNA, each level of control over the MKP-1 protein is a potential therapeutic target. Because MKP-1 inactivates JNK1, p38MAPK, and ERK1/2, it is not surprising that stimulants capable of increasing MAPK activity also augment MKP-1 protein levels. The elegance of the up-regulation of MKP-1 protein levels by MAPK assures that under normal circumstances the MAPKs limit the duration of their activity in space and time. Thus, a circuit comprising a stimulant/MAPK pathway/MKP-1 protein is established after a discrete period of time, assuring the proper response for each cell type.

IV. Overview of the Dual-Specificity Phosphatase Family

The human genome codes for many types of phosphatases, among which are more than 100 protein tyrosine (Alonso et al., 2004c), lipid (Suzuki et al., 2008), carbohydrate (Worby et al., 2006; Vilchez et al., 2007), and RNA (Deshpande et al., 1999) phosphatases. The protein tyrosine phosphatases can be categorized according to sequence similarity, substrate specificity, subcellular localization, and other factors. Depending on how the tyrosine phosphatases are categorized into subgroups, the exact number and identity of the members vary within each subgroup (Alonso et al., 2004c; Farooq and Zhou, 2004). Reviewing all of the members of the protein tyrosine phosphatase family is beyond the scope of this review. However, here we summarize the MKP family and the atypical dual-specificity phosphatases, which have MAPKs as substrates, in terms of function, tissue distribution, and role in cancer (Tables 1 and 2). The atypical dual-specificity phosphatases differ from the MKPs in that the members of the latter subgroup have N-terminal domains that determine substrate specificity, whereas the former lack this domain (Alonso et al., 2004c). In general, phosphatases also differ by whether they are in an active conformation before ligand binding (e.g., VHR/DUSP3, HVH3/DUSP5, and MKP-5/DUSP10) or whether ligand binding mediates a change in conformation of the phosphatase, which enables the formation of a functional catalytic site (e.g., MKP-1/DUSP1, MKP-3/DUSP6, MKP-4/DUSP9, and PAC-1/DUSP2) (Camps et al., 1998; Alonso et al., 2004c; Farooq and Zhou, 2004; Owens and Keyse, 2007).

Kinetic studies revealed that the MKP-mediated dephosphorylation of MAPKs, which are dually phosphorylated at the T-X-Y motif (where X is Glu for ERK1/2/5, Gly for p38MAPKs, and Pro for JNKs), proceeds through a two-step dephosphorylation process: 1) binding of MAPK to MKP, targeting phospho-tyrosine, as this residue is first to be dephosphorylated by the catalytic site, and then release of the MKP-MAPK complex; and 2) binding of MAPK to MKP, dephosphorylation of the phospho-threonine residue, and then release of both proteins (Alonso et al., 2004c; Farooq and Zhou, 2004).

Although substrate specificity varies depending on the phosphatase, there is obvious overlap between the specificities. This overlap can be explained in terms of gene duplication and evolutionary divergence (Alonso et al., 2004c). However, these overlaps may be reduced if we consider subcellular localization, tissue distribution, or other factors. Unfortunately, most of the reports on the tissue expression concern only mRNA and not the protein product of the phosphatases. When substrate specificity was investigated, only one representative member of the most common MAPKs were used, for obvious practical considerations, leaving open the possibility that the other isoforms and splice forms of MAPKs might interact differently with each phosphatase. Other observations suggested that a given phosphatase was not a simple "off switch" for its kinase substrate. This was exemplified by the following observations, which will be developed shortly: 1) a DUSP2 (PAC-1) variant, without intrinsic phosphatase activity, enhanced MKP-1 phosphatase activity; 2) absence of DUSP2 increased JNK activity and decreased ERK and p38MAPK activities; 3) DUSP5 transported and sequestered unphosphorylated ERK2 to the nucleus; 4) the DUSP7 (Pyst2) catalytically inactive short form (Pyst2-S) can regulate the function of the long form (Pyst2-L); 5) DUSP22 activated the JNK pathway; and 6) DUSP23 enhanced p38MAPK and JNK activities (Tables 1 and 2; Fig. 2).

Although the exact mechanisms by which the previously mentioned observations occurred remain to be determined, there were some concepts relating to each that had been unveiled. The first of these was that absence of DUSP2 increased JNK activity leading to decreased ERK and p38MAPK activities. Whereas the increased JNK activity in the absence of phosphatase activity was a straightforward concept, decreased kinase activities of ERK and p38MAPK were thought to be a JNK-mediated effect on the latter MAPKs (Jeffrey et al., 2006). Indeed, JNK and its signaling module are believed to exert both positive and negative control over the other MAPKs (Fig. 2) (see also section II.E. on MAPK cross-talk). Therefore, when a phosphatase is overexpressed in an attempt to inactivate its preferred MAPK substrate, it would be necessary to determine 1) the activity of other members of the MAPK family, given the cross-talk between the MAPK family and their signaling modules and 2) the possibility that the phosphatase can activate upstream kinases. Of note, JKAP (DUSP22) activated the JNK pathway via activation of MKK7 (but not MKK4) (Chen et al., 2002) and JSP1 (DUSP22) activated MKK4 (not MKK7) (Shen et al., 2001). Conversely, LMW-DSP2 (DUSP22) had been implicated in inactivation of JNK and p38MAPK through an indirect mecha-

nism (Aoyama et al., 2001) (Fig. 2). As the activation status of the upstream kinases was not investigated in the latter study, the difference between these results (Aoyama et al., 2001) and the results of the two former studies (Shen et al., 2001; Chen et al., 2002) remained unclear. Human JKAP differs in its C-terminal sequence, which is slightly longer than that of JSP1 or LMW-DSP2. These three phosphatases are most likely splice forms of the *dusp22* gene (Chen et al., 2002), which could account for their different affinities for MKK7 and MKK4 in different cell types. Similar to DUSP22 protein, DUSP23 protein could activate MKK4 (upstream kinase of p38MAPK and JNK) and MKK6 (upstream kinase of p38MAPK) (Takagaki et al., 2004). In summary, DUSP22 (JKAP, JSP1) and DUSP23 dephosphorylated and activated MKK4, MKK6, and MKK7 (Shen et al., 2001; Chen et al., 2002; Takagaki et al., 2004). Conversely, AKT phosphorylated and inactivated MLK3, ASK, and MEKK4 (Bogoyevitch and Kobe, 2006). How cells manipulate the activity of these phosphatases and kinases in response to specific stimuli in health and disease may be determined by the level of expression of a given phosphatase, which seems to vary according to tumor type, such as MKP-1 (see sections V and VI), and other phosphatases (Tables 1 and 2).

Adding to these observations were the findings that inactive phosphatases were not merely dominant-negative proteins. Whereas Pyst2-S regulates the function and activity of Pyst2-L by competing for effectors and transcription and/or translation factors, among other possible mechanisms (Levy-Nissenbaum et al., 2003a,b, 2004), a PAC-1 inactive variant enhanced the function of MKP-1 (Kothapalli et al., 2003) (Table 1). Moreover, there were at least two phosphatases within the group we are considering in this review, MK-STYX (STYXL1) and STYX, with inactive catalytic sites due to a naturally occurring substitution, Cys→Ser and Cys→Gly, respectively (Wishart and Dixon, 1998). MK-STYX is classified as a member of the MKP subfamily, and STYX is considered an atypical dual-specificity phosphatase, as it had no N-terminal substrate-binding motif. Although the substrates of these phosphatase-dead proteins have as yet to be determined, the consequences of lacking an inactive phosphatase were revealed from results with STYX male null mice (Table 2). The male mice were infertile, whereas their female null counterparts remained fertile, although both seemed to have a "normal" overall phenotype (Wishart and Dixon, 2002). This subtle difference in fertility for a catalytically inactive phosphatase that had a ubiquitously expressed mRNA warrants further investigation. Until the proteomic expression profile of STYX and its function and substrate specificity are known, it will be difficult to determine the reason for this gender-specific infertility. There may be other gender-specific differences in the STYX-null mice. The function of MK-STYX remains unclear although it has been implicated in cancer (Table 1). One role these phosphatase-dead proteins could have is to sequester their MAPK substrates and "preserve" them in active form, protected from dephosphorylation. Alternatively, catalytically inactive "phosphatases" could maintain the unphosphorylated MAPK sequestered in their subcellular localization ready for activation by their upstream kinases. Such an effect was seen with the inactive form of DUSP5, due to an experimentally induced point mutation. Whereas both the active and inactive forms of DUSP5 transported unphosphorylated ERK2 to the nucleus, the inactive form of DUSP5 allowed activation of ERK2 by MEK1/2 in the nucleus in response to mitogen (Mandl et al., 2005).

Another twist in this story lies in the rare event in mammalian cells of two different proteins of the same family coded by the same gene as a result of different open reading frames, as is the case for DUSP13A (MDSP) and DUSP13B (TMDP) (Chen et al., 2004) (Table 2). These two proteins share 42% sequence identity. Interestingly, this locus has an ortholog in mouse and a similar arrangement of phosphatases in puffer fish (*Fugu rubripes*), indicating evolutionary conservation and perhaps importance (Chen et al., 2004). MDSP and TMDP display phosphatase activity toward phosphotyrosine and phosphothreonine when tested against artificial substrates. However, the naturally occurring substrates and the consequence of their function await identification (Nakamura et al., 1999; Chen et al., 2004). Other phosphatases such as M3/6 (DUSP8) and MKP-7 (DUSP16), can bind to the scaffolding protein JIP1, and MKP-7 can decrease MLK3-JNK-JIP1-mediated phosphorylation of c-Jun (Willoughby et al., 2003). As Tables 1 and 2 reveal, there are many phosphatases that await characterization for function, distribution, and involvement in cancer or other diseases. When more phosphatases are characterized, in terms of binding to scaffolding proteins, expression pattern, and involvement during normal and disease processes, a more comprehensive picture will emerge as to how kinases and phosphatases interact to generate cell phenotypes.

In sections V and VI, we will focus our attention on MKP-1 and its role in cancer, both its pro- and anticancer activities (Ducruet et al., 2005), vis-à-vis the MAPK family, cell and animal models, and MKP-1 expression in cancer patients. Finally, chemotherapeutic agents used for the treatment of various tumors in animal models or patients are discussed when they pertain to the expression of MKP-1 or to its function. A recurring theme is that MKP-1 can sometimes be directly linked to tumor phenotypes, whereas at other times it is more of a bystander. Perhaps this is not only a consequence of the complexities associated with MAPK signal transduction but also has a basis in the multiplicity of nuclear and cytosolic phosphatases with overlapping MAPK substrate specificity.

V. Mitogen-Activated Protein Kinase Phosphatase-1, Animal Models, and Cancer

Considering the level of control of the mkp-1 mRNA and protein (see section III.B–C), it is not surprising to find many agents that modulate their expression levels. Indeed, each naturally occurring point of control over the mRNA or protein represents a potential therapeutic target. These agents could modulate MKP-1 enzymatic activity, the rate of protein/mRNA synthesis, the rate of protein/mRNA degradation, unblocking elongation of the mRNA, and the methylation/demethylation of the *mkp-1* gene, among other levels of control. As the pathology of cancer entails a lack of or inappropriate restraint at one or more of these levels, chemotherapeutic agents that target a specific facet of control have been and continue to be sought after in an attempt to exert exogenous management when endogenous mechanism fails. However, as a considerable number of genes and proteins are controlled by a given mechanism, each chemotherapeutic agent, although specific for a certain regulatory mechanism, may target many or all genes and proteins controlled by that mechanism.

Having outlined the mechanisms that control mkp-1 mRNA and protein expression and stability, we will review what is known about *mkp-1* organ-specific transgenic and *mkp-1*-null mice and how this modulation of *mkp-1* affects normal development and adult mouse phenotypes when exogenous stressors are applied.

A. mkp-1 *Transgenic Mice*

mkp-1 constitutive cardiac transgenic mice expressing high levels of MKP-1 (3.7 times normal levels) die between postnatal days 7 and 15 because of a lack of developmental cardiac hypertrophy, whereas mice expressing moderate levels of MKP-1 (1.8 times normal levels) survive to adulthood although with abnormal cardiac morphology as determined with more detailed histological analysis and visualization of function by echocardiography. The founder mice are able to survive because of the mosaic distribution of the transgene (Bueno et al., 2001).

B. mkp-1 *Knockout Mice*

1. Phenotype. *mkp-1* knockout mice have no overt phenotype from histology to behavior. The genotype is expressed in a predictable mendelian distribution. Interestingly, *mkp-1*-null mice do not demonstrate increased ERK1/2 phosphorylation (Dorfman et al., 1996). Moreover, there are no differences between *mkp-1*(−/−) and *mkp-1*(+/+) littermates in the developmental phase or in various organ systems, such as the neurological, cardiac, hematological, and endocrinological systems (Salojin et al., 2006).

2. Immunological Consequences. Although *mkp-1*(−/−) mice seem to be normal overall, the null mice have an exaggerated innate immune response to LPS and increase their serum levels for various cytokines (TNF-α, IL-6, interferon-γ, and IL-10) (Salojin et al., 2006; Zhao et al., 2006) as well as IL-12p70 and monocyte chemotactic protein-1, compared with their *mkp-1*(+/+) littermates (Salojin et al., 2006). This difference in serum cytokine levels underscores the survival of both sets of mice after LPS challenge, with all of the *mkp-1*(−/−) mice dying within 48 h and all the wild-type mice surviving after 72 h after LPS injection. Furthermore, *mkp-1*(−/−) mice demonstrate a greater severity and incidence of arthritis in a chicken type II collagen-mediated model of rheumatoid arthritis (Salojin et al., 2006). Other studies showed similar survival kinetics for LPS-mediated endotoxic shock in *mkp-1*(−/−) mice versus their *mkp-1*(+/+) littermates (Hammer et al., 2006; Zhao et al., 2006).

LPS challenge also causes renal, hepatic, and pulmonary damage (Zhao et al., 2006). This effect was assessed by measuring blood urea nitrogen and blood alanine aminotransferase activity and histological analysis, respectively. The lungs revealed thickening of the alveolar septa, edema, and infiltration by leukocytes in the interstitial space. In LPS-treated *mkp-1*(−/−) mice blood nitrate levels were elevated compared with those in their *mkp-1*(+/+) littermates. This finding was paralleled by reduced blood pressure and hypotension in *mkp-1*(−/−) mice compared with their *mkp-1*(+/+) littermates (Zhao et al., 2006).

Among the genes up-regulated as a result of LPS challenge in *mkp-1*-null versus WT littermates are the chemokines CCL3, CCL4, and CXCL2, also called macrophage inflammatory proteins 1α, 1β, and 2, respectively, at the mRNA and protein levels. As these chemokines have the potential to recruit leukocytes, they may contribute to damage, leading to lethality. In addition, IL-6 and IL-10 were both up-regulated as a result of LPS in *mkp-1*(−/−) mice, leading to SOC3, NFIL3, Ndr1, and Gadd45γ up-regulation (Hammer et al., 2006). MKP-1 may therefore be required to down-regulate LPS- and IL-10-regulated genes (Hammer et al., 2006).

In vitro, bone marrow-derived dendritic cells and peritoneal macrophages stimulated with LPS show differences in levels of secreted cytokines (TNF-α, IL-6, IL-10, and IL-12p70) compared with *mkp-1*(−/−) with *mkp-1*(+/+) mice (Zhao et al., 2006). Peritoneal macrophages from *mkp-1*-null mice have prolonged phosphorylated p38MAPK and JNK in response to LPS compared with wild-type mice (Zhao et al., 2006). Cytokine production is altered in *mkp-1*-null versus wild-type mice upon LPS treatment whether or not they have been primed with interferon-γ or thioglycollate (Zhao et al., 2006). Others found that bone marrow-derived macrophages increased their p38MAPK phosphorylation in response to LPS in *mkp-1*-null mice compared with their wild-type counterparts (Salojin et al., 2006). In both primary and immortalized murine alveolar macrophages (MH-S), LPS induces both mkp-1 mRNA and protein synthesis (Zhao et

al., 2005). This MKP-1 induction correlates with down-regulation of ERK, JNK, and p38MAPK phosphorylation status in primary murine alveolar macrophages as demonstrated when WT and *mkp-1*-null cells are treated with LPS (Zhao et al., 2005). This group also used MH-S cells to evaluate the effect of some steroids on the induction of mkp-1 mRNA versus their anti-inflammatory activity. They found a positive correlation between the ability of a given steroid to induce mkp-1 mRNA and its anti-inflammatory activity (Zhao et al., 2005).

So what has all this to do with cancer? Within the confines of infection/MKP-1 there is a cancer connection. At present there are five observations that merit our attention: 1) the increase in inflammatory cytokine response to an LPS challenge, when *mkp-1*(−/−) mice are compared with their *mkp-1*(+/+) littermates (see section V.B.2); 2) chronic inflammatory processes, caused by either bacteria or viruses, leading to cancer (Karin et al., 2006); 3) the use of fever induced by bacterial infection (Busch-Coley treatment) to induce remission in some cancers (Hobohm, 2001); 4) fever caused by chemotherapeutic agents (Davis and Raebel, 1998); and 5) the number of immunomodulatory-related phosphatases (Tables 1 and 2). Furthermore, retrospective analysis revealed that the success of the Busch-Coley treatment was enhanced with the following four criteria: 1) the cancer was of mesodermal embryonic origin; 2) treatment was given three times per week for at least 6 months; 3) the patient achieved a fever of approximately 39 C for 12 to 24 h after each injection; and 4) the patient has an otherwise healthy immune system that has not been weakened by radiotherapy or chemotherapy (Hobohm, 2001). Taken together, these observations raise a few interesting questions: 1) If bacterial and viral infection induce some cancers, can these tumors be targeted by a modernized version of the Busch-Coley treatment, the mixed bacterial vaccine? 2) If lack of MKP-1 expression increases the production of inflammatory cytokines to the point of death in *mkp-1*-null mice, can judicious targeting of this or another phosphatase involved in reducing inflammation be used to increase efficiency of such a vaccine? 3) More importantly, what are the phosphatase and MAPK profiles for chronic inflammation that does not lead to cancer versus the profiles that lead to chronic inflammation-mediated tumorigenesis or inflammation-induced tumor regression for a given tissue? Understanding these differences will be a first step in 1) dealing with chronic inflammation-induced cancer, 2) increasing the efficacy of a mixed bacterial vaccine targeting cancer of mesodermal origin, 3) manipulating MAPKs and phosphatases to enhance the efficacy of vaccine, and 4) extending these observations outside the confines of cancer from mesodermal origin to tissues deriving from other embryological origins.

3. Diet-Induced Obesity. *mkp-1*-null mice are lean and resistant to diet-induced obesity. Activated p38MAPK, JNK1, and ERK levels are elevated in skeletal muscle and white adipose tissue, compared with levels in similarly treated wild-type mice, and the liver also demonstrates elevated p38MAPK and JNK1 activity whereas ERK activity remains unchanged. Some researchers believed that inactivation of the *mkp-1* gene may be an important element in uncoupling diet-induced obesity from glucose intolerance(Wu et al., 2006a). However, how the activities of p38MAPK, JNK1, and ERK relate to this phenotype await elucidation.

One of the most unexpected findings for a phosphatase that controls p38MAPK, JNK1, ERK1/2, and ERK5 activities is a lack of obvious phenotype in *mkp-1*-null mice. Normal development coupled with normal fertility in null mice is difficult to reconcile considering the ubiquitous expression of the mkp-1 mRNA and protein in virtually all tissues examined. One key piece of information that we lack to resolve this conundrum is which phosphatases compensate for the lack of MKP-1 in null mice during development? More importantly, why are these phosphatase(s) failing to compensate upon stress?

C. Mitogen-Activated Protein Kinase and Cancer: An Overview

MKP-1 and other dual-specificity phosphatases target members of the MAP kinases and inactivate their function in both the cytosol and the nucleus (Tables 1 and 2). To appreciate the possible involvement of members of the MAPK family in cancer, we will present an overview of what is known about their contribution to the process or inhibition of oncogenesis and how this relates to cell and animal models as well as to human tumors in this and the following section.

1. Ras-Raf Cancer Connection. Both Ras, the upstream activator of the Raf-MEK-ERK module, and Raf have been implicated in cancer (Schubbert et al., 2007; Barault et al., 2008). More specifically, the Ras isoforms have been associated with different cancers when they have an activating mutation or developmental diseases when the proteins that control Ras activity are mutated (Roberts and Der, 2007; Schubbert et al., 2007). Of the Rafs, B-Raf has the highest incidence of activating mutations, whereas Raf1 activating mutations are rare and A-Raf activating mutations have not been found (Emuss et al., 2005). Reasons for the discrepancy in frequency of association with cancer include the following: 1) the constitutive phosphorylation of B-Raf at S445 gives it a higher basal activity than Raf1 basal activity (Mason et al., 1999); and 2) the negative charge at the N terminus of B-Raf is due to an aspartic acid residue instead of the tyrosine found at the homologous site in Raf1; the tyrosine requires phosphorylation for activation. Thus, the constitutive phosphorylation combined with the N terminus negative charge is believed to contribute to B-Raf being more easily activated by a point mutation. Thus, the B-Raf mutation is frequently associated with cancers (Schubbert et al., 2007). In contrast, a single point mutation is rarely sufficient to activate A-Raf or Raf1 (Well-

brock et al., 2004). In addition, B-Raf activation by Ras is more direct than that of A-Raf and Raf1. The two latter isoforms require other kinases, scaffolding proteins, or phosphatases for activation (Wellbrock et al., 2004). Finally, it should be noted that Raf is only one of many effectors of Ras, which include: the catalytic subunit of class I PI3K (p110α–δ) (Martin and Vuori, 2004; Olson and Hallahan, 2004; Hannigan et al., 2005; McLean et al., 2005; Cully et al., 2006; Engelman et al., 2006; Holz et al., 2006; Kohno and Pouyssegur, 2006; Goldsmith and Dhanasekaran, 2007; Hehlgans et al., 2007; Mayor et al., 2007; Roberts and Der, 2007; Schubbert et al., 2007), phospholipase Cε, and some RhoGEFs of Rac and Ral that have been implicated in Ras-mediated oncogenesis (Roberts and Der, 2007).

2. Extracellular-Regulated Kinases. Although ERK1/2 functions permeate cell cycle regulation on their own, their functions are not sufficient to enter into the cell cycle. This fact is underscored by the finding that Ras overactivation on its own is insufficient for cell cycle entry and would result in growth arrest (Hirakawa and Ruley, 1988), whereas other results reveal that overexpression of growth factor receptors in PC12 cells leads to differentiation (Marshall, 1995) and sustained ERK activation in fibroblasts leads to proliferation (Marshall, 1995). Thus, the level within the signal transduction pathway, which is responsible for overstimulation, along with cell type may influence phenotypic outcome (see section II.B on ERKs). Furthermore, ERK also increases CDK inhibitor proteins p21, p27, and p53, among others, which results in cell cycle arrest (Smalley, 2003; Dhillon et al., 2007) and phosphorylation of CDC25c, leading to its ubiquitination and proteasomal degradation (Eymin et al., 2006), whereas Raf can also induce cell cycle arrest via p21 (Smalley, 2003). Thus, tumors must counteract this effect and can do so via AKT and RhoGTPase proteins (Dhillon et al., 2007). AKT inhibits the CDK inhibitor proteins p21 and p27 and the cyclin D1 inhibitor glycogen synthase kinase (GSK3β) (Lo-Piccolo et al., 2007; Manning and Cantley, 2007). However, greater levels of ERK mRNA, protein, and phosphotyrosine ERK1/2 were found in primary breast cancer cells compared with surrounding normal tissue (Sivaraman et al., 1997). Considering the level of involvement of ERK1/2 in the cell cycle, a phosphatase capable of inactivating ERK1/2 can have any number of effects, depending on when the phosphatase is expressed and to what extent its effects can be bypassed, assisted, or inhibited via other members of the MAPK family.

Interestingly, ERK3 expression has been associated with inhibition of proliferation and the induction of cell differentiation. Overexpression of ERK3 or ERK7 can inhibit the cell cycle in the S phase (Coulombe and Meloche, 2007). It remains to be determined whether this effect is part of the "normal" function of ERK3 or ERK7 or is due to overexpression. ERK5 has been implicated in cell cycle progression, although the extent of this involvement, the cell type, and the circumstance await further clarification, as *erk5*-null MEFs cycle through the S phase, and the increase in cyclin D1 can be uncoupled from ERK5 activation status, although mitogen signaling through ERK5 and cyclin D1 transcription can be regulated by ERK5. Because ERK1/2 are implicated in many aspects of the cell cycle, ERK5 may serve redundant functions with its more famous family members or it may have a more restricted role. For instance, ERK5 is implicated in neuronal and endothelial cell survival (Wang and Tournier, 2006), whereas leukemia cells increase levels of ERK5 via stabilization of the protein in an Abl kinase-dependent manner, leading to transformation (Buschbeck et al., 2005). Overexpression of shRNA for ERK5 in the EL-4 T-lymphoma cell line failed to develop tumors in mice when injected subcutaneously compared with mice with vector control-treated cells, which did develop tumors (Garaude et al., 2006). Recently, down-regulation of miRNA 143, which targets ERK5 expression levels, has been implicated in human cancers (Akao et al., 2006, 2007). Homeobox gene (*HOXB9*) expression, identified in Hodgkin's lymphoma, corresponds to active ERK5 (Nagel et al., 2007). Conversely, medulloblastoma cell lines increase apoptosis upon MEK5/ERK5 exogenous expression in a MEF2-dependent manner (Sturla et al., 2005). Finally, ERK5 also has a role in angiogenesis and prostate cancer (see section V.D.8 on angiogenesis and vasculogenesis and section VI.E.3 on prostate cancer).

3. c-Jun NH_2-Terminal Kinases. As previously mentioned (see section II on the MAPK family), active JNKs can have both pro- and antiapoptotic activities, whereas active and unphosphorylated JNKs modulate the stability of their effector proteins via polyubiquitination and the proteasomal pathway, although via different mechanisms. This array of functions is accompanied by an equally varied response regarding JNK function and tumorigenesis as revealed in this section.

Jnk2-null mice demonstrated lower 12-*O*-tetradecanoylphorbol-13-acetate-mediated tumorigenesis compared with WT control mice (Chen et al., 2001), whereas *Jnk1*-null mice demonstrated enhanced 12-*O*-tetradecanoylphorbol-13-acetate-mediated tumorigenesis compared with their WT control mice (She et al., 2002). The JNK1/2 double knockouts affect neither ERK, p38MAPK, nor MKK4 or MKK7 protein expression levels. WT MEFs expressed high p46-JNK1 and p54-JNK2 and low levels of p54-JNK1 and p46-JNK2, and JNK3 was not detected (Tournier et al., 2000). p38MAPK, MKK4, and MKK7 kinase activities were down-regulated after stress compared with those in WT MEFs, whereas ERK kinase activity was not visibly affected upon serum stimulation. When stressed, JNK1/2 double knockout fibroblasts fail to show apoptosis in vitro (Tournier et al., 2000). Conversely, no tumors developed with either JNK1/2 double null fibroblasts or WT MEFs when injected into athymic nude mice (Kennedy et al., 2003). Ras-transformed

JNK1/2 double KO MEFs developed greater tumor mass than Ras-transformed-WT MEFs when injected in athymic nude mice, as a result of reduced apoptosis, generating both a greater number and size of nodules (Kennedy et al., 2003). In vivo JNK complementation studies support a role for JNK in interfering with Ras-mediated cell survival, contrary to in vitro results, which suggest that JNK is a positive regulator of Ras-mediated oncogenesis (Kennedy et al., 2003). A constitutively active form of Ras (RasV12) led to persistent JNK1 activity in NCl-H82 human small-cell lung cancer cells, but had a more modest effect on ERK activity. The importance of JNK1 activity was revealed when a dominant-negative form of JNK1 reduced both cell number and colony number in RasV12-transformed NCl-H82 cells in an AP-1-dependent manner (Xiao and Lang, 2000). Other systems reveal that JNK1 is essential for survival of B-lymphoblasts (Hess et al., 2002). Therefore, the involvement of JNK1 and JNK2 in cancer is not uniquely prodeath in either human cell lines or animal models (Bode and Dong, 2007). JNK3 has a more localized distribution than either JNK1 or JNK2. Lack of JNK3 has been associated with human brain tumors (Yoshida et al., 2001). In addition, activation segment mutation in JNK1 and kinase domain mutation in JNK2 have been reported (Greenman et al., 2007), although mutations in the families of Jun and Fos, which are components of the AP-1 transcription factor, are as yet unknown (Verde et al., 2007).

Despite the previously mentioned results, a clear image of exact role of JNKs in tumor development and progression is still wanting. For instance, JNK can activate or inhibit the proapoptotic pathway via phosphorylation and phosphorylate AKT to prime it for interaction with its kinase PDK1 (Bogoyevitch and Kobe, 2006). Active AKT can phosphorylate and inhibit MKK4, ASK, and MLK3, the upstream kinases of JNK (Bogoyevitch and Kobe, 2006) (Fig. 2). In addition, when looking at JNKs to find an answer, we must also consider the function of unphosphorylated JNK as it can target its effectors for degradation (Fuchs et al., 1997, 1998a; Bode and Dong, 2007). Unfortunately, when analyzing the JNKs and their function in many systems, we often relegate nonactive JNK to be part of a loading control for phosphorylated JNK rather than a molecule that has a bona fide function in its own right. Taking the latter into account, the normal function of JNKs (see section II.C on the MAPK family), their possible role in tumorigenesis (this section), the different targets of the Jun and Fos family of transcription factors (Verde et al., 2007), and the contribution of other MAPKs (MAPK cross-talk) and phosphatases in signal transduction (Fig. 2), among other factors, we find it is easy to realize why a definitive image of JNK and cancer is still wanting.

4. p38 Mitogen-Activated Protein Kinases. Increased p38MAPK signaling is associated with MMP-9 in leukemia (Ringshausen et al., 2004) and MMP-2 in prostate cancer (Xu et al., 2006), whereas production of MMP-2 (Kim et al., 2003a) and urokinase plasminogen activator was mediated by H-Ras (Behren et al., 2005). Overall, MMP production is initially associated with the creation of a tumor microenvironment for enhanced growth and later with metastasis (Overall and López-Ot n, 2002). p38α is also involved in angiogenesis; this topic will be covered in section V.D.8 on angiogenesis and vasculogenesis. Although, HSP27 is implicated in p38αMAPK-mediated cell migration (Rousseau et al., 2000, 2006), collectively as a family HSPs can be indicators of poor prognosis in cancer treatment (Ciocca and Calderwood, 2005). p38α/βMAPK is also implicated in H-Ras-mediated invasion of human breast epithelial cells (Kim et al., 2003a), and contact inhibition, a characteristic of nontransformed cells (Hoff et al., 2004), is achieved by sustained p38α activity (Faust et al., 2005), whereas p38γ assists K-Ras-mediated transformation (Tang et al., 2005). p38MAPK is also involved in cell cycle arrest by directly phosphorylating CDC25A, leading to degradation of this phosphatase (Khaled et al., 2005; Kittipatarin et al., 2006) or indirectly phosphorylating CDC25B via MAPKAP-K2 (Manke et al., 2005). The CDCs are a family of phosphatases regulating the cell cycle (Rudolph, 2007a), which are overexpressed in various cancers (Boutros et al., 2007; Rudolph, 2007b) and targeted for therapeutic intervention (Boutros et al., 2007). Although p38MAPK is activated by telomere shortening associated with replicative senescence, it is also linked to replicative senescence that is unrelated to telomere length (Han and Sun, 2007).

Although p38α KO mice are embryonically lethal, as mentioned in section II on the MAPK family, p38α embryo-specific knockouts [placentas are *p38*α(+/+)] develop into pups but most die at 4 days after birth (Hui et al., 2007). Hematopoietic cells and MEFs from these mice are associated with uncontrolled proliferation in vitro (Hui et al., 2007). Another study revealed that mice with an inducible deletion of the *p38*α gene lack differentiation in murine lung progenitor cells (Ventura et al., 2007). Other animal models support a role for p38MAPK and some of its effectors in the prevention of tumorigenesis (Han and Sun, 2007). p38MAPK is also implicated in human tumors, as a lack of p38MAPK signaling is associated with a myosarcoma, a childhood solid tumor characterized by lack of differentiation of myoblasts into myotubes (Puri et al., 2000) and other human tumors are linked to defects in the p38MAPK signal transduction pathway (Han and Sun, 2007).

Collectively members of the p38MAPK family have the potential to influence tumorigenicity on many levels, notwithstanding the lack of phenotype associate with the KO mice for the β, γ, and δ isoforms of p38MAPK during development (see section II.D). This is recognized when the best-characterized isoform, p38αMAPK, is used as an example with its involvement in chemo-

taxis, MMP production, and modulation of mRNA stability, proliferation, differentiation, and so on. Thus, inappropriately expressed or inhibited expression of p38MAPK isoforms can deregulate cell function on multiple levels. Likewise, an inappropriately expressed phosphatase that is capable of dephosphorylating p38αMAPK (Tables 1 and 2), such as MKP-1, could have deleterious consequences for the phenotype of the cell. Conversely, a cell overexpressing p38MAPK or a mutated oncogene, such as H-Ras, could benefit from expressing a p38αMAPK-specific phosphatase. This benefit holds true not only for p38MAPK but also for the other axes of MAPK. Earlier studies revealed that quiescent rat embryonic fibroblast REF-52 cells cease to synthesize DNA in response to the constitutively active form of Ras when MKP-1 is coexpressed (Sun et al., 1994). Conversely, NIH 3T3 cells demonstrate an MKP-1-mediated increase in Raf1 and MEK-1/2 activities, in both a Ras-dependent and -independent manner (Shapiro and Ahn, 1998). This effect could be due to MKP-1 inactivating ERK and preventing its inhibitory phosphorylation of the upstream kinases. In addition, PKA down-regulates Raf1, which can lead to the down-regulation of ERK1/2. As this latter kinase has been known to phosphorylate and stabilize MKP-1 protein (Brondello et al., 1999), growth factor signaling that activates ERK would be counterbalanced by PKA and MKP-1 (Pursiheimo et al., 2002).

Differential ERK activity in the cytosol versus the nucleus was observed in response to a conditionally expressed Ha-Ras in NIH 3T3 cells. Cytosolic ERK2 remained active in response to Ha-Ras for much longer than nuclear ERK2, which was dephosphorylated by MKP-1 (Plows et al., 2002). Others found that when an angiotensin II signaling system is reconstituted with either angiotensin type 1 receptor only (γ2A/AT_1) or in combination with Jak2 (γ2A/AT_1/Jak2), Jak2 inacti-

in the 5′-position of the ring in the decitabine molecule (in place of a carbon atom in the cytosine ring), methylation occurs, but there is no hydrogen at position C-5 to complete the reaction and free the DNA methyltransferase; the enzyme thus becomes "trapped" (Yoo and Jones, 2006). Although the resultant hypomethylation can occur throughout the genome (Oki et al., 2007), loss of methylation in repeat elements near the centromere does not herald the loss of genomic integrity (Bird, 2002).

The prostate cancer cell line PC-3 up-regulated mkp-1 mRNA expression in response to either decitabine or a combination of trichostatin A (a histone deacetylase inhibitor) (Yoshida and Horinouchi, 1999) and decitabine treatment (Rauhala et al., 2005). Overall, this method served to detect the epigenetically down-regulated genes by suppression of subtractive hybridization and cDNA microarray analysis. The results indicate that DNA methylation may be part of a mechanism used by PC-3 cells for the down-regulation of *mkp-1* gene expression. Interestingly, whereas hormone-refractory prostate carcinomas showed lower levels of MKP-1 protein and mRNA expression levels, untreated prostate carcinomas revealed lower MKP-1 protein expression, whereas mRNA levels were no different from those of benign prostate hyperplasia (Rauhala et al., 2005). All together, the results suggest that down-regulation of MKP-1 is an early event in human prostate tumorigenesis (Rauhala et al., 2005).

c. Glucocorticoids. Although the therapeutic potential of endogenous glucocorticoids has been known for some time (Buckingham, 2006), the in vivo functions of both endogenous and exogenously administered glucocorticoids are still being discovered (Distelhorst, 2002; Hayashi et al., 2004b; Stellato, 2004; Buckingham, 2006). Some of these functions include up- or down-regulation of transcription (Hayashi et al., 2004b) and stabilization/destabilization of mRNA (Stellato, 2004) in addition to apoptosis.

Interestingly, whereas glucocorticoids induce apoptosis in lymphocytes (Distelhorst, 2002), pre-B acute lymphoblastic leukemia cell line 697 is more resistant to hydroxyurea-mediated apoptosis than to glucocorticoid-mediated apoptosis when mkp-1 is stably transfected in this cell line (Abrams et al., 2005). Other studies revealed that dexamethasone used before a chemotherapeutic agent, such as paclitaxel or doxorubicin, has an antiapoptotic effect on breast cancer cells (MCF-7 and MDA-MB-231) (Wu et al., 2004b). MDA-MB-231 cells ectopically expressing MKP-1 and treated with paclitaxel have a number of apoptotic cells similar to that of sister cultures expressing MKP-1 and treated with dexamethasone before paclitaxel treatment. Moreover, in the absence of MKP-1 ectopic expression, dexamethasone/paclitaxel-treated cultures have fewer apoptotic cells than cultures treated with paclitaxel only. Interestingly, MKP-1 and dexamethasone offer a similar level of protection to MDA-MB-231 cells against paclitaxel-induced apoptosis (Wu et al., 2004b). When MKP-1 levels are reduced using siRNA, paclitaxel induces the same level of apoptosis in MDA-MB-231 cells, whether or not dexamethasone is present. When endogenous levels of MKP-1 are present, dexamethasone pretreatment reduces the number of apoptotic cells compared with the paclitaxel treatment only paradigm. Thus, it would seem that dexamethasone affords antiapoptotic protection to MDA-MB-231 cells, in part via MKP-1 (Wu et al., 2004b). The same group found that paclitaxel-induced phosphorylation of MAPK (ERK1/2 and JNK) in MDA-MB-231 cells is decreased by pretreatment with dexamethasone; under such conditions (dexamethasone/paclitaxel treatment) MKP-1 protein expression increases. If MKP-1 siRNA is used, both ERK1/2 and JNK phosphorylation levels increase, whereas phospho-p38MAPK levels remain unaffected (Wu et al., 2005b). Although MKP-1 is implicated in the mechanism by which dexamethasone reduces apoptosis in breast cancer cell lines, the exact mechanism awaits further elucidation. As glucocorticoids have pleiotropic effects (Distelhorst, 2002; Hayashi et al., 2004b; Stellato, 2004; Buckingham, 2006), MKP-1 may be one facet by which dexamethasone rescues cells from paclitaxel-induced apoptosis. With the use of dexamethasone as an antiemetic agent or to reduce acute toxicity, its side effect of being an antiapoptotic agent for breast cancer cells in vitro needs to be confirmed in vivo.

d. Fas/Fas ligand. Tumors are known to escape the innate and acquired immune detection systems and avoid death by using an array of responses (Abrams, 2005). Among the escape mechanisms available to tumors, avoidance of Fas/FasL-mediated apoptosis by different methods has been implicated in tumorigenicity. Tumors resist Fas-mediated signaling, by means of 1) down-regulation of Fas expression, 2) synthesis of secreted forms of Fas, 3) synthesis of Fas that lacks signal transduction capabilities, and 4) mutations in downstream effectors of Fas (Abrams, 2005). In addition, mutations in Fas-mediated signaling (Shin et al., 2002) and the lack of Fas-FasL interaction (Owen-Schaub et al., 1998; Koshkina et al., 2007) have been implicated in the ability of tumors to metastasize. Another way tumors could control the immune system is by way of FasL expression on tumor cells, which may induce apoptosis in Fas-expressing immune cells, although this latter model is controversial (Abrams, 2005).

Given the possible involvement of Fas/FasL in cancer, it is not surprising that this system has become a target of study. Mouse embryonic fibroblasts BALB/3T3 stably expressing Fas, designated FH2 cells, showed improved viability when transfected with constitutively activate forms of either K-Ras [constitutively active K-Ras (K-RasV12)], Raf (Raf-CAAX), or MAPKK (SDSE-MEK) when stimulated with anti-Fas antibody, compared with control FH2 cells stimulated anti-Fas antibody only.

When mkp-1 cDNA is cotransfected, the protective effects of the constitutively active forms of K-Ras, Raf, or MEK against anti-Fas antibody are abolished (Kazama and Yonehara, 2000). Thus, sustained MAPK (ERK) activity could inhibit Fas-mediated apoptosis.

DU145 human prostate cancer cells cotransfected with androgen receptor and androgen receptor coactivator, down-regulated their 5α-dihydrotestosterone-induced chloramphenicol acetyltransferase activity (chloramphenicol acetyltransferase activity assay) upon MKP-1 ectopic expression, in the presence or absence of Her-2/Neu receptor (erbB2 receptor; v-erb-b2 erythroblastic leukemia viral oncogene homologue 2 receptor/neuro/glioblastoma derived oncogene homologue receptor) (Yeh et al., 1999). Furthermore, a conditionally expressing MKP-1 construct protected DU145 cells from FasL-induced caspase-1 and caspase-3 activation and mitochondrial membrane depolarization associated with apoptosis (Srikanth et al., 1999). In addition, JNK activation via transfection of ASK1 or a constitutively active MEKK1 into DU145 cells is also down-regulated by overexpression of MKP-1 in these cells. Thus, in the human prostate cancer cell line DU145, MKP-1 overexpression protects against many agents inducing apoptosis (Srikanth et al., 1999).

e. Mitogen-activated protein kinase phosphatase-1 and the proteasome.

i. An overview. The proteasome complex is a multisubunit 26S complex composed of a 20S protease and 19S regulatory complexes. The latter can be further subdivided into base and lid multisubunit complexes (Nandi et al., 2006). Although the proteasome is known for its protein degradation activity via the ubiquitin-proteasomal pathway (Lorentzen and Conti, 2006; Nandi et al., 2006), it is increasingly being recognized as a means of controlling transcription (Muratani and Tansey, 2003). As cancer, among other diseases, is associated with both mutations in the ubiquitin-proteasomal pathway per se and mutations in proteins degraded by this pathway, it is also being targeted for pharmacological intervention (Adams, 2004; Nalepa et al., 2006). At present, which of the proteasomes is responsible for the degradation of MKP-1, the cytosolic (Lorentzen and Conti, 2006; Nandi et al., 2006) or the nuclear proteasome (von Mikecz, 2006), remains undetermined. Conversely, as transcription factors are targeted to the nuclear proteasome (von Mikecz, 2006), MKP-1 may also be targeted by this complex.

Many proteins are degraded by the proteasomal pathway, a minute fraction of which are the kinases and phosphatases. Thus, the effect of proteasomal inhibitors is most likely the result of a complex series of interactions in space and time and not the result of increasing expression of a single protein, such as MKP-1.

ii. Bortezomib. Among the pharmacological arsenal that exists to fight cancer, bortezomib is a synthetic reversible inhibitor of the proteasome-related chymotrypsin-like activity (APEX Trial, 2003; Jagannath et al., 2007; Richardson et al., 2006). Some bortezomib-related functions that have been characterized and retain our attention include 1) induction of apoptosis in tumors (Voorhees et al., 2003), 2) increasing JNK activity in various models (Meriin et al., 1998; Hideshima et al., 2003; Yang et al., 2004), and 3) up-regulation of *mkp-1* transcription levels (Orlowski et al., 2002). In line with some previous observations, transient adenovirus-mediated expression of MKP-1 in myc-transformed human mammary epithelial cells (A1N4-myc) or stable MKP-1 overexpression in BT-474 breast carcinoma cells protected these cell lines from bortezomib-induced apoptosis (Small et al., 2004). In the complementary experiment, disruption of MKP-1 in *MKP-1*-null MEFs or use of siRNA to silence mkp-1 mRNA, in conjunction with proteasome inhibitors, increased the susceptibility of mammary epithelial and breast carcinoma cells to apoptosis (Small et al., 2004). Anthracyclines were used as MKP-1 inhibitors (Small et al., 2003) in conjunction with proteasome inhibitors to augment apoptosis in vitro and in xenograft model (see section V.D.4) (Small et al., 2004).

iii. Bowman-Birk inhibitor. The Bowman-Birk inhibitor (BBI), an 8-kDa soybean-derived serine protease inhibitor, has been used in a phase IIa clinical trial (Armstrong et al., 2000; Meyskens, 2001). This serine protease inhibitor is known to inhibit trypsin from one of the domains in its double-headed structure and chymotrypsin from the other inhibitory domain (Birk, 1985). To better characterize the anticancer activity of the BBI, Chen et al. (2005) treated MCF-7 cells with this inhibitor and found that the antichymotrypsin activity of the BBI blocked the chymotrypsin-like activity of the proteasome. Among the proteins that accumulated in MCF-7 cells as a result of proteasomal inhibition, MKP-1 levels correlated with ERK1/2 inactivation and cell growth inhibition (Chen et al., 2005).

iv. MG132. With use of a hyperosmotic dehydration paradigm on rat hepatoma cell line H4IIE-C3 in conjunction with the proteasome inhibitor MG132, MKP-1 protein stabilization and increased mRNA levels were observed (Lornejad-Schafer et al., 2005). However, it is noteworthy that injection of MG-132 into the substantia nigra compacta of C57 black mice causes apoptosis to dopaminergic neurons (Sun et al., 2006). Such toxicity warrants further testing, because cancer treatment is not usually a one-time event.

*f. (Z)-1-[*N*-(2-Aminoethyl)-*N*-(2-ammonioethyl) amino]diazen-1-ium-1,2-diolate.* Use of DETA-NONOate, a nitric oxide (NO) donor with a 20-h half-life at 37 C, on the breast cancer cell lines MDA-MB-468, ZR 75–30, and MDA-MB-231 led to NO-mediated up-regulation of MKP-1, dephosphorylation of ERK1/2, and subsequent dephosphorylation of Akt (PKB) in the MDA-MB-468 and ZR 75–30 cell lines but not the MDA-MB-231 cell line. This up-regulation of MKP-1 and dephosphoryla-

tion of ERK1/2 and AKT led to apoptosis in the two former cell lines but not the latter (Pervin et al., 2003). The source of the difference in response to DETA-NONOate between these breast cancer cell lines is currently unknown. The MDA-MB-231 cell line can up-regulate MKP-1 expression (Orlowski et al., 2002). As levels of JNK1 were not assayed, it remains possible that one of the differences relating to apoptotic activities lies in levels of phosphorylated JNK1. JNK1 can phosphorylate AKT, a priming phosphorylation, leading to enhanced phosphorylation and activation of AKT by PDK1 (Bogoyevitch and Kobe, 2006) and inhibition of upstream kinases if JNK (section V.C.3). Alternatively, the NO effectors (Bonavida et al., 2006; Hirst and Robson, 2007; Mocellin et al., 2007) may be responsive in MDA-MB-468 and ZR 75–30 cell lines but unresponsive in MDA-MB-231 cells. Although this treatment is not effective for all cell lines, it represents a nonhormonal method of modulating MKP-1 expression.

g. Radiation. In vitro experiments demonstrate that γ-radiation up-regulates mkp-1 mRNA levels (Kasid et al., 1997). More recently, both normal human skin fibroblasts and A431 cells up-regulated ataxia telangiectasia-mutated kinase activity in response to radiation, which led to dephosphorylation of ERK1/2 in an MKP-1-dependant manner. The results of ERK1/2 dephosphorylation by MKP-1 were reprised in a xenograft model using A431 cells and irradiated nude mice (Nyati et al., 2006).

Short-wavelength ultraviolet light (UVC) up-regulates ERK2 and JNK1 activities followed by an increase in MKP-1 protein levels, leading to down-regulation of JNK1 activity in HeLa cells (Liu et al., 1995). In the C3H 10T1/2 murine cell line, p38MAPK is preferentially linked to *mkp-1* gene induction (Li et al., 2001), whereas ectopic expression of MKP-1 in U937 cells protected them from UV-induced apoptotic cell death, inhibited JNK1 activity, and induced caspase-3 activation and DNA degradation (Franklin et al., 1998). Finally, low doses of UVC decrease mkp-1 mRNA in transcription-coupled repair-deficient human fibroblasts (Hamdi et al., 2005). Collectively, these findings demonstrate that MKP-1 can protect cells from chemical- as well as radiation-induced JNK activation. This could be a significant finding as ~50% of patients that receive chemotherapy are also given radiotherapy (Bentzen, 2006).

2. Antiapoptosis. MKP-1 has also been implicated in antiapoptotic effects, by the suppression of caspase-3-mediated apoptosis in MEFs in the presence of anisomycin (Wu and Bennett, 2005). Moreover, cisplatin activated poly(ADP-ribose) polymerase (PARP) and caspase-3 by inducing their cleavage, resulting in an increase in caspase-3 activity in *mkp-1*-null MEFs, whereas wild-type MEFs are less robust in their responses when treated similarly (Wang et al., 2006). Interestingly, the proteasome inhibitor MG132 increased MKP-1 in H4IIE rat hepatoma cells in a hyperosmotic environment and also activated caspase-3 (Lornejad-Schafer et al., 2005). MKP-1 expression in MDA-MB-231 cells via transfection or adenoviral transduction reduced paclitaxel (a microtubule stabilizing agent, which inhibits cell proliferation via reduced mitotic activity) (Jordan and Wilson, 2004)- or mechlorethamine (alkylating agent)-mediated increase in caspase-3/7 activity (Small et al., 2007). Caspase-3/7 can activate MAPKs, such as ERK1/2, p38MAPK, and JNK, via Mst1, an upstream kinase for MAPKKK, in an extrinsic apoptotic pathway-dependent mechanism (Song and Lee, 2008). More specifically, caspase-7 cleaves Mst1, producing a 40-kDa fragment, which activates JNK and p38MAPK, whereas caspase-3 produces a 36-kDa fragment of Mst1, which activates ERK (Song and Lee, 2008). The functional significance of the MAPK activation in this scenario awaits further investigation.

Caspases are characteristically associated with apoptosis and interference with this function is categorized as antiapoptotic (Riedl and Salvesen, 2007). However, caspases also have nonapoptotic functions, including cell maturation, induction of differentiation, and cytokine maturation, among other roles (Lamkanfi et al., 2007). In addition, although PARP activation is a hallmark of apoptosis, as it can curtail the DNA-repair mechanisms, PARP can also be implicated in the inflammatory response by modulation of NF-κB transcriptional activity (Lamkanfi et al., 2007). In turn, NF-κB can inhibit JNK-mediated apoptosis (Papa et al., 2006). However, MEKK1 activates IκBα kinase, which phosphorylates IκBα, leading to its dissociation from NF-κB and proteasomal degradation. Alternatively, JNK phosphorylates IκBα, also leading to polyubiquitination and proteasomal degradation (Ki et al., 2007). In either case, the dissociation of IκBα from NF-κB leads to the activation of NF-κB (Lee et al., 1997). Thus, MEKK1 and JNK can activate a transcription factor, NF-κB, via phosphorylation of IκBα, to potentially synergize with or antagonize JNK activity.

Adenovirus-mediated expression of MKP-1 in BT-474 or A1N4-myc cells reduced their DNA fragmentation after doxorubicin, paclitaxel, or mechlorethamine treatment. MKP-1 expression is also able to reduce DNA fragmentation with combined doxorubicin/mechlorethamine treatment in BT-474 cells. These DNA fragmentation results mirror those derived from treating wild-type MEFs and *mkp-1*-null MEFs with doxorubicin, paclitaxel, or mechlorethamine (Small et al., 2007). The presence of MKP-1 protects human mammary epithelial cells, breast carcinoma cells, and mouse embryonic fibroblasts from various agents inducing DNA fragmentation by a JNK/c-Jun-mediated mechanism (Small et al., 2007).

Considering the potential involvement of MKP-1 in protecting tumor cell lines, it may be necessary to use an antisense or siRNA strategy to down-regulate MKP-1

before use of these chemotherapeutic agents in certain cases. Another possibility is to screen drugs for their ability to induce apoptosis in tumors without inducing the expression of MKP-1.

The preceding sections on apoptosis and antiapoptosis underscore the aforementioned observation that the expression of MKP-1 protein alone cannot predict the biological outcome for a given cell type. The identity of the MAPK that is active at the time of MKP-1 enzymatic function is more predictive although not absolutely so. There are other kinases and phosphatases that may be simultaneously active at the time of MKP-1 expression and activity that will have an impact on the ultimate fate of the cell (Tables 1 and 2; Fig. 2).

3. Differentiation. Activation of the Src kinase family member $p59^{Fyn}$ via mitogen-tyrosine kinase receptor signaling increases both ERK activity and phosphorylation of the melanocyte-specific transcription factor Microphthalmia (Wellbrock et al., 2002). This results in the degradation of the melanocyte differentiation-inducing transcription factor via the proteosomal pathway as well as a decrease in mkp-1 mRNA expression. Conversely, overexpression of MKP-1 decreases Microphthalmia degradation via ERK dephosphorylation and facilitates melanocyte differentiation (Wellbrock et al., 2002). The finding that MKP-1 overexpression can, under certain circumstances, induce cells to differentiate could be interesting if proven true for tumors, as one of the therapeutic axes for the control of tumors relies on inducing them to differentiate to the cell lineage from which they originate, whether the tumors are of hematological origin (Tsiftsoglou et al., 2003) or solid tumors (Honma and Akimoto, 2007).

4. Proliferation. PKCε is implicated in the up-regulation of MKP-1 in bone macrophages when stimulated with macrophage colony-stimulating factor (Valledor et al., 1999) or lipopolysaccharide (Valledor et al., 2000). Although MKP-1 expression did not rely on ERK activation in these studies, inactivation of ERK1/2 coincides with MKP-1 protein expression. When macrophage colony-stimulating factor was used, MKP-1 up-regulation reduced macrophage proliferation without inducing apoptosis. Thus, ERK is neither part of an autoregulatory loop nor does inhibition of proliferation herald apoptosis. MKP-1 negatively regulates cell cycle transition (G_0/G_1) in the absence of DNA damage and in response to growth factors (Li et al., 2003). In addition, constitutive expression of MKP-1 blocks G_1-specific gene expression (Brondello et al., 1995). In line with this result, cyclin D1 expression was decreased in a U28 clonal cell line induced to express MKP-1 (Manzano et al., 2002). These results go hand in hand with the previous findings relating to how *mkp-1* was initially identified, as one of a set of genes that are expressed in cultured murine cells during the G_0/G_1 transition (Lau and Nathans, 1985). Considering the level of involvement of ERK1/2 in cell cycle progression (see section II on the MAPK family), MKP-1 overexpression leading to cell cycle inhibition is in line with its function as an ERK1/2 phosphatase. Expression of the inactive form of MKP-1 (C258A) acts as a dominant-negative, decreasing cell viability in response to both cisplatin and transplatin in 293T and Pam212 cells while not interfering with the ability of JNK1 to phosphorylate c-Jun (Sánchez-Pérez et al., 2000). Lack of MKP-1-phosphatase activity (C258A) may help preserve the kinase activity of JNK1 toward c-Jun, protecting JNK from dephosphorylation by other phosphatases. This protection could also be extended to ERK1/2-mediated activity. As c-Jun can be part of the AP-1 transcription factor (Ozanne et al., 2007) and ERK1/2 mediate inhibition of antiproliferative gene transcription throughout the G_1 phase by an AP-1 dependent mechanism (Yamamoto et al., 2006), among other ERK1/2-related cell cycle mechanisms (see section II on the MAPK family), dominant-negative MKP-1 may cause cells to proceed through the cell cycle via an AP-1-mediated mechanism (Verde et al., 2007). Platinum-based compounds can cross-link DNA, resulting in increased apoptosis (Pascoe and Roberts, 1974a,b; Zwelling et al., 1979; Giraud-Panis and Leng, 2000). In addition, the consequences of JNK1 activity, antiapoptotic versus proapoptotic, relies on its duration (Ventura et al., 2006), which could lead to phosphorylation of proapoptotic proteins and induction of apoptosis (Bogoyevitch and Kobe, 2006; Ventura et al., 2006). Furthermore, *mkp-1*-null MEFs have a higher incidence of apoptosis when deprived of serum for 48 h compared with WT culture and lower proliferation rates in the presence of serum compared with WT cultures (Wu and Bennett, 2005). These results suggest that, in the absence of serum, MKP-1 expression targets JNK1, whereas a lower proliferation rate for mkp-1-null MEFs, in the presence of serum, could be the consequence of overstimulation of the Ras-Raf-MEK-ERK pathway, as this has a tendency to lead to reversible or permanent cell cycle arrest (Meloche and Pouyssegur, 2007).

The previously mentioned in vitro results are reflected by animal models, as studies in nude mice revealed that intraperitoneal but not subcutaneous growth of cell lines U28 and M18, clones of UCI101 and A2780, respectively, conditionally expressing MKP-1, have reduced tumor size in comparison with the same cell lines not induced to express MKP-1 (Manzano et al., 2002). Other results using nude mice revealed delayed and attenuated tumorigenicity when MKP-1 antisense-transfected PANC-1 or T3M4 cells are injected, compared with mock or untransfected cells (Liao et al., 2003). In addition, *nu/nu* mice that developed BT-474 cell-based tumors increased their MKP-1 protein levels 24 h after bortezomib treatment and decreased MKP-1 protein expression when they were injected with doxorubicin (a repair inhibitor and redox-mediating DNA damaging agent) (Minotti et al., 2004) compared with vehicle controls as assessed in tumor tissues.

Moreover, the combination of bortezomib and doxorubicin had intermediate levels of MKP-1 protein coupled to the highest apoptosis and phospho-JNK levels compared with either treatment alone. In addition to higher phospho-JNK levels, higher ERK1/2 levels were also found in this bortezomib/doxorubicin-treated xenograft model. These findings led the authors to conclude that such a combination of treatments could be an effective antitumor therapy, if the potentially self-limiting antiapoptotic effect of ERK could be avoided or inhibited (Small et al., 2004). The experiments with nude mice highlight the behavioral difference between tumor cell lines derived from various tissues. They also reveal that a tumor behaves differently, depending on the tissue that hosts its growth. The degree to which the environment exerts an influence on tumor biology, triggering adaptation, has important consequences for metastases as will be revealed in section V.D.6.

As a nuclear dual-specificity phosphatase, MKP-1 is well poised to dephosphorylate ERK and prevent it from initiating transcriptional activation, among other functions. As indicated by the plethora of factors in previous sections, there is no shortage of reagents that up-regulate MKP-1 protein or mRNA. However, up-regulation of mkp-1 mRNA or stabilization of MKP-1 protein, without interfering with the proapoptotic activity of JNK1 while reducing JNK-mediated antiapoptotic activity would not be straightforward. Another possibility relies on the difference in the MAPK binding site within MKP-1 itself. A modified MKP-1 molecule with a mutated JNK1 binding site to prevent inactivation of this MAPK, while allowing the ERK and p38MAPK (arginine residues 53–55) (Fig. 4) to bind to MKP-1 may be an alternative. An intervention that is more amenable than gene therapy for use in patients relies on the design of a small molecule that inhibits the JNK1-binding site within MKP-1 without affecting the ERK-p38MAPK-binding site. Such a strategy would require a more precise definition of the JNK1-binding site within MKP-1 and the three-dimensional structure of this phosphatase. All of these scenarios rely on identifying not only the MAPK isoform and splice form responsible for the tumorigenic phenotype but also the exact function it is playing at a given time point as the effectors and therefore the function of the kinase can vary across space and time.

5. Anchorage-Independent Growth. Soft agar is used for assaying anchorage-independent growth (Anderson et al., 2007) and also serves as a cloning assay (Agre and Williams, 1983). In an assay for anchorage-independent growth, MKP-1 antisense reduced the ability of PANC1 and T3M4 cells to form colonies in soft agar (Liao et al., 2003). A2780 and UCI101 cells, originating from papil-

across tumor types. If a lack of MKP-1 expression demonstrates a direct correlation with metastasis and not a bystander effect, then reexpression of MKP-1 under appropriate conditions may inhibit or delay proliferation at the site of metastasis. However, as ERK, p38MAPK, and JNK can participate in cell motility (see section V.C on MAPK and cancer) and motile cells are nonproliferative (Eccles and Welch, 2007), overexpression of MKP-1 may serve to curtail the motility aspect of metastasis to the secondary site. It is noteworthy that MKP-1 conditionally expressing cell line U28 decreases formation of lamellipodia and filopodia and cell motility when treated with doxycycline treatment (Manzano et al., 2002). This in vitro result should be confirmed in vivo, using cell lines conditionally expressing MKP-1 as a model of metastasis in animals, to distinguish the level of involvement of MKP-1 in metastasis versus a bystander effect. With timing of MKP-1 expression under control, differentiating variables, such as establishment of a premetastatic niche (Kaplan et al., 2005), proliferation, cell motility, inhibition of anoikis, invasion, and proliferation at the secondary site (Steeg, 2003), would be more feasible. As the microenvironment can affect tumor metastatic potential and the tumor can, in turn, affect its environment (Eccles and Welch, 2007), these studies may have to be repeated for different sites of primary tumor growth and secondary sites of metastases to account for these variables. However, this analysis is not enough. We should not forget that a metastasis is a clonal event and that each colonizing cell must survive from the primary tumor from which it detaches until it reaches the secondary site and proliferates (Eccles and Welch, 2007). Therefore, each metastasis represents a certain signature of genomic and proteomic expression profiles deriving from the primary tumor that can be modified to accommodate the site of metastasis. The use of multiple cell lines should be considered when animal models of metastasis are used to account for interclonal variability. Finally, as the current aim is to identify groups of genes involved in the process of metastasis, in addition to other aspects of tumor biology including survival and proliferation at the secondary site (Eccles and Welch, 2007), we should not be surprised that a given gene or its protein product, be it *mkp-1* or another, is not always involved in the survival, dissemination, or other aspects of metastasis or cancer biology.

7. Hypoxia. Under normoxic conditions (~21% O_2), HIF-1α is constitutively synthesized and degraded, whereas HIF-1β is constitutively synthesized and found in the nucleus and is not subject to regulation by the level of oxygen. Degradation of HIF-1α during normoxia involves a multistep process that includes prolyl hydroxylases, factor inhibiting hypoxia-inducible factor, and polyubiquitination by the von Hippel Lindau protein complex targeted for proteasomal degradation. Prolyl hydroxylation targets HIF-1α for polyubiquitination and proteasomal degradation. Factor inhibiting hypoxia-inducible factor asparagine hydroxylates HIF-1α, preventing its interaction with p300/CBP. One of the long-term consequences of HIF-1 function is angiogenesis, among others, and cancers that prevent the timely degradation of HIF-1α are highly vascularized (Kaelin, 2005).

As mentioned earlier, both hypoxia and ischemia have been associated with mkp-1 mRNA (Takano et al., 1995; Wiessner et al., 1995; Seta et al., 2001; Bernaudin et al., 2002; Liu et al., 2003, 2005a) and protein (Seta et al., 2001; Mishra and Delivoria-Papadopoulos, 2004; Liu et al., 2005a) regulation in various systems. In turn, MKP-1 can limit HIF-1 activity (Liu et al., 2003) via dephosphorylation of ERK (Liu et al., 2005a). More specifically, ERK phosphorylates p300 (Sang et al., 2003), a cofactor for HIF-1 activity (Arany et al., 1996) and HIF-1α (Richard et al., 1999), which increases HIF-1 transcriptional activity. In addition, MKP-1 expression was found to reduce the interaction of p300 with HIF-1 in HepG2 cells (Liu et al., 2005b). Conversely, ERK5 can phosphorylate HIF-1α and target it for polyubiquitination and proteasomal degradation, during hypoxia, in a prolyl hydroxylase-independent manner (Pi et al., 2005). In turn, ERK5 can be dephosphorylated and inactivated by MKP-1 and MKP-3 in an ERK1/2-mediated mechanism (Sarkozi et al., 2007). Adding to these results is the observation that hypoxia activates p38α and increases VEGF synthesis, a prelude to angiogenesis (Rousseau et al., 2000). Overall, this is an elegant example of how phosphatases and kinases interact to regulate each other and a key regulator of hypoxia, namely HIF-1α. This interaction assures an appropriate level of HIF-1 activity and the consequence of its activation.

Phosphorylation is not the only post-translational modification that modulates HIF-1α function. It is noteworthy that one of the possible post-translational modifications of HIF-1α (Brahimi-Horn et al., 2005) is cysteine nitrosation of HIF-1α (Yasinska and Sumbayev, 2003), via inducible nitric-oxide synthase or a NO donor. Nitrosation of HIF-1α activates its interaction with p300, whereas HIF-1α acetylation by ARD1 leads to its destabilization (Jeong et al., 2002). However, nitric oxide has a complex relationship with the components of this pathway, as it can stabilize HIF-1α under normoxia or inhibit its induction under hypoxia while inhibiting prolyl hydroxylase function, among other functions (Kaelin, 2005). As with many components of signal transduction, duration and timing may dictate the outcome of the NO interaction with HIF-1α. These seemingly unrelated observations come into their own when we consider that both p300 and CBP possess acetyltransferase activity (Iyer et al., 2004). Both H3 and H4 histone tails are required for p300-mediated acetylation of histones H2A and H2B, within chromatin and consequent transcriptional activation (An et al., 2002). The stress-related transcriptional activation of the *mkp-1* gene has been related to phosphorylation-acetylation of

histone H3 and chromatin remodeling (Li et al., 2001). Although these results are suggestive, direct evidence of p300 involvement in *mkp-1* gene transcription via chromatin remodeling is still wanting. It should be noted that in a previous study, histone H3 was acetylated on lysine 14 (Li et al., 2001) and that p300/CBP-associated factor is known to acetylate histone H3 (Herrera et al., 2000) on this residue (Lau et al., 2000). Although histone acetylation is a recognized modification at sites of actively transcribed genes (Saunders et al., 2006), histone H3 methylation, one of several histone post-translational modifications (Margueron et al., 2005), is associated with transcriptional elongation (Vakoc et al., 2005), a mechanism that not only controls mkp-1 mRNA (see section III.B.4 on mRNA elongation) but also the elongation of other messengers (Saunders et al., 2006). How ERK1/2, ERK5, and MKP-1, among other phosphatases and kinases, interact with HIF-1α to regulate gene transcription via histone modification and other mechanisms and conversely limit the effect of HIF-1α is a picture that needs further development.

8. Angiogenesis and Vasculogenesis. Adenoviral infection of HUVECs with the constitutively activated form of MEK6, MEK6E, the p38MAPK kinase, increases MKP-1 protein expression via p38MAPK, as pretreatment of these cells with SB203580 abolishes this increase. MEK6E also induces lamellipodia formation and cell migration in HUVECs. Furthermore, dominant-negative p38αMAPK inhibits the effects of MEK6E on lamellipodia formation, cell migration, and cell proliferation. The conclusion was that MKP-1 served, in part, to shift the balance of phosphorylated ERK and p38, which may have led to altered endothelial cell function (McMullen et al., 2005). Interestingly, among the p38MAPK KO mice, p38α is associated with chemotaxis (Rousseau et al., 2006) and with VEGF-mediated endothelial cell migration (Cuenda and Rousseau, 2007). Phosphorylated JNK and low MKP-1 protein expression levels also seem to favor angiogenesis in carcinomas (Shimada et al., 2007).

As MKP-1 inactivates p38α and JNK, its involvement in angiogenesis has been attributed to negative modulation of the MAPK pathway. In contrast, treating sections of descending aorta from WT mice and *mkp-1*-null mice with VEGF-A_{165} or thrombin revealed that endothelial cell sprouting was greatly reduced in *mkp-1*-null aortic sections, compared with similarly treated sections from WT control mice (Kinney et al., 2008). How do we reconcile these contradictory findings? The MAPKs were not equally affected by the absence of MKP-1, and residual activity (3- to 5-fold) was seen 3 h after VEGF stimulation, with JNK having the highest phosphorylation. At present the identity of the MAPK responsible for the failure of endothelial cell sprouting remains unknown. As each member of the MAPK family is involved in a multiplicity of functions, lack of endothelial cell sprouting could be caused by increased apoptosis due to JNK activation for extended periods of time, disruption in actin cytoskeleton remodeling affecting cell motility and chemotaxis, and disruption in p38MAPK-mediated mRNA stability possibly decreasing protein expression, among other normal functions attributed to the MAPK family (see section II on the MAPK family).

It is noteworthy that the *mkp-1*-null mice and their *mkp-1*(+/+) littermates had no overt phenotypic difference in the developmental phase or in various organ systems, such as the neurological, cardiac, hematological, and endocrinological systems (Salojin et al., 2006). At present the difference between the previous results (Kinney et al., 2008) and the in vivo findings in *mkp-1*-null mice (Salojin et al., 2006) are unclear. Although gross anatomy was unaffected, it remains possible that the capillary beds of organs in null mice may have a different cytoarchitecture than that found in WT murine organs. This issue needs to be addressed before any firm conclusion as to the difference between both studies can be drawn. Finally, from a practical standpoint, the use of MKP-1 to inhibit angiogenesis would need to rely on preventing the tumor from initiating the signaling cascade associated with angiogenesis rather than trying to affect endothelial cell biology using an MKP-1-based strategy.

Another player in angiogenesis and vasculogenesis was identified as ERK5. The importance of ERK5 in maintaining vascular integrity was revealed when a conditionally expressed ERK5 was "turned off" in adult animals with different efficiencies in various organs. These animals hemorrhaged from organs as a result of "leaky" capillary vasculature, resulting from large fenestrations and erythrocyte extravasation (Hayashi et al., 2004a). More specifically, the endothelial cells linking the various organs changed morphology and became round and apoptotic. The reduction/absence of ERK5 revealed a dependence of endothelial cells on ERK5 for survival and prevention of apoptosis (Hayashi et al., 2004a). Global (nonconditional) ERK5- or MEK5-null mice revealed cardiovascular developmental problems (Yan et al., 2003; Wang et al., 2005) that were reproducible in endothelial cell-specific ERK5-null mice, which also demonstrated vasculogenic and angiogenic problems (Hayashi et al., 2004a). The endothelial cell-specific ERK5 knockout mice revealed the same deficiency in yolk sac vasculature development as ERK5 global knockout mice (Hayashi et al., 2004a). Conversely, cardiomyocyte-specific ERK5 knockout mice had normal heart development up to 1 year. Thus, the phenotype associated with ERK5 global knockout mice and in endothelial cell-specific ERK5 knockout mice, was due to abnormal development of the endothelial cells lining the interior of the heart and not to the cardiomyocytes per se (Hayashi et al., 2004a). Finally, ventricular hypertrophy was accompanied by increased ERK5 activation (Kacimi and Gerdes, 2003), whereas overexpression of MEK5 also leads to cardiac hypertrophy (Nicol et al., 2001).

Section V.D.7 on hypoxia revealed that ERK5 was part of a network of kinases and phosphatases that regulate HIF-1 activity. ERK5 phosphorylated HIF-1α, leading to its polyubiquitination and proteasomal degradation (Pi et al., 2005). Completing the role of ERK5 in angiogenesis and vasculogenesis are the findings of this section, which reveal that ERK5 is essential for endothelial cell survival even in adult animals (Hayashi et al., 2004a). In addition, phosphatases such as MKP-1 and MKP-3 can dephosphorylate and inactivate ERK5, by an ERK1/2-mediated mechanism in renal epithelial cells (Sarkozi et al., 2007). Thus, in summary, MKP-1 has both proangiogenic, if endothelial cells express normal levels, and antiangiogenic propensities, if the tumor is expressing MKP-1. How all the players interact under normal circumstances and during tumorigenesis within a tissue to maintain, repair, and signal for neoangiogenesis still requires elucidation.

Overall it seems that, depending on the type of tissue the tumor derives from, the environment it is growing in, and the stage, grade, and more specifically the identity of the MAPK that is expressed (JNK1, ERK1/2, and ERK5), MKP-1 seems to have opposite functions depending on levels of expression and the cell type expressing it: 1) MKP-1 can help tumors to avoid apoptosis or can interfere with proliferation and tumor growth and inhibit angiogenesis; or 2) normal levels of MKP-1 are required for endothelial cell sprouting as demonstrated by the lack of MKP-1 being a detriment to endothelial cell sprouting in response to VEGF-A_{165} and thrombin. Adding to the complexity, chemotherapeutic agents can induce *mkp-1* expression. As MKP-1 targets MAPKs for dephosphorylation and given the level of cross-talk between the MAPK family member and their signaling modules (see section II), in addition to the presence of other phosphatases (Tables 1 and 2) (see also section IV), the outcome of a given therapy is anything but straightforward.

However, there is hope of untangling the MKP-1 knot. The finding that *mkp-1*-null mice are viable unless challenged by diet or LPS (see section V.B) allows the possibility of determining whether *mkp-1*(−/−) mice are more susceptible to chemically induced tumors than their *mkp-1*(+/+) littermates. Thereafter, as the tumor develops, it can be monitored in terms of proliferation, anchorage-independent growth, metastasis, apoptosis, and so on. The tumors from *mkp-1*-null mice can be compared with those of their WT littermates in terms of size, the development of capillary beds within the tumor, metastatic potential, and other variables. Such experiments can be fine tuned with conditionally expressed *mkp-1* cell lines injected into nude mice. Determining at what point in tumor biology is MKP-1 necessary and at which crossroads other phosphatases are able to bypass the need for MKP-1 is well within reach.

VI. Mitogen-Activated Protein Kinase Phosphatase, Cancer in Humans, and Relevant Models

This section is an overview of MKP-1 in relation to different tumors, but there are conflicting results regarding the importance of MKP-1 here as there have been in previous sections. These can derive from the activity or lack thereof of other phosphatases and kinases, whose presence was not determined. The absence of such information coupled with the complexities allowed by the interaction between the members of the MAPKs and their signaling modules, in addition to the phosphatases that inactivate them, easily accounts for the discrepant information.

A. Breast Cancer

Human breast cancers, ductal and lobular carcinomas, revealed constitutive levels of mkp-1 mRNA in the surrounding tissue, whereas lesions expressed higher levels and the more aggressive tumors had little to no mkp-1 mRNA expression (Loda et al., 1996). However, there is a strong correlation between the expression of the *neu* oncogene and ERK1/2 and MKP-1 protein expression (Loda et al., 1996). It is also possible that the *neu* oncogene (also called ERBB2 and HER2), by stimulating the Raf-MEK-ERK pathway (Grant et al., 2002), could protect ERK1/2 from inactivation by MKP-1 while this phosphatase inactivates JNK1 and p38MAPK, based on MKP-1 substrate specificity. In this scenario, MKP-1 expression could be deleterious. However, Ras overactivation, Raf, and ERK can induce cell cycle arrest (Hirakawa and Ruley, 1988; Smalley, 2003; Eymin et al., 2006; Dhillon et al., 2007). In this situation in which the *neu* oncogene correlates with MKP-1, phosphatase activity may reduce strong sustained ERK activity, favoring senescence or differentiation, which leads to sustained weak activity or transient ERK activity favoring proliferation (see sections II and V).

B. Gastrointestinal Cancers

1. Colon Cancer. Early lesions of human colon adenomata show robust mkp-1 mRNA expression, which is inversely proportional to tumor grade (Loda et al., 1996). This finding goes hand in hand with the concept that low-grade tumors avoid JNK1-induced death via MKP-1 up-regulation, whereas high-grade tumors down-regulate MKP-1 to allow maximal proliferation and possibly metastasize.

A recent study revealed that although both constitutively active forms of K-$Ras^{G12D/+}$ and N-$Ras^{G12V/+}$ were expressed in the murine colon epithelium, only the active form of K-$Ras^{G12D/+}$ produced hyperplasia but not neoplasia of the colon epithelium by increasing the number of progenitor cells (Haigis et al., 2008). K-$Ras^{G12D/+}$ but not N-$Ras^{G12V/+}$ signaled through the MEK-ERK pathway, whereas neither Ras isoform modulated

JNK activation. Conversely, both K-Ras$^{G12D/+}$ and N-Ras$^{G12V/+}$ decreased levels of phosphorylated AKT (Haigis et al., 2008). More importantly, K-Ras$^{G12D/+}$ activated MEK. However, although phosphorylated MEK was detected from the bottom to the top of the intestinal crypts, only the differentiated cells at the top of the crypts expressed high levels of phosphorylated ERK. This lack of ERK activation in other regions that revealed high phospho-MEK was most likely caused by up-regulation of MKP-3 (Haigis et al., 2008). As ERK can inhibit MEK and Raf (Fig. 2) (see also section II on MAPK cross-talk), increased MKP-3 can inactivate cytosolic ERK, leading to stronger MEK activation as detected (Haigis et al., 2008). MKP-3 is involved in mediating inhibition of cell proliferation and inhibition of cell differentiation, depending on the system being tested (Kim et al., 2004; Marchetti et al., 2004). Because there is hyperplasia in the colon of mice, in the study by Haigis et al. (2008), MKP-3 may have prevented cell differentiation induced by ERK. Other observations from the same study revealed that constitutively active N-Ras$^{G12V/+}$ protects murine colon epithelium from dextran sodium sulfate-induced apoptosis but not from radiation-induced apoptosis, whereas K-Ras$^{G12D/+}$ did not afford as much protection from apoptosis induced by these agents (Haigis et al., 2008). The same study also revealed that mice harboring K-Ras$^{G12D/+}$ or N-Ras$^{G12V/+}$ combined with a conditional allele of *Apc*(−/+) (the adenomatous polyposis coli gene) was sufficient to cause high-grade dysplasia and an increased number of tumors in K-Ras$^{G12D/+}$ *Apc*(−/+) but not in N-Ras$^{G12V/+}$ *Apc*(−/+) mice, compared with mice harboring wild-type K-Ras *Apc*(−/+) or N-Ras *Apc*(−/+). More importantly, MEK activation was shown to be unnecessary in K-Ras$^{G12D/+}$ *Apc*(−/+)-mediated tumorigenesis, even though Raf activity was essential for this transformation (Haigis et al., 2008).

APC is a multidomain protein involved in the development of intestinal and other cancers (Aoki and Taketo, 2007). Although the exact mechanism by which lack of APC or its C-terminal truncated form contributes to the formation of cancer is currently unknown, it is believed to involve mutations in the β-catenin-binding region. Furthermore, loss of APC leads to nuclear accumulation of β-catenin and the activation of transcription factors, part of the Wnt signaling pathway, which in the adult mice leads to cell proliferation and polyp formation (Aoki and Taketo, 2007). To put the previous observations in context, in unstimulated cells, β-catenin is found at adherence junctions, and excess cytosolic β-catenin is phosphorylated, within the context of a complex composed of APC, Axin, CK1α, GSK3β, and PP2A, targeted for ubiquitination and proteasomal degradation (Willert and Jones, 2006). In addition, APC and Axin are found in the nucleus and may regulate β-catenin in this compartment as well. Upon Wnt stimulation, APC, CK1α, andGSK3β are dissociated from their complex, leading to an accumulation of β-catenin. The β-catenin acts as a scaffolding protein for transcription factors and chromatin-remodeling complexes, among other proteins and complexes (Willert and Jones, 2006).

So how does all this tie in with the MAPKKK-MAPKK-MAPK pathway? ERK and Wnt3α/β-catenin can stimulate fibroblast proliferation, and siRNA to either ERK1/2 or β-catenin reduces fibroblast proliferation (Yun et al., 2005). Conversely, β-catenin can lead to down-regulation of activated ERK and decreased proliferation in an Axin-mediated lysosomal-dependent degradation of both WT H-Ras and H-Ras^{L61}. This effect is seen only in cells that have WT β-catenin, not in cells that have the β-catenin S33Y mutation, such as HepG2 and HCT-116 cell lines, because this is a nondegradable form of β-catenin (Jeon et al., 2007). Whereas GSK3β phosphorylates and targets β-catenin for proteasomal degradation (Liu et al., 2002), both AKT (Cross et al., 1995) and p38MAPK (Thornton et al., 2008) phosphorylate GSK3β at the N terminus and C terminus, respectively, inhibiting GSK3β function and allowing β-catenin to accumulate. Adding to these observations, Axin, as a scaffolding protein, can lead to JNK activation and apoptosis when Axin is overexpressed (Luo and Lin, 2004) and induces apoptosis by scaffolding p53, among other proteins (Lin and Li, 2007), whereas, conversely, *p53*-null mice develop spontaneous tumors (Donehower et al., 1992). A study using EB-1 human colon cancer cells revealed p53-dependent regulation of MKP-1, which was reflected by *p53*(+/+) MEFs up-regulating MKP-1 in response to stress, whereas *p53*(−/−) MEFs did not. In this context, MKP-1 overexpression induced apoptosis in colon cancer cells (Liu et al., 2008). These results are more interesting because nonphosphorylated JNK can target p53 for ubiquitination and proteasomal degradation (Fuchs et al., 1998a). Thus, MKP-1 inactivates JNK, inactive JNK targets p53 for degradation, and p53 up-regulates MKP-1 and increases apoptosis. This regulatory loop is itself modulated by other MAPKs, phosphatases, and scaffolding proteins.

Taken together, all these studies imply that the stoichiometry of scaffolding proteins, such as APC, β-catenin, and Axin contributes to the cell fate by determining subcellular localization and/or the activation status of proteins. In turn, kinases and phosphatases regulate each other via transcription factors to modulate the level of expression and activity. The consequence of kinase-phosphatase-transcription factor interaction/regulation, which may also depend on stoichiometry, contributes to the determination of cell phenotype.

2. Gastric Cancer. Human primary gastric tumors, adenocarcinomas, expressed increased MKP-1 protein levels compared with normal gastric tissues (Bang et al., 1998). An evaluation of ERK1/2 activity revealed greater levels in gastric tumors compared with patient-matched normal gastric tissue. The authors believed not only that the increase in MKP-1 expression was a consequence of

increased ERK1/2 activity but also that MKP-1 was contributing to carcinogenesis. Unfortunately, other MAPK family members (JNK1 and p38MAPK) were not evaluated. In light of the previously mentioned observations and experimental data, it is possible that the target of MKP-1 activity is JNK1 or p38MAPK and not ERK1/2. Another possibility is that MKP-1 reduces ERK1/2 activity without abolishing it, favoring proliferation over other outcomes (see section II.B on the ERKs).

3. Hepatocellular Carcinoma. Eighty percent of human hepatocellular carcinomas revealed little if any mkp-1 mRNA labeling by in situ hybridization (Loda et al., 1996). Tumor size and serum levels of α-fetoprotein were increased in MKP-1-negative tumors compared with MKP-1-positive tumors (Tsujita et al., 2005). In addition, disease and overall survival rates after hepatectomy decreased in patients whose tumors were not expressing MKP-1 compared with patients with MKP-1-positive tumors. Overall, the results suggested that MKP-1 could be used as an independent predictor of patient survival outcome after hepatectomy for patients with hepatocellular carcinomas (Tsujita et al., 2005). This is the only human-based study that cites MKP-1 as being an independent predictor of patient survival.

Two murine models of liver-specific p38αMAPK deletion, one noninducible and the other inducible, were used with the following results: 1) spontaneous tumors did not form in livers in either group of mice (noninducible or inducible), over the course of 1 year; and 2) the diethylnitrosamine-induced hepatocellular carcinoma murine model revealed that diethylnitrosamine-increased hepatocyte proliferation was mediated via the JNK/c-Jun pathway (Hui et al., 2007). Phosphatase expression (MKP1–7, Pac1, VHR, VH3, and DUSP8) revealed no difference at the mRNA level, in liver tumors or normal liver tissue of p38α conditional deletion mutant mice compared with liver tumors or normal liver tissue of their "floxed" control counterparts. There was no mention of phosphatase protein expression in this study (Hui et al., 2007). Another study used three murine models of liver specific c-Jun mutations: 1) an inducible c-Jun deletion mutant, 2) a nonconditional c-Jun deletion mutant, and 3) a JunAA mutant that cannot be phosphorylated in the N-terminal position by JNKs (Eferl et al., 2003). This study revealed that c-Jun was required for initiation and progression of chemically induced tumorigenesis, but not for the survival of the tumor in the late phases of tumor progression. It is noteworthy that whereas JunAA was sufficient for the induction of hepatic tumorigenesis (Eferl et al., 2003), N-terminal phosphorylated c-Jun was required for proliferation of adult hepatocytes and regeneration of liver mass after partial hepatectomy (Behrens et al., 2002). This study revealed different requirements for normal hepatocyte proliferation versus those for hepatocellular carcinoma. In addition, hepatocytes isolated from conditional c-*Jun*(−/−) and *p53*-null mice, more specifically c-*Jun*(−/−)*p53*(−/−) and c-*Jun*(−/−)*p53*(−/+) mice, revealed that c-*Jun*(−/−)*p53*(−/−) double null mice had reduced p53-mediated cell death upon TNF-α treatment, as measured by lactate dehydrogenase release, compared with c-*Jun*(−/−)*p53*(−/+) double mutant mice. Thus, c-Jun protects hepatocytes from p53-induced cell death, possibly by preventing transcription of the proapoptotic gene *noxa*, which is not required for tumor cell proliferation (Eferl et al., 2003). Overall, these results demonstrate that a given protein can have different functions in normal versus pathological settings. Even if a protein is not mutated, tumors may coerce proteins to function at atypical times or places. The untimely or inappropriate expression of a normal protein could give rise to gain of function in terms of protein-protein interaction, owing to the different domains, stoichiometric considerations, and subcellular distribution of the protein.

C. Lung Cancer

A comparison of all-*trans* retinoic acid-responsive and -unresponsive non–small-cell lung cancer (NSCLC) cells revealed that the early phase of *t*-RA-mediated inhibition of serum-induced JNK1 activity relied on blocking the activity of MKK4, whereas sustained inhibition of JNK1 activity relied on up-regulation of MKP-1 expression (Lee et al., 1999). Vicent et al. (2004) investigated MKP-1 expression in lung cancer and in cell lines. There was a higher MKP-1 expression in NSCLC versus small-cell lung cancer cell lines. Interestingly, there was no obvious relationship between individual clinicopathological variables or MAPK phosphorylation status, as evaluated via immunohistochemistry, and MKP-1 expression. However, in patients with NSCLC, MAPK activation was associated with stage of tumor. Nuclear JNK activation was associated with early stages of cancer, whereas activated p38MAPK was correlated with phosphorylated ERK in both the nucleus and cytoplasm. In addition, ERK was associated with advanced stages of tumors. Overall, for patients with NSCLC, there is an improved survival rate for those with $>50\%$ of tumor nuclei staining positive for MKP-1, versus those with $<50\%$ of tumor nuclei stained (Vicent et al., 2004). These findings lend support to the hypothesis that tumors survive JNK proapoptotic signaling via MKP-1 expression in early stages and down-regulate MKP-1 when ERK is expressed in late stages. The survival rates of patients expressing nuclear MKP-1 protein reflect this finding: although the overall amount of protein expression is important, the subcellular localization also needs consideration especially when one is determining whether a correlation exists between a given protein and patient survival outcome.

D. Urogenital Cancers

1. Renal Cell Carcinomas. Renal cancer cell lines Caki-1 and KU 20–01 (the latter was derived from clear

cell carcinoma) decreased MKP-1 protein expression and revealed persistent JNK1 activation and apoptosis in response to combined treatment with Ro-31-8220 and anisomycin (Mizuno et al., 2004). In contrast, renal cancer cell lines ACHN and 769P were refractory to such combined treatment, as their MKP-1 levels were not modified and JNK1 was not activated. Renal cell carcinomas that express MKP-1 may escape apoptotic cell death by limiting JNK1 activation (Mizuno et al., 2004).

2. Bladder Cancer. Although normal bladder urothelium expressed low levels of mkp-1 mRNA, the level of expression of this transcript was inversely proportional to the grade of the bladder tumor. These tumors were categorized in situ as high-grade transitional cell dysplasia and carcinomas. EGF receptor was expressed in late stages of bladder cancer, but there was no coexpression with mkp-1 mRNA (Loda et al., 1996). In bladder cancers, the down-regulation of MKP-1 may have coincided with down-regulation of JNK1, although this possibility was not verified. However, absence of MKP-1 may not be required for high ERK levels, as ERK1 activity was consistently higher in prostate, colon, and breast cancer tissues compared with levels in their normal counterparts, even in the presence of mkp-1 mRNA and protein, when detected (Loda et al., 1996). As the MKP-1 protein was not always detected, given the level of mkp-1 mRNA and protein regulation, it could be that the MKP-1 protein was phosphorylated and targeted for proteasomal degradation by ERK1/2.

Other results from human urothelial carcinoma cell lines expressing high JNK-low MKP-1 (UMUC14 cell line) and low JNK-high MKP-1 (UMUC6 cell line) levels revealed that JNK activation and low levels of MKP-1 correlated with the ability of a tumor cell line to induce angiogenesis in a chorioallantoic membrane assay (Shimada et al., 2007). This group also found that phosphorylated JNK and decreasing MKP-1 expression levels correlated with increasing grade, invasiveness, and increased amount of microvasculature in human urothelial carcinomas (Shimada et al., 2007). Thus, in this scenario, high levels of phospho-JNK corresponded to an aggressive tumor phenotype, not apoptosis. The mechanism that caused activated JNK to correlate with tumor grade and stage was unclear, and it was not known how long JNK activity lasted, as duration of JNK activity can have anti- or proapoptotic consequences (see section II.C on the JNKs). Furthermore, as ERK5 is involved in angiogenesis and vasculogenesis (see section V.D.8 on angiogenesis and vasculogenesis), it is presently unknown whether cross-talk exists between JNK and ERK5 or to what extent JNK is required for angiogenesis. In addition, some results clearly indicate that JNK can be prosurvival or prodeath: 1) experiments in nude mice revealed that Ras-transformed JNK1/2 double KO MEFs developed greater tumor mass than Ras-transformed WT MEFs in athymic nude mice as a result of reduced apoptosis generating both greater number and size of nodules (Kennedy et al., 2003); and 2) JNK1 is essential for survival of B-lymphoblasts (Hess et al., 2002).

The apparent contradictions between the previously mentioned results regarding JNK activity require the ability to distinguish between the possible outcomes of active or inactive JNK and to identify the multiple factors that correlate with advanced tumor grade and stage versus induction of apoptosis. In the present case, with JNK, some of these factors include 1) the effectors of active JNK when it is protumorigenic versus antitumorigenic (Bogoyevitch and Kobe, 2006), 2) the targeting of JNK effectors to proteasomal degradation by both unphosphorylated JNK (Fuchs et al., 1997, 1998a; Bode and Dong, 2007) and phosphorylated JNK (Gao et al., 2004; Ki et al., 2007), which regulates the availability of the effectors for cell function, 3) the active JNK-mediated increase in E3 ligase activity, part of protein ubiquitination and degradation, which will decrease effector levels degraded by this pathway (Gao et al., 2004; Chang et al., 2006), 4) the implication of AP-1, an effector of c-Fos and c-Jun, in the control of invasion (Ozanne et al., 2007), 5) the presence of other MAPKs, given the level of cross-talk between them (see section II.E on MAPK cross-talk), 6) the presence of ERK1/2, which can stabilize/destabilize MKP-1 (Brondello et al., 1999; Lin et al., 2003; Lin and Yang, 2006), 7) the presence of phosphatases, DUSP22 and DUSP23, that can activate MKK7, MKK4, and MKK6, the upstream kinases of JNK and p38MAPK (Shen et al., 2001; Chen et al., 2002; Takagaki et al., 2004); 8) scaffolding proteins such as JIP (Raman et al., 2007) that can interact with MKP7 and JNK, resulting in the inactivation of JNK (Willoughby et al., 2003), and 9) the presence of other dual-specificity phosphatases that inactivate JNK and other members of the MAPK family (Tables 1 and 2).

3. Prostate Cancer. Strong mkp-1 mRNA expression was detected in basal cells of the human prostatic acini (Loda et al., 1996), whereas MKP-1 protein localized to normal basal and secretory cells (Magi-Galluzzi et al., 1997). Overall, mkp-1 mRNA expression decreased as the prostate tumor grade advanced from the lower to higher grades (Loda et al., 1996, Magi-Galluzzi, 1997).

activities, there was an inverse correlation between the levels of mkp-1 mRNA expression and JNK1 activity but not ERK1 activity (Magi-Galluzzi et al., 1997). In patients treated with androgen ablation, neoplastic tumors were found to express low to undetectable levels of mkp-1 mRNA, although JNK1 protein expression remained detectable. In addition, an inverse correlation developed between mkp-1 mRNA expression and apoptosis after androgen ablation. In general, mkp-1 mRNA expression decreased after hormonal ablation, across all tumor grades. MKP-1 protein mirrored its mRNA expression. It was believed that MKP-1 expression correlated with the ability of human prostate tumors to evade apoptosis during the early stages of tumorigenesis (Magi-Galluzzi et al., 1997). Similar to the previously mentioned studies, hormone-refractory prostate carcinomas demonstrated lower mkp-1 mRNA and protein levels compared with benign prostate hyperplasia and untreated prostate carcinomas (Rauhala et al., 2005).

Noble rats were used as a model of sex hormone-induced prostatic dysplasia and carcinoma (Leav et al., 1996). ERK protein, mkp-1 mRNA, and protein expression were detected in both the dorsal and the lateral lobes of the prostate of wild-type rats and decreased after castration. Normal patterns of expression returned, with time, after exogenous testosterone treatment for several days (Leav et al., 1996). Interestingly, wild-type rats treated with a combination of testosterone and estradiol-17β, a known inducer of dysplasia in the dorsal and lateral lobes of the prostate (Noble, 1982), showed an overall decrease of mkp-1 mRNA and protein expression within dysplastic legions, whereas ERK levels were unaffected (Leav et al., 1996). It was believed that selective up-regulation of MKP-1 could contribute to the dysplastic phenotype via ERK mitogenic activity, as MKP-1 did not target ERK and decreased JNK activity.

Recently, both MEK5 and ERK5 have been involved in some facets of cancer. MEK5 overexpression in prostate cancer correlated with increased bony metastasis and poor patient survival outcome (Mehta et al., 2003). The same group found that ERK5 nuclear localization corresponded with poor prognosis and hormone-insensitive prostate cancer in a subset of patients (McCracken et al., 2007). In vitro, MEK5 overexpression increased proliferation, motility, and metastasis of prostate cancer cells (Mehta et al., 2003). Expression levels of nuclear phosphatases were not mentioned in these studies.

Adding to the previous findings, human prostate tumors revealed higher c-Fos and c-Jun proteins in nuclei of tumor epithelial cells, which correlated with more advanced stages of the disease. Interestingly, phosphorylated ERK also correlated with the presence of c-Fos and c-Jun. Although c-Jun correlated with disease recurrence in a subset of patients, c-Jun was not an indicator of patient survival outcome (Ouyang et al., 2008). c-Fos and c-Jun can be part of AP-1, which has been associated with control of invasion via up-regulation of genes favoring invasion and down-regulating genes inhibiting invasion (Ozanne et al., 2007). AP-1 is also implicated in proliferation (Shaulian and Karin, 2001). Thus, determining the mRNA levels of AP-1 target genes as well as the levels of their protein products may yield a correlation between the levels of the effectors and patient survival outcome. However, as AP-1 can be composed of Jun family homodimers or Fos and Jun family heterodimers, lack of correlation of one member of the family does not exclude involvement of other family members.

In summary, hormone-refractory prostate carcinomas as well as hormonal ablation down-regulate mkp-1 mRNA and protein across all tumor grades. These results are echoed by observations that the expression of *mkp-1* is inversely proportional to tumor grade. Although these findings confirm the importance of hormonal treatment in *mkp-1* regulation, as revealed in section III, there is no lack of agents that regulate *mkp-1* at the mRNA and protein levels. If MKP-1 modulation can lead to the control of hormone-refractory prostate carcinomas, finding an agent that bypasses the need for hormonal treatment, without stimulating mitogenesis, may be within reach. Furthermore, as overexpression of MEK5 and nuclear localization of ERK5 are associated with poor prognosis, MKP-1 expression or another nuclear phosphatase, may serve to decrease ERK5 activity and improve prognosis. Overall, determining whether MKP-1 or another phosphatase can be used to regulate prostate cancer biology will depend on the telltale signature of prostate cancer and which molecules are absolutely required for tumor development and progression in vivo.

4. Uterine Leiomyoma Cells. Leiomyomata are benign tumors that derive from smooth muscle cells (Cramer and Patel, 1990). Uterine leiomyoma cells (GM10964) down-regulated mkp-1 mRNA and protein levels after 17β-estradiol treatment, whereas normal uterine smooth muscle cells down-regulated only mkp-1 mRNA levels (Swartz et al., 2005). This difference in MKP-1 protein levels in GM10964 cells versus normal smooth muscle cells after 17β-estradiol may underlie the difference in proliferation between both cell lines. It would be interesting to determine whether ERK1/2 were involved in MKP-1 protein down-regulation after 17β-estradiol treatment in the context of uterine leiomyoma cells (GM10964) and other tumors.

5. Ovarian Carcinomas. MKP-1 protein expression was reduced in low-grade malignant tumors compared with benign cysts and normal surface epithelium. Conversely, ovarian carcinomas reveal the full spectrum of expression, from negative to strong MKP-1 protein expression. Although there did not seem to be a correlation between MKP-1 protein expression and overall patient survival, there was a significant correlation between MKP-1 protein expression versus a shorter progression-

free survival (the time it takes to detect the disease clinically or pathologically) (Denkert et al., 2002). It would seem that MKP-1 was permissive to the survival of ovarian carcinomas, based on the shorter progression-free survival data. In contrast, the lack of correlation between overall patient survival and MKP-1 expression would argue against such a conclusion. This seeming contradiction between shorter progression-free survival and overall patient survival outcome could have stemmed from the subcellular localization of MKP-1 protein and the duration of JNK activity, which can be modulated by MKP-1, dictating the antiapoptotic versus proapoptotic outcomes. Other phosphatases and MAPKs could also have contributed to patient survival outcome.

Another study compared the mRNA expression pattern of 68 phosphatases from immortalized ovarian surface epithelium with that of cancer cell lines and found that immortalized ovarian surface epithelium had higher mRNA expression patterns in 4 of the 68 phosphatases. MKP-1 was one of the phosphatases thus identified (Manzano et al., 2002). The MKP-1 expression pattern was characterized in primary ovarian cancers versus normal ovaries, and results revealed that MKP-1 protein expression was decreased in primary ovarian cancers compared with that in normal ovaries. The expression of MKP-1 also decreased with increasing stage of the disease (Manzano et al., 2002). Nude mice injected with ovarian cancer cell lines conditionally expressing MKP-1 had reduced tumor size in comparison with the same cell lines not induced to express MKP-1 (Manzano et al., 2002).

Both the Denkert et al. (2002) and the Manzano et al. (2002) studies revealed a tendency for tumors to lose MKP-1 expression with advancing tumor stage. However, to understand the contribution of MKP-1 to the process of tumorigenesis, the first group used a human survival study, whereas the latter used a nude mouse model and injected mice with cell lines conditionally expressing MKP-1. As both studies used different paradigms, the cause of divergence between their results could be ascribed to 1) different levels of MKP-1 expression, 2) different species, 3) tumors developing at their "natural site" (Denkert et al., 2002) versus tumors developed from cell lines injected at sites determined by experimentation (Manzano et al., 2002), and 4) heterogeneity of naturally occurring tumors versus the more homogeneous clones of cell lines used to inject nude mice.

In summary, in the early phase of cancer (low grade and early lesions), the tumor evades JNK1-induced death by up-regulating MKP-1 and in the more advanced stages of tumorigenesis down-regulates MKP-1 to allow for proliferation and increased tumor mass. As metastases have little if any *mkp-1* expression, it is possible that the absence of *mkp-1* heralds the metastatic process for a given tumor. There are exceptions to this observation, and MKP-1 can be expressed in conjunction with receptors such as Neu and EGF receptor, in advanced tumor grades. However, at this point there is no definitive evidence that MKP-1 can be used as a marker for patient survival in most cancers. The reason is that the consequence of MKP-1 activity depends, to a great extent, on the function of the MAPK at the time that MKP-1 (or another phosphatase) is inactivating it. Thus, if MKP-1 reduces JNK activity, it could favor antiapoptosis or proliferation over cell death. If MKP-1 targets ERK1/2, it may lead to enhanced proliferation instead of differentiation. If ERK5 activity is reduced, it may reduce angiogenesis or endothelial cell survival. If MKP-1 targets p38MAPK, it could prevent p38MAPK-mediated cell cycle arrest. Therefore, depending on the function of a particular MAPK, in addition to the interactions of all the other players at any given time point, MKP-1 or another phosphatase may skew the balance in one direction or another. The combination of these effects, among others, will determine cell fate.

VII. Conclusion

As shown in this review, although MKP-1 has a deceptively simple function as a dual-specificity MAPK phosphatase, the array of stimulants and the level of control at the mkp-1 mRNA and protein levels reveal a scenario that is anything but simple. Adding to this complexity, the ubiquitously expressed MKP-1 does not lead to an obvious phenotype when knockout mice are generated. This observation leads to the logical conclusion that the numerous members of the dual-specificity phosphatase family can compensate for the loss of MKP-1 during the developmental stage or that it is not required during development. In contrast, cardiac transgenic mice expressing approximately 4-fold normal levels are not viable past the 15-day postnatal time point, whereas a 2-fold increase allows the cardiac transgenic mice to survive. Considering these observations and all of the reports on MKP-1 and cancer, how can we use this information to understand the role of MKP-1 in cancer? Is it even relevant for cancer biology? The lack of phenotype in *mkp-1*-null mice and the obvious phenotype in transgenic mice present an interesting dichotomy. Keeping the effect of the genetic background of the mice as a possible explanation for the difference between null and transgenic mice, can overexpressing this phosphatase be used to target tumor proliferation or other aspects of cancer biology such as metastasis, angiogenesis, or differentiation?

Before answering such questions we need to take a hard look at where we stand. First, we should recognize that reports on the importance of MKP-1 in cancer have been divergent, with some implicating MKP-1 as a major player in cancer and others demonstrating that MKP-1 is present only as a bystander. This apparent contradiction stems, in part, from the function of MAPKs, which dictate the biological outcome under normal and patho-

logical circumstances. For instance, activation of ERK1/2 leads to proliferation, differentiation, and cell motility, among other phenotypes (Meloche and Pouyssegur, 2007; Raman et al., 2007). This multiplicity of phenotypes holds true for JNK1, which leads to cell survival or apoptosis (Bogoyevitch and Kobe, 2006), and for p38MAPK, which is implicated in cell differentiation and tumor dormancy, among other phenotypes (Zarubin and Han, 2005; Ranganathan et al., 2006) (sections II and V). Depending on which MAPK is expressed and what function it is implicated in, MKP-1 or other phosphatases will seem to have divergent effects as they inactivate their target MAPK.

Other factors also come into play in the MAPK-MKP-1 saga. For example, the subcellular distribution of active versus unphosphorylated MAPK can be recognized when a specific MAPK, such as ERK, is considered. As ERK is known to stabilize or destabilize MKP-1 via phosphorylation, the net result for a given cell of the ERK-MKP-1 interaction will depend on the mechanisms that determine ERK inactivation by MKP-1 versus ERK-mediated stabilization of MKP-1 or targeting of MKP-1 for degradation via polyubiquitination and the proteasomal pathway. In general, the consequences of phosphorylation are varied and thus far include activation, inactivation, stabilization, targeting for proteasomal degradation, and priming. Therefore the context of phosphorylation is crucial to understanding its ensuing consequences. As a result, how a stimulus affects a given cell type in relation to the ERK-MKP-1 interaction will depend on which of the three outcomes results from an ERK-MKP-1 interaction. In turn, this result will also unveil the effect on JNK1, p38MAPK, and ERK5 as they are also targeted by MKP-1. At present it is unknown whether JNK1, p38MAPK, or ERK5 can stabilize or target MKP-1 for degradation via phosphorylation as ERK1/2 do.

Adding to this complexity, unphosphorylated JNK can bind to and target its effectors for polyubiquitination and proteasomal degradation (Bode and Dong, 2007) (see also section II.C on the JNKs). As JNK has different targets in the cytosol versus the nucleus (Bogoyevitch and Kobe, 2006), the outcome of the function associated with unphosphorylated JNK will depend on chronological as well as spatial distribution. It is currently unknown whether binding to the unphosphorylated form of JNK1 can target MKP-1 for polyubiquitination and consequent degradation or whether ERK and p38MAPK can target their effector when they are in an unphosphorylated state. The subcellular distribution and activation states of ERK, JNK1, and p38MAPK will contribute to activation, inactivation, or degradation of cytosolic and nuclear effectors, gene regulation, and ultimately cell fate.

The situation regarding the axes of MAPK is far from being this linear. Cross-talk exists at every level of the MAPK signal transduction cascade and across MAPK modules(Zhang et al., 2001; Shen et al., 2003; Cuevas et al., 2007) (see also section II on MAPK cross-talk). We need to improve our understanding of the role that the various axes of the MAPK family have in cancer. For instance, there are four splice forms of JNK1 and JNK2 and two of JNK3 (Waetzig and Herdegen, 2005), several forms of p38MAPK (Olson and Hallahan, 2004), and the presence of monomers and dimers of ERK (Khokhlatchev et al., 1998; Adachi et al., 1999; Zambrowicz and Sands, 2003) in addition to all the isoforms of ERK, some of which, such as ERK3/4 and ERK7, await full characterization. As more data concerning all of these factors come to light, our understanding of MKP-1 and the modulatory role of other phosphatases will increase.

We should not forget other factors and observations. The finding that MKP-1 and MKP-3 can limit HIF-1α activity may be an important observation. This result needs to be confirmed in other tumor cell types as well as in xenograft tumor animal models. If proven to be a general effect in tumors, it would suggest, for instance, that MKP-1 is hardly expressed in advanced tumor grades when ERK1/2 are expressed or in metastases. However, ERK5 is involved in tumor proliferation and phosphorylation of HIF-1α and targeting of it for proteasomal degradation. As MKP-1 inactivates ERK5, characterizing how ERK1/2, ERK5, HIF-1α, and phosphatases (MKP-1, MKP-3, or other) function in primary tumor survival and how they "cooperate" during angiogenesis may reveal the mechanisms tumors use to strike a balance between proliferation and mediating angiogenesis among other functions.

We are now in a position to devise a method of answering the questions: Is the amount of MKP-1 relevant for cancer phenotype? More importantly, can MKP-1 overexpression be used to target tumor proliferation, metastases, angiogenesis, or other cancer phenotypes? To answer the first question, malignant tissue can be compared with its normal counterpart. All malignant tissues would need to be categorized according to type, stage, grade, gender, and chemotherapeutic, radiotherapeutic, or other treatments used, as these can also affect gene and protein expression. Thereafter, screening the malignant tissues and their normal control counterparts according to the localization of the MAPK isoform and splice form would need to be accompanied by screening for MKP-1 protein with emphasis on its localization to the nucleus. When unphosphorylated forms of MAPK are localized, the expression level of their effectors should be determined. Tumor banks are well suited for this type of study. However, to determine the activation status of these MAPKs, fresh biopsy specimens of relevant tumors may need to be obtained. For tumors that do not reveal a direct correlation between various aspects of their phenotype and MKP-1 expression, screening for other members of the dual-specificity phosphatase family may be in order, considering that they

not only target the same substrates but also have positive and negative modulatory roles on their target proteins.

For tumors that do demonstrate a correlative effect between their phenotype and MKP-1 expression, animal models can be devised to distinguish between a secondary role versus cause and effect of MKP-1 expression in relation to tumor phenotype. Thereafter, the answer to the question relating to the manipulation of MKP-1 expression levels to modify tumor biology can be addressed using animal models and cells conditionally expressing MKP-1. Cells with different oncogenic phenotypes, such as uncontrolled proliferation, metastasis, and angiogenic potential, may reveal changes in phenotype when expressing MKP-1 at different time points, as they develop into tumor masses within the context of an animal.

Another facet that may yield promising results is the targeting of the immune system. The number of phosphatases involved in immunomodulation (Tables 1 and 2) could represent a potential target for immunologically based antitumor therapy such as the mixed bacterial vaccine (Busch-Coley treatment). However, before such an effort could be undertaken, a more complete identification of the phosphatases involved in immunomodulation, a characterization of their role, and the exact mechanism relating bacterial infection to the ability of the immune system to recognize cancerous cells would need to be revealed in some detail.

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