Protein family review

MAP kinase phosphatases

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Summary

Mitogen-activated protein MAP kinases are key signal-transducing enzymes that are activated by a wide range of extracellular stimuli. They are responsible for the induction of a number of cellular responses, such as changes in gene expression, proliferation, differentiation, cell cycle arrest and apoptosis. Although regulation of MAP kinases by a phosphorylation cascade has long been recognized as significant, their inactivation through the action of specific phosphatases has been less studied. An emerging family of structurally distinct dual-specificity serine, threonine and tyrosine phosphatases that act on MAP kinases consists of ten members in mammals, and members have been found in animals, plants and yeast. Three subgroups have been identified that differ in exon structure, sequence and substrate specificity.

The mitogen-activated protein (MAP) kinases are evolutionary conserved enzymes that play an important role in orchestrating a variety of cellular processes, including proliferation, differentiation and apoptosis [1,2]. To date, four major discrete groups of MAP kinases have been identified in mammalian cells, known as the extracellular signal-regulated kinases (ERK1 and ERK2), the c-Jun amino-terminal kinases (JNK1, JNK2 and JNK3), the p38 kinases (p38α, p38β, p38γ and p38δ) and ERK5/Big MAP kinase 1 (BMK1). MAP kinases are part of a three-tiered cascade consisting of a MAP kinase, a MAP kinase kinase (MAPKK, MKK or MEK) and a MAP kinase kinase kinase (MAPKKK or MEKK). Phosphorylation of the threonine and tyrosine residues in the T-loop of the MAP kinase, by its upstream kinase, results in activation. The activation of MAP kinases is not a simple switch, however, as both the duration and magnitude of activation is crucial in determining the physiological outcome in cells [3]. Thus, it seems likely that dephosphorylation of the MAP kinases is vital for their control. This is achieved by removal of phosphate groups from either the threonine residue or the tyrosine residue, or both. Both protein serine/threonine phosphatases and protein tyrosine phosphatases have been reported to dephosphorylate MAP kinases. In the past decade, however, some dual-specificity phosphatases (DSPs) have been recognized as key players for inactivating different MAP kinase isoforms; this class of phosphatases has been designated MAP kinase phosphatases (MKPs).

Although the MKPs form a structurally and functionally distinct subclass among the large number of protein phosphatases present in eukaryotic genomes, there is a great deal of confusion regarding their classification. In this article we discuss the family of 'classical' MKPs that have been definitively shown to play a role in the de-activation of MAP kinases. Where relevant, we also mention the closely related low-molecular-weight phosphatases, whose role in MAP kinase regulation is controversial. Other more distantly related DSP families, including members of the FYVE family, which have high sequence similarity to myotubularin, and of the Cdc25 family, which dephosphorylate cyclin-dependent kinases, are beyond the scope of this review.

Gene organization and evolutionary history
Gene organization

To date, 10 genes encoding members of the classical MKP family have been isolated and characterized from mammalian

genomes (see Table 1). They all share some common features, including an extended active-site motif with significant sequence similarity to the corresponding region of the VH-1 protein tyrosine phosphatase that was isolated from vaccinia virus [4]. In addition, their amino termini contain two short regions that are homologous to sequences that flank the Cdc25 phosphatase catalytic site [5] and a cluster of basic amino-acid residues that play an important role in binding to the MAP kinases [6]. The MKPs can be further subdivided into groups depending on their substrate specificity for the MAP kinases, subcellular localization (nuclear, cytoplasmic or both) and the structural organization of their genes.

A gene closely related to the MKP family is *STYX*. The protein encoded by *STYX* contains many of the hallmarks of the MKPs but lacks a critically important catalytically active cysteine residue, having a glycine instead [7]. Interestingly, alteration of this glycine residue to cysteine renders the STYX protein catalytically active, suggesting that other critical residues have been retained. STYX appears to function as a phosphotyrosine/phosphoserine/phosphothreonine-binding protein analogous to the SH2 and PTB domains. It is unclear whether it plays any role in signaling through the MAP kinase pathways, however.

Evolutionary history
On the basis of structures predicted from genomic sequence, the MKPs can readily be divided into the following subgroups: subgroup I, DUSP1, DUSP2, DUSP4 and DUSP5; subgroup II, DUSP6, DUSP7, DUSP9 and DUSP10; and subgroup III, DUSP8 and DUSP16 (Figure 1, Table 1). Division into these three subgroups is also supported by phylogenetic

analysis (Figure 2), and to a considerable extent by substrate preference.

The genes of subgroup I consist of four exons [8-11], and the exact positions of the introns are highly conserved. The first exon encodes the amino terminus of each of the three proteins and ends within the second of the Cdc25 homology (CH2) domains. The active-site motif of all four proteins is encoded within exon 4, and the length of exon 3 is identical for all these DUSPs, suggesting that they arose from a common ancestral gene.

Subgroup II genes (*DUSP6*, *7* and *9*, and provisionally *10*) consist of three exons [12]. As with subgroup I, exon 1 encodes the amino terminus and the 3′-most exon encodes the active site. Unlike subgroup I, however, the central portion of the protein is encoded by a single exon. *DUSP10* has an intron/exon structure consistent with a placement within subgroup II, but it shares some features with members of subgroup III, such as a similar MAP kinase docking-site motif and similar substrate specificity. In addition, it has an extended amino-terminal region that is not present in any of the other DUSPs. Assignment of *DUSP10* to subgroup II or III or to its own subgroup may need re-evaluation in the future.

The genes of subgroup III (*DUSP8* and *DUSP16*) consist of six exons [13-15]. The lengths of exons 2, 3, 4 and 5 are identical in the two genes. Unlike the two previous subgroups, however, exon 1 encodes only the first of the CH2 domains. Exon 2 ends within the same region of the second CH2 domain as does exon 1 of subgroups I and II, however. The

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Table 1

Classification and chromosomal and subcellular localization of DUSP genes

DUSP	Subgroup	Human protein	Species ortholog	Chromosomal localization	Subcellular localization	References
DUSP1	I	hVH1/CL100	MKP-1/3CH134 (m)	5q35	Nuclear	[8,60-62]
DUSP2	I	hPAC-1	—-	2p11.2-2q11	Nuclear	[10,42,62]
DUSP4	I	hVH2/TYP1	MKP-2 (r)	8p12-p11	Nuclear	[42,63-65]
DUSP5	I	hVH3/B23	Cpg21 (r)	10q25	Nuclear	[48,62,66]
DUSP6	II	PYST1	MKP-3/rVH6 (r)	12q22-q23	Cytosolic	[49-51,65]
DUSP7	II	PYST2/B59	MKP-X (r)	3p21	Cytosolic	[49,51,65,67]
DUSP8	III	hVH5	M3/6 (m)	11p15.5	Nuclear /cytosolic	[13,43,44]
DUSP9	II	MKP-4	—-	Xq28	Nuclear /cytosolic	[45]
DUSP10	II(?)	MKP-5	—-	1q32	Nuclear /cytosolic	[46,52]
DUSP16	III	MKP-7	MKP-M (m)	12p12	Cytosolic	[14,15,36]

Compilation of DUSP genes identified in the human and other mammalian genomes. The DUSPs are classified into subgroups I, II or III (see text for details). The various alternative names for each gene in human (h) or rat (r) or mouse (m) are given. The chromosomal location of each gene and the subcellular localization of the protein product of each gene are also indicated. Multiple names are used in the literature for each member of the MAP kinase phosphatase gene family. To avoid confusion in this article, we have used the gene names recommended by the human gene nomenclature committee, which uses the root DUSP (for dual specificity phosphatase). Note that the designation of DUSP16 is provisional.
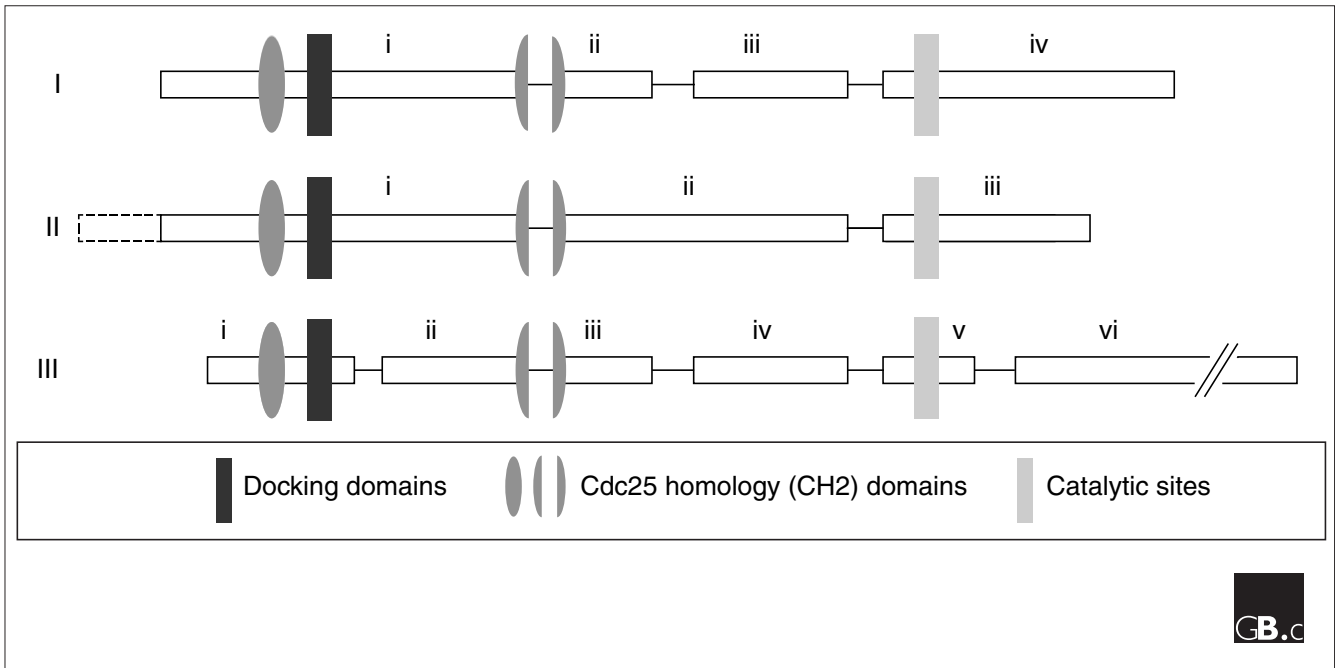
Figure 1
Gene structure of the DUSPs. Three distinct gene structures have been described for the DUSPs, allowing their classification into subgroups I, II and III. Structural motifs (CH2 domains, docking domain and catalytic domain) in the encoded proteins are indicated by shaded shapes and exons by boxes and roman numerals. The dashed box in subgroup II is an alternatively spliced exon.
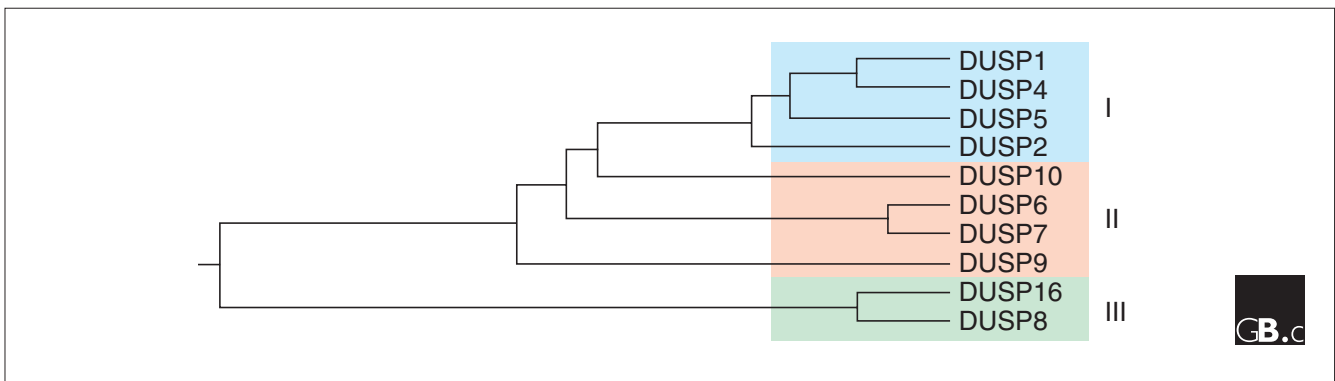

Figure 2
Phylogenetic analysis of DUSP sequences. Human DUSP amino-acid sequences were aligned and a phylogenetic tree derived using Clustal W [68] on DNASTAR. The length of the branches is proportional to sequence divergence between proteins. Subgroups I, II, and III are shaded (see text for further details).

central portion of the protein is encoded by two exons, as in subgroup I, rather than the single exon of subgroup II. This suggests that subgroup II genes may have diverged earlier and that subgroup I and III genes may be more closely related to each other. Exon 5 encodes the catalytic site of these DUSPs but not the carboxyl terminus; the sixth exon encodes the large extended carboxyl terminus, with the intron between the catalytic domain and the carboxyl terminus of the protein being diagnostic of this subgroup.

Overall, the high conservation of some intron/exon boundaries within all three subgroups of DUSPs indicates a common ancestral gene for each subgroup. The similarities of substrate specificity among subgroup members suggest that these ancestral genes are themselves likely to have shown some substrate preference.

Dual specificity phosphatases have been found in a wide variety of phylogenetically distinct eukaryotes, including

yeasts, worms, flies and plants, as well as mammals. Although budding yeast does not encode any predicted protein tyrosine kinases, it has 16 dual specificity and low-molecular-weight phosphatases and five phosphatase-like *STYX* genes. A direct role has been demonstrated for the budding yeast dual specificity phosphatase gene *MSG5* in the regulation of the MAP kinase Fus3 [16]. Six MKPs have been noted in the *Drosophila melanogaster* genome sequence [17]. Of these, two have been characterized: *puckered* has been shown to encode a MKP that inactivates *basket*, the *Drosophila* equivalent of mammalian JNK [18]; in addition, a *Drosophila DUSP6*-like gene has recently been identified [19]. The sequence of the *Caenorhabditis elegans* genome includes 26 predicted DSPs, including some MKPs [20]. One of these, LIP-1, has been suggested to inactivate MAP kinase, mediating inhibition by the ligand Notch of a signaling pathway involving the GTPase Ras, during *C. elegans* vulval development [21]. Moreover, at least two MKPs have been identified in *Arabidopsis thaliana*, one of which is required for relief from genotoxic stress [22].

Characteristic structural features
Catalytic domain
All the DUSPs share strong amino-acid sequence identity over their catalytic domains (37%-50%), with members of certain subgroups sharing up to 75% identity (for example, DUSP6 with DUSP7 and DUSP9 or DUSP8 with DUSP16). The catalytic domain invariably contains the highly conserved consensus sequence **D**X$_{26}$(V/L)X(V/I)H**C**XAG(I/V)-S**R**SXT(I/V)XXAY(L/I)M, in the single-letter amino-acid code where X is any amino acid. The three amino acids indicated in bold have been shown to be absolutely essential for catalysis. The cysteine is required for the nucleophilic attack of the phosphorus of the substrate and the formation of the thiol-phosphate intermediate; the conserved arginine binds the phosphate group of the phosphotyrosine or phosphothreonine, enabling transition-state stabilization; whereas the aspartate enhances catalysis by protonating the leaving group oxygen [23].

Determination of the crystal structure of the catalytic domain of DUSP6 [24] has revealed that this domain adopts a shallow cleft conformation, similar to that of VH1-related phosphatase (VHR) [25]. Within this cleft, the phosphatase is able to accommodate both phosphotyrosine and phosphothreonine side chains. In the absence of substrate, DUSP6 exists in a low-activity state, with key residues (Arg299 and Asp262 in DUSP6) disengaged from interaction. Upon binding of the substrate ERK2, however, the active-site residues are rearranged, adopting a catalytically active conformation [24] and enabling the dephosphorylation of both Thr183 and Tyr185 in ERK2 (Figure 3b). In particular, Asp262 (the conserved aspartate in the consensus sequence) is located in a loop, 5.5 Å away from the nucleophilic cysteine and arginine in the active site. This suggests that the loop must undergo con-

formational rearrangement for activation. Upon binding of the substrate ERK2, closure of the Asp262 loop over the active site occurs, positioning the aspartate residue for catalysis. Thus DUSP6 adopts a catalytically active conformation [24].

CH2 domains
The amino terminus is much less conserved in the DUSPs than the catalytic domain. Nevertheless, all DUSPs contain at their amino terminus two conserved regions that show similarity to the Ccd25 phosphatase, designated CH2 domains. In Cdc25, these domains flank the catalytic site of the enzyme, whereas in the DUSPs they are found upstream of the active site [5]. They have been suggested to play a role in substrate binding, but this has yet to be formally demonstrated.

Docking sites
All DUSPs have, near to their amino termini, a MAP kinase docking site, which consists of a cluster of positively charged
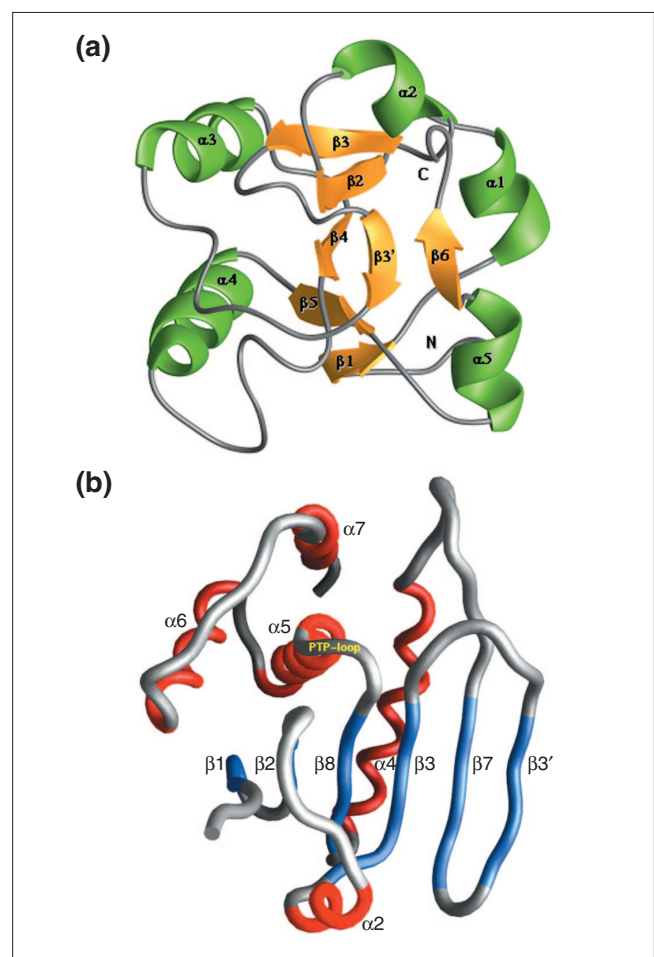

Figure 3
Three-dimensional structure of DUSP6. **(a)** The amino-terminal Erk-binding (EB) domain, reproduced with permission from [38]. **(b)** The catalytic domain, reproduced with permission from [24].

amino acids [6]. It has been proposed that the number of consecutive positively charged residues in this docking site of MKPs may play a role in determining binding specificity and therefore catalytic activity (Figure 4) [14]. The corresponding docking site on the MAP kinases has been shown to consist of negatively charged residues (known as the common docking domain, CD), suggesting that electrostatic interactions are critical for the binding of MAP kinases and MKPs. Consistent with this suggestion, an altered form of ERK2 (D319N) corresponding to the *Drosophila sevenmaker* mutation is highly compromised in its ability to bind to, and activate, DUSP6 [26,27]. The low-molecular-weight DSPs do not have the MAP kinase docking site present in MKPs, but VHR (a low-molecular-weight DSP) has been shown to be capable of inactivating ERK in cells [28,29], suggesting that it may have a distinct mechanism of interaction.

Further regions that are potentially important for docking to and activation by MAPKs have now been defined in certain subsets of MKPs. The FXFP motif, and variants of this sequence (present in DUSP1, DUSP4, DUSP6, DUSP7 and DUSP9) has been shown to mediate ERK binding [30-32]. Another such region is the D-domain (or D-box), which comprises a cluster of basic residues amino-terminal to an L/I-X-L/I motif [30,32,33]. The consensus for this site was derived from alignment of the sequence of the delta domain of c-Jun, the docking site for JNK, with similar motifs found in other transcription factors that are known targets of the MAP kinases. This domain is present in all MKPs identified to date [34] and is thought to mediate binding to both JNK

and ERK MAP kinases [30,32]. Given the diversity of docking sites, the prevailing view is that the affinity of the MKPs for their substrates may be governed by the number, type and accessibility of docking sites.

PEST sequences
The subgroup III DUSPs, DUSP8 and DUSP16, have an extended carboxyl terminus containing PEST sequences (abundant in proline, glutamate, serine and threonine residues) that are frequently found in rapidly degraded proteins [35]. Removal of the PEST sequences from these proteins can result in their stabilization ([36] and our unpublished observations), consistent with a role for the domain in rapid turnover.

Regulation of MKPs
In 1998, Arkinstall's group [27] demonstrated that binding of DUSP6 to purified ERK2 enhances the catalytic activity of the phosphatase. Since then, several papers have attempted to elucidate the mechanism of catalytic activation of DUSP6 [37,38] and to define the regions of interaction between DUSP6 and ERK2. The derivation of the solution structure of the ERK2-binding (EB) domain of DUSP6 [38] has provided considerable insight into this issue (Figure 3a). The EB domain of DUSP6 contains a positively charged cluster of arginine residues that binds to a highly negatively charged region of ERK2. This binding causes a conformational change in DUSP6, affecting the association of its EB domain with its catalytic domain, which results in the re-positioning of the conserved aspartate residue (Asp262 in DUSP6).

MKP	MAP kinase docking site	MAP kinase substrates
DUSP1	FSTIVRRRAKGAKGA	
DUSP2	WNALLRRRARARGPP	JNK/SAPK p38 (ERK)
DUSP4	CNTIVRRRAKGSVSL	
DUSP5	NSVVLRRARGGAVSA	
DUSP6	PGIMLRRLQKGNLPV	ERK
DUSP7	PALLLRRLRRGSLSV	
DUSP9	PGLMLRRLRKGNLPI	
DUSP8	SKLVKRRLQQGKVTI	
DUSP10	DKISRRRLQQGKITV	JNK/SAPK p38
DUSP16	SKLMKRRLQQIKVLI	

Figure 4
Classification of DUSPs on the basis of MAP kinase docking site. The sequence thought to be responsible for MAP kinase interaction in DUSPs is shown, as is substrate preference. This results in a DUSP subclassification similar to that obtained by analysis of gene structure (Figure 1) or amino-acid sequence similarity (Figure 2). Adapted from [14].

These conformational effects, in conjunction with the catalytic domain binding to phosphorylated ERK2, allosterically trigger the rearrangement of DUSP6 active-site residues, resulting in a high-activity state of the phosphatase (Figure 5). This mechanism of activation of DUSP6 by its substrate may be generally applicable to the DUSPs; recent data support a similar model for both DUSP4 and DUSP9 [27,39], and binding of DUSP1 to p38 results in the catalytic activation of the phosphatase [40].

An alternative regulatory mechanism is exemplified by the binding of DUSP1 to ERK1, which results in phosphorylation of the phosphatase [41]. This phosphorylation does not directly affect the phosphatase activity of DUSP1, but instead leads to its stabilization, by reducing the rate of proteosome-mediated degradation of this rather labile protein [41].

Localization and function

Most DUSPs display wide tissue distribution but some show a rather restricted expression pattern. These include: DUSP2, which is predominantly expressed in hematopoietic tissues [42]; DUSP8, which is expressed mainly in brain, as well as in heart and lung [43,44]; DUSP9, which is detected in placenta, kidney and fetal liver [45]; and DUSP10, which

appears to be restricted to liver and skeletal muscle [46]. Detailed information on expression patterns during development is not available for most DUSPs.

The DUSPs vary in their subcellular locations and can be subdivided into three major groups on the basis of location. One group consists of members expressed exclusively in the nucleus, including DUSP1, DUSP2, DUSP4 and DUSP5 [42,47,48] whereas another group includes DUSP6, DUSP7 and DUSP16, which are predominantly expressed in the cytoplasm [12,14,15,49-51]. Some DUSPs (DUSP8, DUSP9 and DUSP10) show both cytoplasmic and nuclear localizations [44-46,52]. It is of interest that DUSP9 shows punctate nuclear staining, which is reminiscent of the pattern displayed by promyelocytic leukemia (PML) protein [45]. Moreover, DUSP9 and PML co-localize in a significant proportion of cells, suggesting a potential role for DUSP9 as a regulator of kinases that phosphorylate PML. The recently identified DUSP16 has been shown to act as a shuttle protein [15]; it has both an active nuclear localization signal (NLS) and a nuclear export signal (NES), and it has been suggested to function by translocating into the nucleus, where it interacts with and dephosphorylates its substrates (JNK and/or p38) and then subsequently transports them to the cytoplasm. The sequence of the NES region of DUSP8 is very
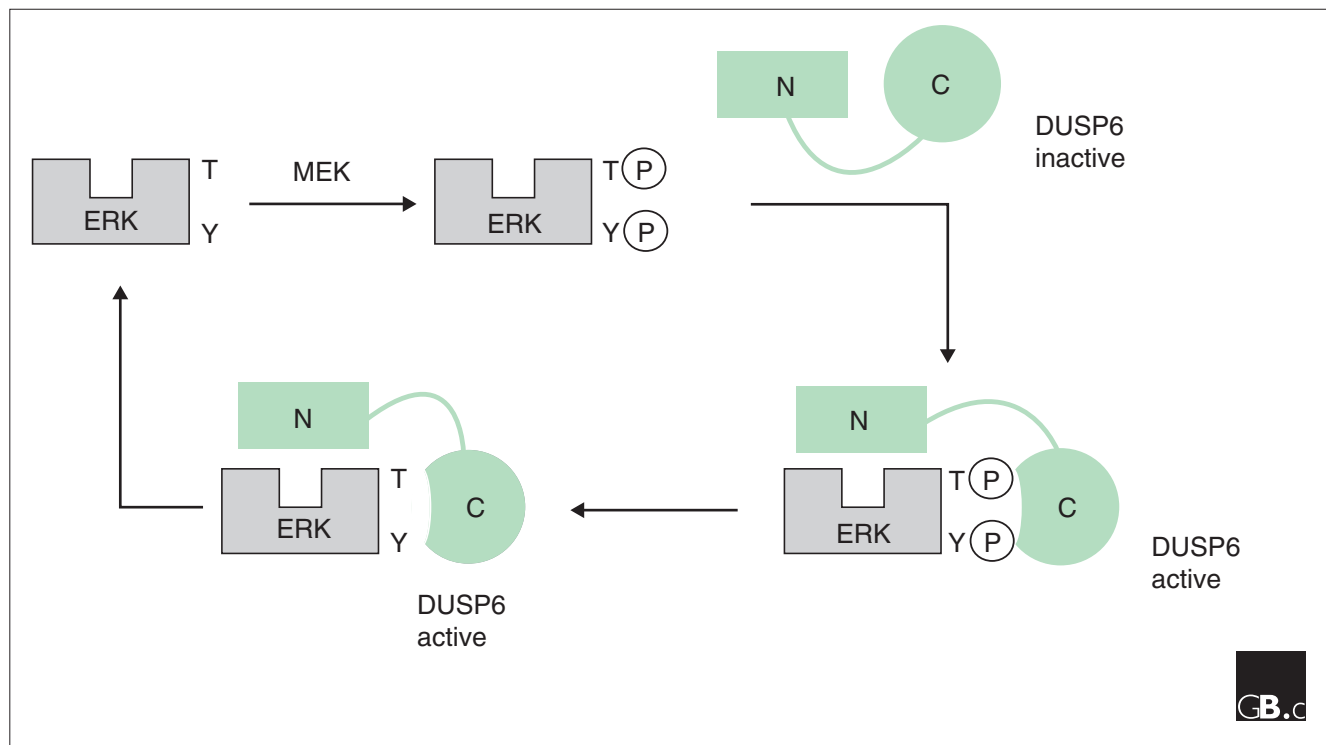

Figure 5
Activation of DUSP6 phosphatase by interaction with substrate ERK. This is a simplified schematic representation of ERK binding to DUSP6, resulting in catalytic activation. The carboxy-terminal catalytic domain is represented by a circle and the amino terminus containing the ERK binding (EB) domain by a rectangle. DUSP6 appears to exist in a low-activity state until binding to ERK through the EB domain results in a conformational change, triggering activation of the phosphatase. This results in ERK dephosphorylation and subsequent dissociation of the complex.

similar to that of DUSP16, and it therefore seems possible that DUSP8 also shuttles between cytoplasm and nucleus.

Despite the wealth of data supporting the role of MKPs in the regulation of MAP kinases in cell-culture systems, there is as yet no direct *in vivo* evidence confirming this in mammals. Disruption of the *DUSP1* gene in mice causes no obvious phenotype [53]. Moreover, *DUSP1*-deficient embryonic fibroblasts and stem cells display no abnormalities in the regulation of MAP kinases. Genetic studies in yeasts, *Drosophila* and *Arabidopsis* have provided evidence for a role for MKPs in the regulation of MAP kinases *in vivo*, however. In *Drosophila*, mutations in *puckered* (encoding an MKP) cause severe developmental defects, resulting in embryonic lethality due to hyperactivation of *Drosophila* JNK and failure of dorsal closure [18]. Moreover, a mutant has been identified in *Arabidopsis* that develops normally but is hypersensitive to genotoxic stress as a result of disruption of a gene that encodes a MKP, *AtMKP1*. AtMKP1 is required for maintaining the MAP kinase activity at levels that contribute to genotoxic stress resistance rather than those that trigger cell death [22].

Substrate specificity

Assessment of substrate specificity for the DUSPs has proved problematic because many of the assays used do not reflect the situation *in vivo*. From data published so far, however, it seems that many of the DUSPs have preferred substrates. For example, after transfection into mammalian cells, DUSP6 completely inactivates ERK1 and ERK2, even at low concentrations, but not JNK or p38 kinases [49,54]; similarly, DUSP9 also displays some specificity towards ERK [45]. In contrast, DUSP8 and DUSP16 appear highly specific for the inactivation of JNK and p38 kinases but fail to inhibit ERK activation [14,15,54]; similarly, DUSP10 displays specificity for p38 and JNK kinases [46,52]. These 'specificities' should be treated with caution, as they may be specific to a certain cell type or physiological status [26,55,56]. Some of the specificity may reside in the docking sites present on the various MKPs, but it must be emphasized that access to substrate may be an equally important factor in regulating specificity *in vivo*.

Frontiers

MAP kinase activation has been implicated in oncogenic transformation, tumor formation and metastasis [57-59]. This suggests that the MKPs could have a role in tumorigenesis, perhaps as tumor suppressors. Several MKPs have been mapped to regions of the human genome altered in human cancer, but direct evidence for such a role is at present lacking. It will be interesting to see whether this family of genes plays a part in the development of cancer or other diseases.

The lack of relevant mammalian models makes it difficult to assign a physiological role to these phosphatases. Given the overlapping substrate specificity displayed by members of the various subgroups, in addition to the similar tissue distributions, it seems likely that there will be a certain degree of redundancy. This issue could be addressed by combined gene targeting of the members of each subgroup. Such an approach, in conjunction with further genetic biochemical and cell biological analyses of other model organisms should result in the elucidation of the precise biological roles of these enzymes in controlling key signaling pathways. Further insight into the regulation of these phosphatases by their substrates will be gained by determining the three-dimensional structures of representative members of each subgroup, both free and in complex with their targets.

Finally, the adoption by the community of a common nomenclature (such as the DUSP system used here and recommended by the Human Gene Nomenclature committee) will considerably facilitate progress in this important area.

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