Diverse physiological functions for dual-specificity MAP kinase phosphatases

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Summary

A structurally distinct subfamily of ten dual-specificity (Thr/Tyr) protein phosphatases is responsible for the regulated dephosphorylation and inactivation of mitogen-activated protein kinase (MAPK) family members in mammals. These MAPK phosphatases (MKPs) interact specifically with their substrates through a modular kinase-interaction motif (KIM) located within the N-terminal non-catalytic domain of the protein. In addition, MAPK binding is often accompanied by enzymatic activation of the C-terminal catalytic domain, thus ensuring specificity of action. Despite our knowledge of the biochemical and structural basis for the catalytic mechanism of the MKPs, we know much less about their regulation and physiological functions in mammalian cells and tissues. However, recent studies employing a range of model systems have begun to reveal essential non-redundant roles for the MKPs in determining the outcome of MAPK signalling in a variety of physiological contexts. These include development, immune system function, metabolic homeostasis and the regulation of cellular stress responses. Interestingly, these functions may reflect both restricted subcellular MKP activity and changes in the levels of signalling through multiple MAPK pathways.

Key words: MAPK, MKP, Signal transduction, Phosphorylation

Introduction

Mitogen-activated protein kinases (MAPKs) constitute a conserved family of enzymes that regulate a large number of physiological processes, including proliferation, differentiation, development, immune function, stress responses and apoptosis (Chang and Karin, 2001; Davis, 2000; Johnson and Lapadat, 2002; Pearson et al., 2001; Wada and Penninger, 2004). Mammalian MAPKs comprise three major groups, which are classified on the basis of sequence similarity, differential activation by agonists and substrate specificity. These are the p42/p44 MAPKs or extracellular signal-regulated kinases (ERKs), the Jun N-terminal kinases (JNK1, JNK2, JNK3) and the p38 MAPKs (α, β, δ and γ) (Cohen, 1997).

MAPKs are activated by phosphorylation on threonine and tyrosine residues within a conserved signature sequence TxY by a MAPK kinase (MKK or MEK), which is in turn phosphorylated and activated by a MAPK kinase kinase (MKKK or MEKK) (Marshall, 1994; Qi and Elion, 2005). The component kinases of the MAPK module may interact sequentially, but can also be organised into signalling complexes via interactions with specific scaffold proteins (Garrington and Johnson, 1999; Morrison and Davis, 2003).

One conserved property of MAPK signalling is that the magnitude and duration of MAPK activation is a crucial determinant of biological outcome (Ebisuya et al., 2005; Marshall, 1995; Sabbagh, Jr et al., 2001). This highlights the importance of negative regulatory mechanisms in determining the output of MAPK pathways, and it is now clear that a major point of control occurs at the level of the MAPK itself, via the intervention of protein phosphatases. Because modification of both the threonine residue and the tyrosine residue within the activation motif is required for MAPK activity, dephosphorylation of either residue inactivates these enzymes. This can be achieved by tyrosine-specific phosphatases, serine-threonine phosphatases or dual-specificity (Thr/Tyr) phosphatases, and work using a variety of model organisms has demonstrated that all three classes of protein phosphatase can regulate MAPKs in vivo (Keyse, 2000; Saxena and Mustelin, 2000). However, the largest group of phosphatases dedicated to the regulation of MAPK signalling in vertebrates are the dual-specificity MAPK phosphatases (MKPs).

The MKP subfamily of dual-specificity phosphatases

The MKPs constitute a structurally distinct subgroup of 11 proteins within the larger family of dual-specificity protein phosphatases (DUSP or VHR-like cysteine-dependent protein phosphatases) encoded in the human genome (Alonso et al., 2004). The subgroup comprises ten active enzymes and one protein, DUSP24/MK-STYX, that has many features of an MKP but lacks the active site cysteine that is essential for catalysis (Wishart and Dixon, 1998). All MKPs share a common structure (see Fig. 1A), comprising a C-terminal catalytic domain that shows sequence similarity to the prototypic dual-specificity protein phosphatase VH-1 of vaccinia virus and an N-terminal domain containing two regions of sequence similarity to the catalytic domain of the Cdc25 phosphatase (Keyse and Ginsburg, 1993). The latter similarity reflects a common evolutionary origin for this domain of the MKPs and Cdc25 in the rhodanese family of sulphotransferases (Bordo and Bork, 2002). The N-terminal MKP domain also contains a cluster of basic amino-acid residues, which play an important role in MAPK substrate

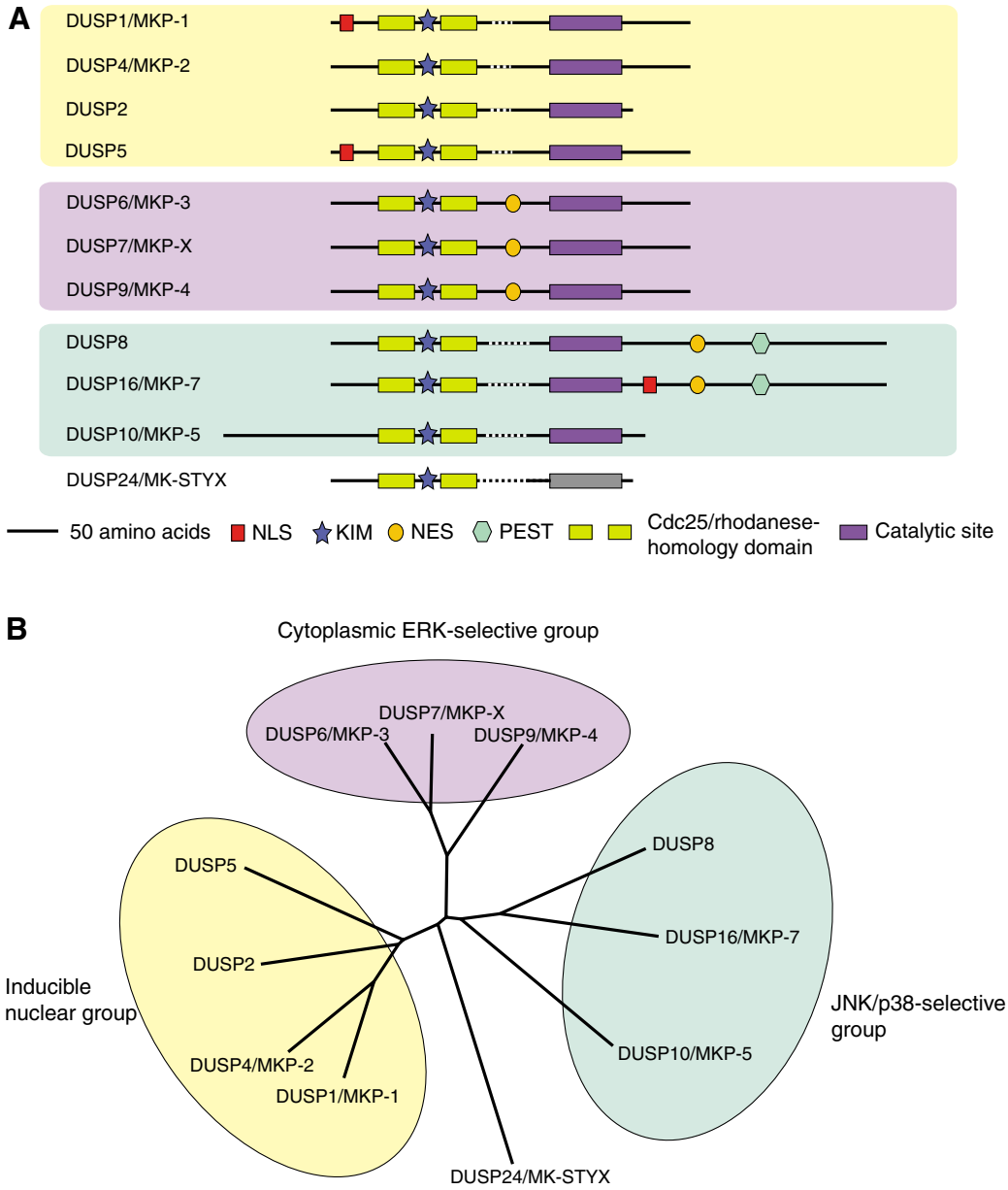

Fig. 1. Classification, domain structure and phylogenetic analysis of the dual-specificity MAPK phosphatases. (A) Domain structures of the ten catalytically active DUSP proteins and MK-STYX. In addition to the N-terminal non-catalytic domain containing the Cdc25/rhodanese-homology region and the catalytic site, the positions of the conserved kinase-interaction motif (KIM), nuclear localisation signals (NLS), nuclear export signals (NES) and PEST sequences are indicated. The three subgroups revealed by the phylogenetic analysis are indicated by the background colour. (B) DUSP sequence analysis. Human DUSP amino acid sequences were aligned using CLUSTALW (http://align.genome.jp/) and a phylogenetic tree was generated. The three subgroups of DUSP proteins together with defining properties are indicated by the coloured ovals.

recognition and binding (Tanoue et al., 2000; Tanoue et al., 2002).

On the basis of sequence similarity, gene structure, substrate specificity and subcellular localisation, the MKPs can be further subdivided into three groups (Fig. 1B). The first comprises four inducible nuclear MKPs encoded by *DUSP1* (MKP-1), *DUSP2* (PAC-1), *DUSP4* (MKP-2) and *DUSP5* (hVH3). The second group contains three closely related cytoplasmic ERK-specific MKPs encoded by *DUSP6* (MKP-3), *DUSP7* (MKP-X) and *DUSP9* (MKP-4). The third group contains three MKPs encoded by *DUSP8* (hVH5), *DUSP10* (MKP-5) and *DUSP16* (MKP-7); these selectively inactivate the stress-activated MAPK isoforms (Camps et al., 2000; Keyse, 2000; Theodosiou and Ashworth, 2002).

While our knowledge of the biochemical and structural basis of MKP catalysis and substrate specificity has greatly increased over the past few years, progress in our understanding of the physiological roles of these enzymes in mammalian cells and tissues has been somewhat slow. This is due to the large number of MKPs with overlapping substrate specificities,

combined with a lack of relevant mammalian models. Here, we focus on recent progress using a variety of approaches, including the generation of mouse gene knockouts, which has begun to yield insights into the regulation and functions of the MKPs and will provide experimental platforms to enable us to determine whether these enzymes play a part in the development of human diseases including immune disorders, diabetes and cancer.

MKPs in immune function, stress responses and metabolic homeostasis

DUSP10/MKP-5 and JNK in innate and adaptive immunity

The first MKP to be identified as specific for the stress-activated MAPKs JNK and p38 in mammalian cells was DUSP8 (also known as hVH5 or M3/6) (Muda et al., 1996). This subgroup of MKPs was expanded by the subsequent characterisation of the related phosphatases DUSP10/MKP-5 and DUSP16/MKP-7 (Masuda et al., 2001; Tanoue et al., 1999; Tanoue et al., 2001; Theodosiou et al., 1999).

Only DUSP10/MKP-5 has been studied genetically in mammals. DUSP10/MKP-5 was originally identified as a phosphatase capable of regulating JNK and p38 MAPKs, but not ERK1 or ERK2, both in vitro and in vivo (Tanoue et al., 1999; Theodosiou et al., 1999). Interestingly, DUSP10/MKP-5 mRNA is induced in a mouse macrophage cell line by bacterial lipopolysaccharide (LPS). In T cells, it is constitutively expressed in naive CD4 T cells but downregulated 24 hours after T-cell activation (Zhang et al., 2004). Deletion of the murine gene encoding DUSP10/MKP-5 does not cause developmental defects, nor is this enzyme essential for the development of the immune system. However, both the Th1 and Th2 subsets of T-helper cells lacking DUSP10/MKP-5 have significantly higher levels of JNK activity, whereas p38 MAPK activity is unaffected. Increased JNK activity is also seen in DUSP10/MKP-$5^{-/-}$ macrophages after LPS treatment, which induces innate responses via Toll-like receptor (TLR) 4. This indicates that the inactivation of JNK by DUSP10/MKP-5 regulates innate or adaptive immune responses. Indeed, peritoneal macrophages from DUSP10/MKP-$5^{-/-}$ animals, when challenged with LPS, produce increased levels of the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor (TNF), and TLR2 and TLR3 signalling also results in increased levels of these cytokines. Moreover, injection of LPS into DUSP10/MKP-$5^{-/-}$ mice leads to increased serum TNF production (Zhang et al., 2004).

Another function of the innate immune system is the activation of antigen-specific T cells. In this regard, the T-cell-priming activity of LPS-treated antigen-presenting cells (APCs) lacking DUSP10/MKP-5 is greatly enhanced. DUSP10/MKP-5 is therefore a negative regulator of innate immunity (Zhang et al., 2004). In the adaptive immune response, CD4 T cells from DUSP10/MKP-$5^{-/-}$ mice show reduced proliferative responses to activating stimuli, which indicates that DUSP10/MKP-5 is required for proper T cell expansion. To examine the role of DUSP10/MKP-5 in effector T-helper-cell function, wild-type or knockout CD4 T cells were differentiated into Th1 and Th2 cells. These T-helper-cell subtypes are defined on the basis of the specific cytokines they produce. Th1 cells produce interferon γ (IFNγ) and IL12 whereas Th2 cells produce IL3, IL4 and IL13. When compared with wild-type cells, DUSP10/MKP-$5^{-/-}$ Th1 and Th2 cells were found to produce increased levels of IFNγ and IL-4, respectively. DUSP10/MKP-$5^{-/-}$ CD8 T cells (also known as cytotoxic or killer T cells) also produce more IFNγ and TNF in vitro. These results indicate that cytokine expression in effector T cells is negatively regulated by DUSP10/MKP-5.

Finally, the effects of loss of DUSP10/MKP-5 in several in vivo immune and autoimmune models have been characterised. Immunisation with myelin oligodendrocyte glycoprotein (MOG) peptide induces experimental autoimmune encephalomyelitis (EAE). Compared with wild-type mice, DUSP10/MKP-$5^{-/-}$ animals exhibit a markedly reduced incidence and severity of disease, which suggests that this phosphatase plays a crucial role in the generation and/or expansion of autoreactive T cells in EAE (Zhang et al., 2004). This finding, together with the reduced proliferative capacity of naive T-helper cells in these animals, indicates a positive regulatory role for DUSP10/MKP-5 in these cells. DUSP10/MKP-5 may therefore have distinct roles depending on the immune cell population studied. Note also that one cannot assign JNK as the preferred target for DUSP10/MKP-5 activity unless the levels of activation for each MAPK pathway are measured in all cell types.

To examine the role of DUSP10/MKP-5 during T-cell-mediated immunity to infection, Zhang et al. also infected animals with lymphocytic choriomeningitis virus (LCMV). Although DUSP10/MKP-$5^{-/-}$ mice clear virus and exhibit little difference in the primary T-cell response, they respond to secondary LCMV challenge by producing significantly elevated levels of serum TNF. This is probably responsible for the immune-mediated death of these animals 2-4 days after challenge.

DUSP10/MKP-5 thus negatively regulates the JNK signalling pathway and serves important roles as a negative regulator of innate immune responses and a regulator of proper T cell function. It is not yet clear whether, as suggested by biochemical studies, DUSP10/MKP-5 is also involved in the regulation of p38 MAPK activity or plays any additional role in the regulation of mammalian stress responses. No doubt these aspects of DUSP10/MKP-5 function will be the subject of future studies as will the generation and study of mice lacking the genes encoding the other MKPs that selectively inactivate the stress-activated MAPKs.

Inducible nuclear MKPs as regulators of immune function

The inducible nuclear MKPs constitute a subfamily of four enzymes: DUSP1/MKP-1, DUSP2/PAC-1, DUSP4/MKP-2 and DUSP5. An early disappointment in the genetic dissection of MKP function came with a report that mice lacking the prototypic inducible nuclear phosphatase DUSP1/MKP-1 appear normal and fertile (Dorfman et al., 1996). Furthermore, cells derived from these animals show no evidence of abnormal regulation of the ERK pathway. However, although DUSP1/MKP-1 was originally shown to dephosphorylate the ERK1/2 MAPKs in vitro, it can also target the JNKs and p38 MAPKs (Franklin and Kraft, 1997; Groom et al., 1996; Slack et al., 2001). Despite this, neither the activities of JNK or p38 nor stress-induced endpoints were originally studied in DUSP1/MKP-1-null animals. However, several groups have

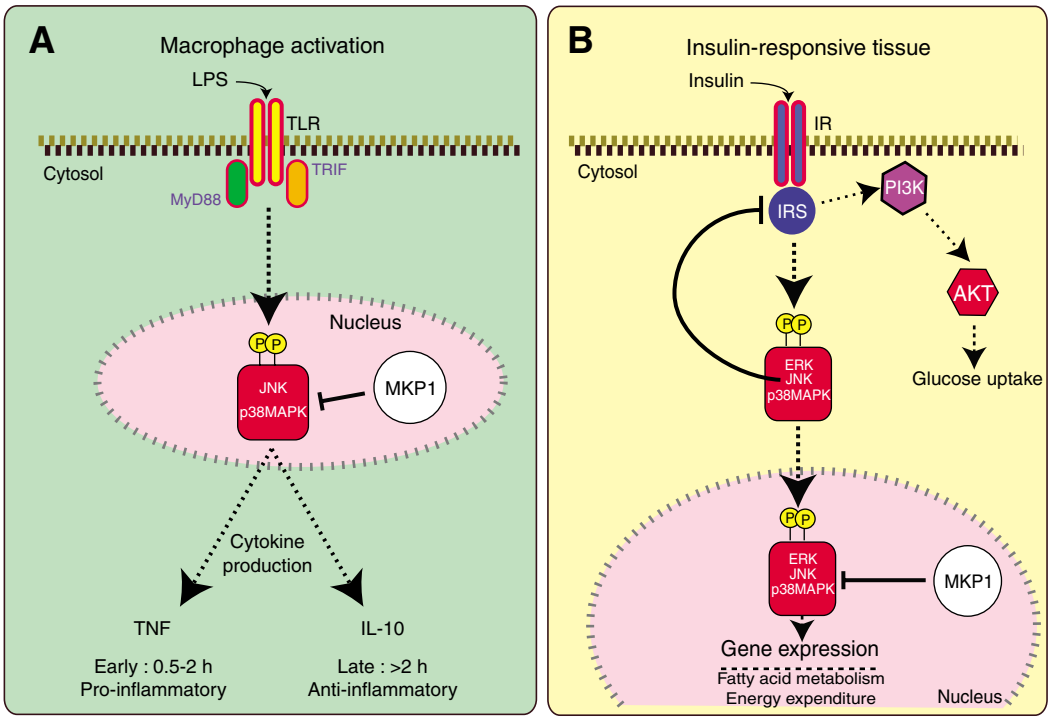

Fig. 2. Physiological roles of DUSP1/MKP-1 in immunity and metabolic regulation. (A) In macrophages, LPS signals through Toll-like receptor (TLR), which engages the adaptor proteins MyD88 and TRIF. This leads to an early response, in which p38 MAPK and JNK are activated and contribute to the expression of TNF, and is followed by induction of MKP-1 expression, which serves to downregulate p38/JNK activities and TNF production. At later times, as MKP-1 expression declines, the levels of p38 MAPK and JNK activity remain higher than in unstimulated cells and promote IL10 expression. (B) In insulin-responsive tissues, MKP-1 regulates multiple MAPK pathways in the nucleus, and thus controls the expression of genes involved in fatty acid metabolism and energy expenditure. The activities of MAPKs in the cytosol are not subject to regulation by MKP-1. Therefore, negative feedback controls, such as the phosphorylation of IRS by JNK, are unaffected.

recently addressed this question and provided compelling evidence that DUSP1/MKP-1 plays a key role in regulation of the physiological output of signalling through the ERK, JNK and p38 MAPK pathways.

First, both mouse embryo fibroblasts (MEFs) and primary alveolar macrophages from mice lacking DUSP1/MKP-1 show increased JNK and p38 activities in response to a range of activating stimuli compared with wild-type cells (Wu and Bennett, 2005; Zhao et al., 2005). Furthermore, MEFs lacking DUSP1/MKP-1 display lower growth rates owing to increased cell death and are also more sensitive to anisomycin- or hydrogen-peroxide-induced apoptosis (Wu and Bennett, 2005; Zhou et al., 2006). These results demonstrate that DUSP1/MKP-1 promotes cell survival by attenuating signalling through the stress-activated MAPK pathways.

The finding that loss of DUSP1/MKP-1 greatly increases the duration of p38 activation in LPS-treated macrophages suggested that this phosphatase might regulate inflammation during the innate immune response (Zhao et al., 2005). Recent work now confirms such a role, because mice lacking DUSP1/MKP-1 respond to LPS by overproducing a subset of cytokines, including TNF, IL6 and IL10, and are acutely sensitive to lethal endotoxic shock. They also exhibit a marked increase in both the incidence and severity of experimentally induced autoimmune arthritis (Chi et al., 2006; Hammer et al., 2006; Salojin et al., 2006; Zhao et al., 2006). Thus DUSP1/MKP-1 controls the levels of both pro-inflammatory (TNF) and anti-inflammatory (IL10) cytokines in response to LPS (Fig. 2A). The overproduction of TNF in DUSP1/MKP-$1^{-/-}$ macrophages is seen only at early time points after exposure to LPS. By contrast, elevated levels of IL10 are seen at all time points following LPS challenge. The reduction in TNF levels seen at these later time points most probably reflects the known function of IL10 in suppressing production of TNF, macrophages responding to the IL10 in an autocrine and/or paracrine fashion. In support of this mechanism, exposure of DUSP1/MKP-$1^{-/-}$ macrophages to a neutralising antibody directed against IL10 prior to LPS challenge enhances TNF mRNA levels (Chi et al., 2006). This indicates a dynamic balance between MAPK activation and the activity of DUSP1/MKP-1 in the temporal regulation of pro- and anti-inflammatory mediators.

Finally, it has been known for some time that synthetic glucocorticoids such as dexamethasone are potent inducers of DUSP1/MKP-1 expression (Kassel et al., 2001; Lasa et al., 2002). Because these agents inhibit the expression of pro-inflammatory genes and are widely used in the treatment of inflammatory disease, it was suggested that at least part of the therapeutic action of these drugs might be mediated by induction of DUSP1/MKP-1 (Clark, 2003). A recent study has now shown that the suppression of a subset of pro-inflammatory genes including those encoding TNF, cyclooxygenase-2, IL-1α and IL-1β in response to dexamethasone is reduced in macrophages from DUSP1/MKP-

1-knockout mice and that the in vivo anti-inflammatory effects of dexamethasone on zymosan-induced inflammation are also impaired in these animals. These results demonstrate that the expression of DUSP1/MKP-1 is necessary for the inhibition of pro-inflammatory signalling and that this is at least partly responsible for the anti-inflammatory effects of dexamethasone in vivo (Abraham et al., 2006).

It is interesting to compare the immune defects seen in the DUSP1/MKP-1$^{-/-}$ animals with those in the DUSP10/MKP-5 knockout (Zhang et al., 2004). Both phosphatases have essential, non-redundant functions in regulating immune function despite being able to dephosphorylate and inactivate the same subset of MAPKs. However, there may be significant differences in the way that these two enzymes differentially regulate MAPK pathways. Loss of DUSP10/MKP-5 has a greater effect on signalling through the JNK pathway, whereas the effects of knocking out DUSP1/MKP-1 in immune cells are more consistent with increased signalling through the p38 MAPK pathway. For instance, both the overproduction of cytokines and expression of co-stimulatory molecules in LPS-stimulated DUSP1/MKP-1$^{-/-}$ macrophages are abrogated by SB203580, a specific inhibitor of p38 MAPK, which indicates that this pathway is a major target of DUSP1/MKP-1 in these cells (Chi et al., 2006; Hammer et al., 2006). Note, however, that one group has observed attenuation of IL12 levels in DUSP1/MKP-1$^{-/-}$ macrophages stimulated with LPS (Zhao et al., 2006). This is surprising, because the production of this cytokine has been closely linked to levels of p38 MAPK activity (Lu et al., 1999). Finally, it should be remembered that a major difference between DUSP10/MKP-5 and DUSP1/MKP-1 is the ability of the latter to influence the activity of all three classes of MAPK and the combinatorial regulation of these pathways by DUSP1/MKP-1 relative to DUSP10/MKP-5 may account for the distinct effects of gene deletion on endpoints such as cytokine production.

Of the inducible MKPs, the enzyme most closely associated with cells of the immune system is DUSP2/PAC-1. This phosphatase was originally identified as a mitogen-inducible gene in human T cells that encodes a dual-specificity phosphatase with activity towards ERK1/2 and p38 MAPKs in vitro and in vivo (Chu et al., 1996; Rohan et al., 1993; Ward et al., 1994). More recently, DUSP2/PAC-1 was found to be a transcriptional target of the p53 pathway in response to either growth-factor withdrawal or oxidative stress. The resulting inactivation of ERK2 MAPK has been implicated in p53-mediated apoptosis (Yin et al., 2003). Animals lacking DUSP2/PAC-1 have a complex phenotype with respect to the regulation of MAPK activities in immune effector cells and this has revealed an unexpected positive regulatory function for this enzyme in the induction and maintenance of certain inflammatory responses (Jeffrey et al., 2006).

Micro-array analysis of gene expression in wild-type versus DUSP2/PAC-1-null macrophages following LPS stimulation revealed a significant deficit in the expression of pro-inflammatory mediators and cytokines including IL-6, IL12α, cyclooxygenase-2, IL-1β and inflammatory chemokines in the null cells. Furthermore, this deficit can be rescued by re-expression of wild-type but not a catalytically inactive mutant of DUSP2/PAC-1 (Jeffrey et al., 2006). To test the physiological role of DUSP2/PAC-1, Jeffrey et al. assessed basic features of the immune system such as lymphoid tissue development and lymphocyte/granulocyte numbers in blood and bone marrow and found them to be normal. They then assessed the null mice in an autoimmune (K/BxN serum-induced) model of inflammatory arthritis in which pathology is driven by effector leukocytes such as mast cells, neutrophils and macrophages. Whereas wild-type mice injected with arthritogenic K/BxN serum develop inflammatory arthritis within 2 days and show inflammatory cell infiltration and eventual joint destruction, DUSP2/PAC-1-null littermates are protected and show greatly diminished pathological features of arthritis.

Despite biochemical evidence linking DUSP2/PAC-1 to negative regulation of ERK and p38 MAPKs in vitro, both mast cells and macrophages derived from animals lacking PAC-1 display reduced levels of activated ERK and p38. By contrast, levels of activated JNK, which had not previously been identified as a DUSP2/PAC-1 substrate, are considerably higher in LPS-stimulated macrophages (Jeffrey et al., 2006). Pharmacological inhibition of JNK activity in these cells leads to a significant increase in ERK activity, which suggests that, by elevating JNK activity, DUSP2/PAC-1 loss may indirectly decrease the activity of ERK through an undefined mechanism. With respect to the latter, it is now clear that direct crosstalk between MAPK pathways can occur in cells during both stress- and growth-factor-induced signalling (Shen et al., 2003; Zhang et al., 2001). Despite this apparent selectivity of DUSP2/PAC-1 for inactivation of JNK, co-immunoprecipitation experiments using an inactive mutant of DUSP2/PAC-1 detected interactions with ERK, p38 and JNK1 in EGF-stimulated cells. However, a recent study has indicated that, although DUSP2/PAC-1 interacts efficiently with p38, it fails to dephosphorylate and inactivate this MAPK in vitro (Zhang et al., 2005).

The recently generated knockouts thus have provided contrasting and apparently contradictory immune system phenotypes. Loss of DUSP10/MKP-5 causes elevation of JNK activity and enhanced cytokine production, whereas loss of DUSP1/MKP-1 elevates both p38 and JNK activities, resulting in defects in innate immune cell responses. By contrast, the combination of increased JNK activation, combined with a reduction of ERK and p38 activities seen in DUSP2/PAC-1-null animals, translates into a quite different outcome: a deficit in cytokine gene expression accompanied by protection from inflammatory arthritis. It will be important to explore the apparent discordance between the effects of loss of genes encoding MKPs on MAPK signalling and the biochemical studies of DUSP10/MKP-5 and DUSP2/PAC-1 substrate specificity. These studies give a strong indication that MKP function is complex and that the ability of these enzymes to differentially modulate multiple MAPK pathways can give rise to quite distinct biological outcomes.

MKP-1 and metabolic homeostasis

Given that DUSP1/MKP-1 is widely expressed during development and in adult tissues (Carrasco and Bravo, 1993; Kwak et al., 1994), it would be surprising if its functions were restricted to cells of the immune system. Recent work, again using the DUSP1/MKP-1$^{-/-}$ mice, indicates that this phosphatase also plays a key role in regulating MAPK

signalling in insulin-responsive tissues. The effects of DUSP1/MKP-1 deletion on MAPK signalling in adult tissues are complex. Hyperactivation of JNK and p38 is seen in white adipose tissue (WAT), skeletal muscle and liver whereas ERK activity is elevated only in WAT and muscle (Wu et al., 2006). Furthermore, DUSP1/MKP-1-null animals gain weight following weaning onto a chow diet at a significantly lower rate than wild-type animals and are also resistant to obesity induced by a high-fat diet. Surprisingly, despite enhanced MAPK activities in insulin-responsive tissues, DUSP1/MKP-1$^{-/-}$ mice remain susceptible to the development of glucose intolerance and hyperinsulinemia caused by high-fat feeding. Thus, DUSP1/MKP-1 must regulate body mass independently of the regulation of glucose homeostasis.

At first sight these results seem difficult to reconcile with recent work on the role of JNK signalling in animal models, because elevated JNK activity has been associated with obesity and insulin resistance, and mice lacking JNK1 are both resistant to diet-induced obesity and also exhibit insulin sensitivity (Hirosumi et al., 2002). However, two features of the regulation of MAPK activities by DUSP1/MKP-1 could account for the uncoupling of diet-induced obesity from glucose intolerance (Fig. 2B).

First, all three of the MAPKs targeted by DUSP1/MKP-1 are capable of affecting metabolic regulation. p38 MAPK can regulate energy expenditure via activation of peroxisome proliferator-activated receptor gamma (PPARγ) coactivator 1α (PGC-1α) (Fan et al., 2004) and mice lacking ERK1 display resistance to diet-induced obesity coupled with protection from the development of insulin resistance (Bost et al., 2005). Thus, the phenotype of the DUSP1/MKP-1$^{-/-}$ mice probably reflects complex changes in the activities of all three major classes of MAPK in target tissues rather than hyperactivation of one MAPK pathway in isolation (Wu et al., 2006).

Second, DUSP1/MKP-1 is a nuclear phosphatase and it may not inactivate the cytosolic pools of activated MAPK, which participate in the control of insulin-responsive glucose uptake. Indeed Wu et al. did not see any changes in the phosphorylation of insulin receptor substrate 1 (IRS-1), which is targeted by JNK, in DUSP1/MKP-1$^{-/-}$ mice. They also observed the nuclear accumulation of phosphorylated JNK in livers from these animals and this correlated with enhanced nuclear Jun phosphorylation and activation in DUSP1/MKP-1$^{-/-}$ hepatocytes (Wu et al., 2006). Thus, the increased activity of all three classes of MAPK in the nucleus might increase the transcription of genes that regulate fatty acid metabolism and energy expenditure.

There are alternative explanations, which require further investigation. First, a role for the activity of DUSP1/MKP-1 in tissues other than those studied cannot be excluded. In particular, DUSP1/MKP-1 is expressed in regions of the brain including the pineal gland and hypothalamus, where changes in MAPK signalling might lead to altered behavior and thus affect energy expenditure (Price et al., 2004). Second, the mixed genetic background may play a role in the expression of both the metabolic and immune phenotypes seen in the DUSP1/MKP-1 knockout. It will certainly be interesting to study the effects of DUSP1/MKP-1 deletion in an inbred strain and also to employ tissue-specific deletion of this gene to dissect the target tissue(s) and signalling pathways responsible for this clinically interesting phenotype.

The cytoplasmic ERK-specific MKPs play important roles during early development

DUSP9/MKP-4 is essential for placental development and function

The subfamily of cytoplasmic ERK-specific MKPs comprises three closely related proteins encoded by *DUSP6* (MKP-3), *DUSP7* (MKP-X) and *DUSP9* (MKP-4). DUSP6/MKP-3 specifically inactivates ERK1 and ERK2, whereas biochemical studies indicate that both DUSP7/MKP-X and DUSP9/MKP-4 may also be able to inactivate p38 MAPK (Camps et al., 1998; Dickinson et al., 2002b; Dowd et al., 1998; Groom et al., 1996). Deletion of the murine DUSP9/MKP-4 gene causes embryonic lethality at mid-gestation (Christie et al., 2005). This is a consequence of placental insufficiency caused by a failure of labyrinth development. The appearance of this defect correlates exactly with the timing of expression of DUSP9/MKP-4 in the normal placenta, in which it is first detected in the ectoplacental cone and chorion, and later appears in the trophoblast giant cells, spongiotrophoblast and labyrinth as these lineages differentiate (Christie et al., 2005; Dickinson et al., 2002b).

Despite this clear developmental defect, no evidence could be found for abnormal phosphorylation or activation of either ERK or p38 MAPKs in the placenta. This indicates that either the timing of any abnormal signalling events is restricted or that the magnitude of the changes in the affected cells and tissues is too small to detect. Interestingly, disruption of either MEK1 (Giroux et al., 1999), an upstream activator of ERK1/2, or p38α MAPK (Adams et al., 2000) gives rise to placental phenotypes that are strikingly similar to that seen following loss of DUSP9/MKP-4. This indicates that abnormal regulation of either or both of these signalling cascades is involved. Further studies involving an examination of well-characterised patterns of marker gene expression during placental development may shed some light on the underlying defect in tissues lacking DUSP9/MKP-4.

Tetraploid aggregation has been used to bypass the placental defect caused by loss of this phosphatase. Despite the normally high levels of DUSP9/MKP-4 expression in the developing liver and in the kidneys and testes of adult mice, the DUSP/MKP-4-null animals generated in this way develop to term, have no obvious phenotype and are fertile (Christie et al., 2005). Furthermore, DUSP9/MKP-4-expressing tissues such as the embryonic liver, kidney and testis, show no abnormal morphology in the null animals. Thus, essential non-redundant functions for DUSP9/MKP-4 appear to be restricted to the extra-embryonic tissues during development. However, it is possible that DUSP9/MKP-4 is required to regulate MAPK signalling in adult tissues, perhaps during as-yet-undefined conditions of stress.

DUSP6/MKP-3 is a negative feedback regulator of fibroblast growth factor signalling

The first clues about the regulation and physiological function of DUSP6/MKP-3 came from studies of the pattern of its expression in developing mouse embryos (Dickinson et al., 2002a). Dynamic patterns of DUSP6/MKP-3 mRNA expression are detected in limb bud and branchial arch mesenchyme, midbrain/hindbrain isthmus, and nasal, dental, hair and mammary placodes. Most of these are known sites of fibroblast growth factor (FGF) signalling, which indicates that

Table 1. Nomenclature, key properties and mouse knockout phenotypes for the dual-specificity MAP kinase phosphatases

Gene	MKP	Trivial names	Localisation	Substrate MAPKs	Knockout phenotype
DUSP1	MKP-1	CL100, 3CH134, erp, hVH1	Nuclear	ERK1/2, p38, JNK	Immune defects and resistance to obesity
DUSP4	MKP-2	hVH2, TYP1, STY8	Nuclear	ERK1/2, p38, JNK	N/D
DUSP2	N/A	PAC1	Nuclear	ERK1/2, p38	Resistant to immune inflammation
DUSP5	N/A	hVH3, B23	Nuclear	ERK1/2	N/D
DUSP6	MKP-3	PYST1, rVH6	Cytoplasmic	ERK1/2	N/D
DUSP7	MKP-X	PYST2, B59	Cytoplasmic	ERK1/2	N/D
DUSP9	MKP-4	PYST3	Cytoplasmic	ERK1/2 > p38	Midgestation lethality – placental insufficiency
DUSP8	N/A	hVH5, HB5, M3/6	Nuclear/cytoplasmic	JNK, p38	N/D
DUSP10	MKP-5	N/A	Nuclear/cytoplasmic	JNK, p38	Defects in innate and adaptive immunity
DUSP16	MKP-7	N/A	Nuclear/cytoplasmic	JNK, p38	N/D

both the expression and function of DUSP6/MKP-3 could be related to this key pathway during early development. Subsequent studies have reinforced the link between FGF signalling and DUSP6/MKP-3 function.

In chicken embryos, tissue ablation and transplantation have revealed that the apical ectodermal ridge (AER) and Henson's node, both of which produce FGFs, are essential for DUSP6/MKP-3 expression in the developing limb and nervous system, respectively (Eblaghie et al., 2003; Kawakami et al., 2003). Furthermore, expression of DUSP6/MKP-3 is also regulated by FGF in the murine isthmic organiser during neural tube development and in the developing chick somite (Echevarria et al., 2005; Smith et al., 2005). MKP-3 could therefore be a crucial negative feedback regulator of FGF signalling. However, it is less clear which intracellular signalling pathway(s) downstream of the FGF receptor promote the expression of DUSP6/MKP-3. Essential roles have been proposed for both ERK-MAPK (Eblaghie et al., 2003; Gomez et al., 2005; Smith et al., 2005; Tsang et al., 2004) and PI3-kinase (Echevarria et al., 2005; Kawakami et al., 2003) signalling. These studies have involved a range of tissues from different model systems, often using different pharmacological inhibitors of the ERK and PI3-kinase pathways, and this may explain the apparently contradictory data (Smith et al., 2006). In addition, evidence for the involvement of retinoic acid and maternal β-catenin has been obtained (Moreno and Kintner, 2004; Tsang et al., 2004). Detailed studies of the interface between intracellular signalling pathways and the DUSP6/MKP-3 promoter will be required to clarify these issues.

Overexpression of DUSP6/MKP-3 in the chick limb and neural plate causes developmental abnormalities, whereas knocking it down by using RNAi results in increased levels of apoptosis in limb-bud mesenchyme (Eblaghie et al., 2003; Kawakami et al., 2003). DUSP6/MKP-3 might therefore regulate the developmental outcome of FGF signalling by setting an appropriate level of activated ERK MAPK. In support of this notion, both gain- and loss-of function studies in zebrafish embryos reveal an essential role for DUSP6/MKP-3 in limiting the extent of ERK-MAPK signalling in response to FGF, interference with this negative regulation causing disruption of dorsoventral patterning at the onset of gastrulation (Tsang et al., 2004).

Concluding remarks and perspectives

It is now clear that, rather than comprising a group of related enzymes with overlapping and redundant functions, the MKPs have distinct and essential roles, which impinge on a wide range of physiological outputs of MAPK signalling. These include immune function, stress responses and metabolic regulation (Table 1). Interestingly, the phenotypes observed in animals lacking a single MKP may reflect complex changes in the activities of multiple MAPK pathways, suggesting MAPK signalling is integrated at the level of regulation by MKPs. In addition, the differential subcellular localisation of MKPs may facilitate spatio-temporal regulation of MAPK activities. The demonstration that deletion of MKP-1 selectively affects nuclear JNK activity to regulate energy expenditure demonstrates the importance of spatially restricted MKP activity in determining the physiological outcome of signalling.

Clearly, these studies are only a beginning in terms of providing us with a full understanding of MKP function. There is no doubt that, as the animal models are more widely studied and additional knockouts are produced, the repertoire of known MKP functions will expand. In the immediate future there are several issues to be resolved. These include the reasons for the apparent discordance between the biochemical activities of MKPs such as DUSP2/PAC-1 and the observed changes in MAPK activities in animals lacking this gene, and why elevation of JNK signalling on deletion of this phosphatase produces quite different effects on immune function compared with loss of other JNK-specific MKPs, such as DUSP10/MKP-5. In addition to different combinatorial effects on MAPK signalling, one explanation for the latter may be that, although distinct MKPs share common MAPK substrates, their activities may be directed through selective recruitment to scaffolding proteins. Finally, the effects of loss of MKP function on the immune system and metabolic regulation raise the possibility that selective pharmacological inhibition of these enzymes has therapeutic potential and should greatly stimulate interest in this class of dual-specificity protein phosphatases as candidates for drug targets.

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