

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

TAREK BOUTROS, ERIC CHEVET, AND PETER METRAKOS

Departments of Surgery (T.B., E.C., P.M.) and Anatomy and Cell Biology (T.B.), McGill University, Montreal, Quebec, Canada; Team Avenir, Institut National de la Santé et de la Recherche Médicale, U889, Bordeaux, France (E.C.); and Université Bordeaux 2, Bordeaux, France (E.C.)

Abstract	262
I. Introduction	262
II. Mitogen-activated protein kinase family: an overview	263
A. Ras-Raf-mitogen-activated protein kinase kinase/extracellular signal-regulated kinase kinase-extracellular-regulated kinase interactions	263
B. Extracellular-regulated kinases	264
C. c-Jun NH ₂ -terminal kinases	267
D. p38 mitogen-activated protein kinases	271
E. Mitogen-activated protein kinase cross-talk	274
III. Regulation of mitogen-activated protein kinase phosphatase-1 expression and activity	275
A. <i>mkp-1</i> gene, structure, promoter, and enhancer	275
B. <i>mkp-1</i> mRNA	276
1. Regulation of <i>mkp-1</i> mRNA expression levels	276
2. mRNA stability	277
3. De novo transcription	277
4. mRNA elongation	277
C. Mitogen-activated protein kinase phosphatase-1 protein function and affinity	278
1. Regulation of mitogen-activated protein kinase phosphatase protein expression levels	279
2. Protein stability and de novo protein synthesis	280
3. Protein activity	280
IV. Overview of the dual-specificity phosphatase family	281
V. Mitogen-activated protein kinase phosphatase, animal models, and cancer	283
A. <i>mkp-1</i> transgenic mice	283
B. <i>mkp-1</i> knockout mice	283
1. Phenotype	283
2. Immunological consequences	283
3. Diet-induced obesity	284
C. Mitogen-activated protein kinase and cancer: an overview	284
1. Ras-Raf cancer connection	284
2. Extracellular-regulated kinases	285
3. c-Jun NH ₂ -terminal kinases	285
4. p38 mitogen-activated protein kinases	286
D. Cancer-related mechanisms	287
1. Apoptosis	287
a. Platinum	287
b. Deacylation/demethylation	287
c. Glucocorticoids	288
d. Fas/Fas ligand	288
e. Mitogen-activated protein kinase phosphatase-1 and the proteasome	289
f. (Z)-1-[N-(2-Aminoethyl)-N-(2-ammonioethyl) amino]diazen-1-ium-1,2-diolate	289
g. Radiation	290

Address correspondence to: Tarek Boutros, Department of Surgery, Royal Victoria Hospital, McGill University, 687 Pine Ave. W., Montreal, QC, Canada H3A 1A1. E-mail: tarek.boutros@mail.mcgill.ca
This article is available online at <http://pharmrev.aspetjournals.org>.
doi:10.1124/pr.107.00106.

2. Antiapoptosis	290
3. Differentiation	291
4. Proliferation	291
5. Anchorage-independent growth	292
6. Metastasis	292
7. Hypoxia	293
8. Angiogenesis and vasculogenesis	294
VI. Mitogen-activated protein kinase phosphatase, cancer in humans, and relevant models	295
A. Breast cancer	295
B. Gastrointestinal cancers	295
1. Colon cancer	295
2. Gastric cancer	296
3. Hepatocellular carcinoma	297
C. Lung cancer	297
D. Urogenital cancers	297
1. Renal cell carcinomas	297
2. Bladder cancer	298
3. Prostate cancer	298
4. Uterine leiomyoma cells	299
5. Ovarian carcinomas	299
VII. Conclusion	300
Acknowledgments	302
References	302

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 progres-

sion, 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

MAP¹ 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; I κ B α , inhibitor of nuclear factor- κ B α ; 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)1H-imidazole; cisplatin, (cis-diaminedichloroplatinum II); LPS, lipopolysaccharide; Ro 31-8220, 3-[1-3-(amidinothio)propyl-1H-indol-3-yl]-3-(1-methyl-1H-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 G₀/G₁ 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; Sarközi 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; γ 2A/AT₁, 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]diazene-1-ium-1,2-diolate; NO, nitric oxide; PARP, poly(ADP-ribose) polymerase; NF- κ B, nuclear factor κ B; 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).

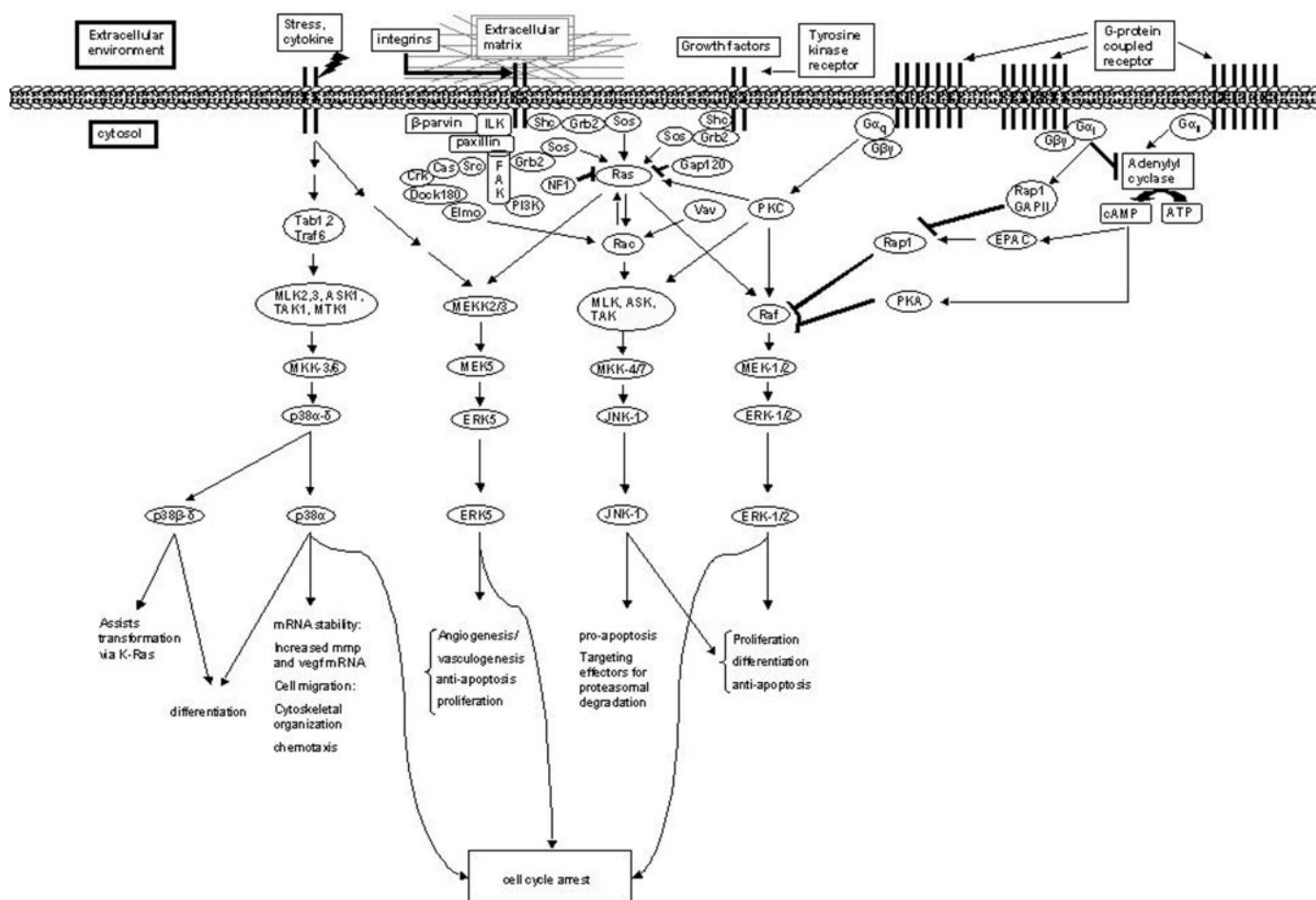


FIG. 1. Linear aspect of MAPK signal transduction. This figure summarizes some of the components in the MAPK signal transduction pathway from the extracellular milieu to the phenotypes associated with the more "classic" MAPK family members, with an emphasis on a linear interaction. SOS, Vav, and exchange 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 GAP II 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 uncou-

pled. 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-

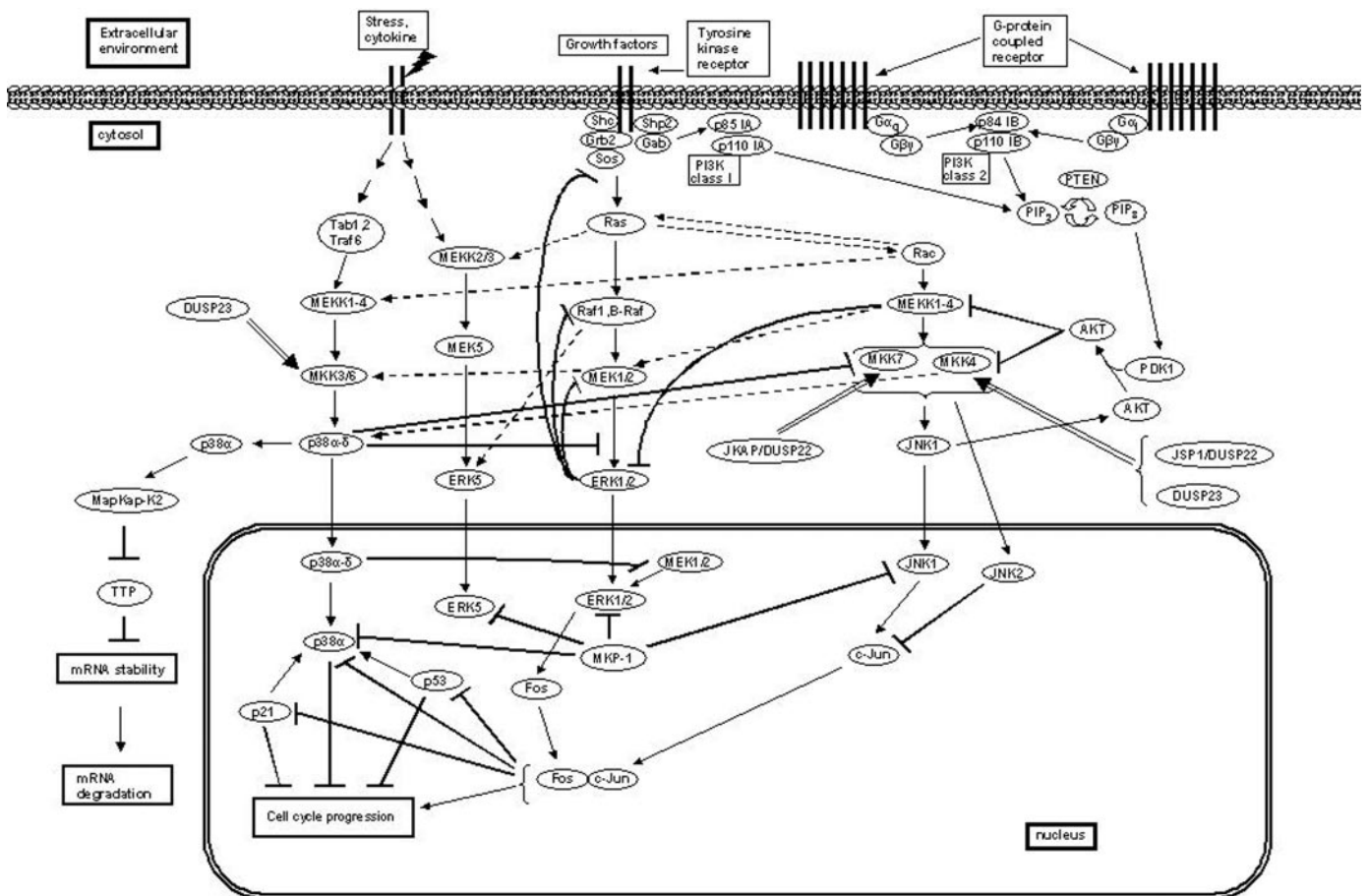


FIG. 2. Cross-talk of the MAPK signal transduction pathway. Summary of the cross-talk and feedback inhibition implicating the MAPK signal transduction pathway. Solid single arrows indicate activating signal transduction within a module (except JNK → Akt which is a priming phosphorylation). Hatched arrows 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; Hehlhans 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).

- **G₀-G₁ 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 G₀ to late G₁ (Jones and Kazlauskas, 2001).
- **G₁-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 G₁ phase by an AP-1-dependent mechanism (Yamamoto et al., 2006).

- **G₂-M transition:** ERK1/2 activity is required during the early G₂ phase and DNA damage checkpoints during G₂ (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 inter-

action 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, MEK5 α and MEK5 β . 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 (Hoefflich 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 p38 γ MAPK, 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* NH₂-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 NH₂-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: α 1 and β 1 splice forms (p46) and α 2 and β 2 splice forms (p54). The α 1 and β 1 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 α 2 and β 2 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 (α 1, α 2, β 1, and β 2) and molecular masses analogous to that of JNK1, whereas JNK3 has only the α 1 (p46) and α 2 (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 \rightarrow 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
<i>DUSP1, MKP-1, erp, CL100, HVH1, PTPN10, Ptpn16, 3CHL34</i>	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
<i>DUSP2, PAC-1</i>	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 Increased MKP-2 protein if Raf-1/MEK is active	Develop normally: <i>Dusp2</i> ^{-/-} mice protected from arthritis; increased JNK1 activity with decreased ERK and p38 activities	Rohan et al., 1998; 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
<i>DUSP4, MKP-2, HVH2, TYP</i>	Nuclear	ERK ~ JNK > p38 C-terminal domain of MKP-2 reduces its phosphatase activity	Ubiquitous, inducible	Exocrine pancreatic tumor cell lines inverse correlation between survival of rats and <i>mip-2</i> 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; Sjaban et al., 2005; Shen et al., 2006
<i>DUSP5, HVH3, Cpg21, Gm337</i>	Nuclear	ERK >> JNK ~ p38 ERK2 ~ 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 reveals 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
<i>DUSP6, MKP-3, PYST1, rVH6</i>	Cytosolic	ERK \gg JNK \sim p38 Monomer ERK binds to monomer MKP-3 ERK2 binding to MKP-3 \rightarrow rearrangement MKP-3 catalytic site MKP-3 (C ²⁹³ \rightarrow 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: presagittal paraxial mesoderm, limb bud, brachial arch mesenchyme, midbrain/hindbrain isthmus, nasal, dental, hair, and mammary placodes	Expression of <i>DUSP6</i> 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 MCF-7 cells 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 \rightarrow lack of <i>DUSP6</i> 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) 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	1) At embryonic days 8.5–17.5 F1 heterozygous offspring of germline chimeras: <i>DUSP6</i> (<i>MKP-3</i> -null mice have normal mendelian frequency with no obvious phenotype) 2) Postnatal development: <i>DUSP6</i> (<i>MKP-3</i> -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; Cur et al., 2006; Li et al., 2007; Arkell et al., 2008; Bermudez et al., 2008; Chan et al., 2008
<i>DUSP7, PYST2, MKPX, B59</i>	Cytosolic	ERK $>$ p38 \gg 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	Expression of <i>DUSP6</i> 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 MCF-7 cells 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 \rightarrow lack of <i>DUSP6</i> 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) 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	
<i>DUSP8, HVH-5, HVH8 Ntp1, M3/6, HB5</i>	Nuclear and cytosolic	JNK \sim p38 \gg ERK M3/6 binds to JIP1 to dephosphorylate JNK M3/6 can also bind to JIP2 JNK phosphorylates M3/6 \rightarrow 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	Expression of <i>DUSP6</i> 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 MCF-7 cells 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 \rightarrow lack of <i>DUSP6</i> 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) 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	Martell et al., 1995; Muda et al., 1996; Theodosou et al., 1996; Nesbit et al., 1997; Bernabei 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
<i>DUSP9, MKP-4, Pyst3</i>	Cytosolic Dependent on nuclear export signal and nuclear export protein CRM1	ERK \gg p38 $>$ JNK1 ERK2 $>$ ERK1 \gg ERK3, ERK5, ERK7 p38 α \gg p38 γ ; p38 δ	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 \rightarrow 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 γ/γ are born healthy and fertile males γ/γ derive from females γ/γ \times males γ/γ	Muda et al., 1997; Dickinson et al., 2002b; Christie et al., 2005; Liu et al., 2007
<i>DUSP10, MKP-5</i>	Nuclear and cytosolic	p38 $>$ JNK \gg ERK p38 α , β \gg p38 γ , δ	Inducible Some expression in human liver and skeletal muscle; mouse heart, lung, liver, skeletal muscle, and kidney.	Decreased up-regulation of <i>mkp-5</i> mRNA in human prostate cancer and squamous cell carcinoma; low 1,25-D is associated with increased risk of prostate cancer in older men Overexpression of <i>mkp-5</i> reduces apoptosis in nonstressed p53-null cells expressing WT p53, constitutively active JNKK, and <i>mkp-5</i> 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; Theodorosou et al., 1999; Buschmann et al., 2001; Zhang et al., 2004; Nomn et al., 2006
<i>DUSP16, MKP-7, MlepM</i>	Cytosolic	JNK \sim p38 \gg ERK p38 α , β \gg p38 γ , δ MKP-7 binds to JIP1 to phosphorylate JNK2 MKP-7 can also bind to JIP2 MKP-7 binds to β -arrestin 2 to phosphorylate 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 Phosphorylation: 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 Presumed catalytically inactive Cys \rightarrow Ser substitution at catalytic site	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; Matsunuchi et al., 2001; Fanoue et al., 2001; Froomaert et al., 2003; Masuda et al., 2003; Willoughby et al., 2003; Katagiri et al., 2005; Willoughby and Collins, 2005	
<i>STYXL1, DUSP24, MK-STYX</i>			Expression is increased in Ewing's sarcoma family of tumors	Expression is increased in Ewing's sarcoma family of tumors		Wishart and Dixon, 1998; Shigan et al., 2005

mTOR, mammalian target of rapamycin; FGF, fibroblast growth factor; 1,25-D, 1,25-dihydroxyvitamin-D₃; 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 I κ B α (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 non-stimulated 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 p38 α MAPK 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. p38 α MAPK, which is the most extensively characterized of its family members, has a wide expression pattern and so does p38 β MAPK (Cuenda and Rousseau, 2007; Mayor et al., 2007). p38 γ MAPK is expressed in skeletal muscle, whereas p38 δ MAPK 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 p38 α MAPK 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 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.

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 in Tumor Cell Lines and in Vivo Tumors	Knockout Mice Phenotype	References
<i>DUSP3, VHR, T-DSP11</i>	Nuclear	ERK ~ JNK >> p38 STAT5 dephosphorylation VHR (Y ¹⁶⁵) may create binding for STAT5 SH2 domain VHR (C ¹²⁴ →S); VHR (Y ¹³⁸ →F); either mutation → dominant negative for STAT5 dephosphorylation VHR (Y ¹³⁸) autodephosphorylation intra- or intermolecular	Constitutive Human: heart, brain, skeletal muscle, breast, ovary	VHR siRNA-mediated decrease induced cell-cycle arrest in HeLa cells VHR highly expressed in HeLa, SHa, C636, C33, and HT3 cervical cancer cell lines with nuclear localization compared with HT3 primary keratinocytes (from hysterectomies) that have no VHR nuclear staining High-grade squamous intraepithelial lesions and squamous cell carcinomas are associated with strong nuclear and cytoplasmic VHR protein Primary cervical adenocarcinomas and adenocarcinoma in situ are also associated with high VHR staining in cytoplasm, but no nuclear immunoreactivity VHR may be a marker for cervix carcinoma		Kamb et al., 1994; Todd et al., 1999; 2002; Rahmouni et al., 2006; Hoyt et al., 2007; Henkens et al., 2008
<i>DUSP12, GKAP, HYVH-1, LMW-DSP4, T-DSP4, VHI, mVHI</i>	Cytosolic	No specificity toward MAPK. May increase level of phospho-ERK1 Dephosphorylates pNPP and a phosphotyrosine-containing peptide "RAYTIDE" but not a phosphoserine-containing peptide Dephosphorylates and accelerates glucokinase activity No specificity toward MAPK MDSP has phosphatase activity toward P-Tyr and P-Thr Unknown substrate No MAPK specificity Dephosphorylates P-Tyr (MBP) and P-Thr (MBP) with similar efficiency Possibly active before substrate binding JNK > ERK ~ p38	Inducible: expression follows cyclin D1 accumulating during G ₁ /S phase in NIH 3T3 Some expression in mouse kidney, liver, lung, heart, thymus, spleen, brain, and skeletal muscle Skeletal muscle (protein) Highest levels in adult mouse Testis: highest levels in adult mouse (protein)	Increased expression owing to increased gene copy number in LS3x, LS21, LS43, and MS8x sarcomas		Munoz-Alonso et al., 2000; Zhang et al., 2000; Aoki et al., 2001; Kresse et al., 2005
<i>DUSP13A, MDSP, BEDP</i>	Cytosolic					Chen et al., 2004
<i>DUSP13B, TMDF, TS-DSP6</i>						Nakamura et al., 1999; Chen et al., 2004; Kim et al., 2007
<i>DUSP14, MKP6, MKP-L</i>	At the plasma membrane		Ubiquitous	HCT-116 cell lines transfected with Kruppel-type zinc-finger protein (ZBP-89) up-regulates JNK1/2 phosphorylation by down-regulating MKP-6 (DUSP14) expression, leading to enhanced apoptosis		Marti et al., 2001; Bai et al., 2004
<i>DUSP15, VHY, LMW-DSP10, T-DSP10</i> <i>DUSP18, DUSP20, LMWDSP20</i>	Cytoplasmic face of plasma Membrane (myristoylated) Cytosolic and nuclear	JNK >> p38 ~ ERK	mRNA Testis protein (pachytene spermatocytes) Adult: ubiquitous Fetal: all tissues tested Interaction between C-terminal domain of DUSP18 and its catalytic domain may enhance stabilization of catalytic domain and account for its thermal stability: the optimum activity for DUSP18 is at 55°C and 80% of WT activity is retained at 70°C	All carcinoma cell lines and cell "normal" lines tested		Alonso et al., 2004b Wu et al., 2003, 2006b; Jeong et al., 2006

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
<i>DUSP19, LDP-2, SKRP1, TS-DSP1, DUSP17, LMW-DSP3</i>	Cytosolic	JNK >> ERK ~ p38 SKRP1 also interacts directly with MKK7 and ASK1	Mouse: ubiquitous Human adult: heart, lung, liver, pancreas Testis	LMW-DSP2 interacts directly and dephosphorylates STAT3 in murine testicular GC-1 cells; LMW-DSP2 also prevents STAT3 translocation to the nucleus	JKAP-null mice are borne with predicted mendelian ratios and are healthy throughout adult life	Nakamura et al., 2002; Zama et al., 2002a,b; Cheng et al., 2003 Hood et al., 2002
<i>DUSP21, LMWDSF21</i>	Cytosolic and nuclear	p38 >> JNK > ERK VHX inactivates ERK-2 JKAP associates with MKK7 JKAP-null cells have reduced JNK activation in response to cytokines	Lymphoid cells adult murine tissue: heart, brain, liver, kidney and testis	DUSP22 mRNA increases in response to estrogen in ER α -positive human breast cancer cell lines MCF-7 and T47D Luciferase reporter assay in MCF-7 or HeLa cells: 1) DUSP22 siRNA enhanced estrogen/ER α -induced luciferase activation (HeLa cells were ER α -transfected, MCF-7 endogenous ER α); 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		Aoyama et al., 2001; Shen et al., 2001; Alonso et al., 2002; Chen et al., 2002; Sekine et al., 2006, 2007
<i>DUSP22, LMW-DSP2, TS-DSP-2, VHX, JSPI, JKAP, MKPX</i>	Cytosolic and nuclear (myristolated)	JSP1 activates MKK4 LMW-DSP2 dephosphorylates STAT3 DUSP22 dephosphorylates ER α at Ser-118		Pull-down assays indicate that DUSP22 interacts directly with ER α		
<i>DUSP23, MOSP, VHX, LDP-3, DUSP25, FLJ20442</i>	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	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		Alonso et al., 2004a; Takagaki et al., 2004, 2007; Wu et al., 2004a; Agarwal et al., 2008
<i>DUSP26, MKP8, NATA1, SKRP3, LDP-4</i>	Nuclear and Golgi apparatus	p38 >> JNK ~ ERK	Mouse: skeletal muscle and brain, cerebellum Mouse LDP-4 expressed in brain except hippocampus			Vasudevan et al., 2005; Hu and Mivechi, 2006; Takagaki et al., 2007; Yu et al., 2007
<i>DUPD1, DUSP27, FMDSP</i>	Cytosolic		Skeletal muscle, liver and adipose tissue			Friedberg et al., 2007
<i>DUSP28, VHP</i>						
<i>PTPMT1, MOSP, PLIP, PNAS-129</i>	Mitochondrial: anchored to the matrix face of the inner mitochondrial membrane	Currently unknown Knockdown of <i>PTPMT1</i> 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 <i>PTPMT1</i> but not the exocrine-derived cell line PANC-1	Knockdown of <i>PTPMT1</i> 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		Strausberg et al., 2002 Pagliarini et al., 2005
<i>STYX, hStyxb</i>		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; pNPP, *p*-nitrophenyl phosphate; P, phospho; MBP, myelin basic protein; ER α , estrogen receptor- α ; VHP, VHL-like phosphatase.

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, p38 α MAPK and p38 β MAPK 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, p38 δ is associated with keratinocyte maturation (Efimova et al., 2003; Cuenda and Rousseau, 2007). In the same line of thought, p38 α MAPK also participates in inhibition of cellular proliferation (Mikhailov et al., 2005; Heasley and Han, 2006; Perdiguero et al., 2007).

p38 α MAPK 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 p38 β , p38 γ , or p38 δ MAPK reveal no overt phenotype and develop normally. Null mice for p38 α MAPK 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 p38 α MAPK. Results from a recent study revealed that p38 α embryo-specific knockout mice [placentas are p38 α (+/+)] 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 *p38 α* 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 \rightarrow MAPKK \rightarrow MAPK model. However, evi-

dence 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 p38 α - and p38 β MAPK-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 *p38 α MAPK* genes revealed normal liver regeneration (Stepniak et al., 2006; Wada et al., 2008). Together p53 and p21 increase phosphorylation levels of p38 α , and all three inhibit cell cycle progression, whereas c-Jun, the downstream effector of JNK, inhibits p38 α , 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 p38 α 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 p38 α 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) p38 α 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

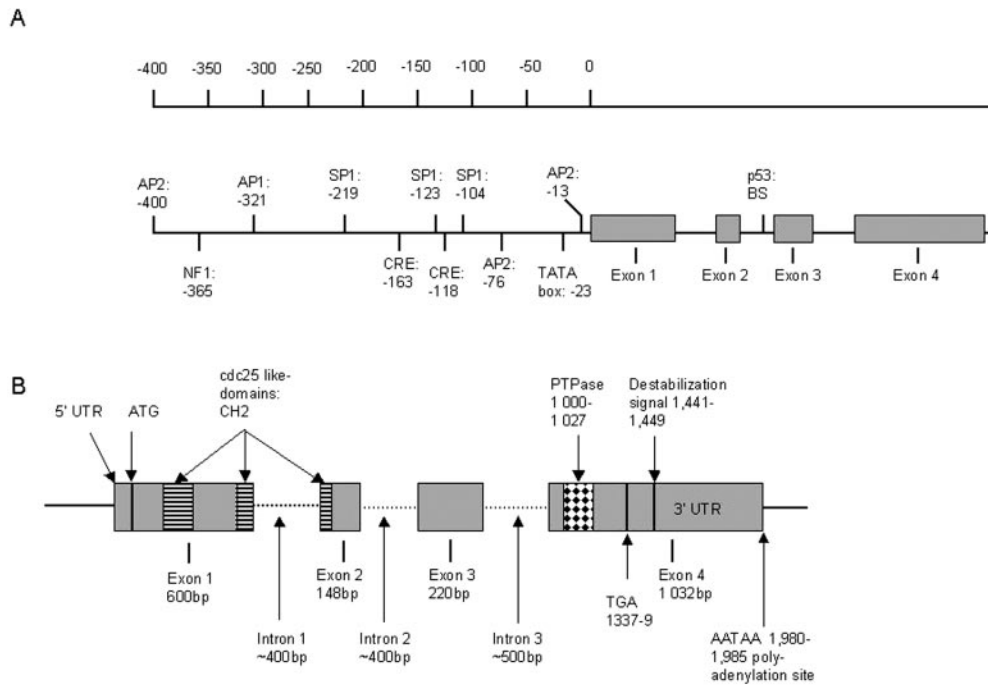


FIG. 3. MKP-1 promoter and gene. A, although the MKP-1 promoter demonstrates the potential for binding many transcription factors, caution should be used when inferring the binding of a transcription factor even when its consensus sequence is present in the promoter region, because flanking regions may 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) (Görlach 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 (Görlach 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; Görlach 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 mRNA (Dean et al., 2004), does so in a microRNA (miRNA)-dependent manner (Jing et al., 2005). Furthermore, TTP is involved in mRNA decapping (Fenger-Gron et al., 2005; Simon et al., 2006), which is an important step for mRNA degradation (Chan and Slack, 2006; Simon et al., 2006). At present neither the decapping mechanism nor the involvement of miRNA have been confirmed as mechanisms for *mkp-1* mRNA degradation. Modulating the *mkp-1* mRNA is not presently considered for therapy, whereas modulating the stability of other mRNAs is being considered (Eberhardt et al., 2007). As the MKP-1 protein has a short-half-life (see section III.C.2 on protein stability), targeting the mRNA expression could represent a therapeutic target. The potential of using *mkp-1* mRNA stability as a means of control is further

highlighted by observations demonstrating the involvement of MKP-1 substrates, p38MAPK, JNK1, and ERK1/2, in the signal transduction cascade relating to the stabilization of mRNAs (Eberhardt et al., 2007).

3. De Novo Transcription. Although previously mentioned studies using either hypoxia or ischemia did not reveal the mechanism of *mkp-1* mRNA regulation, in the human cervical carcinoma cell line SiHa, hypoxia up-regulates *mkp-1* mRNA expression without stabilization; this mechanism is thought to protect hypoxic cells against apoptosis (Laderoute et al., 1999). Treatment of mesangial cells with hydrogen peroxide induced transcription of *mkp-1* messenger, in an AP-1-dependent manner, without affecting stability (Xu et al., 2004). Of note, although the ectopic expression of MKP-1 induced apoptosis in untreated mesangial cells, it also reduced hydrogen peroxide-mediated apoptosis. Conversely, catalytically inactive MKP-1 neither induced apoptosis in untreated cells nor protected cells from apoptosis upon hydrogen peroxide treatment. Thus, although MKP-1 reduced oxidative stress-mediated apoptosis, inactivation of basal levels of ERK1/2 activity may account for the apoptosis induced by ectopic expression of MKP-1 in untreated cells (Xu et al., 2004). Using MKP-1 expression to inactivate the MAPK member that is most expressed in the cell is an interesting prospect. However, once the levels of the targeted MAPK become controlled or the stressor is inactivated, MKP-1 may target other members of the MAPK family if it continues to be expressed. This should be considered in the event of therapeutic targeting of MKP-1. A possible resolution to this problem may lie in the flexibility of the system. The ability to up-regulate mRNA without its concomitant stabilization not only allows greater flexibility in manipulating the system but also permits the assaying of the gene where transcription is concerned. Lack of efficient transcription can be distinguished from lack of stabilization of the mRNA; therefore, a more precise therapeutic intervention can be envisaged should the need arise.

4. mRNA Elongation. The calcium-mediated regulation of gene transcription is quite complex, as it can depend on the frequency of Ca²⁺ oscillation (Dolmetsch et al., 1998), the subcellular localization (Hardingham et al., 1997; Hardingham and Bading, 1999), and the amplitude and duration (Dolmetsch et al., 1997). In line with the previously mentioned complexity, a prolonged Ca²⁺ signal induces a much greater MKP-1 up-regulation compared with a transient Ca²⁺ signal in the neuronal-like CA-77 cell line (Durham and Russo, 2000). Although the importance of calcium-mediated *mkp-1* gene regulation in Rat-1 fibroblasts (Cook et al., 1997; Scimeca et al., 1997) is known, the mechanism by which Ca²⁺ mediates this regulation remained elusive.

A possible mechanism by which Ca²⁺ up-regulates *mkp-1* mRNA is described for the rat neuroendocrine cell line GH4C1 (Ryser et al., 2001). When the GH4C1 cell line is treated with thyrotropin-releasing hormone, a

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 (Görlach et al., 2006; Høyer-Hansen and Jäättelä, 2007), could the calcium-sensitive block in elongation also be part of an ER stress response (Görlach 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 (Sarközi et al., 2007). However, MAPK binds to different sites within MKP-1; indeed, whereas MKP-1 binding of ERK2, ERK1, and p38 α MAPK 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 p38 α (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 p38 α MAPK 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-

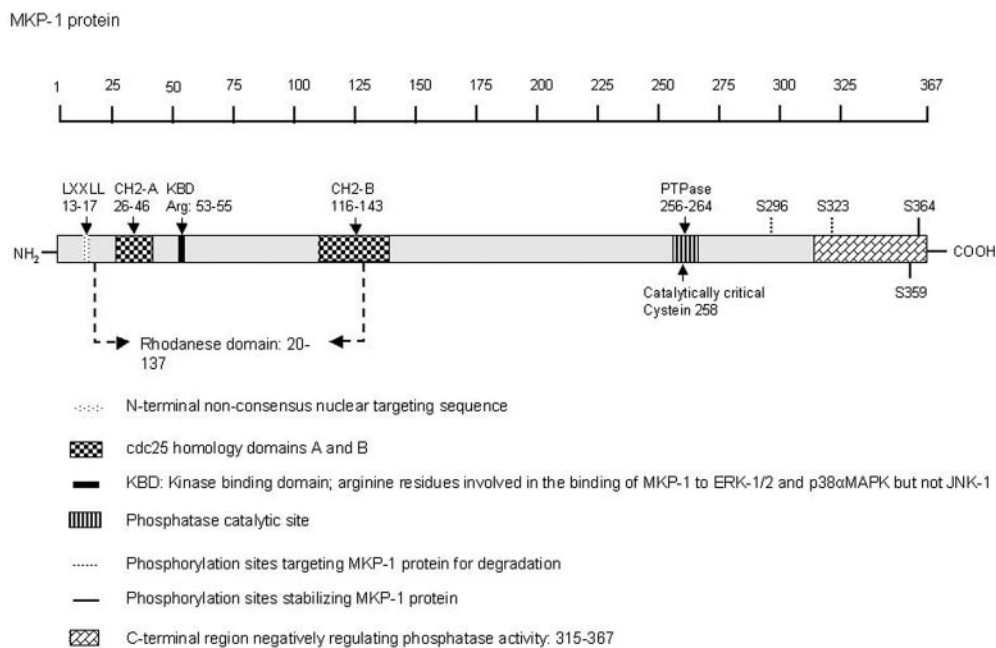


FIG. 4. MKP-1 protein. Although the N-terminal aspect of the protein is responsible for the nuclear localization and the binding of the MAPK (ERK1/2 and p38 α MAPK, the residues responsible for binding JNK-1 await identification), the C-terminal part contains the catalytic and stabilization/destabilization 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 NH₂ 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 ³⁵⁹Ser and ³⁶⁴Ser 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 ²⁹⁶Ser and ³²³Ser 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 PKC δ 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 p38 α /p38 β MAPK, 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 such as benzofuran block MKP-1 protein function (Lazo et al., 2006), whereas others, such as Ro 31-8220, inhibit kinase activity (McKenna and Hanson, 1993; Hers et al., 1999; Chepurny et al., 2002; Hofmann, 2004) and also prevent MKP-1 protein expression (Beltman et al., 1996; Zhang et al., 2003). Sanguinarine, an alkaloid plant poison (Garcia et al., 2006) known for its antibacterial activity (Vollmer, 2006), has also been characterized to inhibit MKP-1 activity and, as a result, increases levels of phospho-ERK and phospho-JNK after a 30-min treatment in the human pancreatic cancer cell line PANC1 (Vogt et al., 2005). Nevertheless, a direct link associating sanguinarine-induced cell death (Ding et al., 2002) and reduced MKP-1 activity is still wanting.

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 res-

idue 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 chemoattractant 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 1 α , 1 β , 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 Gadd45 γ 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 skel-

etal 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 (p110 α - 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; Hehlhans et al., 2007; Mayor et al., 2007; Roberts and Der, 2007; Schubert et al., 2007), phospholipase C ϵ , 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 (GSK3 β) (LoPiccolo 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 MEK2-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* NH₂-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 NCI-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 NCI-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 leuke-

mia (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-Otín, 2002). p38 α is also involved in angiogenesis; this topic will be covered in section V.D.8 on angiogenesis and vasculogenesis. Although, HSP27 is implicated in p38 α MAPK-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). p38 α / β MAPK 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 p38 α activity (Faust et al., 2005), whereas p38 γ 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; Kittipattarin 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 p38 α KO mice are embryonically lethal, as mentioned in section II on the MAPK family, p38 α 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, p38 α MAPK, 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 p38 α MAPK (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 p38 α MAPK-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 (γ 2A/AT₁) or in combination with Jak2 (γ 2A/AT₁/Jak2), Jak2 inactivates ERK2 in 30 min in an MKP-1-dependent manner. In the absence of Jak2, ERK2 inactivation requires 120 min (Sandberg et al., 2004). As cytosolic MAPK has effectors different from those of nuclear MAPK (Yoon and Seger, 2006), by selectively inactivating the latter, MKP-1 can inhibit MAPK-mediated transcription of genes while allowing MAPK to maintain signal transduction capabilities within the cytosol.

D. Cancer-Related Mechanisms

Many of the chemotherapeutic agents mentioned in this section have been approved by the Food and Drug Administration for certain types of cancers when they are used alone or in conjunction with other chemotherapeutic agents, radiotherapy, or surgical intervention. The following list serves as an example (for a more complete listing the reader is directed to the Web site <http://www.cancer.gov/cancertopics/druginfo/alphalist>). Among uses of the reagents, 1) bortezomib has been

approved for multiple myeloma, 2) cisplatin for ovarian cancer that has metastasized and for bladder cancer, 3) decitabine for myelodysplastic syndrome, 4) doxorubicin to treat several types of cancers, and 5) paclitaxel for metastatic and recurrent breast cancer. In addition, as this section is divided into different segments according to cancer phenotype, the chemotherapeutic agents have been cross-referenced accordingly.

1. Apoptosis. Because the increase in MKP-1 expression is linked to a decrease in JNK activity and a corresponding increase in cancer cell survival via a decrease in JNK-mediated apoptosis (Mizuno et al., 2004), various molecules have been used to increase apoptosis in an attempt to thwart the growth of tumors. It should be emphasized that none of the following compounds or any stress or growth factor previously mentioned, targets *mkp-1* exclusively except *mkp-1* siRNA/shRNA. The following compounds usually possess pleiotropic effects, and MKP-1 regulation is only one facet when it occurs. How the presence of MKP-1, whether endogenous or exogenously introduced, affects the function of these compounds will be addressed.

a. Platinum. Platinum-based drugs such as cisplatin and transplatin are well established chemotherapeutic agents that act (in part) by cross-linking DNA (Pascoe and Roberts, 1974a,b; Zwelling et al., 1979; Giraud-Panis and Leng, 2000). The difference in method of cross-linking is believed to underlie the effects of the *cis*-isomer, in its ability to block transcription and DNA replication in some tumors, whereas the *trans*-isomer is ineffective (Boudvillain et al., 1995; Mymryk et al., 1995; Siddik, 2003; Wang and Lippard, 2005). The result of the interaction between cisplatin and the molecular machinery of a tumor is multifactorial and incompletely understood (Siddik, 2003; Wang and Lippard, 2005). Recently, cisplatin was found to up-regulate MKP-1 in human lung H460 and ovarian OVCAR3 cancer cell lines (Wang et al., 2006). Furthermore, *mkp-1*-null MEFs were more sensitive to cisplatin-induced apoptosis in a JNK-dependent manner, whereas the wild-type counterparts were more resistant. Indeed, the JNK inhibitor SP600125 blocked cisplatin-mediated phosphorylation of JNK and increases cell survival by decreasing cisplatin-induced apoptosis (Wang et al., 2006). In addition, cisplatin is a strong inducer of JNK1 activity but weak inducer of MKP-1 expression, whereas transplatin up-regulates MKP-1 and weakly induces JNK1 activity (Sánchez-Pérez et al., 1998, 2000). These results mirror the survival of Pam212 and 293T cells, as cisplatin induces more cell death than transplatin.

b. Deacetylation / demethylation. 5-Aza-2'-deoxycytidine (decitabine) can cause reexpression of a gene that was hypermethylated and, as a result, inactivated as long as the treatment continues (Baylin, 2005). Decitabine is a deoxycytidine analog that is capable of being incorporated into DNA after phosphorylation by deoxycytidine kinase (Oki et al., 2007). As a nitrogen atom is

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 pro-

tection 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 5 α -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 Tansy, 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 chymo-

trypsin-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]diazene-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 hy-

perosmotic 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- κ B transcriptional activity (Lamkanfi et al., 2007). In turn, NF- κ B can inhibit JNK-mediated apoptosis (Papa et al., 2006). However, MEKK1 activates I κ B α kinase, which phosphorylates I κ B α , leading to its dissociation from NF- κ B and proteasomal degradation. Alternatively, JNK phosphorylates I κ B α , also leading to polyubiquitination and proteasomal degradation (Ki et al., 2007). In either case, the dissociation of I κ B α from NF- κ B leads to the activation of NF- κ B (Lee et al., 1997). Thus, MEKK1 and JNK can activate a transcription factor, NF- κ B, via phosphorylation of I κ B α , 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 (Tsiftoglou et al., 2003) or solid tumors (Honma and Akimoto, 2007).

4. Proliferation. PKC ϵ 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 (G₀/G₁) in the absence of DNA damage and in response to growth factors (Li et al., 2003). In addition, constitutive expression of MKP-1 blocks G₁-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 G₀/G₁ 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 G₁ 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, anti-apoptotic 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-mediated 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 papillary serous adenocarcinomas and stably transfected with MKP-1 cDNA, gave similar results (Manzano et al., 2002). In addition, the lung tumor cell line HCC2429, showed increased *mkp-1* mRNA levels when transfected with a Notch3 dominant-negative receptor upon serum stimulation, with concomitant reduction in ERK1/2

phosphorylation, a reduction of growth in soft agar, and an increase in both apoptosis and dependence on exogenous growth factors (Haruki et al., 2005).

Upon cell-matrix or cell-cell detachment, a type of apoptosis called anoikis is usually triggered, preventing normally adherent cells from adherence-independent survival (Reddig and Juliano, 2005; Eccles and Welch, 2007). The Raf-MEK-ERK pathway, activated by mitogens, can inhibit anoikis in certain transformed cells but not in primary cells or “normal” cell lines (Reddig and Juliano, 2005). In addition, ERK1/2, JNK, and p38MAPK are involved in cell motility, focal adhesion, and cell proliferation, among other functions. However, given the level of cross-talk (Fig. 2) and MKP-1 phosphorylates these MAPKs and ERK5, distinguishing between a direct effect of MKP-1 cell growth in soft agar, for instance, versus an indirect effect becomes problematic. A high-throughput soft agar assay (Anderson et al., 2007), will accelerate the resolution of this dilemma concerning information on the identity of the MAPKs involved and the timing of their involvement in anchorage-independent growth and other mechanisms.

6. Metastasis. Eighty percent of metastases derived from tumors of epithelial origin (bladder, breast, colon, and prostate) revealed little if any *mkp-1* mRNA expression by in situ hybridization (Loda et al., 1996). Interestingly, the metastases that were positive for *mkp-1* mRNA also overexpressed either *neu* or *egfr* mRNA (Loda et al., 1996). In accordance with the previous study, metastasis to the lymph nodes derived from prostate adenocarcinomas revealed no *mkp-1* mRNA expression (Magi-Galluzzi et al., 1997). In addition, increased expression of ERK mRNA and protein was found in lymph node metastases derived from human breast cancer, compared with surrounding normal lymph node tissue (Sivaraman et al., 1997). Lymph node metastases derived from human prostate cancer revealed c-Jun and c-Fos protein expression and phosphorylated ERK (Ouyang et al., 2008). These results reflect the increasing recognition of the involvement of AP-1 transcription factor in positively modulating tumor invasion by up-regulating proinvasive genes and down-regulating anti-invasive genes (Ozanne et al., 2007).

These findings can be appreciated in light of reports on the following proteins being potential metastasis suppressors: MKK4, MKK6, and MKK7, which activate p38MAPK and JNK, p38MAPK, or JNK, respectively; and Raf kinase inhibitor protein, which is an inhibitor of Raf-mediated ERK phosphorylation. Interestingly, tumors that express metastasis suppressor genes can complete all aspects of metastasis with the exception of proliferation at the secondary site (Eccles and Welch, 2007). Thus, MKP-1 expression would be required if p38MAPK or JNK activity would be deleterious to tumor survival or proliferation at the site of metastasis.

These observations on metastasis are interesting but need to be confirmed for a greater number of cases and

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 ($\sim 21\% \text{O}_2$), HIF-1 α is constitutively synthesized and degraded, whereas HIF-1 β is constitutively synthesized and found in the nucleus and is not subject to regulation by the level of oxygen. Degradation of HIF-1 α 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-1 α for polyubiquitination and proteasomal degradation. Factor inhibiting hy-

poxia-inducible factor asparagine hydroxylates HIF-1 α , 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-1 α 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-1 α (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-1 α 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 (Sarközi et al., 2007). Adding to these results is the observation that hypoxia activates p38 α 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-1 α . 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-1 α function. It is noteworthy that one of the possible post-translational modifications of HIF-1 α (Brahimi-Horn et al., 2005) is cysteine nitrosation of HIF-1 α (Yasinska and Sumbayev, 2003), via inducible nitric-oxide synthase or a NO donor. Nitrosation of HIF-1 α activates its interaction with p300, whereas HIF-1 α 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-1 α 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-1 α . 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-1 α to regulate gene transcription via histone modification and other mechanisms and conversely limit the effect of HIF-1 α 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 p38 α MAPK 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, p38 α 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 p38 α 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-A₁₆₅ 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-1 α , 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 (Sarközi 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-A₁₆₅ 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, CK1 α , GSK3 β , 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, CK1 α , and GSK3 β 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 Wnt3 α / 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 GSK3 β 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 GSK3 β at the N terminus and C terminus, respectively, inhibiting GSK3 β 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 (Tsujiata 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 (Tsujiata 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 p38 α MAPK 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 p38 α 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). Interestingly, all areas of preinvasive prostate lesions or intraepithelial neoplasia expressed high levels of *mkp-1* mRNA and ERK1 and JNK1 proteins. A high *mkp-1* mRNA level of expression was assessed as being >50% of cells that are *mkp-1*-positive. It was believed that the mechanism that accounted for transformation via the Ras pathway was also responsible for the up-regulation in MKP-1 synthesis. For patients with invasive adenocarcinomas that were not treated with androgen ablation, the less differentiated the carcinoma was (using the Gleason score system) the fewer the tumors that showed high levels of *mkp-1* mRNA expression. Interestingly, when tumor-normal prostate tissue pairs were assessed for *mkp-1* mRNA expression versus JNK1 and ERK1

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-17 β , 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 lesions, 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 17 β -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 17 β -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 17 β -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 con-

junction 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-1 α 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-1 α and targeting of it for proteasomal degradation. As MKP-1 inactivates ERK5, characterizing how ERK1/2, ERK5, HIF-1 α , 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.

Acknowledgments. We thank Dr. de Lamirande for critical reading of the manuscript. This work was funded by Astellas Pharma Canada, Inc.

REFERENCES

- Abe MK, Kuo WL, Hershenson MB, and Rosner MR (1999) Extracellular signal-regulated kinase 7 (ERK7), a novel ERK with a C-terminal domain that regulates its activity, its cellular localization, and cell growth. *Mol Cell Biol* **19**:1301–1312.
- Abe MK, Saelzler MP, Espinosa R 3rd, Kahle KT, Hershenson MB, Le Beau MM, and Rosner MR (2002) ERK8, a new member of the mitogen-activated protein kinase family. *J Biol Chem* **277**:16733–16743.
- Abrams MT, Robertson NM, Litwack G, and Wickstrom E (2005) Evaluation of glucocorticoid sensitivity in 697 pre-B acute lymphoblastic leukemia cells after overexpression or silencing of MAP kinase phosphatase-1. *J Cancer Res Clin Oncol* **131**:347–354.
- Abrams SI (2005) Positive and negative consequences of Fas/Fas ligand interactions in the antitumor response. *Front Biosci* **10**:809–821.
- Adachi M, Fukuda M, and Nishida E (1999) Two co-existing mechanisms for nuclear import of MAP kinase: passive diffusion of a monomer and active transport of a dimer. *EMBO J* **18**:5347–5358.
- Adams J (2004) The proteasome: a suitable antineoplastic target. *Nat Rev Cancer* **4**:349–360.
- Agarwal R, Burley SK, and Swaminathan S (2008) Structure of human dual specificity protein phosphatase 23, VHZ, enzyme-substrate/product complex. *J Biol Chem* **283**:8946–8953.
- Agell N, Bachs O, Rocamora N, and Villalonga P (2002) Modulation of the Ras/Raf/MEK/ERK pathway by Ca²⁺ and calmodulin. *Cell Signal* **14**:649–654.
- Agre P and Williams TE (1983) The human tumor cloning assay in cancer drug development: a review. *Invest New Drugs* **1**:33–45.
- Akao Y, Nakagawa Y, Kitade Y, Kinoshita T, and Naoe T (2007) Downregulation of microRNAs-143 and -145 in B-cell malignancies. *Cancer Sci* **98**:1914–1920.
- Akao Y, Nakagawa Y, and Naoe T (2006) MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. *Oncol Rep* **16**:845–850.
- Akool el-S, Kleinert H, Hamada F, Abdelwahab MH, Förstermann U, Pfeilschifter J, and Eberhardt W (2003) Nitric oxide increases the decay of matrix metalloproteinase 9 mRNA by inhibiting the expression of mRNA-stabilizing factor HuR. *Mol Cell Biol* **23**:4901–4916.
- Alonso A, Burkhalter S, Sasin J, Tautz L, Bogetz J, Huynh H, Bremer MC, Holsinger LJ, Godzik A, and Mustelin T (2004a) The minimal essential core of a cysteine-based protein-tyrosine phosphatase revealed by a novel 16-kDa VHI-like phosphatase, VHZ. *J Biol Chem* **279**:35768–35774.
- Alonso A, Merlo JJ, Na S, Kholod N, Jaroszewski L, Kharitonov A, Williams S, Godzik A, Posada JD, and Mustelin T (2002) Inhibition of T cell antigen receptor signaling by VHR-related MKPX (VHX), a new dual specificity phosphatase related to VHI1 related (VHR). *J Biol Chem* **277**:5524–5528.
- Alonso A, Narisawa S, Bogetz J, Tautz L, Hadzic R, Huynh H, Williams S, Gjørloff-Wingren A, Bremer MC, Holsinger LJ, et al. (2004b) VHY, a novel myristoylated testis-restricted dual specificity protein phosphatase related to VHX. *J Biol Chem* **279**:32586–32591.
- Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, and Mustelin T (2004c) Protein tyrosine phosphatases in the human genome. *Cell* **117**:699–711.
- Alonso MT, Villalobos C, Chamero P, Alvarez J, and Garcia-Sancho J (2006) Calcium microdomains in mitochondria and nucleus. *Cell Calcium* **40**:513–525.
- Ambrosino C, Mace G, Galban S, Fritsch C, Vintersten K, Black E, Gorospe M, and Nebreda AR (2003) Negative feedback regulation of MKK6 mRNA stability by p38 α mitogen-activated protein kinase. *Mol Cell Biol* **23**:370–381.
- An W, Palhan VB, Karymov MA, Leuba SH, and Roeder RG (2002) Selective requirements for histone H3 and H4 N termini in p300-dependent transcriptional activation from chromatin. *Mol Cell* **9**:811–821.
- Anderson SN, Towne DL, Burns DJ, and Warrior U (2007) A high-throughput soft agar assay for identification of anticancer compound. *J Biomol Screen* **12**:938–945.
- Aoki K and Taketo MM (2007) Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. *J Cell Sci* **120**:3327–3335.
- Aoki N, Aoyama K, Nagata M, and Matsuda T (2001) A growing family of dual specificity phosphatases with low molecular masses. *J Biochem* **130**:133–140.
- Aouadi M, Binetruy B, Caron L, Le Marchand-Brustel Y, and Bost F (2006) Role of MAPKs in development and differentiation: lessons from knockout mice. *Biochimie* **88**:1091–1098.
- Aoyama K, Nagata M, Oshima K, Matsuda T, and Aoki N (2001) Molecular cloning and characterization of a novel dual specificity phosphatase, LMW-DSP2, that lacks the cdc25 homology domain. *J Biol Chem* **276**:27575–27583.
- APEX (Assessment of Proteasome Inhibition for Extending Remissions) Trial (2003) Phase III randomized, multicenter, placebo-controlled trial to evaluate the efficacy and safety of bortezomib versus dexamethasone in patients with recurrent or treatment-resistant multiple myeloma. *Clin Adv Hematol Oncol* **1**:190.
- Arany Z, Huang LE, Eckner R, Bhattacharya S, Jiang C, Goldberg MA, Bunn HF, and Livingston DM (1996) An essential role for p300/CBP in the cellular response to hypoxia. *Proc Natl Acad Sci U S A* **93**:12969–12973.
- Arena S, Benvenuti S, and Bardelli A (2005) Genetic analysis of the kinome and phosphatome in cancer. *Cell Mol Life Sci* **62**:2092–2099.
- Arkell RS, Dickinson RJ, Squires M, Hayat S, Keyse SM, and Cook SJ (2008) DUSP6/MKP-3 inactivates ERK1/2 but fails to bind and inactivate ERK5. *Cell Signal* **20**:836–843.
- Armes JE, Hammet F, de Silva M, Ciciulla J, Ramus SJ, Soo WK, Mahoney A, Yarovaya N, Henderson MA, Gish K, et al. (2004) Candidate tumor-suppressor genes on chromosome arm 8p in early-onset and high-grade breast cancers. *Oncogene* **23**:5697–5702.
- Armstrong WB, Kennedy AR, Wan XS, Taylor TH, Nguyen QA, Jensen J, Thompson W, Lagerberg W, and Meyskens FL Jr (2000) Clinical modulation of oral leukoplakia and protease activity by Bowman-Birk inhibitor concentrate in a phase IIa chemoprevention trial. *Clin Cancer Res* **6**:4684–4691.
- Bai L, Yoon SO, King PD, and Merchant JL (2004) ZBP-89-induced apoptosis is p53-independent and requires JNK. *Cell Death Differ* **11**:663–673.
- Bang YJ, Kwon JH, Kang SH, Kim JW, and Yang YC (1998) Increased MAPK activity and MKP-1 overexpression in human gastric adenocarcinoma. *Biochem Biophys Res Commun* **250**:43–47.
- Bar-Sagi D and Hall A (2000) Ras and Rho GTPases: a family reunion. *Cell* **103**:227–238.
- Barault L, Veyrie N, Jooste V, Lecorre D, Chapusot C, Ferraz JM, Lièvre A, Cortet M, Bouvier AM, Rat P, et al. (2008) Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. *Int J Cancer* **122**:2255–2259.
- Baylin SB (2005) DNA methylation and gene silencing in cancer. *Nat Clin Pract Oncol* **2** (Suppl 1):S4–S11.
- Behren A, Binder K, Vucelic G, Herberhold S, Hirt B, Loewenheim H, Preyer S, Zenner HP, and Simon C (2005) The p38 SAPK pathway is required for Ha-ras induced in vitro invasion of NIH3T3 cells. *Exp Cell Res* **303**:321–330.
- Behrens A, Sibilla M, David JP, Möhle-Steinlein U, Tronche F, Schütz G, and Wagner EF (2002) Impaired postnatal hepatocyte proliferation and liver regeneration in mice lacking *c-jun* in the liver. *EMBO J* **21**:1782–1790.
- Beltman J, McCormick F, and Cook SJ (1996) The selective protein kinase C inhibitor, Ro-31-8220, inhibits mitogen-activated protein kinase phosphatase-1 (MKP-1) expression, induces c-Jun expression, and activates Jun N-terminal kinase. *J Biol Chem* **271**:27018–27024.
- Benedetti C, Haynes CM, Yang Y, Harding HP, and Ron D (2006) Ubiquitin-like protein 5 positively regulates chaperone gene expression in the mitochondrial unfolded protein response. *Genetics* **174**:229–239.
- Bentzen SM (2006) Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. *Nat Rev Cancer* **6**:702–713.
- Bermudez O, MARCHETTI S, Pages G, and Gimond C (2008) Post-translational regulation of the ERK phosphatase DUSP6/MKP3 by the mTOR pathway. *Oncogene* **27**:3685–3691.
- Bernabeu R, Di Scala G, and Zwiler J (2000) Odor regulates the expression of the mitogen-activated protein kinase phosphatase gene *hVH-5* in bilateral entorhinal cortex-lesioned rats. *Brain Res Mol Brain Res* **75**:113–120.
- Bernaudin M, Tang Y, Reilly M, Petit E, and Sharp FR (2002) Brain genomic response following hypoxia and re-oxygenation in the neonatal rat: identification of genes that might contribute to hypoxia-induced ischemic tolerance. *J Biol Chem* **277**:39728–39738.
- Bird A (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* **16**:6–21.
- Birk Y (1985) The Bowman-Birk inhibitor: trypsin- and chymotrypsin-inhibitor from soybeans. *Int J Pept Protein Res* **25**:113–131.

- Bode AM and Dong Z (2007) The functional contrariety of JNK. *Mol Carcinog* **46**:591–598.
- Bogoyevitch MA and Court NW (2004) Counting on mitogen-activated protein kinases—ERKs 3, 4, 5, 6, 7 and 8. *Cell Signal* **16**:1345–1354.
- Bogoyevitch MA and Kobe B (2006) Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. *Microbiol Mol Biol Rev* **70**:1061–1095.
- Bokemeyer D, Sorokin A, Yan M, Ahn NG, Templeton DJ, and Dunn MJ (1996) Induction of mitogen-activated protein kinase phosphatase 1 by the stress-activated protein kinase signaling pathway but not by extracellular signal-regulated kinase in fibroblasts. *J Biol Chem* **271**:639–642.
- Bollig F, Wizen R, Gaestel M, Kostka S, Resch K, and Holtmann H (2003) Affinity purification of ARE-binding proteins identifies polyA-binding protein 1 as a potential substrate in MK2-induced mRNA stabilization. *Biochem Biophys Res Commun* **301**:665–670.
- Bonavida B, Khineche S, Huerta-Yepez S, and Garbán H (2006) Therapeutic potential of nitric oxide in cancer. *Drug Resist Updat* **9**:157–173.
- Bordo D and Bork P (2002) The rhodanese/Cdc25 phosphatase superfamily: sequence-structure-function relations. *EMBO Rep* **3**:741–746.
- Boudvillain M, Dalbiès R, Aussourd C, and Leng M (1995) Intrastrand cross-links are not formed in the reaction between transplatin and native DNA: relation with the clinical inefficiency of transplatin. *Nucleic Acids Res* **23**:2381–2388.
- Boulton TG, Nye SH, Robbins DJ, Ip NY, Radziejewska E, Morgenbesser SD, DePinto RA, Panayotatos N, Cobb MH, and Yancopoulos GD (1991) ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* **65**:663–675.
- Boulton TG, Yancopoulos GD, Gregory JS, Slaughter C, Moomaw C, Hsu J, and Cobb MH (1990) An insulin-stimulated protein kinase similar to yeast kinases involved in cell cycle control. *Science* **249**:64–67.
- Boutros R, Lobjois V, and Ducommun B (2007) CDC25 phosphatases in cancer cells: key players? Good targets? *Nat Rev Cancer* **7**:495–507.
- Brahimi-Horn C, Mazure N, and Pouyssegur J (2005) Signalling via the hypoxia-inducible factor-1 α requires multiple posttranslational modifications. *Cell Signal* **17**:1–9.
- Brancho D, Tanaka N, Jaeschke A, Ventura JJ, Kelkar N, Tanaka Y, Kyuuma M, Takeshita T, Flavell RA, and Davis RJ (2003) Mechanism of p38 MAP kinase activation in vivo. *Genes Dev* **17**:1969–1978.
- Briata P, Forcales SV, Ponassi M, Corte G, Chen CY, Karin M, Puri PL, and Gherzi R (2005) p38-dependent phosphorylation of the mRNA decay-promoting factor KSRP controls the stability of select myogenic transcripts. *Mol Cell* **20**:891–903.
- Brondello JM, McKenzie FR, Sun H, Tonks NK, and Pouyssegur J (1995) Constitutive MAP kinase phosphatase (MKP-1) expression blocks G₁ specific gene transcription and S-phase entry in fibroblasts. *Oncogene* **10**:1895–1904.
- Brondello JM, Pouyssegur J, and McKenzie FR (1999) Reduced MAP kinase phosphatase-1 degradation after p42/p44MAPK-dependent phosphorylation. *Science* **286**:2514–2517.
- Brunet A, Roux D, Lenormand P, Dowd S, Keyse S, and Pouyssegur J (1999) Nuclear translocation of p42/p44 mitogen-activated protein kinase is required for growth factor-induced gene expression and cell cycle entry. *EMBO J* **18**:664–674.
- Buckingham JC (2006) Glucocorticoids: exemplars of multi-tasking. *Br J Pharmacol* **147** (Suppl 1):S258–S268.
- Bueno OF, De Windt LJ, Lim HW, Tymitz KM, Witt SA, Kimball TR, and Molkenin JD (2001) The dual-specificity phosphatase MKP-1 limits the cardiac hypertrophic response in vitro and in vivo. *Circ Res* **88**:88–96.
- Bukau B, Weissman J, and Horwich A (2006) Molecular chaperones and protein quality control. *Cell* **125**:443–451.
- Buschbeck M, Hofbauer S, Di Croce L, Keri G, and Ullrich A (2005) Abl-kinase-sensitive levels of ERK5 and its intrinsic basal activity contribute to leukaemia cell survival. *EMBO Rep* **6**:63–69.
- Buschbeck M and Ullrich A (2005) The unique C-terminal tail of the mitogen-activated protein kinase ERK5 regulates its activation and nuclear shuttling. *J Biol Chem* **280**:2659–2667.
- Buschmann T, Potapova O, Bar-Shira A, Ivanov VN, Fuchs SY, Henderson S, Fried VA, Minamoto T, Alarcon-Vargas D, Pincus MR, et al. (2001) Jun NH₂-terminal kinase phosphorylation of p53 on Thr-81 is important for p53 stabilization and transcriptional activities in response to stress. *Mol Cell Biol* **21**:2743–2754.
- Cameron SJ, Abe J, Malik S, Che W, and Yang J (2004) Differential role of MEK5 α and MEK5 β in BMK1/ERK5 activation. *J Biol Chem* **279**:1506–1512.
- Camps M, Nichols A, and Arkininstall S (2000) Dual specificity phosphatases: a gene family for control of MAP kinase function. *FASEB J* **14**:6–16.
- Camps M, Nichols A, Gillieron C, Antonsson B, Muda M, Chabert C, Boschert U, and Arkininstall S (1998) Catalytic activation of the phosphatase MKP-3 by ERK2 mitogen-activated protein kinase. *Science* **280**:1262–1265.
- Cartier R and Reszka R (2002) Utilization of synthetic peptides containing nuclear localization signals for nonviral gene transfer systems. *Gene Ther* **9**:157–167.
- Cerhan JR, Ansell SM, Fredericksen ZS, Kay NE, Liebow M, Call TG, Dogan A, Cunningham JM, Wang AH, Liu-Mares W, et al. (2007) Genetic variation in 1253 immune and inflammation genes and risk of non-Hodgkin lymphoma. *Blood* **110**:4455–4463.
- Chambard JC, Lefloch R, Pouyssegur J, and Lenormand P (2007) ERK implication in cell cycle regulation. *Biochim Biophys Acta* **1773**:1299–1310.
- Chan DW, Liu VW, Tsao GS, Yao KM, Furukawa T, Chan KK, and Ngan HY (2008) Loss of MKP3 mediated by oxidative stress enhances tumorigenicity and chemoresistance of ovarian cancer cells. *Carcinogenesis*, in press.
- Chan SP and Slack FJ (2006) microRNA-mediated silencing inside P-bodies. *RNA Biol* **3**:97–100.
- Chang L, Kamata H, Solinas G, Luo JL, Maeda S, Venuprasad K, Liu YC, and Karin M (2006) The E3 ubiquitin ligase itch couples JNK activation to TNF α -induced cell death by inducing c-FLIP_L turnover. *Cell* **124**:601–613.
- Charles CH, Abler AS, and Lau LF (1992) cDNA sequence of a growth factor-inducible immediate early gene and characterization of its encoded protein. *Oncogene* **7**:187–190.
- Chen AJ, Zhou G, Juan T, Colicos SM, Cannon JP, Cabrera-Hansen M, Meyer CF, Jurecic R, Copeland NG, Gilbert DJ, et al. (2002) The dual specificity JKAP specifically activates the c-Jun N-terminal kinase pathway. *J Biol Chem* **277**:36592–36601.
- Chen HH, Luche R, Wei B, and Tonks NK (2004) Characterization of two distinct dual specificity phosphatases encoded in alternative open reading frames of a single gene located on human chromosome 10q22.2. *J Biol Chem* **279**:41404–41413.
- Chen N, Nomura M, She QB, Ma WY, Bode AM, Wang L, Flavell RA, and Dong Z (2001) Suppression of skin tumorigenesis in c-Jun NH₂-terminal kinase-2-deficient mice. *Cancer Res* **61**:3908–3912.
- Chen TH, Kao YC, Chen BC, Chen CH, Chan P, and Lee HM (2006) Dipyrindamole activation of mitogen-activated protein kinase phosphatase-1 mediates inhibition of lipopolysaccharide-induced cyclooxygenase-2 expression in RAW 264.7 cells. *Eur J Pharmacol* **541**:138–146.
- Chen YW, Huang SC, Lin-Shiau SY, and Lin JK (2005) Bowman-Birk inhibitor abates proteasome function and suppresses the proliferation of MCF7 breast cancer cells through accumulation of MAP kinase phosphatase-1. *Carcinogenesis* **26**:1296–1306.
- Cheng H, Gao Q, Jiang M, Ma Y, Ni X, Guo L, Jin W, Cao G, Ji C, Ying K, et al. (2003) Molecular cloning and characterization of a novel human protein phosphatase, LMW-DSP3. *Int J Biochem Cell Biol* **35**:226–234.
- Chepurny OG, Hussain MA, and Holz GG (2002) Exendin-4 as a stimulator of rat insulin I gene promoter activity via bZIP/CRE interactions sensitive to serine/threonine protein kinase inhibitor Ro 31–8220. *Endocrinology* **143**:2303–2313.
- Choi BH, Hur EM, Lee JH, Jun DJ, and Kim KT (2006) Protein kinase C δ -mediated proteasomal degradation of MAP kinase phosphatase-1 contributes to glutamate-induced neuronal cell death. *J Cell Sci* **119**:1329–1340.
- Christie GR, Williams DJ, Macisaac F, Dickinson RJ, Rosewell I, and Keyse SM (2005) The dual-specificity protein phosphatase DUSP9/MKP-4 is essential for placental function but is not required for normal embryonic development. *Mol Cell Biol* **25**:8323–8333.
- Christophe D, Christophe-Hobertus C, and Pichon B (2000) Nuclear targeting of proteins: how many different signals? *Cell Signal* **12**:337–341.
- Chu Y, Solski PA, Khosravi-Far R, Der CJ, and Kelly K (1996) The mitogen-activated protein kinase phosphatases PAC1, MKP-1, and MKP-2 have unique substrate specificities and reduced activity in vivo toward the ERK2 sevenmaker mutation. *J Biol Chem* **271**:6497–6501.
- Ciocca DR and Calderwood SK (2005) Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. *Cell Stress Chaperones* **10**:86–103.
- Clafey KP, Shih SC, Mullen A, Dziennis S, Cusick JL, Abrams KR, Lee SW, and Detmar M (1998) Identification of a human VPF/VEGF 3' untranslated region mediating hypoxia-induced mRNA stability. *Mol Biol Cell* **9**:469–481.
- Cook SJ, Beltman J, Cadwallader KA, McMahon M, and McCormick F (1997) Regulation of mitogen-activated protein kinase phosphatase-1 expression by extracellular signal-related kinase-dependent and Ca²⁺-dependent signal pathways in Rat-1 cells. *J Biol Chem* **272**:13309–13319.
- Cook SJ and Lockyer PJ (2006) Recent advances in Ca²⁺-dependent Ras regulation and cell proliferation. *Cell Calcium* **39**:101–112.
- Coulombe P and Meloche S (2007) Atypical mitogen-activated protein kinases: structure, regulation and functions. *Biochim Biophys Acta* **1773**:1376–1387.
- Cramer SF and Patel A (1990) The frequency of uterine leiomyomas. *Am J Clin Pathol* **94**:435–438.
- Cross DA, Alessi DR, Cohen P, and Andjelkovich M, and Hemmings BA (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* **378**:785–789.
- Cuenda A and Rousseau S (2007) p38 MAP-kinases pathway regulation, function and role in human diseases. *Biochim Biophys Acta* **1773**:1358–1375.
- Cuevas BD, Abell AN, and Johnson GL (2007) Role of mitogen-activated protein kinase kinases in signal integration. *Oncogene* **26**:3159–3171.
- Cui Y, Parra I, Zhang M, Hilsenbeck SG, Tsimelzon A, Furukawa T, Horii A, Zhang ZY, Nicholson RI, and Fuqua SA (2006) Elevated expression of mitogen-activated protein kinase phosphatase 3 in breast tumors: a mechanism of tamoxifen resistance. *Cancer Res* **66**:5950–5959.
- Cully M, You H, Levine AJ, and Mak TW (2006) Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer* **6**:184–192.
- Davis DD and Raebel MA (1998) Ambulatory management of chemotherapy-induced fever and neutropenia in adult cancer patients. *Ann Pharmacother* **32**:1317–1323.
- Dean JL, Sully G, Clark AR, and Saklatvala J (2004) The involvement of AU-rich element-binding proteins in p38 mitogen-activated protein kinase pathway-mediated mRNA stabilisation. *Cell Signal* **16**:1113–1121.
- Denkert C, Schmitt WD, Berger S, Reles A, Pest S, Siegart A, Lichtenegger W, Diel M, and Hauptmann S (2002) Expression of mitogen-activated protein kinase phosphatase-1 (MKP-1) in primary human ovarian carcinoma. *Int J Cancer* **102**:507–513.
- Deshpande T, Takagi T, Hao L, Buratowski S, and Charbonneau H (1999) Human PIR1 of the protein-tyrosine phosphatase superfamily has RNA 5'-triphosphatase and diphosphatase activities. *J Biol Chem* **274**:16590–16594.
- Dhillon AS, Hagan S, Rath O, and Kolch W (2007) MAP kinase signalling pathways in cancer. *Oncogene* **26**:3279–3290.
- Dickinson RJ, Eblaghie MC, Keyse SM, and Morriss-Kay GM (2002a) Expression of the ERK-specific MAP kinase phosphatase PYST1/MKP3 in mouse embryos during morphogenesis and early organogenesis. *Mech Dev* **113**:193–196.
- Dickinson RJ and Keyse SM (2006) Diverse physiological functions for dual-specificity MAP kinase phosphatases. *J Cell Sci* **119**:4607–4615.
- Dickinson RJ, Williams DJ, Slack DN, Williamson J, Seternes OM, and Keyse SM (2002b) Characterization of a murine gene encoding a developmentally regulated cytoplasmic dual-specificity mitogen-activated protein kinase phosphatase. *Biochem J* **364**:145–155.

- Ding Z, Tang SC, Weerasinghe P, Yang X, Pater A, and Liepins A (2002) The alkaloid sanguinarine is effective against multidrug resistance in human cervical cells via bimodal cell death. *Biochem Pharmacol* **63**:1415–1421.
- Disanza A, Steffen A, Hertzog M, Frittoli E, Rotner K, and Scita G (2005) Actin polymerization machinery: the finish line of signaling networks, the starting point of cellular movement. *Cell Mol Life Sci* **62**:955–970.
- Distelhorst CW (2002) Recent insights into the mechanism of glucocorticosteroid-induced apoptosis. *Cell Death Differ* **9**:6–19.
- Dolmetsch RE, Lewis RS, Goodnow CC, and Healy JI (1997) Differential activation of transcription factors induced by Ca^{2+} response amplitude and duration. *Nature* **386**:855–858.
- Dolmetsch RE, Xu K, and Lewis RS (1998) Calcium oscillations increase the efficiency and specificity of gene expression. *Nature* **392**:933–936.
- Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS, and Bradley A (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**:215–221.
- Dorfman K, Carrasco D, Gruda M, Ryan C, Lira SA, and Bravo R (1996) Disruption of the *erp/mkp-1* gene does not affect mouse development: normal MAP kinase activity in ERP/MKP-1-deficient fibroblasts. *Oncogene* **13**:925–931.
- Dowd S, Sneddon AA, and Keyse SM (1998) Isolation of the human genes encoding the pyst1 and Pyst2 phosphatases: characterisation of Pyst2 as a cytosolic dual-specificity MAP kinase phosphatase and its catalytic activation by both MAP and SAP kinases. *J Cell Sci* **111**:3389–3399.
- Ducruet AP, Vogt A, Wipf P, and Lazo JS (2005) Dual specificity protein phosphatases: therapeutic targets for cancer and Alzheimer's disease. *Annu Rev Pharmacol Toxicol* **45**:725–750.
- Duff JL, Monia BP, and Berk BC (1995) Mitogen-activated protein (MAP) kinase is regulated by the MAP kinase phosphatase (MKP-1) in vascular smooth muscle cells: effect of actinomycin D and antisense oligonucleotides. *J Biol Chem* **270**:7161–7166.
- Durham PL and Russo AF (2000) Differential regulation of mitogen-activated protein kinase-responsive genes by the duration of a calcium signal. *Mol Endocrinol* **14**:1570–1582.
- Eberhardt W, Doller A, Akool el-S, and Pfeilschifter J (2007) Modulation of mRNA stability as a novel therapeutic approach. *Pharmacol Ther* **114**:56–73.
- Eccles SA and Welch DR (2007) Metastasis: recent discoveries and novel treatment strategies. *Lancet* **369**:1742–1757.
- Eferl R, Ricci R, Kenner L, Zenz R, David JP, Rath M, and Wagner EF (2003) Liver tumor development. c-Jun antagonizes the proapoptotic activity of p53. *Cell* **112**:181–192.
- Eferl R, Sibilia M, Hilberg F, Fuchsbichler A, Kufferath I, Guertl B, Zenz R, Wagner EF, and Zatloukal K (1999) Functions of c-Jun in liver and heart development. *J Cell Biol* **145**:1049–1061.
- Efimova T, Broome AM, and Eckert RL (2003) A regulatory role for p38 δ MAPK in keratinocyte differentiation. Evidence for p38 δ -ERK1/2 complex formation. *J Biol Chem* **278**:34277–34285.
- Emuss V, Garnett M, Mason C, and Marais R (2005) Mutations of C-RAF are rare in human cancer because C-RAF has a low basal kinase activity compared with B-RAF. *Cancer Res* **65**:9719–9726.
- Engelman JA, Luo J, and Cantley LC (2006) The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* **7**:606–619.
- English JM, Pearson G, Hockenberry T, Shivakumar L, White MA, and Cobb MH (1999) Contribution of the ERK5/MEK5 pathway to Ras/Raf signaling and growth control. *J Biol Chem* **274**:31588–31592.
- Ermak G and Davies KJ (2002) Calcium and oxidative stress: from cell signaling to cell death. *Mol Immunol* **38**:713–721.
- Eymin B, Claverie P, Salon C, Brambilla C, Brambilla E, and Gazzeri S (2006) p14ARF triggers G₂ arrest through ERK-mediated Cdc25C phosphorylation, ubiquitination and proteasomal degradation. *Cell Cycle* **5**:759–765.
- Farooq A and Zhou MM (2004) Structure and regulation of MAPK phosphatases. *Cell Signal* **16**:769–779.
- Faust D, Dolado I, Cuadrado A, Oesch F, Weiss C, Nebreda AR, and Dietrich C (2005) p38 α MAPK is required for contact inhibition. *Oncogene* **24**:7941–7945.
- Fenger-Grøn M, Fillman C, Norrild B, and Lykke-Andersen J (2005) Multiple processing body factors and the ARE binding protein TTP activate mRNA decapping. *Mol Cell* **20**:905–915.
- Franklin CC and Kraft AS (1997) Conditional expression of the mitogen-activated protein kinase (MAPK) phosphatase MKP-1 preferentially inhibits p38 MAPK and stress-activated protein kinase in U937 cells. *J Biol Chem* **272**:16917–16923.
- Franklin CC, Srikanth S, and Kraft AS (1998) Conditional expression of mitogen-activated protein kinase phosphatase-1, MKP-1, is cytoprotective against UV-induced apoptosis. *Proc Natl Acad Sci U S A* **95**:3014–3019.
- Friedberg I, Nika K, Tautz L, Saito K, Cerignoli F, Friedberg I, Godzik A, and Mustelin T (2007) Identification and characterization of DUSP27, a novel dual-specific protein phosphatase. *FEBS Lett* **581**:2527–2533.
- Fuchs SY, Adler V, Buschmann T, Yin Z, Wu X, Jones SN, and Ronai Z (1998a) JNK targets p53 ubiquitination and degradation in nonstressed cells. *Genes Dev* **12**:2658–2663.
- Fuchs SY, Adler V, Pincus MR, and Ronai Z (1998b) MEKK1/JNK signaling stabilizes and activates p53. *Proc Natl Acad Sci U S A* **95**:10541–10546.
- Fuchs SY, Dolan L, Davis RJ, and Ronai Z (1996) Phosphorylation-dependent targeting of c-Jun ubiquitination by Jun N-kinase. *Oncogene* **13**:1531–1535.
- Fuchs SY, Xie B, Adler V, Fried VA, Davis RJ, and Ronai Z (1997) c-Jun NH₂-terminal kinases target the ubiquitination of their associated transcription factors. *J Biol Chem* **272**:32163–32168.
- Fujita T, Ryser S, Tortola S, Piuze I, and Schlegel W (2007) Gene-specific recruitment of positive and negative elongation factors during stimulated transcription of the MKP-1 gene in neuroendocrine cells. *Nucleic Acids Res* **35**:1007–1017.
- Fürst R, Brueckl C, Kuebler WM, Zahler S, Krötz F, Görlach A, Vollmar AM, and Kierner AK (2005) Atrial natriuretic peptide induces mitogen-activated protein kinase phosphatase-1 in human endothelial cells via Rac1 and NAD(P)H oxidase/Nox2-activation. *Circ Res* **96**:43–53.
- Furukawa T, Fujisaki R, Yoshida Y, Kanai N, Sunamura M, Abe T, Takeda K, Matsuno S, and Horii A (2005) Distinct progression pathways involving the dysfunction of DUSP6/MKP-3 in pancreatic intraepithelial neoplasia and intraductal papillary-mucinous of the pancreas. *Mod Pathol* **18**:1034–1042.
- Furukawa T, Sunamura M, Motoi F, Matsuno S, and Horii A (2003) Potential tumor suppressive pathway involving DUSP6/MKP-3 in pancreatic cancer. *Am J Pathol* **162**:1807–1815.
- Gao M, Labuda T, Xia Y, Gallagher E, Fang D, Liu YC, and Karin M (2004) Jun turnover is controlled through JNK-dependent phosphorylation of the E3 ligase Itch. *Science* **306**:271–275.
- Garaude J, Cherni S, Kaminski S, Delepine E, Chable-Bessia C, Benkirane M, Borges J, Pandiella A, Iniguez MA, Fresno M, et al. (2006) ERK5 activates NF- κ B in leukemic T cells and is essential for their growth in vivo. *J Immunol* **177**:7607–7617.
- García VP, Valdés F, Martín R, Luis JC, Afonso AM, and Ayala JH (2006) Biosynthesis of antitumoral and bactericidal sanguinarine. *J Biomed Biotechnol* **2006**:63518.
- Gerits N, Kostenko S, and Moens U (2007) In vivo functions of mitogen-activated protein kinases: conclusions from knock-in and knock-out mice. *Transgenic Res* **16**:281–314.
- Giraud-Panis M and Leng M (2000) Transplatin-modified oligonucleotides as modulators of gene expression. *Pharmacol Ther* **85**:175–181.
- Givart-Horwitz V, Davidson B, Goderstad JM, Nesland JM, Tropé CG, and Reich R (2004) The PAC-1 dual specificity phosphatase predicts poor outcome in serous ovarian carcinoma. *Gynecol Oncol* **93**:517–523.
- Goedert M, Hasegawa M, Jakes R, Lawler S, Cuenda A, and Cohen P (1997) Phosphorylation of microtubule-associated protein tau by stress-activated protein kinases. *FEBS Lett* **409**:57–62.
- Goldsmith ZG and Dhanasekaran DN (2007) G protein regulation of MAPK networks. *Oncogene* **26**:3122–3142.
- Görlach A, Klappa P, and Kietzmann T (2006) The endoplasmic reticulum: folding, calcium homeostasis, signaling, and redox control. *Antioxid Redox Signal* **8**:1391–1418.
- Grant S, Qiao L, and Dent P (2002) Roles of ERBB family receptor tyrosine kinases, and downstream signaling pathways, in the control of cell growth and survival. *Front Biosci* **7**:d376–389.
- Greenman C, Stephens P, Smith R, Dalgleish GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, et al. (2007) Patterns of somatic mutation in human cancer genomes. *Nature* **446**:153–158.
- Groom LA, Sneddon AA, Alessi DR, Dowd S, and Keyse SM (1996) Differential regulation of the MAP, SAP and RK/p38 kinases by Pyst1, a novel cytosolic dual-specificity phosphatase. *EMBO J* **15**:3621–3632.
- Guan KL and Butch E (1995) Isolation and characterization of a novel dual specific phosphatase, HVH2, which selectively dephosphorylates the mitogen-activated protein kinase. *J Biol Chem* **270**:7197–7203.
- Gupta S, Barrett T, Whitmarsh AJ, Cavanagh J, Sluss HK, Dérjard B, and Davis RJ (1996) Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J* **15**:2760–2770.
- Haeseleer F, Imanishi Y, Sokal I, Filipek S, and Palczewski K (2002) Calcium-binding proteins: intracellular sensors from the calmodulin superfamily. *Biochem Biophys Res Commun* **290**:615–623.
- Haigis KM, Kendall KR, Wang Y, Cheung A, Haigis MC, Glickman JN, Niwa-Kawakita M, Sweet-Cordero A, Sebolt-Leopold J, Shannon KM, et al. (2008) Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. *Nat Genet* **40**:600–608.
- Hamdi M, Kool J, Cornelissen-Steijger P, Carlotti F, Popeijus HE, van der Burgt C, Janssen JM, Yasui A, Hoeben RC, Terlath C, et al. (2005) DNA damage in transcribed genes induces apoptosis via the JNK pathway and the JNK-phosphatase MKP-1. *Oncogene* **24**:7135–7144.
- Hammer M, Mages J, Dietrich H, Servatius A, Howells N, Cato AC, and Lang R (2006) Dual specificity phosphatase 1 (DUSP1) regulates a subset of LPS-induced genes and protects mice from lethal endotoxin shock. *J Exp Med* **203**:15–20.
- Hammond EM, Kaufmann MR, and Giaccia AJ (2007) Oxygen sensing and the DNA-damage response. *Curr Opin Cell Biol* **19**:680–684.
- Han J and Sun P (2007) The pathways to tumor suppression via route p38. *Trends Biochem Sci* **32**:364–371.
- Hancock JF (2003) Ras proteins: different signals from different locations. *Nat Rev Mol Cell Biol* **4**:373–384.
- Hannigan G, Troussard AA, and Dedhar S (2005) Integrin-linked kinase: a cancer therapeutic target unique among its ILK. *Nat Rev Cancer* **5**:51–63.
- Hardingham GE and Bading H (1999) Calcium as a versatile second messenger in the control of gene expression. *Microsc Res Tech* **46**:348–355.
- Hardingham GE, Chawla S, Johnson CM, and Bading H (1997) Distinct functions of nuclear and cytoplasmic calcium in the control of gene expression. *Nature* **385**:260–265.
- Hartl FU and Hayer-Hartl M (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* **295**:1852–1858.
- Haruki N, Kawaguchi KS, Eichenberger S, Massion PP, Olson S, Gonzalez A, Carbone DP, and Dang TP (2005) Dominant-negative Notch3 receptor inhibits mitogen-activated protein kinase pathway and the growth of human lung cancers. *Cancer Res* **65**:3555–3561.
- Hayashi M, Kim SW, Imanaka-Yoshida K, Yoshida T, Abel ED, Eliceiri B, Yang Y, Ulevitch RJ, and Lee JD (2004a) Targeted deletion of BMK1/ERK5 in adult mice perturbs vascular integrity and leads to endothelial failure. *J Clin Invest* **113**:1138–1148.
- Hayashi R, Wada H, Ito K, and Adcock IM (2004b) Effects of glucocorticoids on gene transcription. *Eur J Pharmacol* **500**:51–62.
- Hesley LE and Han SY (2006) JNK regulation of oncogenesis. *Mol Cell* **21**:167–173.

- Hehlgans S, Haase M, and Cordes N (2007) Signalling via integrins: implications for cell survival and anticancer strategies. *Biochim Biophys Acta* **1775**:163–180.
- Henkens R, Delvenne P, Arafa M, Moutschen B, Zeddou M, Tautz L, Boniver J, Mustelin T, and Rahmouni S (2008) Cervix carcinoma is associated with an up-regulation and nuclear localization of the dual-specificity protein phosphatase VHR. *BMC Cancer* **8**:147.
- Herrera JE, Schiltz RL, and Bustin M (2000) The accessibility of histone H3 tails in chromatin modulates their acetylation by P300/CBP-associated factor. *J Biol Chem* **275**:12994–12999.
- Hers I, Tavaré JM, and Denton RM (1999) The protein kinase C inhibitors bisindolylmaleimide I (GF 109203x) and IX (Ro 31–8220) are potent inhibitors of glycogen synthase kinase-3 activity. *FEBS Lett* **460**:433–436.
- Hess P, Pihan G, Sawyers CL, Flavell RA, and Davis RJ (2002) Survival signaling mediated by c-Jun NH₂-terminal kinase in transformed B lymphoblasts. *Nat Genet* **32**:201–205.
- Hideshima T, Richardson PG, and Anderson KC (2003) Targeting proteasome inhibition in hematologic malignancies. *Rev Clin Exp Hematol* **7**:191–204.
- Hilberg F, Aguzzi A, Howells N, and Wagner EF (1993) c-Jun is essential for normal mouse development and hepatogenesis. *Nature* **365**:179–181.
- Hindley A and Kolch W (2002) Extracellular signal regulated kinase (ERK)/mitogen activated protein kinase (MAPK)-independent functions of Raf kinases. *J Cell Sci* **115**:1575–1581.
- Hirakawa T and Ruley HE (1988) Rescue of cells from ras oncogene-induced growth arrest by a second, complementing, oncogene. *Proc Natl Acad Sci U S A* **85**:1519–1523.
- Hirst D and Robson T (2007) Targeting nitric oxide for cancer therapy. *J Pharm Pharmacol* **59**:3–13.
- Hobohm U (2001) Fever and cancer in perspective. *Cancer Immunol Immunother* **50**:391–396.
- Hochedlinger K, Wagner EF, and Sabapathy K (2002) Differential effects of JNK1 and JNK2 on signal specific induction of apoptosis. *Oncogene* **21**:2441–2445.
- Hoeflich KP, Eby MT, Forrest WF, Gray DC, Tien JY, Stern HM, Murray LJ, Davis DP, Modrusan Z, and Seshagiri S (2006) Regulation of ERK3/MAPK6 expression by BRAF. *Int J Oncol* **29**:839–849.
- Hoff H, Belletti B, Zhang H, and Sell C (2004) The transformed phenotype. *Methods Mol Biol* **285**:95–104.
- Hofmann J (2004) Protein kinase C isozymes as potential targets for anticancer therapy. *Curr Cancer Drug Targets* **4**:125–146.
- Holly SP, Larson MK, and Parise LV (2005) The unique N-terminus of R-ras is required for Rac activation and precise regulation of cell migration. *Mol Biol Cell* **16**:2458–2469.
- Holz GG, Kang G, Harbeck M, Roe MW, and Chepurny OG (2006) Cell physiology of cAMP sensor Epac. *J Physiol* **577**:5–15.
- Honma Y and Akimoto M (2007) Therapeutic strategy using phenotypic modulation of cancer cells by differentiation-inducing agents. *Cancer Sci* **98**:1643–1651.
- Hood KL, Tobin JF, and Yoon C (2002) Identification and characterization of two novel low-molecular-weight dual specificity phosphatases. *Biochem Biophys Res Commun* **298**:545–551.
- Hoornaert I, Marynen P, Goris J, Sciort R, and Baens M (2003) MAPK phosphatase DUSP16/MKP-7, a candidate tumor suppressor for chromosome region 12p12–13, reduces BCR-ABL-induced transformation. *Oncogene* **22**:7728–7736.
- Høyer-Hansen M and Jäättelä M (2007) Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium. *Cell Death Differ* **14**:1576–1582.
- Hoyt R, Zhu W, Cerignoli F, Alonso A, Mustelin T, and David M (2007) Cutting edge: Selective tyrosine dephosphorylation of interferon-activated nuclear STAT5 by the VHR phosphatase. *J Immunol* **179**:3402–3406.
- Hu Y and Mivechi NF (2006) Association and regulation of heat shock transcription factor 4b with both extracellular signal-regulated kinase mitogen-activated protein kinase and dual-specificity tyrosine phosphatase DUSP26. *Mol Cell Biol* **26**:3282–3294.
- Huang C, Jacobson K, and Schaller MD (2004) MAP kinases and cell migration. *J Cell Sci* **117**:4619–4628.
- Huang C, Rajfur Z, Borchers C, Schaller MD, and Jacobson K (2003) JNK phosphorylates paxillin and regulates cell migration. *Nature* **424**:219–223.
- Hui L, Bakiri L, Mairhorfer A, Schweifer N, Haslinger C, Kenner L, Komnenovic V, Scheuch H, Beug H, and Wagner EF (2007) p38 α suppresses normal and cancer cell proliferation by antagonizing the JNK-c-Jun pathway. *Nat Genet* **39**:741–749.
- Hutter D, Chen P, Barnes J, and Liu Y (2000) Catalytic activation of mitogen-activated protein (MAP) kinase phosphatase-1 by binding to p38 MAP kinase: critical role of the p38 C-terminal domain in its negative regulation. *Biochem J* **352**:155–163.
- Hutter D, Chen P, Li J, Barnes J, and Liu Y (2002) The carboxyl-terminal domains of MKP-1 and MKP-2 have inhibitory effects on their phosphatase activity. *Mol Cell Biochem* **233**:107–117.
- Huwiler A, Akool el-S, Aschrafi A, Hamada FM, Pfeilschifter J, and Eberhardt W (2003) ATP potentiates interleukin-1 β -induced MMP-9 expression in mesangial cells via recruitment of the ELAV protein HuR. *J Biol Chem* **278**:51758–51769.
- Innocenti M, Frittoli E, Ponzanelli I, Falck JR, Brachmann SM, Di Fiore PP, and Scita G (2003) Phosphoinositide 3-kinase activates Rac by entering in a complex with Eps8, Abi1, and Sos-1. *J Cell Biol* **160**:17–23.
- Iyer NG, Ozdag H, and Caldas C (2004) p300/CBP and cancer. *Oncogene* **23**:4225–4231.
- Jaganath S, Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmayer EA, Facon T, Harousseau JL, Cowan JM, and Anderson KC (2007) Bortezomib appears to overcome the poor prognosis conferred by chromosome 13 deletion in phase 2 and 3 trials. *Leukemia* **21**:151–157.
- Jeffrey KL, Brummer T, Rolph MS, Liu SM, Callejas NA, Grumont RJ, Gillieron C, Mackay F, Grey S, Camps M, et al. (2006) Positive regulation of immune cell function and inflammatory responses by phosphatase PAC-1. *Nat Immunol* **7**:274–283.
- Jeon SH, Yoon JY, Park YN, Jeong WJ, Kim S, Jho EH, Surh YJ, and Choi KY (2007) Axin inhibits extracellular signal-regulated kinase pathway by Ras degradation via β -catenin. *J Biol Chem* **282**:14482–14492.
- Jeong DG, Cho YH, Yoon TS, Kim JH, Ryu SE, and Kim SJ (2007) Crystal structure of the catalytic domain of human DUSP5, a dual specificity MAP kinase protein phosphatase. *Proteins* **66**:253–258.
- Jeong DG, Cho YH, Yoon TS, Kim JH, Son JH, Ryu SE, and Kim SJ (2006) Structure of human DSP18, a member of the dual-specificity protein tyrosine phosphatase family. *Acta Crystallogr D Biol Crystallogr* **62**:582–588.
- Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, Yoo MA, Song EJ, Lee KJ, and Kim KW (2002) Regulation and destabilization of HIF-1 α by ARD1-mediated acetylation. *Cell* **111**:709–720.
- Jing Q, Huang S, Guth S, Zarubin T, Motoyama A, Chen J, Di Padova F, Lin SC, Gram H, and Han J (2005) Involvement of microRNA in AU-rich element-mediated mRNA instability. *Cell* **120**:623–634.
- Johnson TR, Biggs JR, Winbourn SE, and Kraft AS (2000) Regulation of dual-specificity phosphatases M3/6 and hVH5 by phorbol esters: analysis of a δ -like domain. *J Biol Chem* **275**:31755–31762.
- Jones SM and Kazlauskas A (2001) Growth-factor-dependent mitogenesis requires two distinct phases of signalling. *Nat Cell Biol* **3**:163–172.
- Jordan MA and Wilson L (2004) Microtubules as a target for anticancer drugs. *Nat Rev Cancer* **4**:253–265.
- Kacimi R and Gerdes AM (2003) Alterations in G protein and MAP kinase signaling pathways during cardiac remodeling in hypertension and heart failure. *Hypertension* **41**:968–977.
- Kaelin WG (2005) Proline hydroxylation and gene expression. *Annu Rev Biochem* **74**:115–128.
- Kaiser RA, Bueno OF, Lips DJ, Doevendans PA, Jones F, Kimball TF, and Molkenin JD (2004) Targeted inhibition of p38 mitogen-activated protein kinase antagonizes cardiac injury and cell death following ischemia-reperfusion in vivo. *J Biol Chem* **279**:15524–15530.
- Kamb A, Futreal PA, Rosenthal J, Cochran C, Harshman KD, Liu Q, Phelps RS, Tavtigian SV, Tran T, and Hussey C (1994) Localization of the VHR phosphatase gene and its analysis as a candidate for BRCA1. *Genomics* **23**:163–167.
- Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, et al. (2005) VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* **438**:820–827.
- Karin M, Lawrence T, and Nizet V (2006) Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. *Cell* **124**:823–835.
- Karlsson M, Mathers J, Dickinson RJ, Mandl M, and Keyse SM (2004) Both nuclear-cytoplasmic shuttling of the dual specificity phosphatase MKP-3 and its ability to anchor MAP kinase in the cytoplasm are mediated by a conserved nuclear export signal. *J Biol Chem* **279**:41882–41891.
- Kasid U, Wang FH, and Whiteside TL (1997) Ionizing radiation and TNF- α stimulate gene expression of a Thr/Tyr-protein phosphatase HVH1 and inhibitory factor I κ B α in human squamous carcinoma cells. *Mol Cell Biochem* **173**:193–197.
- Kassel O, Sancono A, Krätzschmar J, Kreft B, Stassen M, and Cato AC (2001) Glucocorticoids inhibit MAP kinase via increased expression and decreased degradation of MKP-1. *EMBO J* **20**:7108–7116.
- Katagiri C, Masuda K, Urano T, Yamashita K, Araki Y, Kikuchi K, and Shima H (2005) Phosphorylation of Ser-446 determines stability of MKP-7. *J Biol Chem* **280**:14716–14722.
- Kazama H and Yonehara S (2000) Oncogenic K-Ras and basic fibroblast growth factor prevent Fas-mediated apoptosis in fibroblasts through activation of mitogen-activated protein kinase. *J Cell Biol* **148**:557–566.
- Kennedy NJ, Sluss HK, Jones SN, Bar-Sagi D, Flavell RA, and Davis RJ (2003) Suppression of Ras-stimulated transformation by the JNK signal transduction pathway. *Genes Dev* **17**:629–637.
- Keyse SM (2000) Protein phosphatases and the regulation of mitogen-activated protein kinase signalling. *Curr Opin Cell Biol* **12**:186–192.
- Keyse SM and Emslie EA (1992) Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. *Nature* **359**:644–647.
- Khaled AR, Bulavin DV, Kittipatirin C, Li WQ, Alvarez M, Kim K, Young HA, Fornace AJ, and Durum SK (2005) Cytokine-driven cell cycling is mediated through Cdc25A. *J Cell Biol* **169**:755–763.
- Khokhlatchev AV, Canagarajah B, Wilsbacher J, Robinson M, Atkinson M, Goldsmith E, and Cobb MH (1998) Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. *Cell* **93**:605–615.
- Kholodenko BN (2006) Cell-signalling dynamics in time and space. *Nat Rev Mol Cell Biol* **7**:165–176.
- Ki SH, Choi MJ, Lee CH, and Kim SG (2007) G α_{12} specifically regulates COX-2 induction by sphingosine 1-phosphate: role for JNK-dependent ubiquitination and degradation of I κ B α . *J Biol Chem* **282**:1938–1947.
- Kim M, Cha GH, Kim S, Lee JH, Park J, Koh H, Choi KY, and Chung J (2004) MKP-3 has essential roles as a negative regulator of the Ras/mitogen-activated protein kinase pathway during *Drosophila* development. *Mol Cell Biol* **24**:573–583.
- Kim MS, Lee EJ, Kim HR, and Moon A (2003a) p38 kinase is a key signaling molecule for H-Ras-induced cell motility and invasive phenotype in human breast epithelial cells. *Cancer Res* **63**:5454–5461.
- Kim SC, Hahn JS, Min YH, Yoo NC, Ko YW, and Lee WJ (1999) Constitutive activation of extracellular signal-regulated kinase in human acute leukemias: combined role of activation of MEK, hyperexpression of extracellular signal-regulated kinase, and downregulation of a phosphatase, PAC1. *Blood* **93**:3893–3899.
- Kim SJ, Jeong DG, Yoon TS, Son JH, Cho SK, Ryu SE, and Kim JH (2007) Crystal structure of human TMDP, a testis-specific dual specificity protein phosphatase: implications for substrate specificity. *Proteins* **66**:239–245.
- Kim Y, Rice AE, and Denu JM (2003b) Intramolecular dephosphorylation of ERK by MKP3. *Biochemistry* **42**:15197–15207.
- King AG, Ozanne BW, Smythe C, and Ashworth A (1995) Isolation and characteri-

- sation of a uniquely regulated threonine, tyrosine phosphatase (TYP 1) which inactivates ERK2 and p54^{lck}. *Oncogene* **11**:2553–2563.
- Kinney CM, Chandrasekharan UM, Mavrakis L, and DiCorleto PE (2008) VEGF and thrombin induce MKP-1 through distinct signaling pathways: role for MKP-1 in endothelial cell migration. *Am J Physiol Cell Physiol* **294**:C241–C250.
- Kittipatrin C, Li WQ, Bulavin DV, Durum SK, and Khaled AR (2006) Cell cycling through Cdc25A: transducer of cytokine proliferative signals. *Cell Cycle* **5**:907–912.
- Kohno M and Pouyssegur J (2006) Targeting the ERK signaling pathway in cancer therapy. *Ann Med* **38**:200–211.
- Kolch W (2005) Coordinating ERK/MAPK signalling through scaffolds and inhibitors. *Nat Rev Mol Cell Biol* **6**:827–837.
- Konstantinopoulos PA, Karamouzis MV, and Papavassiliou AG (2007) Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. *Nat Rev Drug Discov* **6**:541–555.
- Koshkina NV, Khanna C, Mendoza A, Guan H, DeLauter L, and Kleinerman ES (2007) Fas-negative osteosarcoma tumor cells are selected during metastasis to the lungs: the role of the Fas pathway in the metastatic process of osteosarcoma. *Mol Cancer Res* **5**:991–999.
- Kothapalli R, Yoder SJ, Kusmartseva I, and Loughran TP Jr (2003) Characterization of a variant of PAC-1 in large granular lymphocyte leukemia. *Protein Expr Purif* **32**:52–60.
- Kresse SH, Berner JM, Meza-Zepeda LA, Gregory SG, Kuo WL, Gray JW, Forus A, and Myklebost O (2005) Mapping and characterization of the amplicon near APOA2 in 1q23 in human sarcomas by FISH and array CGH. *Mol Cancer* **4**:39.
- Kuan CY, Yang DD, Samanta Roy DR, Davis RJ, Rakic P, and Flavell RA (1999) The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron* **22**:667–676.
- Kwak SP and Dixon JE (1995) Multiple dual specificity protein tyrosine phosphatases are expressed and regulated differentially in liver cell lines. *J Biol Chem* **270**:1156–1160.
- Kwak SP, Hakes DJ, Martell KJ, and Dixon JE (1994) Isolation and characterization of a human dual specificity protein-tyrosine phosphatase gene. *J Biol Chem* **269**:3596–3604.
- Laderoute KR, Mendonca HL, Calaoagan JM, Knapp AM, Giaccia AJ, and Stork PJ (1999) Mitogen-activated protein kinase phosphatase-1 (MKP-1) expression is induced by low oxygen conditions found in solid tumor microenvironments: a candidate MKP for the inactivation of hypoxia-inducible stress-activated protein kinase/c-Jun N-terminal protein kinase activity. *J Biol Chem* **274**:12890–12897.
- Lambert JM, Lambert QT, Reuther GW, Malliri A, Siderovski DP, Sondek J, Collard JG, and Der CJ (2002) Tiam1 mediates Ras activation of Rac by a PI(3)K-independent mechanism. *Nat Cell Biol* **4**:621–625.
- Lamkanfi M, Festjens N, Declercq W, Vandenberghe T, and Vandennebe P (2007) Caspases in cell survival, proliferation and differentiation. *Cell Death Differ* **14**:44–55.
- Lau LF and Nathans D (1985) Identification of a set of genes expressed during the G₀/G₁ transition of cultured mouse cells. *EMBO J* **4**:3145–3151.
- Lau OD, Courtney AD, Vassilev A, Marzilli LA, Cotter RJ, Nakatani Y, and Cole PA (2000) p300/CBP-associated factor histone acetyltransferase processing of a peptide substrate: kinetic analysis of the catalytic mechanism. *J Biol Chem* **275**:21953–21959.
- Lazo JS, Nunes R, Skoko JJ, Queiroz de Oliveira PE, Vogt A, and Wipf P (2006) Novel benzofuran inhibitors of human mitogen-activated protein kinase phosphatase-1. *Bioorg Med Chem* **14**:5643–5650.
- Leav I, Galluzzi CM, Ziar J, Stork PJ, Ho SM, and Loda M (1996) Mitogen-activated protein kinase and mitogen-activated kinase phosphatase-1 expression in the Noble rat model of sex hormone-induced prostatic dysplasia and carcinoma. *Lab Invest* **75**:361–370.
- Lee FS, Hagler J, Chen ZJ, and Maniatis T (1997) Activation of the IκBα kinase complex by MEKK1, a kinase of the JNK pathway. *Cell* **88**:213–222.
- Lee HY, Sueoka N, Hong WK, Mangelsdorf DJ, Claret FX, and Kurie JM (1999) All-trans-retinoic acid inhibits Jun N-terminal kinase by increasing dual-specificity phosphatase activity. *Mol Cell Biol* **19**:1973–1980.
- Lee KH, Lee CT, Kim YW, Han SK, Shim YS, and Yoo CG (2005) Preheating accelerates mitogen-activated protein (MAP) kinase inactivation post-heat shock via a heat shock protein 70-mediated increase in phosphorylated MAP kinase phosphatase-1. *J Biol Chem* **280**:13179–13186.
- Levy-Nissenbaum O, Sagi-Assif O, Kapon D, Hantisteanu S, Burg T, Raanani P, Avigdor A, Ben-Bassat I, and Witz IP (2003a) Dual-specificity phosphatase Pyst2-L is constitutively highly expressed in myeloid leukemia and other malignant cells. *Oncogene* **22**:7649–7660.
- Levy-Nissenbaum O, Sagi-Assif O, Raanani P, Avigdor A, Ben-Bassat I, and Witz IP (2003b) cDNA microarray analysis reveals an overexpression of the dual-specificity MAPK phosphatase PYST2 in acute leukemia. *Methods Enzymol* **366**:103–113.
- Levy-Nissenbaum O, Sagi-Assif O, and Witz IP (2004) Characterization of the dual-specificity phosphatase PYST2 and its transcripts. *Genes Chromosomes Cancer* **39**:37–47.
- Li C, Scott DA, Hatch E, Tian X, and Mansour SL (2007) Dusp6 (Mkp3) is a negative feedback regulator of FGF-stimulated ERK signaling during mouse development. *Development* **134**:167–176.
- Li J, Gorospe M, Hutter D, Barnes J, Keyse SM, and Liu Y (2001) Transcriptional induction of MKP-1 in response to stress is associated with histone H3 phosphorylation-acetylation. *Mol Cell Biol* **21**:8213–8224.
- Li M, Zhou JY, Ge Y, Matherly LH, and Wu GS (2003) The phosphatase MKP1 is a transcriptional target of p53 involved in cell cycle regulation. *J Biol Chem* **278**:41059–41068.
- Liao Q, Guo J, Kleeff J, Zimmermann A, Büchler MW, Korc M, and Friess H (2003) Down-regulation of the dual-specificity phosphatase MKP-1 suppresses tumorigenicity of pancreatic cancer cells. *Gastroenterology* **124**:1830–1845.
- Lim KH and Counter CM (2005) Reduction in the requirement of oncogenic Ras signaling to activation of PI3K/AKT pathway during tumor maintenance. *Cancer Cell* **8**:381–392.
- Lin NY, Lin CT, and Chang CJ (2008) Modulation of immediate early gene expression by tristetraprolin in the differentiation of 3T3–L1 cells. *Biochem Biophys Res Commun* **365**:69–74.
- Lin SC and Li Q (2007) Axin bridges Daxx to p53. *Cell Res* **17**:301–302.
- Lin YW, Chuang SM, and Yang JL (2003) ERK1/2 achieves sustained activation by stimulating MAPK phosphatase-1 degradation via the ubiquitin-proteasome pathway. *J Biol Chem* **278**:21534–21541.
- Lin YW and Yang JL (2006) Cooperation of ERK and SCF^{Skp2} for MKP-1 destruction provides a positive feedback regulation of proliferating signaling. *J Biol Chem* **281**:915–926.
- Linder CC (2006) Genetic variables that influence phenotype. *ILAR J* **47**:132–140.
- Liu C, Li Y, Semenov M, Han C, Baeg GH, Tan Y, Zhang Z, Lin X, and He X (2002) Control of β-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell* **108**:837–847.
- Liu C, Shi Y, Du Y, Ning X, Liu N, Huang D, Liang J, Xue Y, and Fan D (2005a) Dual-specificity phosphatase DUSP1 protects overactivation of hypoxia-inducible factor 1 through inactivating ERK MAPK. *Exp Cell Res* **309**:410–418.
- Liu C, Shi Y, Han Z, Pan Y, Liu N, Han S, Chen Y, Lan M, Qiao T, and Fan D (2003) Suppression of the dual-specificity phosphatase MKP-1 enhances HIF-1 transactivation and increases expression of EPO. *Biochem Biophys Res Commun* **312**:780–786.
- Liu CJ, Shi YQ, Du YL, Pan YL, Liang J, Han S, and Fan DM (2005b) Inhibition of the in vitro interaction between hypoxia inducible factor 1α and p300 by mitogen-activated protein kinase phosphatase 1. *Zhonghua Yi Xue Za Zhi* **85**:2344–2348.
- Liu J and Lin A (2005) Role of JNK activation in apoptosis: a double-edged sword. *Cell Res* **15**:36–42.
- Liu Y, Gorospe M, Yang C, and Holbrook NJ (1995) Role of mitogen-activated protein kinase phosphatase during the cellular response to genotoxic stress: inhibition of c-Jun N-terminal kinase activity and AP-1-dependent gene activation. *J Biol Chem* **270**:8377–8380.
- Liu Y, Lagowski J, Sundholm A, Sundberg A, and Kulesz-Martin M (2007) Microtubule disruption and tumor suppression by mitogen-activated protein kinase phosphatase 4. *Cancer Res* **67**:10711–10719.
- Liu YX, Wang J, Guo J, Wu J, Lieberman HB, and Yin Y (2008) DUSP1 is controlled by p53 during the cellular response to oxidative stress. *Mol Cancer Res* **6**:624–633.
- Loda M, Capodiceci P, Mishra R, Yao H, Corless C, Grigioni W, Wang Y, Magi-Galluzzi C, and Stork PJ (1996) Expression of mitogen-activated protein kinase phosphatase-1 in the early phases of human epithelial carcinogenesis. *Am J Pathol* **149**:1553–1564.
- LoPiccolo J, Granville CA, Gills JJ, and Dennis PA (2007) Targeting Akt in cancer therapy. *Anticancer Drugs* **18**:861–874.
- Lorentzen E and Conti E (2006) The exosome and the proteasome: nanocompartments for degradation. *Cell* **125**:651–654.
- Lornejad-Schäfer MR, Schäfer C, Richter L, Grune T, Häussinger D, and Schliess F (2005) Osmotic regulation of MG-132-induced MAP-kinase phosphatase MKP-1 expression in H4IIE rat hepatoma cells. *Cell Physiol Biochem* **16**:193–206.
- Lornejad-Schäfer MR, Schäfer C, Graf D, Häussinger D, and Schliess F (2003) Osmotic regulation of insulin-induced mitogen-activated protein kinase phosphatase (MKP-1) expression in H4IIE rat hepatoma cells. *Biochem J* **371**:609–619.
- Lu Z, Xu S, Joazeiro C, Cobb MH, and Hunter T (2002) The PHD domain of MEKK1 acts as an E3 ubiquitin ligase and mediates ubiquitination and degradation of ERK1/2. *Mol Cell* **9**:945–956.
- Luo W and Lin SC (2004) Axin: a master scaffold for multiple signaling pathways. *Neurosignals* **13**:99–113.
- Magi-Galluzzi C, Mishra R, Fiorentino M, Montironi R, Yao H, Capodiceci P, Wishnow K, Kaplan I, Stork PJ, and Loda M (1997) Mitogen-activated protein kinase phosphatase 1 is overexpressed in prostate cancers and is inversely related to apoptosis. *Lab Invest* **76**:37–51.
- Mandl M, Slack DN, and Keyse SM (2005) Specific inactivation and nuclear anchoring of extracellular signal-regulated kinase 2 by the inducible dual-specificity protein phosphatase DUSP5. *Mol Cell Biol* **25**:1830–1845.
- Manke IA, Nguyen A, Lim D, Stewart MQ, Elia AE, and Yaffe MB (2005) MAPKAP kinase-2 is a cell cycle checkpoint kinase that regulates the G₂/M transition and S phase progression in response to UV irradiation. *Mol Cell* **17**:37–48.
- Manning BD and Cantley LC (2007) AKT/PKB signaling: navigating downstream. *Cell* **129**:1261–1274.
- Manzano RG, Montuenga LM, Dayton M, Dent P, Kinoshita I, Vicent S, Gardner GJ, Nguyen P, Choi YH, Trepel J, et al. (2002) CL100 expression is down-regulated in advanced epithelial ovarian cancer and its re-expression decreases its malignant potential. *Oncogene* **21**:4435–4447.
- Marchetti S, Gimond C, Chambard JC, Touboul T, Roux D, Pouyssegur J, and Pagès G (2005) Extracellular signal-regulated kinases phosphorylate mitogen-activated protein kinase phosphatase 3/DUSP6 at serines 159 and 197, two sites critical for its proteasomal degradation. *Mol Cell Biol* **25**:854–864.
- Marchetti S, Gimond C, Roux D, Gothié E, Pouyssegur J, and Pagès G (2004) Inducible expression of a MAP kinase phosphatase-3-GFP chimera specifically blunts fibroblast growth and ras-dependent tumor formation in nude mice. *J Cell Physiol* **199**:441–450.
- Margueron R, Trojer P, and Reinberg D (2005) The key to development: interpreting the histone code? *Curr Opin Genet Dev* **15**:163–176.
- Marshall CJ (1995) Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* **80**:179–185.
- Martell KJ, Seasholtz AF, Kwak SP, Clemens KK, and Dixon JE (1995) hVH-5: a protein tyrosine phosphatase abundant in brain that inactivates mitogen-activated protein kinase. *J Neurochem* **65**:1823–1833.
- Marti F, Krause A, Post NH, Lyddane C, Dupont B, Sadelain M, and King PD (2001) Negative-feedback regulation of CD28 costimulation by a novel mitogen-activated protein kinase phosphatase, MKP6. *J Immunol* **166**:197–206.

- Martin SS and Vuori K (2004) Regulation of Bcl-2 proteins during anoikis and amorphosis. *Biochim Biophys Acta* **1692**:145–157.
- Mason CS, Springer CJ, Cooper RG, Superti-Furga G, Marshall CJ, and Marais R (1999) Serine and tyrosine phosphorylations cooperate in Raf-1, but not B-Raf activation. *EMBO J* **18**:2137–2148.
- Masuda K, Shima H, Katagiri C, and Kikuchi K (2003) Activation of ERK induces phosphorylation of MAPK phosphatase-7, a JNK specific phosphatase, at Ser-446. *J Biol Chem* **278**:32448–32456.
- Matsuguchi T, Musikacharoen T, Johnson TR, Kraft AS, and Yoshikai Y (2001) A novel mitogen-activated protein kinase phosphatase is an important negative regulator of lipopolysaccharide-mediated c-Jun N-terminal kinase activation in mouse macrophage cell lines. *Mol Cell Biol* **21**:6999–7009.
- Maudrell K, Antonsson B, Magnenat E, Camps M, Muda M, Chabert C, Gillieron C, Boschert U, Vial-Knecht E, Martinou J, et al. (1997) Bcl-2 undergoes phosphorylation by c-Jun N-terminal kinase/stress-activated protein kinases in the presence of the constitutively active GTP-binding protein Rac1. *J Biol Chem* **272**:25238–25242.
- Mayor F Jr, Jurado-Pueyo M, Campos PM, and Murga C (2007) Interfering with MAP kinase docking interactions: implications and perspective for the p38 route. *Cell Cycle* **6**:528–533.
- McCaw BJ, Chow SY, Wong ES, Tan KL, Guo H, and Guy GR (2005) Identification and characterization of mErk5-T, a novel Erk5/Bmk1 splice variant. *Gene* **345**:183–190.
- McCracken SR, Ramsay A, Heer R, Mathers ME, Jenkins BL, Edwards J, Robson CN, Marquez R, Cohen P, and Leung HY (2008) Aberrant expression of extracellular signal-regulated kinase 5 in human prostate cancer. *Oncogene* **27**:2978–2988.
- McDonald PH, Chow CW, Miller WE, Laporte SA, Field ME, Lin FT, Davis RJ, and Lefkowitz RJ (2000) β -Arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3. *Science* **290**:1574–1577.
- McKay MM and Morrison DK (2007) Integrating signals from RTKs to ERK/MAPK. *Oncogene* **26**:3113–3121.
- McKenna JP and Hanson PJ (1993) Inhibition by Ro 31-8220 of acid secretory activity induced by carbachol indicates a stimulatory role for protein kinase C in the action of muscarinic agonists on isolated rat parietal cells. *Biochem Pharmacol* **46**:583–588.
- McLean GW, Carragher NO, Avizienyte E, Evans J, Brunton VG, and Frame MC (2005) The role of focal-adhesion kinase in cancer—a new therapeutic opportunity. *Nat Rev Cancer* **5**:505–515.
- McMullen ME, Bryant PW, Glembotski CC, Vincent PA, and Pugliese KM (2005) Activation of p38 has opposing effects on the proliferation and migration of endothelial cells. *J Biol Chem* **280**:20995–21003.
- Mehta PB, Jenkins BL, McCarthy L, Thilak L, Robson CN, Neal DE, and Leung HY (2003) MEK5 overexpression is associated with metastatic prostate cancer, and stimulates proliferation, MMP-9 expression and invasion. *Oncogene* **22**:1381–1389.
- Meloche S and Pouyssegur J (2007) The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G₁- to S-phase transition. *Oncogene* **26**:3227–3239.
- Menges CW and McCance DJ (2008) Constitutive activation of the Raf-MAPK pathway causes negative feedback inhibition of Ras-PI3K-AKT and cellular arrest through the EphA2 receptor. *Oncogene* **27**:2934–2940.
- Meriin AB, Gabai VL, Yaglom J, Shifrin VI, and Sherman MY (1998) Proteasome inhibitors activate stress kinases and induce Hsp72. Diverse effects on apoptosis. *J Biol Chem* **273**:6373–6379.
- Metzler B, Hu Y, Sturm G, Wick G, and Xu Q (1998) Induction of mitogen-activated protein kinase phosphatase-1 by arachidonic acid in vascular smooth muscle cells. *J Biol Chem* **273**:33320–33326.
- Meyskens FL (2001) Development of Bowman-Birk inhibitor for chemoprevention of oral head and neck cancer. *Ann N Y Acad Sci* **952**:116–123.
- Mikhailov A, Shinohara M, and Rieder CL (2005) The p38-mediated stress-activated checkpoint: A rapid response system for delaying progression through antepause and entry into mitosis. *Cell Cycle* **4**:57–62.
- Minotti G, Menna P, Salvatorelli E, Cairo G, and Gianni L (2004) Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol Rev* **56**:185–229.
- Mishra OP and Delivoria-Papadopoulos M (2004) Effect of hypoxia on the expression and activity of mitogen-activated protein (MAP) kinase-phosphatase-1 (MKP-1) and MKP-3 in neuronal nuclei of newborn piglets: the role of nitric oxide. *Neuroscience* **129**:665–673.
- Mizuno R, Oya M, Shiomi T, Marumo K, Okada Y, and Murai M (2004) Inhibition of MKP-1 expression potentiates JNK related apoptosis in renal cancer cells. *J Urol* **172**:723–727.
- Mocellin S, Bronte V, and Nitti D (2007) Nitric oxide, a double edged sword in cancer biology: searching for therapeutic opportunities. *Med Res Rev* **27**:317–352.
- Mody N, Campbell DG, Morrice N, Peggie M, and Cohen P (2003) An analysis of the phosphorylation and activation of extracellular-signal-regulated protein kinase 5 (ERK5) by mitogen-activated protein kinase kinase 5 (MKK5) in vitro. *Biochem J* **372**:567–575.
- Mor A and Philips MR (2006) Compartmentalized Ras/MAPK signaling. *Annu Rev Immunol* **24**:771–800.
- Muda M, Boschert U, Smith A, Antonsson B, Gillieron C, Chabert C, Camps M, Martinou I, Ashworth A, and Arkinstall S (1997) Molecular cloning and functional characterization of a novel mitogen-activated protein kinase phosphatase, MKP-4. *J Biol Chem* **272**:5141–5151.
- Muda M, Theodosiou A, Rodrigues N, Boschert U, Camps M, Gillieron C, Davies K, Ashworth A, and Arkinstall S (1996) The dual specificity phosphatases M3/6 and MKP-3 are highly selective for inactivation of distinct mitogen-activated protein kinases. *J Biol Chem* **271**:27205–27208.
- Muñoz-Alonso MJ, Guillemain G, Kassis N, Girard J, Burnol AF, and Leturque A (2000) A novel cytosolic dual specificity phosphatase, interacting with glucokinase, increases glucose phosphorylation rate. *J Biol Chem* **275**:32406–32412.
- Muratani M and Tansey WP (2003) How the ubiquitin-proteasome system controls transcription. *Nat Rev Mol Cell Biol* **4**:192–201.
- Murphy LO and Blenis J (2006) MAPK signal specificity: the right place at the right time. *Trends Biochem Sci* **31**:268–275.
- Mymryk JS, Zaniewski E, and Archer TK (1995) Cisplatin inhibits chromatin remodeling, transcription factor binding, and transcription from the mouse mammary tumor virus promoter in vivo. *Proc Natl Acad Sci U S A* **92**:2076–2080.
- Nagel S, Burek C, Venturini L, Scherr M, Quentmeier H, Meyer C, Rosenwald A, Drexler HG, and MacLeod RA (2007) Comprehensive analysis of homeobox genes in Hodgkin lymphoma cell lines identifies dysregulated expression of HOXB9 mediated via ERK5 signaling and BMI1. *Blood* **109**:3015–3023.
- Nakamura K, Shima H, Watanabe M, Haneji T, and Kikuchi K (1999) Molecular cloning and characterization of a novel dual-specificity protein phosphatase possibly involved in spermatogenesis. *Biochem J* **344**:819–825.
- Nakamura K, Tanoue K, Satoh T, Takekawa M, Watanabe M, Shima H, and Kikuchi K (2002) A novel low-molecular-mass dual-specificity phosphatase, LDP-2, with a naturally occurring substitution that affects substrate specificity. *J Biochem* **132**:463–470.
- Nalepa G, Rolfe M, and Harper JW (2006) Drug discovery in the ubiquitin-proteasome system. *Nat Rev Drug Discov* **5**:596–613.
- Nandi D, Tahiliani P, Kumar A, and Chandu D (2006) The ubiquitin-proteasome system. *J Biosci* **31**:137–155.
- Nesbit MA, Hodges MD, Campbell L, de Meulemeester TM, Alders M, Rodrigues NR, Talbot K, Theodosiou AM, Mannens MA, Nakamura Y, et al. (1997) Genomic organization and chromosomal localization of a member of the MAP kinase phosphatase gene family to human chromosome 11p15.5 and a pseudogene to 10q11.2. *Genomics* **42**:284–294.
- Nicol RL, Frey N, Pearson G, Cobb M, Richardson J, and Olson EN (2001) Activated MEK5 induces serial assembly of sarcomeres and eccentric cardiac hypertrophy. *EMBO J* **20**:2757–2767.
- Noble RL (1982) Prostate carcinoma of the Nb rat in relation to hormones. *Int Rev Exp Pathol* **23**:113–159.
- Noguchi T, Metz R, Chen L, Mattéi MG, Carrasco D, and Bravo R (1993) Structure, mapping, and expression of erp, a growth factor-inducible gene encoding a non-transmembrane protein tyrosine phosphatase, and effect of ERP on cell growth. *Mol Cell Biol* **13**:5195–5205.
- Nonn L, Peng L, Feldman D, and Peehl DM (2006) Inhibition of p38 by vitamin D reduces interleukin-6 production in normal prostate cells via mitogen-activated protein kinase phosphatase 5: implications for prostate cancer prevention by vitamin D. *Cancer Res* **66**:4516–4524.
- Nyati MK, Feng FY, Maheshwari D, Varambally S, Zielske SP, Ahsan A, Chun PY, Arora VA, Davis MA, Jung M, et al. (2006) Ataxia telangiectasia mutated down-regulates phospho-extracellular signal-regulated kinase 1/2 via activation of MKP-1 in response to radiation. *Cancer Res* **66**:11554–11559.
- Oki Y, Aoki E, and Issa JP (2007) Decitabine—bedside to bench. *Crit Rev Oncol Hematol* **61**:140–152.
- Olson JM and Hallahan AR (2004) p38 MAP kinase: a convergence point in cancer therapy. *Trends Mol Med* **10**:125–129.
- Orlowski RZ, Small GW, and Shi YY (2002) Evidence that inhibition of p44/42 mitogen-activated protein kinase signaling is a factor in proteasome inhibitor-mediated apoptosis. *J Biol Chem* **277**:27864–27871.
- Ortega-Paino E, Fransson J, Ek S, and Borrebaeck CA (2008) Functionally associated targets in mantle cell lymphoma as defined by DNA microarrays and RNA interference. *Blood* **111**:1617–1624.
- Ouyang X, Jessen WJ, Al-Ahmadie H, Serio AM, Lin Y, Shih WJ, Reuter VE, Scardino PT, Shen MM, Aronow BJ, et al. (2008) Activator protein-1 transcription factors are associated with progression and recurrence of prostate cancer. *Cancer Res* **68**:2132–2144.
- Overall CM and López-Otín C (2002) Strategies for MMP inhibition in cancer: innovations for the post-trial era. *Nat Rev Cancer* **2**:657–672.
- Owen-Schaub LB, van Golen KL, Hill LL, and Price JE (1998) Fas and Fas ligand interactions suppress melanoma lung metastasis. *J Exp Med* **188**:1717–1723.
- Owens DM and Keyse SM (2007) Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. *Oncogene* **26**:3203–3213.
- Ozanne BW, Spence HJ, McGarry LC, and Hennigan RF (2007) Transcription factors control invasion: AP-1 the first among equals. *Oncogene* **26**:1–10.
- Pagliarini DJ, Wiley SE, Kimple ME, Dixon JR, Kelly P, Worby CA, Casey PJ, and Dixon JE (2005) Involvement of a mitochondrial phosphatase in the regulation of ATP production and insulin secretion in pancreatic beta cells. *Mol Cell* **19**:197–207.
- Papa S, Bubici C, Zazzeroni F, Pham CG, Kuntzen C, Knabb JR, Dean K, and Franzoso G (2006) The NF- κ B-mediated control of the JNK cascade in the antagonism of programmed cell death in health and disease. *Cell Death Differ* **13**:712–729.
- Pascoe JM and Roberts JJ (1974a) Interactions between mammalian cell DNA and inorganic platinum compounds. I. DNA interstrand cross-linking and cytotoxic properties of platinum(II) compounds. *Biochem Pharmacol* **23**:1359–1365.
- Pascoe JM and Roberts JJ (1974b) Interactions between mammalian cell DNA and inorganic platinum compounds. II. Interstrand cross-linking of isolated and cellular DNA by platinum(IV) compounds. *Biochem Pharmacol* **23**:1345–1357.
- Perdiguero E, Ruiz-Bonilla V, Serrano AL, and Muñoz-Cánoves P (2007) Genetic deficiency of p38 α reveals its critical role in myoblast cell cycle exit: the p38 α -JNK connection. *Cell Cycle* **6**:1298–1303.
- Pervin S, Singh R, Freije WA, and Chaudhuri G (2003) MKP-1-induced dephosphorylation of extracellular signal-regulated kinase is essential for triggering nitric oxide-induced apoptosis in human breast cancer cell lines: implications in breast cancer. *Cancer Res* **63**:8853–8860.
- Pi X, Garin G, Xie L, Zheng Q, Wei H, Abe J, Yan C, and Berk BC (2005) BMK1/

- ERK5 is a novel regulator of angiogenesis by destabilizing hypoxia inducible factor 1 α . *Circ Res* **96**:1145–1151.
- Plows D, Briassoulis P, Owen C, Zoumpourlis V, Garrett MD, and Pintzas A (2002) Ecdysone-inducible expression of oncogenic Ha-Ras in NIH 3T3 cells leads to transient nuclear localization of activated extracellular signal-regulated kinase regulated by mitogen-activated protein kinase phosphatase-1. *Biochem J* **362**:305–315.
- Potente M, Michaelis UR, Fisslthaler B, Busse R, and Fleming I (2002) Cytochrome P450 2C9-induced endothelial cell proliferation involves induction of mitogen-activated protein (MAP) kinase phosphatase-1, inhibition of the c-Jun N-terminal kinase, and up-regulation of cyclin D1. *J Biol Chem* **277**:15671–15676.
- Price DM, Wloka MT, Chik CL, and Ho AK (2007) Mitogen-activated protein kinase phosphatase-1 (MKP-1) preferentially dephosphorylates p42/44MAPK but not p38MAPK in rat pinealocytes. *J Neurochem* **101**:1685–1693.
- Pullikuth AK and Catling AD (2007) Scaffold mediated regulation of MAPK signaling and cytoskeletal dynamics: a perspective. *Cell Signal* **19**:1621–1632.
- Puri PL, Wu Z, Zhang P, Wood LD, Bhakta KS, Han J, Feramisco JR, Karin M, and Wang JY (2000) Induction of terminal differentiation by constitutive activation of p38 MAP kinase in human rhabdomyosarcoma cells. *Genes Dev* **14**:574–584.
- Pursiheimo JP, Kieksi A, Jalkanen M, and Salmivirta M (2002) Protein kinase A balances the growth factor-induced Ras/ERK signaling. *FEBS Lett* **521**:157–164.
- Rahmouni S, Cerignoli F, Alonso A, Tsutji T, Henkens R, Zhu C, Louis-dit-Sully C, Moutschen M, Jiang W, and Mustelin T (2006) Loss of the VHR dual-specific phosphatase causes cell-cycle arrest and senescence. *Nat Cell Biol* **8**:524–531.
- Raman M, Chen W, and Cobb MH (2007) Differential regulation and properties of MAPKs. *Oncogene* **26**:3100–3112.
- Ranganathan AC, Adam AP, Zhang L, and Aguirre-Ghiso JA (2006) Tumor cell dormancy induced by p38SAPK and ER-stress signaling: an adaptive advantage for metastatic cells? *Cancer Biol Ther* **5**:729–735.
- Rauhala HE, Porkka KP, Tolonen TT, Martikainen PM, Tammela TL, and Visakorpi T (2005) Dual-specificity phosphatase 1 and serum/glucocorticoid-regulated kinase are downregulated in prostate cancer. *Int J Cancer* **117**:738–745.
- Reddig PJ and Juliano RL (2005) Clinging to life: cell to matrix adhesion and cell survival. *Cancer Metastasis Rev* **24**:425–439.
- Richard DE, Berra E, Góthié E, Roux D, and Pouyssegur J (1999) p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1 α (HIF-1 α) and enhance the transcriptional activity of HIF-1. *J Biol Chem* **274**:32631–32637.
- Richardson PG, Mitsiades C, Hideshima T, and Anderson KC (2006) Bortezomib: proteasome inhibition as an effective anticancer therapy. *Annu Rev Med* **57**:33–47.
- Riedl SJ and Salvesen GS (2007) The apoptosome: signalling platform of cell death. *Nat Rev Mol Cell Biol* **8**:405–413.
- Ringshausen I, Dechow T, Schneller F, Weick K, Oelsner M, Peschel C, and Decker T (2004) Constitutive activation of the MAPkinase p38 is critical for MMP-9 production and survival of B-CLL cells on bone marrow stromal cells. *Leukemia* **18**:1964–1970.
- Roberts PJ and Der CJ (2007) Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene* **26**:3291–3310.
- Rohan PJ, Davis P, Moskaluk CA, Kearns M, Krutzsch H, Siebenlist U, and Kelly K (1993) PAC-1: a mitogen-induced nuclear protein tyrosine phosphatase. *Science* **259**:1763–1766.
- Rosini P, De Chiara G, Bonini P, Lucibello M, Marcocci ME, Garaci E, Cozzolino F, and Torcia M (2004) Nerve growth factor-dependent survival of CESS B cell line is mediated by increased expression and decreased degradation of MAPK phosphatase 1. *J Biol Chem* **279**:14016–14023.
- Rousseau S, Dolado I, Beardmore V, Shapiro N, Marquez R, Nebreda AR, Arthur JS, Case LM, Tessier-Lavigne M, Gaestel M, et al. (2006) CXCL12 and C5a trigger cell migration via a PAK1/2-p38 α MAPK-MAPKAP-K2-HSP27 pathway. *Cell Signal* **18**:1897–1905.
- Rousseau S, Houle F, and Huot J (2000) Integrating the VEGF signals leading to actin-based motility in vascular endothelial cells. *Trends Cardiovasc Med* **10**:321–327.
- Rousseau S, Morrice N, Peggie M, Campbell DG, Gaestel M, and Cohen P (2002) Inhibition of SAPK2/p38 prevents hnRNP A0 phosphorylation by MAPKAP-K2 and its interaction with cytokine mRNAs. *EMBO J* **21**:6505–6514.
- Rudolph J (2007a) Cdc25 phosphatases: structure, specificity, and mechanism. *Biochemistry* **46**:3595–3604.
- Rudolph J (2007b) Inhibiting transient protein-protein interactions: lessons from the Cdc25 protein tyrosine phosphatases. *Nat Rev Cancer* **7**:202–211.
- Ryser S, Massiha A, Piuz I, and Schlegel W (2004) Stimulated initiation of mitogen-activated protein kinase phosphatase-1 (MKP-1) gene transcription involves the synergistic action of multiple cis-acting elements in the proximal promoter. *Biochem J* **378**:473–484.
- Ryser S, Tortola S, van Haasteren G, Muda M, Li S, and Schlegel W (2001) MAP kinase phosphatase-1 gene transcription in rat neuroendocrine cells is modulated by a calcium-sensitive block to elongation in the first exon. *J Biol Chem* **276**:33319–33327.
- Saelzler MP, Spackman CC, Liu Y, Martinez LC, Harris JP, and Abe MK (2006) ERK8 down-regulates transactivation of the glucocorticoid receptor through Hic-5. *J Biol Chem* **281**:16821–16832.
- Salojin KV, Owusu IB, Millerberg KA, Potter M, Platt KA, and Oravecz T (2006) Essential role of MAPK phosphatase-1 in the negative control of innate immune responses. *J Immunol* **176**:1899–1907.
- Sánchez-Pérez I, Martínez-Gomariz M, Williams D, Keyse SM, and Perona R (2000) CL100/MKP-1 modulates JNK activation and apoptosis in response to cisplatin. *Oncogene* **19**:5142–5152.
- Sánchez-Pérez I, Murguía JR, and Perona R (1998) Cisplatin induces a persistent activation of JNK that is related to cell death. *Oncogene* **16**:533–540.
- Sandberg EM, Ma X, VonDerLinden D, Godeny MD, and Sayeski PP (2004) Jak2 tyrosine kinase mediates angiotensin II-dependent inactivation of ERK2 via induction of mitogen-activated protein kinase phosphatase 1. *J Biol Chem* **279**:1956–1967.
- Sang N, Stiehl DP, Bohensky J, Leshchinsky I, Srinivas V, and Caro J (2003) MAPK signaling up-regulates the activity of hypoxia-inducible factors by its effects on p300. *J Biol Chem* **278**:14013–14019.
- Sarközi R, Miller B, Pollack V, Feifel E, Mayer G, Sorokin A, and Schramek H (2007) ERK1/2-driven and MKP-mediated inhibition of EGF-induced ERK5 signaling in human proximal tubular cells. *J Cell Physiol* **211**:88–100.
- Sasaki AT, Chun C, Takeda K, and Firtel RA (2004) Localized Ras signaling at the leading edge regulates PI3K, cell polarity, and directional cell movement. *J Cell Biol* **167**:505–518.
- Sasaki AT and Firtel RA (2006) Regulation of chemotaxis by the orchestrated activation of Ras, PI3K, and TOR. *Eur J Cell Biol* **85**:873–895.
- Saunders A, Core LJ, and Lis JT (2006) Breaking barriers to transcription elongation. *Nat Rev Mol Cell Biol* **7**:557–567.
- Schliess F, Heinrich S, and Häussinger D (1998) Hyperosmotic induction of the mitogen-activated protein kinase phosphatase MKP-1 in H4IIE rat hepatoma cells. *Arch Biochem Biophys* **351**:35–40.
- Schliess F, Kurz AK, and Häussinger D (2000) Glucagon-induced expression of the MAP kinase phosphatase MKP-1 in rat hepatocytes. *Gastroenterology* **118**:929–936.
- Schubert S, Shannon K, and Bollag G (2007) Hyperactive Ras in developmental disorders and cancer. *Nat Rev Cancer* **7**:295–308.
- Schwacke JH and Voit EO (2007) The potential for signal integration and processing in interacting MAP kinase cascades. *J Theor Biol* **246**:604–620.
- Scimeca JC, Servant MJ, Dyer JO, and Meloche S (1997) Essential role of calcium in the regulation of MAP kinase phosphatase-1 expression. *Oncogene* **15**:717–725.
- Sekine Y, Ikeda O, Hayakawa Y, Tsuji S, Imoto S, Aoki N, Sugiyama K, and Matsuda T (2007) DUSP2/LMW-DSP2 regulates estrogen receptor- α -mediated signaling through dephosphorylation of Ser-118. *Oncogene* **26**:6038–6049.
- Sekine Y, Tsuji S, Ikeda O, Sato N, Aoki N, Aoyama K, Sugiyama K, and Matsuda T (2006) Regulation of STAT3-mediated signaling by LMW-DSP2. *Oncogene* **25**:5801–5806.
- Seta KA, Kim R, Kim HW, Millhorn DE, and Beitner-Johnson D (2001) Hypoxia-induced regulation of MAPK phosphatase-1 as identified by subtractive suppression hybridization and cDNA microarray analysis. *J Biol Chem* **276**:44405–44412.
- Shapiro PS and Ahn NG (1998) Feedback regulation of Raf-1 and mitogen-activated protein kinase (MAP) kinase kinases 1 and 2 by MAP kinase phosphatase-1 (MKP-1). *J Biol Chem* **273**:1788–1793.
- Shaulian E and Karin M (2001) AP-1 in cell proliferation and survival. *Oncogene* **20**:2390–2400.
- She QB, Chen N, Bode AM, Flavell RA, and Dong Z (2002) Deficiency of c-Jun-NH₂-terminal kinase-1 in mice enhances skin tumor development by 12-*O*-tetradecanoylphorbol-13-acetate. *Cancer Res* **62**:1343–1348.
- Shen WH, Wang J, Wu J, Zhurkin VB, and Yin Y (2006) Mitogen-activated protein kinase phosphatase 2: a novel transcription target of p53 in apoptosis. *Cancer Res* **66**:6033–6039.
- Shen Y, Luche R, Wei B, Gordon ML, Diltz CD, and Tonks NK (2001) Activation of the Jnk signaling pathway by a dual-specificity phosphatase, JSP-1. *Proc Natl Acad Sci U S A* **98**:13613–13618.
- Shen YH, Godlewski J, Zhu J, Sathyanarayana P, Leaner V, Birrer MJ, Rana A, and Tzivion G (2003) Cross-talk between JNK/SAPK and ERK/MAPK pathways: sustained activation of JNK blocks ERK activation by mitogenic factors. *J Biol Chem* **278**:26715–26721.
- Shimada K, Nakamura M, Ishida E, Higuchi T, Tanaka M, Ota I, and Konishi N (2007) c-Jun NH2 terminal kinase activation and decreased expression of mitogen-activated protein kinase phosphatase-1 play important roles in invasion and angiogenesis of urothelial carcinomas. *Am J Pathol* **171**:1003–1012.
- Shin DY, Ishibashi T, Choi TS, Chung E, Chung IY, Aaronson SA, and Bottaro DP (1997) A novel human ERK phosphatase regulates H-ras and v-raf signal transduction. *Oncogene* **14**:2633–2639.
- Shin I, Kim S, Song H, Kim HR, and Moon A (2005) H-Ras-specific activation of Rac-MKK3/6-p38 pathway: its critical role in invasion and migration of breast epithelial cells. *J Biol Chem* **280**:14675–14683.
- Shin MS, Kim HS, Lee SH, Lee JW, Song YH, Kim YS, Park WS, Kim SY, Lee SN, Park JY, et al. (2002) Alterations of Fas-pathway genes associated with nodal metastasis in non-small cell lung cancer. *Oncogene* **21**:4129–4136.
- Shinohara M, Mikhailov AV, Aguirre-Ghiso JA, and Rieder CL (2006) Extracellular signal-regulated kinase 1/2 activity is not required in mammalian cells during late G₂ for timely entry into or exit from mitosis. *Mol Biol Cell* **17**:5227–5240.
- Siddik ZH (2003) Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene* **22**:7265–7279.
- Sieben NL, Oosting J, Flanagan AM, Prat J, Roemen GM, Kolkman-Uljee SM, van Eijk R, Cornelisse CJ, Fleuren GJ, and van Engeland M (2005) Differential gene expression in ovarian tumors reveals Dusp 4 and Serpin 5 as key regulators for benign behavior of serous borderline tumors. *J Clin Oncol* **23**:7257–7264.
- Siligan C, Ban J, Bachmaier R, Spahn L, Kreppel M, Schaefer KL, Poremba C, Arvey DN, and Kovar H (2005) EWS-FL11 target genes recovered from Ewing's sarcoma chromatin. *Oncogene* **24**:2512–2524.
- Simon E, Camier S, and Séraphin B (2006) New insights into the control of mRNA decapping. *Trends Biochem Sci* **31**:241–243.
- Sivaraman VS, Wang H, Nuovo GJ, and Malbon CC (1997) Hyperexpression of mitogen-activated protein kinase in human breast cancer. *J Clin Invest* **99**:1478–1483.
- Slack DN, Seternes OM, Gabrielsen M, and Keyse SM (2001) Distinct binding determinants for ERK2/p38 α and JNK map kinases mediate catalytic activation and substrate selectivity of map kinase phosphatase-1. *J Biol Chem* **276**:16491–16500.
- Small GW, Shi YY, Edmund NA, Somasundaram S, Moore DT, and Orłowski RZ (2004) Evidence that mitogen-activated protein kinase phosphatase-1 induction by proteasome inhibitors plays an antiapoptotic role. *Mol Pharmacol* **66**:1478–1490.
- Small GW, Shi YY, Higgins LS, and Orłowski RZ (2007) Mitogen-activated protein

- kinase phosphatase-1 is a mediator of breast cancer chemoresistance. *Cancer Res* **67**:4459–4466.
- Small GW, Somasundaram S, Moore DT, Shi YY, and Orlowski RZ (2003) Repression of mitogen-activated protein kinase (MAPK) phosphatase-1 by anthracyclines contributes to their antiapoptotic activation of p44/42-MAPK. *J Pharmacol Exp Ther* **307**:861–869.
- Smalley KS (2003) A pivotal role for ERK in the oncogenic behaviour of malignant melanoma? *Int J Cancer* **104**:527–532.
- Sommer A, Burkhardt H, Keyse SM, and Lüscher B (2000) Synergistic activation of the *mkp-1* gene by protein kinase A signaling and USF, but not c-Myc. *FEBS Lett* **474**:146–150.
- Song JJ and Lee YJ (2008) Differential cleavage of Mst1 by caspase-7/-3 is responsible for TRAIL-induced activation of the MAPK superfamily. *Cell Signal* **20**:892–906.
- Srikanth S, Franklin CC, Duke RC, and Kraft RS (1999) Human DU145 prostate cancer cells overexpressing mitogen-activated protein kinase phosphatase-1 are resistant to Fas ligand-induced mitochondrial perturbations and cellular apoptosis. *Mol Cell Biochem* **199**:169–178.
- Steeg PS (2003) Metastasis suppressors alter the signal transduction of cancer cells. *Nat Rev Cancer* **3**:55–63.
- Stellato C (2004) Post-transcriptional and nongenomic effects of glucocorticoids. *Proc Am Thorac Soc* **1**:255–263.
- Stepniak E, Ricci R, Eferl R, Sumara G, Sumara I, Rath M, Hui L, and Wagner EF (2006) c-Jun/AP-1 controls liver regeneration by repressing p53/p21 and p38 MAPK activity. *Genes Dev* **20**:2306–2314.
- Stewart AE, Dowd S, Keyse SM, and McDonald NQ (1999) Crystal structure of the MAPK phosphatase Pyst1 catalytic domain and implications for regulated activation. *Nat Struct Biol* **6**:174–181.
- Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, Wagner L, Shenmen CM, Schuler GD, Altschul SF, et al. (2002) Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc Natl Acad Sci USA* **99**:16899–16903.
- Sturla LM, Cowan CW, Guenther L, Castellino RC, Kim JY, and Pomeroy SL (2005) A novel role for extracellular signal-regulated kinase 5 and myocyte enhancer factor 2 in medulloblastoma cell death. *Cancer Res* **65**:5683–5689.
- Sun F, Anantharam V, Zhang D, Latchoumycandane C, Kanthasamy A, and Kanthasamy AG (2006) Proteasome inhibitor MG-132 induces dopaminergic degeneration in cell culture and animal models. *Neurotoxicology* **27**:807–815.
- Sun H, Charles CH, Lau LF, and Tonks NK (1993) MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase in vivo. *Cell* **75**:487–493.
- Sun H, Tonks NK, and Bar-Sagi D (1994) Inhibition of Ras-induced DNA synthesis by expression of the phosphatase MKP-1. *Science* **266**:285–288.
- Suzuki A, Nakano T, Mak TW, and Sasaki T (2008) Portrait of PTEN: messages from mutant mice. *Cancer Sci* **99**:209–213.
- Suzuki C, Unoki M, and Nakamura Y (2001) Identification and allelic frequencies of novel single-nucleotide polymorphisms in the DUSP1 and BTG1 genes. *J Hum Genet* **46**:155–157.
- Swartz CD, Afshari CA, Yu L, Hall KE, and Dixon D (2005) Estrogen-induced changes in IGF-I, Myb family and MAP kinase pathway genes in human uterine leiomyoma and normal uterine smooth muscle cell lines. *Mol Hum Reprod* **11**:441–450.
- Takagaki K, Satoh T, Tanuma N, Masuda K, Takekawa M, Shima H, and Kikuchi K (2004) Characterization of a novel low-molecular-mass dual-specificity phosphatase-3 (LDP-3) that enhances activation of JNK and p38. *Biochem J* **383**:447–455.
- Takagaki K, Shima H, Tanuma N, Nomura M, Satoh T, Watanabe M, and Kikuchi K (2007) Characterization of a novel low-molecular-mass dual specificity phosphatase-4 (LDP-4) expressed in brain. *Mol Cell Biochem* **296**:177–184.
- Takano S, Fukuyama H, Fukumoto M, Hirashimizu K, Higuchi T, Takenawa J, Nakayama H, Kimura J, and Fujita J (1995) Induction of CL100 protein tyrosine phosphatase following transient forebrain ischemia in the rat brain. *J Cereb Blood Flow Metab* **15**:33–41.
- Takehara N, Kawabe J, Aizawa Y, Hasebe N, and Kikuchi K (2000) High glucose attenuates insulin-induced mitogen-activated protein kinase phosphatase-1 (MKP-1) expression in vascular smooth muscle cells. *Biochim Biophys Acta* **1497**:244–252.
- Tang J, Qi X, Mercola D, Han J, and Chen G (2005) Essential role of p38gamma in K-Ras transformation independent of phosphorylation. *J Biol Chem* **280**:23910–23917.
- Tanoue T, Moriguchi T, and Nishida E (1999) Molecular cloning and characterization of a novel dual specificity phosphatase, MKP-5. *J Biol Chem* **274**:19949–19956.
- Tanoue T, Yamamoto T, Maeda R, and Nishida E (2001) A novel MAPK phosphatase MKP-7 acts preferentially on JNK/SAPK and p38 α and β MAPKs. *J Biol Chem* **276**:26629–26639.
- Temple R, Allen E, Fordham J, Phipps S, Schneider HC, Lindauer K, Hayes I, Lockey J, Pollock K, and Jupp R (2001) Microarray analysis of eosinophils reveals a number of candidate survival and apoptosis genes. *Am J Respir Cell Mol Biol* **25**:425–433.
- Theodosiou A and Ashworth A (2002) MAP kinase phosphatases. *Genome Biol* **3**:REVIEWS3009.
- Theodosiou A, Smith A, Gillieron C, Arkininstall S, and Ashworth A (1999) MKP5, a new member of the MAP kinase phosphatase family, which selectively dephosphorylates stress-activated kinases. *Oncogene* **18**:6981–6988.
- Theodosiou AM, Rodrigues NR, Nesbit MA, Ambrose HJ, Paterson H, McLellan-Arnold E, Boyd Y, Leversha MA, Owen N, Blake DJ, et al. (1996) A member of the MAP kinase phosphatase gene family in mouse containing a complex trinucleotide repeat in the coding region. *Hum Mol Genet* **5**:675–684.
- Thornton TM, Pedraza-Alva G, Deng B, Wood CD, Aronshtam A, Clements JL, Sabio G, Davis RJ, Matthews DE, Doble B, et al. (2008) Phosphorylation by p38 MAPK as an alternative pathway for GSK3 β inactivation. *Science* **320**:667–670.
- Tierno MB, Johnston PA, Foster C, Skoko JJ, Shiinde SN, Shun TY, and Lazo JS (2007) Development and optimization of high-throughput in vitro protein phosphatase screening assays. *Nat Protoc* **2**:1134–1144.
- Todd JL, Rigas JD, Rafta LA, and Denu JM (2002) Dual-specificity protein tyrosine phosphatase VHR down-regulates c-Jun N-terminal kinase (JNK). *Oncogene* **21**:2573–2583.
- Todd JL, Tanner KG, and Denu JM (1999) Extracellular regulated kinases (ERK) 1 and ERK2 are authentic substrates for the dual-specificity protein-tyrosine phosphatase VHR: a novel role in down-regulating the ERK pathway. *J Biol Chem* **274**:13271–13280.
- Tonks NK (2005) Redox redux: revisiting PTPs and the control of cell signaling. *Cell* **121**:667–670.
- Tournier C, Hess P, Yang DD, Xu J, Turner TK, Nimmual A, Bar-Sagi D, Jones SN, Flavell RA, and Davis RJ (2000) Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science* **288**:870–874.
- Tsiftoglou AS, Pappas IS, and Vizirianakis IS (2003) Mechanisms involved in the induced differentiation of leukemia cells. *Pharmacol Ther* **100**:257–290.
- Tsuiki H, Tnani M, Okamoto I, Kenyon LC, Emlet DR, Holgado-Madruga M, Lanham JS, Joynes CJ, Vo KT, and Wong AJ (2003) Constitutively active forms of c-Jun NH2-terminal kinase are expressed in primary glial tumors. *Cancer Res* **63**:250–255.
- Tsujita E, Taketomi A, Gion T, Kuroda Y, Endo K, Watanabe A, Nakashima H, Aishima S, Kohnoe S, and Maehara Y (2005) Suppressed MKP-1 is an independent predictor of outcome in patients with hepatocellular carcinoma. *Oncology* **69**:342–347.
- Turjanski AG, Vaqué JP, and Gutkind JS (2007) MAP kinases and the control of nuclear events. *Oncogene* **26**:3240–3253.
- Ueda K, Arakawa H, and Nakamura Y (2003) Dual-specificity phosphatase 5 (DUSP5) as a direct transcriptional target of tumor suppressor p53. *Oncogene* **22**:5586–5591.
- Vakoc CR, Mandat SA, Olenchock BA, and Blobel GA (2005) Histone H3 lysine 9 methylation and HP1 γ are associated with transcription elongation through mammalian chromatin. *Mol Cell* **19**:381–391.
- Valledor AF, Xaus J, Comalada M, Soler C, and Celada A (2000) Protein kinase C ϵ is required for the induction of mitogen-activated protein kinase phosphatase-1 in lipopolysaccharide-stimulated macrophages. *J Immunol* **164**:29–37.
- Valledor AF, Xaus J, Marqués L, and Celada A (1999) Macrophage colony-stimulating factor induces the expression of mitogen-activated protein kinase phosphatase-1 through a protein kinase C-dependent pathway. *J Immunol* **163**:2452–2462.
- Vasudevan SA, Skoko J, Wang K, Burlingame SM, Patel PN, Lazo JS, Nuchtern JG, and Yang J (2005) MKP-8, a novel MAPK phosphatase that inhibits p38 kinase. *Biochem Biophys Res Commun* **330**:511–518.
- Ventura JJ, Hübner A, Zhang C, Flavell RA, Shokat KM, and Davis RJ (2006) Chemical genetic analysis of the time course of signal transduction by JNK. *Mol Cell* **21**:701–710.
- Ventura JJ, Tenbaum S, Perdiguer E, Huth M, Guerra C, Barbacid M, Pasparakis M, and Nebreda AR (2007) p38 α MAP kinase is essential in lung stem and progenitor cell proliferation and differentiation. *Nat Genet* **39**:750–758.
- Verde P, Casalino L, Talotta F, Yaniv M, and Weitzman JB (2007) Deciphering AP-1 function in tumorigenesis: fraternizing on target promoters. *Cell Cycle* **6**:2633–2639.
- Vicent S, Garayoa M, López-Picazo JM, Lozano MD, Toledo G, Thunnissen FB, Manzano RG, and Montuenga LM (2004) Mitogen-activated protein kinase phosphatase-1 is overexpressed in non-small cell lung cancer and is an independent predictor of outcome in patients. *Clin Cancer Res* **10**:3639–3649.
- Vilchez D, Ros S, Cifuentes D, Pujadas L, Vallés J, García-Fojeda B, Criado-García O, Fernández-Sánchez E, Medraño-Fernández I, Dominguez J, et al. (2007) Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. *Nat Neurosci* **10**:1407–1413.
- Vogt A, Tamewitz A, Skoko J, Sikorski RP, Giuliano KA, and Lazo JS (2005) The benzo[c]phenanthridine alkaloid, sanguinarine, is a selective, cell-active inhibitor of mitogen-activated protein kinase phosphatase-1. *J Biol Chem* **280**:19078–19086.
- Vollmer W (2006) The prokaryotic cytoskeleton: a putative target for inhibitors and antibiotics? *Appl Microbiol Biotechnol* **73**:37–47.
- von Mikecz A (2006) The nuclear ubiquitin-proteasome system. *J Cell Sci* **119**:1977–1984.
- Voorhees PM, Dees EC, O'Neil B, and Orlowski RZ (2003) The proteasome as a target for cancer therapy. *Clin Cancer Res* **9**:6316–6325.
- Wada T, Stepniak E, Hui L, Leibbrandt A, Katada T, Nishina H, Wagner EF, and Penninger JM (2008) Antagonistic control of cell fates by JNK and p38-MAPK signaling. *Cell Death Differ* **15**:89–93.
- Waetzig V and Herdgen T (2005) Context-specific inhibition of JNKs: overcoming the dilemma of protection and damage. *Trends Pharmacol Sci* **26**:455–461.
- Wang D and Lippard SJ (2005) Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov* **4**:307–320.
- Wang HY, Cheng Z, and Malbon CC (2003) Overexpression of mitogen-activated protein kinase phosphatases MKP1, MKP2 in human breast cancer. *Cancer Lett* **191**:229–237.
- Wang J, Shen WH, Jin YJ, Brandt-Rauf PW, and Yin Y (2007a) A molecular link between E2F-1 and the MAPK cascade. *J Biol Chem* **282**:18521–18531.
- Wang J, Yin DP, Liu YX, Baer R, and Yin Y (2007b) Dual specificity phosphatase 1/CL100 is a direct transcriptional target of E2F-1 in the apoptotic response to oxidative stress. *Cancer Res* **67**:6737–6744.
- Wang X, Merritt AJ, Seyfried J, Guo C, Papadakis ES, Finegan KG, Kayahara M, Dixon J, Boot-Handford RP, Cartwright EJ, et al. (2005) Targeted deletion of mek5 causes early embryonic death and defects in the extracellular signal-regulated kinase 5/myocyte enhancer factor 2 cell survival pathway. *Mol Cell Biol* **25**:336–345.
- Wang X and Tournier C (2006) Regulation of cellular functions by the ERK5 signaling pathway. *Cell Signal* **18**:753–760.

- Wang Z, Xu J, Zhou JY, Liu Y, and Wu GS (2006) Mitogen-activated protein kinase phosphatase-1 is required for cisplatin resistance. *Cancer Res* **66**:8870–8877.
- Ward Y, Gupta S, Jensen P, Wartmann M, Davis RJ, and Kelly K (1994) Control of MAP kinase activation by the mitogen-induced threonine/tyrosine phosphatase PAC1. *Nature* **367**:651–654.
- Wellbrock C, Karasarides M, and Marais R (2004) The RAF proteins take centre stage. *Nat Rev Mol Cell Biol* **5**:875–885.
- Wellbrock C, Weisser C, Geissinger E, Troppmaier J, and Scharlt M (2002) Activation of p59(Fyn) leads to melanocyte dedifferentiation by influencing MKP-1-regulated mitogen-activated protein kinase signaling. *J Biol Chem* **277**:6443–6454.
- Wiessner C, Neumann-Haefelin T, Vogel P, Back T, and Hossmann KA (1995) Transient forebrain ischemia induces an immediate-early gene encoding the mitogen-activated protein kinase phosphatase 3CH134 in the adult rat brain. *Neuroscience* **64**:959–966.
- Wiland AM, Denu JM, Mourey RJ, and Dixon JE (1996) Purification and kinetic characterization of the mitogen-activated protein kinase phosphatase rVH6. *J Biol Chem* **271**:33486–33492.
- Willert K and Jones KA (2006) Wnt signaling: is the party in the nucleus? *Genes Dev* **20**:1394–1404.
- Willoughby EA and Collins MK (2005) Dynamic interaction between the dual specificity phosphatase MKP7 and the JNK3 scaffold protein β -arrestin 2. *J Biol Chem* **280**:25651–25658.
- Willoughby EA, Perkins GR, Collins MK, and Whitmarsh AJ (2003) The JNK-interacting protein-1 scaffold protein targets MAPK phosphatase-7 to dephosphorylate JNK. *J Biol Chem* **278**:10731–10736.
- Wishart MJ, Denu JM, Williams JA, and Dixon JE (1995) A single mutation converts a novel phosphotyrosine binding domain into a dual-specificity phosphatase. *J Biol Chem* **270**:26782–26785.
- Wishart MJ and Dixon JE (1998) Gathering STYX: phosphatase-like form predicts functions for unique protein-interaction domains. *Trends Biochem Sci* **23**:301–306.
- Wishart MJ and Dixon JE (2002) The archetype STYX/dead-phosphatase complexes with a spermatid mRNA-binding protein and is essential for normal sperm production. *Proc Natl Acad Sci U S A* **99**:2112–2117.
- Wong HR, Dunsmore KE, Page K, and Shanley TP (2005) Heat shock-mediated regulation of MKP-1. *Am J Physiol Cell Physiol* **289**:C1152–C1158.
- Worby CA, Gentry MS, and Dixon JE (2006) Laforin, a dual specificity phosphatase that dephosphorylates complex carbohydrates. *J Biol Chem* **281**:30412–30418.
- Wu J, Jin YJ, Calaf GM, Huang WL, and Yin Y (2007) PAC1 is a direct transcription target of E2F-1 in apoptotic signaling. *Oncogene* **26**:6526–6535.
- Wu JJ and Bennett AM (2005) Essential role for mitogen-activated protein (MAP) kinase phosphatase-1 in stress-responsive MAP kinase and cell survival signaling. *J Biol Chem* **280**:16461–16466.
- Wu JJ, Roth RJ, Anderson EJ, Hong EG, Lee MK, Choi CS, Neuffer PD, Shulman GI, Kim JK, and Bennett AM (2006a) Mice lacking MAP kinase phosphatase-1 have enhanced MAP kinase activity and resistance to diet-induced obesity. *Cell Metab* **4**:61–73.
- Wu JJ, Zhang L, and Bennett AM (2005a) The noncatalytic amino terminus of mitogen-activated protein kinase phosphatase 1 directs nuclear targeting and serum response element transcriptional regulation. *Mol Cell Biol* **25**:4792–4803.
- Wu Q, Gu S, Dai J, Dai J, Wang L, Li Y, Zeng L, Xu J, Ye X, Zhao W, et al. (2003) Molecular cloning and characterization of a novel *Dual-specificity Phosphatase 18* gene from human fetal brain. *Biochim Biophys Acta* **1625**:296–304.
- Wu Q, Huang S, Sun Y, Gu S, Lu F, Dai J, Yin G, Sun L, Zheng D, Dou C, et al. (2006b) Dual specificity phosphatase 18, interacting with SAPK, dephosphorylates SAPK and inhibits SAPK/JNK signal pathway in vivo. *Front Biosci* **11**:2714–2724.
- Wu Q, Li Y, Gu S, Li N, Zheng D, Li D, Zheng Z, Ji C, Xie Y, and Mao Y (2004a) Molecular cloning and characterization of a novel dual-specificity phosphatase 23 gene from human fetal brain. *Int J Biochem Cell Biol* **36**:1542–1553.
- Wu W, Chaudhuri S, Brickley DR, Pang D, Karrison T, and Conzen SD (2004b) Microarray analysis reveals glucocorticoid-regulated survival genes that are associated with inhibition of apoptosis in breast epithelial cells. *Cancer Res* **64**:1757–1764.
- Wu W, Pew T, Zou M, Pang D, and Conzen SD (2005b) Glucocorticoid receptor-induced MAPK phosphatase-1 (MPK-1) expression inhibits paclitaxel-associated MAPK activation and contributes to breast cancer cell survival. *J Biol Chem* **280**:4117–4124.
- Xiao L and Lang W (2000) A dominant role for the c-Jun NH2-terminal kinase in oncogenic Ras-induced morphologic transformation of human lung carcinoma cells. *Cancer Res* **60**:400–408.
- Xu L, Chen S, and Bergan RC (2006) MAPKAPK2 and HSP27 are downstream effectors of p38 MAP kinase-mediated matrix metalloproteinase type 2 activation and cell invasion in human prostate cancer. *Oncogene* **25**:2987–2998.
- Xu Q, Konta T, Nakayama K, Furusu A, Moreno-Manzano V, Lucio-Cazana J, Ishikawa Y, Fine LG, Yao J, and Kitamura M (2004) Cellular defense against H₂O₂-induced apoptosis via MAP kinase-MKP-1 pathway. *Free Radic Biol Med* **36**:985–993.
- Xu S, Furukawa T, Kanai N, Sunamura M, and Horii A (2005) Abrogation of DUSP6 by hypermethylation in human pancreatic cancer. *J Hum Genet* **50**:159–167.
- Yaglom J, O'Callaghan-Sunol C, Gabai V, and Sherman MY (2003) Inactivation of dual-specificity phosphatases is involved in the regulation of extracellular signal-regulated kinases by heat shock and hsp72. *Mol Cell Biol* **23**:3813–3824.
- Yamaguchi Y, Wada T, and Handa H (1998) Interplay between positive and negative elongation factors: drawing a new view of DRB. *Genes Cells* **3**:9–15.
- Yamamoto T, Ebisuya M, Ashida F, Okamoto K, Yonehara S, and Nishida E (2006) Continuous ERK activation downregulates antiproliferative genes throughout G₁ phase to allow cell-cycle progression. *Curr Biol* **16**:1171–1182.
- Yan C, Luo H, Lee JD, Abe J, and Berk BC (2001) Molecular cloning of mouse ERK5/BMK1 splice variants and characterization of ERK5 functional domains. *J Biol Chem* **276**:10870–10878.
- Yan L, Carr J, Ashby PR, Murry-Tait V, Thompson C, and Arthur JS (2003) Knockout of ERK5 causes multiple defects in placental and embryonic development. *BMC Dev Biol* **3**:11.
- Yang SH, Whitmarsh AJ, Davis RJ, and Sharrocks AD (1998) Differential targeting of MAP kinases to the ETS-domain transcription factor Elk-1. *EMBO J* **17**:1740–1749.
- Yang Y, Ikezoe T, Saito T, Kobayashi M, Koeffler HP, and Taguchi H (2004) Proteasome inhibitor PS-341 induces growth arrest and apoptosis of non-small cell lung cancer cells via the JNK/c-Jun/AP-1 signaling. *Cancer Sci* **95**:176–180.
- Yasinska IM and Sumbayev VV (2003) S-nitrosation of Cys-800 of HIF-1 α protein activates its interaction with p300 and stimulates its transcriptional activity. *FEBS Lett* **549**:105–109.
- Yeh S, Lin HK, Kang HY, Thin TH, Lin MF, and Chang C (1999) From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. *Proc Natl Acad Sci U S A* **96**:5458–5463.
- Yip-Schneider MT, Lin A, and Marshall MS (2001) Pancreatic tumor cells with mutant K-ras suppress ERK activity by MEK-dependent induction of MAP kinase phosphatase-2. *Biochem Biophys Res Commun* **280**:992–997.
- Yokoyama A, Karasaki H, Urushibara N, Nomoto K, Imai Y, Nakamura K, Mizuno Y, Ogawa K, and Kikuchi K (1997) The characteristic gene expressions of MAPK phosphatases 1 and 2 in hepatocarcinogenesis, rat ascites hepatoma cells, and regenerating rat liver. *Biochem Biophys Res Commun* **239**:746–751.
- Yoo CB and Jones PA (2006) Epigenetic therapy of cancer: past, present and future. *Nat Rev Drug Discov* **5**:37–50.
- Yoon S and Seger R (2006) The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. *Growth Factors* **24**:21–44.
- Yoshida M and Horinouchi S (1999) Trichostatin and leptomycin: inhibition of histone deacetylation and signal-dependent nuclear export. *Ann N Y Acad Sci* **886**:23–36.
- Yoshida S, Fukino K, Harada H, Nagai H, Imoto I, Inazawa J, Takahashi H, Teramoto A, and Emi M (2001) The c-Jun NH2-terminal kinase3 (JNK3) gene: genomic structure, chromosomal assignment, and loss of expression in brain tumors. *J Hum Genet* **46**:182–187.
- Yoshiaki A and Moriwaki K (2006) Mouse phenome research: implications of genetic background. *ILAR J* **47**:94–102.
- Yu C, Minemoto Y, Zhang J, Liu J, Tang F, Bui TN, Xiang J, and Lin A (2004) JNK suppresses apoptosis via phosphorylation of the proapoptotic Bcl-2 family protein BAD. *Mol Cell* **13**:329–340.
- Yu W, Imoto I, Inoue J, Onda M, Emi M, and Inazawa J (2007) A novel amplification target, DUSP26, promotes anaplastic thyroid cancer cell growth by inhibiting p38 MAPK activity. *Oncogene* **26**:1178–1187.
- Yun MS, Kim SE, Jeon SH, Lee JS, and Choi KY (2005) Both ERK and Wnt/ β -catenin pathways are involved in Wnt3a-induced proliferation. *J Cell Sci* **118**:313–322.
- Zama T, Aoki R, Kamimoto T, Inoue K, Ikeda Y, and Hagiwara M (2002a) A novel dual specificity phosphatase SKRP1 interacts with the MAPK kinase MKK7 and inactivates the JNK MAPK pathway: implication for the precise regulation of the particular MAPK pathway. *J Biol Chem* **277**:23909–23918.
- Zama T, Aoki R, Kamimoto T, Inoue K, Ikeda Y, and Hagiwara M (2002b) Scaffold role of a mitogen-activated protein kinase phosphatase, SKRP1, for the JNK signaling pathway. *J Biol Chem* **277**:23919–23926.
- Zambrowicz BP and Sands AT (2003) Knockouts model the 100 best-selling drugs—will they model the next 100? *Nat Rev Drug Discov* **2**:38–51.
- Zarubin T and Han J (2005) Activation and signaling of the p38 MAP kinase pathway. *Cell Res* **15**:11–18.
- Zhang B, Hosaka M, Sawada Y, Torii S, Mizutani S, Ogata M, Izumi T, and Takeuchi T (2003) Parathyroid hormone-related protein induces insulin expression through activation of MAP kinase-specific phosphatase-1 that dephosphorylates c-Jun NH₂-terminal kinase in pancreatic β -cells. *Diabetes* **52**:2720–2730.
- Zhang H, Shi X, Hampong M, Blanis L, and Pelech S (2001) Stress-induced inhibition of ERK1 and ERK2 by direct interaction with p38 MAP kinase. *J Biol Chem* **276**:6905–6908.
- Zhang XM, Dormady SP, Chung W, and Basch RS (2000) mVH1, a dual-specificity phosphatase whose expression is cell cycle regulated. *Mamm Genome* **11**:1154–1156.
- Zhang Y, Blattman JN, Kennedy NJ, Duong J, Nguyen T, Wang Y, Davis RJ, Greenberg PD, Flavell RA, and Dong C (2004) Regulation of innate and adaptive immune responses by MAP kinase phosphatase 5. *Nature* **430**:793–797.
- Zhao Q, Shepherd EG, Manson ME, Nelin LD, Sorokin A, and Liu Y (2005) The role of mitogen-activated protein kinase phosphatase-1 in the response of alveolar macrophages to lipopolysaccharide: attenuation of proinflammatory cytokine biosynthesis via feedback control of p38. *J Biol Chem* **280**:8101–8108.
- Zhao Q, Wang X, Nelin LD, Yao Y, Matta R, Manson ME, Baliga RS, Meng X, Smith CV, Bauer JA, et al. (2006) MAP kinase phosphatase 1 controls innate immune responses and suppresses endotoxin shock. *J Exp Med* **203**:131–140.
- Zugaza JL, Caloca MJ, and Bustelo XR (2004) Inverted signaling hierarchy between RAS and RAC in T-lymphocytes. *Oncogene* **23**:5823–5833.
- Zwelling LA, Anderson T, and Kohn KW (1979) DNA-protein and DNA interstrand cross-linking by *cis*- and *trans*-platinum(II) diamminedichloride in L1210 mouse leukemia cells and relation to cytotoxicity. *Cancer Res* **39**:365–369.