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REVIEW ARTICLE

# Dual-specificity phosphatases: critical regulators with diverse cellular targets

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DUSPs (dual-specificity phosphatases) are a heterogeneous group of protein phosphatases that can dephosphorylate both phosphotyrosine and phosphoserine/phosphothreonine residues within the one substrate. DUSPs have been implicated as major modulators of critical signalling pathways that are dysregulated in various diseases. DUSPs can be divided into six subgroups on the basis of sequence similarity that include slingshots, PRLs (phosphatases of regenerating liver), Cdc14 phosphatases (Cdc is cell division cycle), PTENs (phosphatase and tensin homologues deleted on chromosome 10), myotubularins, MKPs (mitogen-activated protein kinase phosphatases) and atypical DUSPs. Of these subgroups, a great deal of research has focused on the characterization of the MKPs. As their name suggests, MKPs dephosphorylate MAPK (mitogen-activated protein kinase) proteins ERK (extracellular-signal-regulated kinase), JNK (c-Jun N-terminal kinase) and p38 with specificity distinct from that of individual MKP proteins. Atypical DUSPs are mostly of low-molecular-mass and lack the N-terminal CH2 (Cdc25 homology 2) domain common to MKPs. The discovery of most atypical DUSPs has occurred in the last 6 years, which has initiated a large amount of interest in their role and regulation. In the past, atypical DUSPs have generally been grouped together with the MKPs and characterized for their role in MAPK signalling cascades. Indeed, some have been shown to dephosphorylate MAPKs. The current literature hints at the potential of the atypical DUSPs as important signalling regulators, but is crowded with conflicting reports. The present review provides an overview of the DUSP family before focusing on atypical DUSPs, emerging as a group of proteins with vastly diverse substrate specificity and function.

Key words: dual-specificity phosphatase (DUSP), mitogen-activated protein kinase phosphatase (MKP), protein phosphatase, protein tyrosine kinase (PTK), protein tyrosine phosphatase (PTP).

## INTRODUCTION

It is well recognized that reversible phosphorylation of proteins by PTKs (protein tyrosine kinases) and PTPs (protein tyrosine phosphatases) regulates a broad spectrum of fundamental signalling pathways and physiological processes [1–6]. Significantly more research has focused on PTKs partly because the first PTP was purified [7] and cloned [8] 10 years after the first PTK [9]. However, in recent years, research has also concentrated on the PTP superfamily and its individual subgroups, which has led to an increased understanding of the tightly controlled balance of reversible protein phosphorylation, with PTPs now being recognized as critical components of multiple signalling networks and important regulators of fundamental physiological processes. Aberrations in protein phosphorylation play a major role in the pathogenesis of numerous diseases, including cancer, diabetes and immune deficiencies. Understanding how aberrations in the precise balance and regulation of PTPs and PTKs affects critical signalling pathways that underpin the molecular pathogenesis of disease is key to the development of new diagnostic and therapeutic strategies, and therefore improved patient outcome. PTPs and their role in human disease has been reviewed elsewhere [2,10–16].

PTPs can be broadly grouped into four separate subfamilies on the basis of the amino acid sequences of their catalytic domains [2,3] (Figure 1). The first, and largest, PTP subfamily contains type-I cysteine-based PTPs. These phosphatases can be divided further into subgroups depending on the similarity of the catalytic domains, comprising either tyrosine-specific classical PTPs or the heterogeneous DUSPs (dual-specificity phosphatases), which are discussed in further detail below. The second PTP subfamily contains only one phosphatase, LMWPTP (low-molecular-mass PTP), that is type-II cysteine-based and tyrosine-specific. It is related to low-molecular-mass bacterial phosphatases that are evolutionarily conserved [2,3]. The third PTP subfamily consists of rhodanese-related type-III cysteine-based phosphatases that are both tyrosine- and threonine-specific [2,17]. This group consists of three cell cycle regulators: Cdc25A, Cdc25B and Cdc25C (Cdc is cell division cycle), which dephosphorylate cyclin-dependent kinases [18]. The fourth PTP subfamily consists of aspartic acid-based PTPs which comprises the EyA (eyes absent) tyrosine-specific phosphatases and HAD (haloacid dehalogenase) family

Abbreviations used: ADF, actin-depolymerization factor; ASK1, apoptosis signal-regulating kinase 1; ATF-2, activating transcription factor 2; Cdc, cell division cycle; Cdk, cyclin-dependent kinase; CH2, Cdc25 homology 2; DUSP, dual-specificity phosphatase; ER, oestrogen receptor; ERK, extracellular-signal-regulated kinase; GLP-1, glucagon-like peptide 1; GST, glutathione transferase; HA, haemagglutinin; HAD, haloacid dehydrogenase; HEK, human embryonic kidney; HIRIP5, HIRA (histone cell cycle regulation defective homologue A)-interacting protein 5; Hsf4b, heat-shock factor 4b; HUGO, Human Genome Organisation; IL, interleukin; JNK, c-Jun N-terminal kinase; KAP, kinase-associated phosphatase; KIM, kinase-interacting motif; LIMK1, LIM domain kinase 1; MAPK, mitogen-activated protein kinase; MKK, MAPK kinase; MKP, MAPK phosphatase; MK-STYX, MAPK serine-, threonine- and tyrosine-specific phosphatase; NSCLC, non-small-cell lung carcinoma; pNPP, *p*-nitrophenyl phosphate; PRL, phosphatase of regenerating liver; PTEN, phosphatase and tensin homologue deleted on chromosome 10; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; RT, reverse transcription; siRNA, short interfering RNA; SSH, slingshot; STAT, signal transducer and activator of transcription; TLR, Toll-like receptor; VH1, vaccinia virus open reading frame H1.

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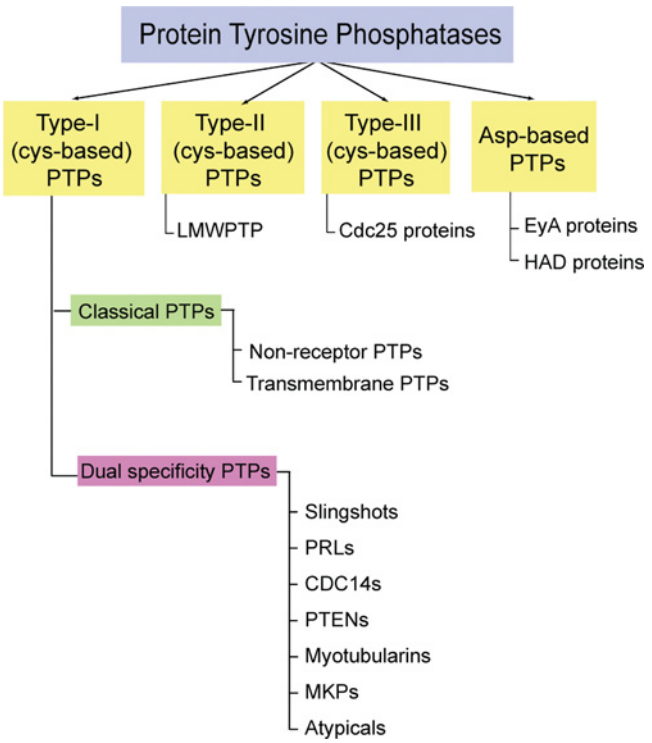


**Figure 1 Classification of PTPs**

PTPs can be classified into subgroups on the basis of their sequence similarity and presence of functional or binding domains. EyA, eyes absent; LMWPTP, low-molecular-mass PTP.

phosphatases [3,19]. The HAD family is a heterogeneous group of phosphatases that can be either tyrosine- [20] or serine- [21,22] specific and have various substrates, including proteins, phospholipids, sugars and nucleotides [23–25].

## DUSPs

A large and heterogeneous subgroup of the type-I cysteine-based PTP superfamily consists of phosphatases broadly referred to as DUSPs. The unique feature that characterizes DUSPs is their ability to dephosphorylate both tyrosine and serine/threonine residues within the one substrate. Both classical PTPs and DUSPs share a similar mechanism of catalysis for the hydrolysis of phosphorylated substrates that involves the formation of a stable phosphoryl-intermediate [26,27]. The highly conserved catalytic domain contains the consensus sequence HCXXXXXR and the critical cysteine residue is positioned at the base of the catalytic cleft and the arginine residue folds back towards the phosphate-binding pocket to assist with catalysis. There is also a conserved aspartic acid residue upstream of this conserved motif that acts as a general acid/base catalyst and helps to stabilize the phosphoryl-intermediate. The catalytic pocket of DUSPs is shallow, but broader than that of classical PTPs, which is thought to be the mechanism by which DUSPs can simultaneously accommodate more than one phosphorylated residue [26].

There are 61 DUSPs of heterogeneous form and function, which can be grouped on the basis of the presence of specific domains and sequence similarity. However, there is some confusion surrounding the classification of some individual DUSPs, which share sequence similarity with one subgroup, but substrate specificity or physiological role with another. In general, DUSPs are classified into one of the subgroups discussed below.

### Slingshot phosphatases

Slingshot phosphatases are represented by three genes (*SSH1L*, *SSH2L* and *SSH3L*), for which biological role and regulation are still poorly understood. Slingshot was first identified in 2002 as a *Drosophila* phosphatase, and is responsible for the dephosphorylation of proteins in the ADF (actin-depolymerization factor)/cofilin group. Mutation of *Drosophila* slingshot results in disorganized epidermal cell morphogenesis [28]. Mammalian slingshots can dephosphorylate phosphoserine and phosphothreonine residues and contain the conserved PTP catalytic domain as well as 14-3-3-binding motifs, a C-terminal F-actin (filamentous actin)-binding site and a SH3 (Src homology 3)-binding motif [29,30]. The three slingshots are widely expressed, but have contrasting subcellular localizations, which suggests they have related but distinct functions in the regulation of actin polymerization [31,32]. Slingshot phosphatases dephosphorylate phospho-ADF and phospho-cofilin and suppress actin filament assembly induced by the kinases TESK1 (testis-specific kinase 1) and LIMK1 (LIM domain kinase 1) [29]. Recently, slingshot *SSH1L* was shown to dephosphorylate LIMK1 directly [30], adding complexity to the regulation of actin polymerization.

### PRL (phosphatase of regenerating liver)

There are three PRLs (PRL-1, PRL-2 and PRL-3) and these have been the subject of reviews [33–35]. PRLs share 78–86 % sequence identity [36], but exhibit specific tissue distribution [34,37]. PRLs contain the conserved PTP catalytic domain as well as a C-terminal CAAX box which allows PRLs to be post-translationally farnesylated [34] and potentially localize to membrane structures [38]. PRLs can be regulated at multiple levels and are early-response genes [39]. Basic amino acids in close proximity to the farnesylation site are thought to facilitate binding to membrane lipids or act as a nuclear localization signal [40], and, like other PTPs, PRLs are susceptible to inactivation by reversible oxidation, which leads to the formation of disulfide bonds within the catalytic cleft [41,42]. PRLs have been mainly studied for their role in cancer metastasis, but their exact biological functions and physiological substrates are poorly understood. It has been demonstrated that PRL-3 is associated with metastasis and is overexpressed in metastatic colorectal cancer compared with primary colorectal tumours or normal colon. However, all three PRLs are overexpressed in a variety of cancer cells [33]. It has also been demonstrated that overexpression of PRL-1 or PRL-3 affects the proliferation, migration and invasion of epithelial cells [39,43]. Although the specific substrates of PRLs remain to be elucidated, PRLs may play a role in epithelial–mesenchymal transition [33,44]. Further work is being directed at identifying inhibitors of PRLs as potential cancer therapeutics [34,45].

### Cdc14 phosphatases

There are four Cdc14 phosphatases [KAP (kinase-associated phosphatase), Cdc14A, Cdc14B and uncharacterized PTP9Q22], all related to the type-III cysteine-based PTPs Cdc25 phosphatases which regulate the initiation of mitosis and DNA damage checkpoint control [2]. KAP dephosphorylates Cdk (cyclin-dependent kinase) 2, leading to the inhibition of cell cycle progression. Paradoxically, it has recently been shown to be overexpressed in malignant glioblastomas, and this expression is associated with poor outcome. However, it was demonstrated that aberrant splicing of KAP occurs in these tumours which leads to increased mRNA expression, but decreased protein expression, consistent with a tumour-suppressor role [46]. Cdc14

**Table 1 Nomenclature of MKPs**

MKPs are listed here according using their official HUGO Gene Nomenclature Committee name. Alternative names are also listed. The MAPK substrate specificity is listed in order of preference; however, for DUSP2, preference *in vivo* differs from that *in vitro*. *In vivo*, DUSP2 inhibits JNK preferentially. Note that MK-STYX is catalytically inactive. The subcellular localization of these MKPs is also listed, with the nuclear MKPs most likely to be inducible. LPS, lipopolysaccharide; ND, not determined.

| Name | Molecular mass (kDa) | Chromosomal location | Alternative names | Substrate specificity | Subcellular localization | Inducible/early-response genes |
|---|---|---|---|---|---|---|
| DUSP1 | 39.30 | 5q34 | MKP-1, CL100, hVH1, 3CH134, PTPN10erp | p38 = JNK > ERK | Nuclear | LPS, hypoxia, dexamethasone, heat shock, oxidative stress |
| DUSP2 | 34.40 | 2q11 | PAC-1 | ERK = p38 > JNK | Nuclear | Growth factors, heat shock, LPS |
| DUSP4 | 42.95 | 8p11-12 | MKP-2, hVHR-2, TYP1 | ERK = JNK > p38 | Nuclear | Mitogens, growth factors |
| DUSP5 | 42.05 | 10q25 | hVHR3, B23 | ERK | Nuclear | IL-2, growth factors, heat shock |
| DUSP6 | 43.32 | 12q22-23 | MKP-3, Pyst1, rVH6 | ERK > JNK = p38 | Cytosolic | No |
| DUSP7 | 40.55 | 3p21 | MKP-X, Pyst2, B59 | ERK > JNK = p38 | Cytosolic | No |
| DUSP8 | 65.84 | 11p15.5 | hVH5, M3/6, HB5 | JNK = p38 > ERK | Nuclear and cytosolic | No |
| DUSP9 | 41.87 | Xq28 | MKP-4, Pyst3 | ERK > p38 > JNK | Nuclear and cytosolic | No |
| DUSP10 | 52.64 | 1q41 | MKP-5 | p38 = JNK > ERK | Nuclear and cytosolic | LPS, peptidoglycans |
| DUSP16 | 73.10 | 12p13 | MKP-7 | JNK = p38 > ERK | Cytosolic | No |
| MK-STYX | 35.82 | 7q11.23 | STYX-L1 | ND | ND | ND |

phosphatases also dephosphorylate specific Cdks and regulate late mitotic events and exit from mitosis in yeast [47]. Mammalian Cdc14 phosphatases are not well understood, but have been shown to play a role in centrosome maturation and spindle stability, cytokinesis and cell cycle progression (reviewed in [48,49]). The regulation of Cdc14 phosphatases is primarily by sequestration in the nucleolus during interphase [50].

### PTEN (phosphatase and tensin homologue deleted on chromosome 10)-like and myotubularin phosphatases

There are five PTEN-like phosphatases, which dephosphorylate D3-phosphorylated inositol phospholipids, and 16 myotubularin phosphatases, some of which also dephosphorylate D3-phosphorylated inositol phospholipids [2]. Reversible phosphorylation of inositol lipids is a fundamental component of many critical signalling cascades. Most of the PTEN-like and myotubularin phosphatases are only partially characterized; however, an enormous amount of research has led to a detailed understanding of the physiological roles and regulation of the protein leading this group of phosphatases, PTEN. PTEN is frequently mutated, silenced or deleted in human cancers, leading to loss of function and aberrant proliferative and survival signalling. PTEN regulation and function has been reviewed thoroughly elsewhere [51–55]. Particular myotubularins also dephosphorylate specific inositol lipids and are highly conserved from yeast to humans. The physiological role of these phosphatases is not completely understood, but a great deal of interest has been generated from the discovery that nearly half the myotubularins are catalytically inactive (reviewed in [51,56–58]). Myotubularins contain a PTP catalytic domain, although, in some, the critical cysteine, arginine and aspartic acid residues are not represented, which renders the phosphatase inactive. In addition to this domain, myotubularins commonly contain additional motifs thought to mediate protein–protein or protein–lipid interactions such as an N-terminal PH (pleckstrin homology) domain which binds particular lipids and targets the protein to the plasma membrane [59,60], a C-terminal coiled-coil domain and a FYVE domain [51]. Although the exact role of myotubularins is unclear, mutations in these proteins have been linked to neuromuscular disease [61]. The inactive myotubularins are conserved from yeast to humans and are not well characterized, but it is possible that they act in conjunction with active myotubularins to regulate the levels of phosphoinositol lipids [61,62].

### MKPs (mitogen-activated protein kinase phosphatases)

One of the best-characterized DUSP subgroups contains ten proteins that can dephosphorylate MAPKs (mitogen-activated protein kinases) at both phosphothreonine and phosphotyrosine residues simultaneously within the MAPK TXY (Thr-Xaa-Tyr) activation motif, and thereby act as antagonists of associated signalling cascades. These DUSPs are listed in Table 1 and are referred to as MKPs. MKPs contain a highly conserved C-terminal catalytic domain that contains the extended consensus sequence **D**$X_{26}$(V/L)X(V/I)H**C**XAG(I/V)S**R**SXT(I/V)XXAY(L/I)M where X is any amino acid and the critical residues for catalysis are in bold. MKPs also contain an N-terminal rhodanese or Cdc25-like domain (also termed CH2 domain for Cdc25 homology 2) thought to contain critical KIMs (kinase-interacting motifs) which confer specific MAPK substrate specificity (Figure 2). On the basis of sequence alignment and domain structure, we have also included MK-STYX (MAPK serine-, threonine- and tyrosine-specific phosphatase) in this group, although this protein contains a naturally occurring substitution of serine for cysteine in the catalytic domain, and is therefore catalytically inactive. MKPs contain little primary sequence similarity to classical PTPs; however, like other DUSPs, they share similar structural folding at the catalytic site [11] and mutation of the critical cysteine residue within the consensus catalytic domain generally abolishes their phosphatase activity [27,63–66]. Individual MKPs show substrate specificity preference for one or more of the MAPKs ERK (extracellular-signal-regulated kinase), JNK (c-Jun N-terminal kinase) or p38 that are phosphorylated and activated in response to extracellular stimuli (Table 1), although the MAPK substrate preference for individual MKPs *in vitro* does not always reflect that *in vivo*. MAPK activation cascades mediate various physiological processes, including cellular proliferation, apoptosis, differentiation and stress responses. Dysregulation of MAPK activation cascades has been implicated in various diseases and has been the focus of extensive research (reviewed in [4,67–69]).

Just as MAPKs are regulated by MKPs, in turn MKPs are also regulated at multiple levels (Figure 3), which is an important characteristic of proteins that play a role in the fine-tuning of major signalling cascades. Many MKPs are inducible or early-response genes and demonstrate low expression in resting or unstressed cells, with their expression rapidly increasing upon appropriate stimulation, e.g. with various cytokines, growth factors or serum. However, the kinetics and magnitude of the

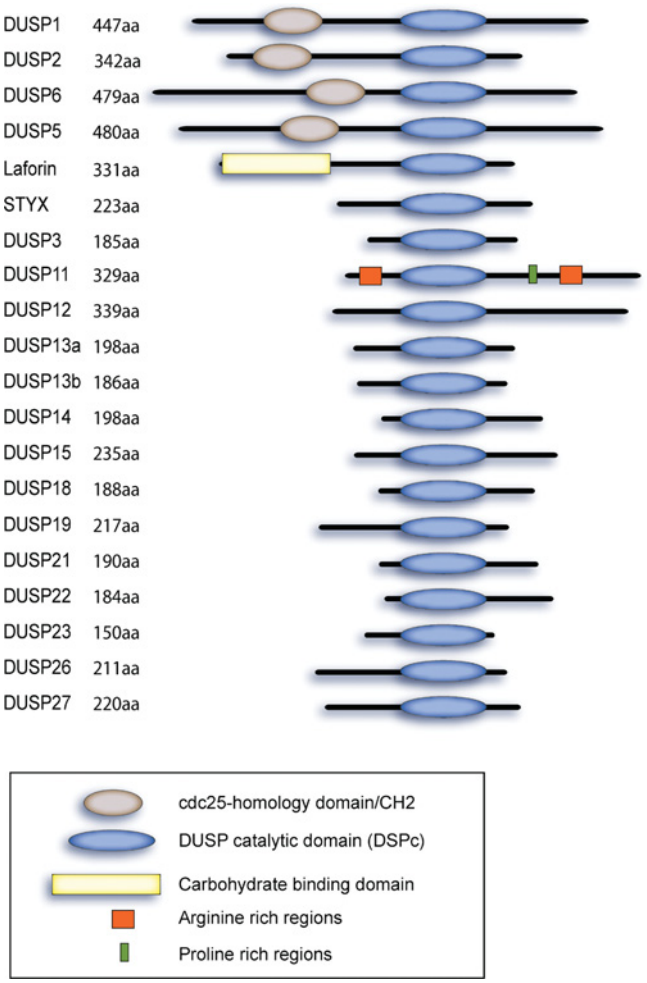


**Figure 2 Schematic representation of atypical DUSP proteins**

Atypical DUSPs are generally smaller and lack the CH2 domain in the N-terminus that is common to the typical DUSPs.

response of individual MKPs to these stimuli can be cell-type- and context-specific [70–74]. This induction is often dependent on MAPK activation and hence is thought to be a negative-feedback mechanism for down-regulating mitogenic signalling. In addition, MKPs can be subject to post-translational modification. Owing to the presence of the catalytic cysteine residue in the catalytic cleft, the enzymatic activity of PTPs including MKPs is sensitive to reversible oxidation and inactivation. Reversible oxidation of the conserved cysteine within the DUSP catalytic domain abolishes its nucleophilic properties and leads to profound changes in the conformation of the catalytic cleft, rendering the phosphatase inactive [75]. This may be mediated by the regulated production of reactive oxygen species such as $H_2O_2$ within the cell [16,76,77]. The regulation of this process under normal physiological conditions, as well as in particular disease states, requires further clarification. The catalytic activity of some DUSPs can be enhanced upon binding to their MAPK substrates [78,79]. Determining the crystal structure of certain MKPs has led to the understanding that this is likely to be due to conformational changes around the catalytic site which involves the critical aspartic acid residue [80]. Also, MKPs can be phosphorylated directly by their substrate(s), which leads to increased stabilization and prolonged half-life of the MKP. This is achieved via reduced ubiquitination and degradation, as is the case when DUSP1 is phosphorylated by its substrate ERK at $Ser^{359}$ and $Ser^{364}$ [81] or when DUSP16 is phosphorylated by p38 at $Ser^{446}$ [82]. MKPs can also be epigenetically regulated by either methylation or chromatin modification. For example, the loss of DUSP6 expression in pancreatic cancer is due to hypermethylation of its promoter [83], and it has been demonstrated recently that histones associated with *DUSP1* are acetylated subsequent to TLR (Toll-like receptor) stimulation [84]. Activation of *DUSP1* gene expression in response to TLR activation acts as a negative-feedback mechanism to attenuate p38 activity and decrease the innate immune response.

As major regulators of MAPK signalling, MKPs have also been implicated in multiple diseases. One study implicates DUSP1 in metabolic control, as mice lacking DUSP1 are resistant to diet-induced obesity [85]; however, there has been particular focus on the role of MKPs in immune regulation (reviewed in [86–89]) and cancer (reviewed in [90–92]). DUSP1, DUSP2 and DUSP10 have been well characterized as important regulators of immune function. DUSP1 is an important negative-feedback regulator of macrophage function and the inflammatory response to TLR signalling, and plays key regulatory roles in both innate and adaptive immune responses via inactivation of p38 and JNK [93–97]. Although DUSP2 negatively regulates p38 and ERK *in vitro* [98], *in vivo* studies in knockout mice demonstrated a role for DUSP2 as a negative regulator of JNK activity and positive regulator of immune responses via cross-talk between JNK and ERK [99]. DUSP10 modulates gene expression in innate immune cells, and is a negative regulator of inflammatory responses via inactivation of JNK [100].

Interestingly, some MKPs are encoded at chromosomal regions which exhibit loss of heterozygosity, and these MKPs exhibit reduced expression in particular cancers. These include DUSP4, DUSP6, DUSP7, DUSP10 and DUSP16, and functional studies support a role for some of these MKPs as tumour suppressors [101–107]. In this context, the best-characterized is DUSP6 for its role in pancreatic cancer. DUSP6 can be up-regulated in specific cancers exhibiting aberrant RTK (receptor tyrosine kinase) and Ras/Raf signalling such as NSCLC (non-small-cell lung carcinoma), potentially as a negative-feedback regulator of mitogenic signalling [108]. However, in pancreatic cancer, DUSP6 expression is reduced owing to promoter hypermethylation and this loss of expression is associated with the progression from pancreatic intraepithelial neoplasia to invasive ductal carcinoma [83]. In addition, loss of DUSP6 in ovarian cancer cells enhances tumorigenicity [109]. However, some MKPs may play a positive role in cancer progression. For example, DUSP1 expression is increased in pancreatic cancer, and it has been demonstrated that down-regulating the expression of DUSP1 leads to reduced tumorigenicity of pancreatic cancer cells in a nude mouse model [110]. Also, particular MKPs may have contradicting roles in different tumour types, such as DUSP1, which has increased expression in prostate [111], glial [112] and gastric [113] cancer cell lines, but decreased expression in ovarian and testicular cancer [114,115]. Furthermore, an individual MKP may have either increased or decreased expression, depending on the stage of carcinogenesis, as is the case with DUSP1 which is overexpressed in early stages of colon, prostate and bladder cancer, but expression is lost as the tumour becomes more aggressive [116,117]. Importantly, MKPs may provide biomarkers of therapeutic responsiveness and patient outcome in specific cancers. For example, expression of DUSP1 and DUSP2 in ovarian cancer is associated with poor outcome [118,119], but expression of DUSP1 in NSCLC [120] and hepatocellular carcinoma [121] is associated with improved prognosis. Also, DUSP1 sensitizes cancer cells to cisplatin-induced apoptosis [122–124]

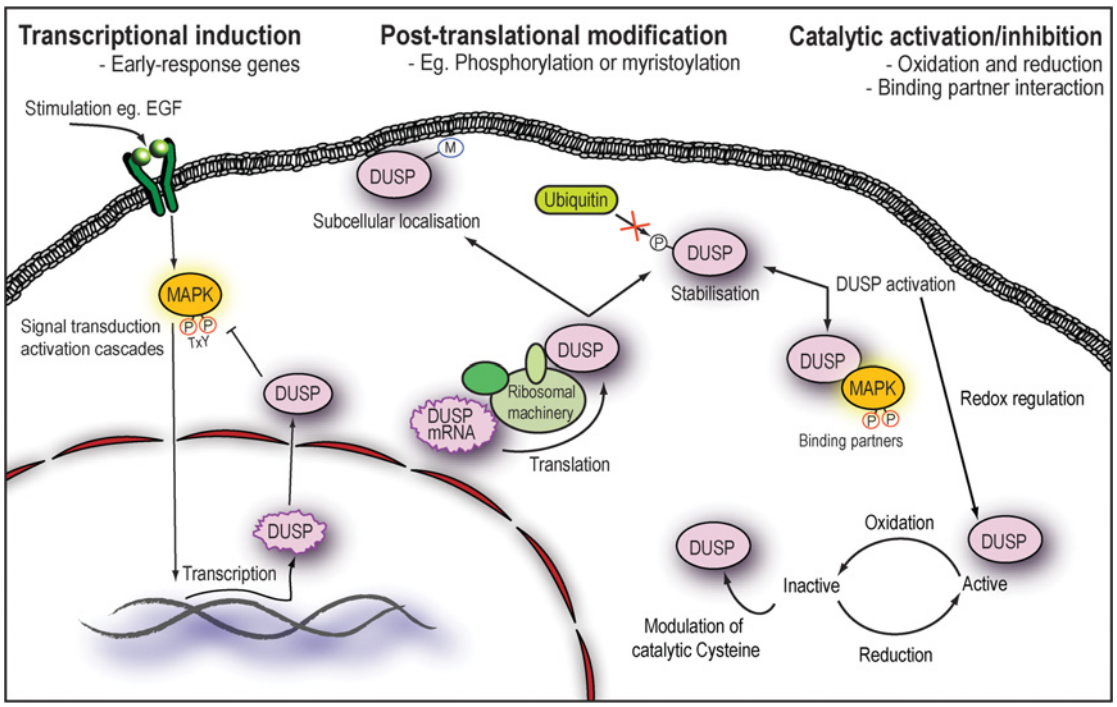


**Figure 3 Regulation of DUSPs**

Many DUSPs are inducible or early-response genes and demonstrate low expression in resting or unstressed cells, with their expression increasing rapidly upon appropriate stimulation. Many DUSPs are also subject to post-translational modification such as myristoylation or phosphorylation. Owing to the presence of a critical cysteine residue in the catalytic cleft, PTPs including DUSPs are sensitive to reversible oxidation and inactivation [16,77]. EGF, epidermal growth factor. An animated version of this Figure can be seen at http://www.BiochemJ.org/bj/418/0475/bj4180475add.htm.

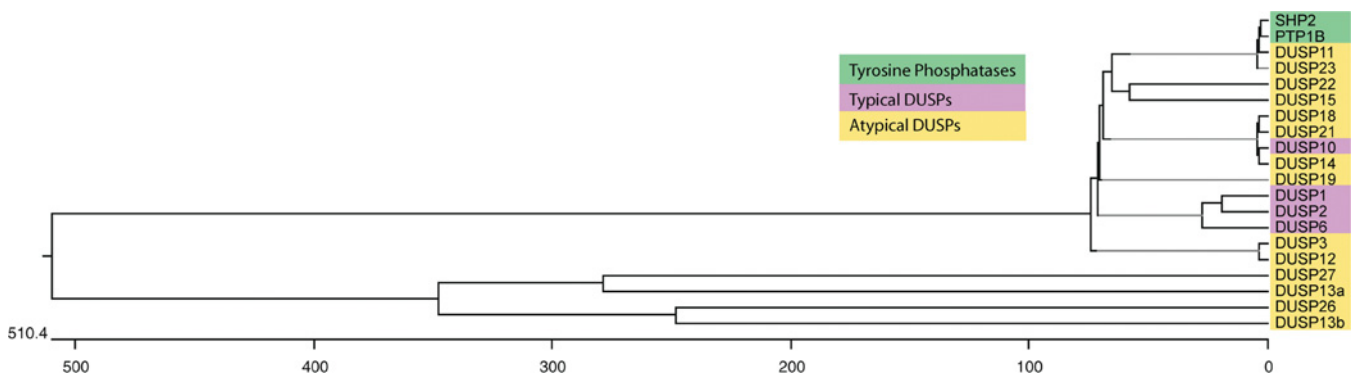


**Figure 4 Phylogenetic tree of atypical DUSPs**

Phylogenetic tree generated using MegAlign software and J Hein method with a PAM 250 residue weight table. Atypical DUSPs are phylogenetically distinct from classical PTPs and also from the majority of MKPs. According to this analysis, the atypical DUSPs appear not to be derived from a common proximal ancestor, which may explain the variety of atypical DUSP substrate specificities. SHP2, Src homology 2 domain-containing protein tyrosine phosphatase 2.

and overexpression of DUSP6 in ER (oestrogen receptor)-positive breast cancer cells confers resistance to the growth inhibitory effects of tamoxifen [125]. However, loss of DUSP6 expression in ovarian cancer cells increases chemoresistance [109].

## Atypical DUSPs

There are at least 16 atypical DUSPs that share some characteristics of the MKPs and are most similar to the VH1 (vaccinia virus open reading frame H1) phosphatase present in vaccinia virus [126]; however, they do not share a common proximal ancestor and phylogenetically are quite distinct from classical PTPs and MKPs (Figure 4). Of these phosphatases, 13 are less than 27 kDa and the remaining three are larger owing to additional functional or binding domains (see Table 2 and Figure 2).

Atypical DUSPs contain the consensus DUSP catalytic domain, but lack the N-terminal CH2 domain found in MKPs. These DUSPs are listed in Table 2. A large number of studies have been reported on atypical DUSPs since the last review in 2001 [127] and the literature is crowded with conflicting reports. Some of these atypical DUSPs were originally grouped with the typical MKPs and were thought to play a similar role regulating MAPK cascades. Although some atypical DUSPs have been shown to regulate MAPKs, it is becoming clear that atypical DUSPs can have vastly different substrate specificities and physiological

**Table 2 Nomenclature of atypical DUSPs**

The nomenclature of the PTP superfamily is crowded, and is particularly confusing within the atypical DUSP subgroup. Although all DUSPs contain the conserved catalytic domain, their function and substrate specificity is often very different from what is predicted, and hence many DUSPs have been renamed following their detailed characterization. In addition, owing to a substantial increase in research directed towards elucidating the physiological role of PTPs including DUSPs in the last 10 years, some individual DUSPs have been cloned and characterized simultaneously by independent groups. On these occasions, it is not uncommon to find the same DUSP named differently by independent groups. In the present review, where possible and unless stated otherwise, each DUSP will be referred to using its official HUGO Gene Nomenclature Committee name. Alternative names are listed.

| Name | Year discovered | Molecular mass (kDa) | Chromosomal location | Alternative names | Subcellular localization | Cell type [reference] |
|---|---|---|---|---|---|---|
| Laforin | 1998 | 38 | 6q24 | EPM2A (epilepsy, progressive myoclonus type 2A) | Cytoplasm | COS1 [130] |
| STYX | 1997 | 25.49 | 14 | Serine/threonine/tyrosine-interacting protein | Not determined | – |
| DUSP3 | 1992 | 20.4 | 17q21 | VHR (VH1-related) | Nucleolus | COS1 [141] |
| DUSP11 | 1998 | 38.94 | 2p13.2 | PIR1 (phosphatase that interacts with RNA–ribonucleoprotein complex 1) | Nucleus | HeLa [147] |
| DUSP12 | 2000 | 37.69 | 1q21-q22 | YVH1 [protein tyrosine phosphatase (*Saccharomyces cerevisiae*) orthologue], GKAP (glucokinase-associated phosphatase) | Cytoplasm and nucleus | mhAT3F [149] |
| DUSP13A | 1999 | 22.21 | 10q22.2 | TMDP (testis- and skeletal muscle-specific DUSP) | Not determined | – |
| DUSP13B | 2004 | 22.21 | 10q22.2 | MDSP (muscle-restricted DUSP), SKRP4 (stress-activated protein kinase pathway-regulating phosphatase-4), BEDP (branching-enzyme-interacting DUSP) | Cytoplasm | HeLa, COS1, myotubules [151] |
| DUSP14 | 2001 | 22.26 | 7q12 | MKP-L (MKP-1-like protein tyrosine phosphatase), MKP-6 | Cytoplasm | T-cells [154] |
| DUSP15 | 2004 | 26.15 | 20q11.21 | VHY (VH1-like member Y) | Cytoplasm (plasma membrane) | NIH 3T3 [158] |
| DUSP18 | 2002 | 21.07 | 22q12.2 | DUSP20 and LMWDSP20 (low-molecular-mass DUSP20) | Cytoplasm and nucleus<br>Mitochondria | COS6M [160]<br>COS7 [163] |
| DUSP19 | 2002 | 24.19 | 2q32.1 | DUSP17 and SKRP1 (stress-activated protein kinase pathway-regulating phosphatase 1) | Cytoplasm | NIH 3T3 [165] |
| DUSP21 | 2002 | 21.52 | Xp11.4-p11.23 | LMWDSP21 (low-molecular-mass DUSP21) | Cytoplasm and nucleus<br>Mitochondria | COS6M [160]<br>COS7 [163] |
| DUSP22 | 2001 | 20.91 | 6p25.3 | LMWDSP2 (low-molecular-mass DUSP2), JSP1 (JNK-stimulating phosphatase 1), JKAP (JNK pathway-associated phosphatase), MKP-X, VHX (VH1-related phosphatase-related X) | Nucleus and cytoplasm (enriched in Golgi)<br>Cytoplasm | COS7 [167]<br>T-cells [169] |
| DUSP23 | 2004 | 16.59 | 1q23.22 | DUSP25, LDP-3 (low-molecular-mass DUSP3), VHZ (VH1-related phosphatase Z) | Cytoplasm | HEK-293 [161,177] |
| DUSP26 | 2005 | 23.94 | 8p12 | DUSP24, LDP-4 (low-molecular-mass DUSP4) MKP-8, NEAP (neuroendocrine-associated phosphatase), SKRP3 (stress-activated protein kinase pathway-regulating phosphatase 3) | Cytoplasm<br>Cytoplasm and nucleus<br>Perinuclear and Golgi | H1299 [179]<br>COS7 [182]<br>COS7 [178,181] |
| DUSP27 | 2007 | 25.33 | 10q22.2 | DUPD1 (DUSP and pro-isomerase domain-containing 1) | Cytoplasm | HeLa [183] |

roles from those of the typical MKPs. In the literature, it is not uncommon to find individual atypical DUSPs with multiple alternative names, but in the present review, each DUSP will be referred to with its official HUGO (Human Genome Organisation) Gene Nomenclature Committee name with alternative names listed in Table 2.

## Laforin

Progressive myoclonus epilepsy or Lafora's disease is an autosomal recessive disease characterized by intracellular polyglucosan inclusions called Lafora bodies. Lafora's disease is caused by one or more of 30 known missense mutations in *EPM2A*, the gene encoding Laforin (reviewed in [128,129]), leading to the accumulation of intracellular polyglucosan [130,131]. As well as a DUSP catalytic domain, Laforin contains a CBM20 (carbohydrate-binding module 20) or starch-binding domain in its N-terminal region which is responsible for its direct association with glycogen *in vitro* and *in vivo* [132]. Mutations leading to Lafora's disease can occur in both the catalytic and starch-binding domains and, interestingly, mutations in the starch-binding domain not only prevent Laforin from binding to glycogen, but also reduce its phosphatase activity by up to 50% [132]. Wild-type Laforin exhibits punctate and cytoplasmic subcellular localization [133], which is disrupted by mutation of the starch-binding domain [132]. Recombinant Laforin can dephosphorylate both phosphotyrosine and phosphoserine/phosphothreonine peptides [134], but there are only a few studies documenting potential substrates of Laforin. One study has demonstrated direct binding and dephosphorylation of the cytoplasmic protein HIRIP5

[HIRA (histone cell cycle regulation defective homologue A)-interacting protein 5] *in vitro* [135]. HIRIP5 contains a NifU-like domain which suggests it may be involved in iron homoeostasis; however, this has not been studied further [135]. An independent study identified an alternative substrate, R5, as binding directly to Laforin [136]. R5 is a glycogen-targeting regulatory subunit of protein phosphatase 1 that acts as a scaffold to facilitate the interaction of this phosphatase and its substrate, glycogen synthase [136], consistent with a role for Laforin in glycogen metabolism.

### STYX

To date there have been no studies characterizing STYX. However, interestingly, STYX contains a naturally occurring substitution of glycine for cysteine in the DUSP catalytic domain, which probably renders the phosphatase inactive, in a similar manner to some myotubularin proteins.

### DUSP3

DUSP3 was originally named VHR (VH1-related) owing to its restricted sequence similarity to VH1, a key gene in vaccinia virus [137] and poxvirus [126]. DUSP3 can dephosphorylate both phosphotyrosine and phosphoserine/phosphothreonine residues [138], but with a preference for diphosphorylated phosphotyrosine and phosphoserine/phosphothreonine peptides over monophosphorylated peptides, confirming the role of DUSP3 as a dual-specificity phosphatase [139]. In the presence of monophosphorylated substrates, however, DUSP3 shows preference for phosphotyrosine over phosphoserine/phosphothreonine, a property characteristic of other PTPs, and it has been suggested that this reflects tight binding of the phosphotyrosine residue within the MAPK pTXpY activation motif to an exposed, but deep, catalytic DUSP3 cleft, while the phosphothreonine residue binds only loosely to an adjacent shallow groove [140,141].

The MAPK substrate specificity of DUSP3 remains controversial. Detailed *in vitro* studies on the kinetics of ERK2 dephosphorylation by DUSP3 suggested that ERK2 was an unlikely physiological substrate of DUSP3 [142]. However, an independent study which included affinity chromatography, enzyme kinetics and cell transfection techniques identified ERK1/2 as specific substrates of DUSP3 with the phosphatase acting on $pTyr^{185}$ of the TXY ERK activation loop [143]. It is possible that DUSPs can also act as single-specificity phosphatases to reduce the activity of MAPKs or that multiple phosphatases work together to inactivate ERK. Recently, it has been shown that the VRK3 (vaccinia-related kinase 3) binds to and activates DUSP3 and that this direct interaction regulates ERK signalling [144], adding another layer of complexity to the regulation of the ERK MAPK cascade. JNK has also been identified as a specific substrate of DUSP3 [145]. *In vitro* and *in vivo* studies confirmed that DUSP3 can dephosphorylate JNK in response to stress stimuli and this was inhibited by the presence of the JNK substrate c-Jun, presumably because c-Jun binds JNK and prevents access of DUSP3 to the JNK active site [145]. In summary, it appears that, under certain conditions, DUSP3 can dephosphorylate ERK and JNK directly and that the N-terminal KIM domain responsible for MAPK specificity in MKPs is not necessary to facilitate binding and dephosphorylation of these MAPKs by DUSP3.

Since closely related phosphatases commonly share a similar repertoire of substrates, and VH1 can dephosphorylate STAT (signal transducer and activator of transcription) 1 [146], it is perhaps not surprising that STAT5 was recently identified as an additional substrate for DUSP3 [147]. Further work will reveal the physiological consequences of this interaction of DUSP3 with STAT5, but, on the basis of these reports, it appears that the substrate specificity for DUSP3 (and potentially other DUSPs) can be variable and highly cell- and micro-environment-specific.

DUSP3 is regulated during the cell cycle [148–150]. In particular, cells lacking DUSP3 arrest at $G_1$/S and $G_2$/M, and demonstrate morphological signs of senescence. The cell cycle arrest seen in these cells lacking DUSP3 is dependent on the activation of the DUSP3 substrates ERK and JNK [149,150]. Unlike some other DUSPs, however, DUSP3 does not appear to be an early-response gene, and its expression does not increase in response to treatment with mitogenic stimuli such as EGF (epidermal growth factor) or serum [143].

### DUSP11

DUSP11 was first named PIR1 (phosphatase that interacts with RNA–ribonucleoprotein complex 1) owing to its high affinity for RNA [151], and was independently cloned after being identified as a human homologue of BVP (baculovirus protein phosphatase), a DUSP of the *Autographa californica* nuclear polyhedrosis virus which also has activity against RNA [152].

The human tissue distribution of DUSP11 has not been studied in detail, but it appears to be expressed in several human cell lines, including those derived from keratinocytes, epidermoid cells and myeloblastic cells [151]. DUSP11 associates with the splicing factors 9G8 and SRp30C in a yeast two-hybrid system; however, this interaction has not been confirmed in mammalian cells [151]. Affinity-purified GST (glutathione transferase)-tagged DUSP11 fusion protein has intrinsic phosphatase activity *in vitro* against phosphotyrosine residues [151,152], but no [151] or weak [152] activity against phosphoserine/phosphothreonine residues. The activity of DUSP11 as a mRNA triphosphatase is 2–3 orders of magnitude greater than as a protein phosphatase [152]. DUSP11 contains two arginine-rich regions found in some other RNA-binding proteins and also a proline-rich region (Figure 2). It is possible that DUSP11 acts in conjunction with other mRNA phosphatases to facilitate mRNA maturation; however, further studies are required to clarify its physiological function.

### DUSP12

DUSP12 contains the consensus DUSP catalytic domain as well as an extended C-terminal domain of unknown function, thought to be related to its potential role in glucokinase regulation. It is expressed in many rat tissues with highest expression in pancreas, brain and lung, and its expression in glucokinase-containing pancreatic $\beta$-cells has been confirmed by RT (reverse transcription)–PCR [153]. DUSP12 was identified as a binding partner of glucokinase and has been shown to dephosphorylate glucokinase *in vitro* [153]. Although *in vitro* GST-tagged DUSP12 showed specificity for phosphotyrosine, but not phosphoserine, residues, dephosphorylation of glucokinase occurred mainly on phosphoserine residues. As only high concentrations of DUSP12 are able to dephosphorylate glucokinase *in vitro* [153], additional protein-binding partners may be required *in vivo* to potentiate its phosphatase activity.

*DUSP12* is located at 1q21-q23 which has been identified as a potential Type 2 diabetes susceptibility chromosomal locus in Caucasian populations using linkage analysis [154]. Although it is likely that other genes in the region contribute to susceptibility to Type 2 diabetes, the predicted glucokinase-regulation function of DUSP12 highlights this enzyme as a strong candidate. Nonetheless, the expression of DUSP12 is wide-ranging and includes tissues without glucokinase activity, suggesting that DUSP12 may have additional functions.

### DUSP13a/DUSP13b

The *DUSP13* gene encodes two distinct atypical DUSPs in alternative reading frames. In eukaryotes, it is extremely rare for two distinct proteins to be encoded by alternative reading frames from a single gene and, in most cases, the two proteins are unrelated, as in the case of INK4 (inhibitor of cyclin-dependent kinase 4)/ARF (alternative reading frame) on chromosome 9p21. *DUSP13*, however, encodes two closely related atypical DUSPs, originally called TMDP (testis- and skeletal muscle-specific DUSP) and MDSP (muscle-restricted DUSP). These are now officially called DUSP13a and DUSP13b respectively. DUSP13a is expressed highly in the testis [155]. In the mouse testis, DUSP13a expression is first evident at 3 weeks of age and increases to peak at 10 weeks of age [155] and this enzyme is most highly expressed in spermatocytes and round spermatids [156], suggesting that it may play a role in spermatogenesis. The crystal structure of human DUSP13a was determined in 2006 and reveals a catalytic domain similar to that of DUSP3 [157]. DUSP13a can dephosphorylate phosphotyrosine and phosphoserine/phosphothreonine residues [156], but its cellular targets are yet to be characterized.

In 2004, a search for novel DUSPs in the human genome identified a second DUSP catalytic domain encoded in close proximity to *DUSP13a* [155]. The two proteins have no overlapping coding exons, and the second transcript is expressed in muscle only. Interestingly, similarly to the pattern of expression of DUSP13a in testis, the expression of endogenous DUSP13b protein in mouse muscle is first evident from approx. 3 weeks of age and increases with tissue maturation [155]. DUSP13b can dephosphorylate phosphotyrosine and phosphoserine/threonine residues, but seems to have substrates other than MAPKs since when DUSP13b is expressed in COS1 cells, there is no change in the phosphorylation of ERK, JNK or p38 [155]. Furthermore, DUSP13b does not reduce the activity of the MAPKs as determined by an *in vitro* kinase assay [155]. Determination of the substrate specificity of DUSP13a and DUSP13b will provide further insight into the physiological role of each protein.

### DUSP14

DUSP14 was first characterized using a yeast two-hybrid system to identify novel proteins that interact with the T-cell co-stimulatory factor CD28 [158]. An interaction of DUSP14 with CD28 was confirmed using co-immunoprecipitation techniques in both COS7 and Jurkat T-lymphoma cells, but only upon introduction of exogenous DUSP14 [159]. Although GST-tagged DUSP14 can dephosphorylate ERK, JNK and p38 *in vitro*, in T-cells expressing the catalytically inactive mutant DUSP14 (C111S), only the phosphorylation of ERK and JNK is enhanced, whereas that of p38 remains unchanged [158]. The production of IL (interleukin)-2 is also enhanced in these T-cells which is consistent with previous findings implicating ERK and JNK in the production of IL-2 [160,161]. Consequently, p38 may not be a substrate of DUSP14 *in vivo*. It has been suggested that DUSP14 may be the NSF ('non-specific suppressor factor') known to play a role in regulating delayed-type hypersensitivity and contact hypersensitivity by an unknown mechanism [159]. DUSP14 seems to function as a negative regulator of co-stimulatory signalling in T-cells [158], and it is possible that DUSP14 relies on direct interaction with CD28 near the plasma membrane to gain closer proximity to MAPKs activated early in response to specific stimuli. Indeed, there is some evidence that DUSP14 may act as an early-response gene. In peripheral blood T-cells, *DUSP14* mRNA and protein expression are rapidly induced after simultaneous stimulation with CD3 monoclonal antibody [directed to the TCR (T-cell receptor) complex] and CD28 monoclonal antibody [158].

Recently, DUSP14 has been shown to play a role in the proliferation of pancreatic $\beta$-cells by modulating ERK activity [162]. It is known that GLP-1 (glucagon-like peptide 1) can act as a growth and differentiation factor in mature $\beta$-cells, but that the proliferative effect is limited. Augmenting this proliferative effect has therapeutic implications for patients with diabetes. In pancreatic $\beta$-cells, DUSP14 expression increases in response to GLP-1 in a similar pattern to that of ERK activation and knockdown of DUSP14 in these cells results in increased $\beta$-cell proliferation in response to GLP-1 [162]. Given that DUSP14 acts as a negative regulator of $\beta$-cell proliferation by modulating ERK1/2 activity, it would be interesting to determine the effect of DUSP14 on cellular growth, proliferation and differentiation in other cell types, including cancer cells.

### DUSP15

DUSP15 was initially named VHY (VH1-like member Y) as it was closely related to DUSP22/VHX (VH1-like member X) [163]. DUSP15 has a N-terminal consensus myristoylation signal similar to that of the Src family kinases and is post-translationally modified by the attachment of myristic acid, presumably to $Gly^2$ [163] (Figure 3). This modification is required for targeting DUSP15 to the plasma membrane. DUSP15 is highly expressed in the testis, and in the mouse, this expression is mostly in developing spermatocytes. DUSP15 can also be detected in low amounts in other tissues such as brain, spinal cord and thyroid. GST-tagged DUSP15 can dephosphorylate pNPP (*p*-nitrophenyl phosphate); however, no other phosphorylated substrates have been tested [163]. The crystal structure of DUSP15 has been determined and, although there are characteristics in common with other members of the DUSP family, several distinct surface properties suggest that DUSP15 may have unique substrate specificity and regulation mechanisms [164].

### DUSP18

DUSP18 was identified by two independent research groups by searching the dbEST for novel tyrosine phosphatase proteins [165] and was also cloned from human fetal brain during large-scale cDNA sequencing [166]. The tissue distribution of DUSP18 appears to be wide, but individual studies report conflicting results. Multiple-tissue Northern blots showed that DUSP18 expression was restricted to the testis [165]; however, RT–PCR demonstrated that *DUSP18* mRNA is constitutively expressed in most adult [70,166] and fetal [70] tissues, with highest expression in testis, liver, brain and ovary [166]. *DUSP18* mRNA expression has also been detected in primary tumours and cancer cell lines, including those derived from the breast, lung, colon, prostate, ovary and pancreas [70]. *DUSP18* mRNA increases rapidly within 15 min in HEK (human embryonic kidney)-293 cells treated with serum [70], activity consistent with that of an early-response gene. This dynamic regulation may partly explain the varied tissue distribution reported for DUSP18. The subcellular localization of DUSP18 can also be variable and, although in COS6M cells DUSP18 is present in both the nucleus and the cytoplasm [165], it is restricted to the mitochondria in COS7 cells, which is dependent on amino acids 95–141 [167]. Furthermore, DUSP18 is released from the mitochondria during apoptosis in a manner similar to that of cytochrome *c* [168].

DUSP18 has phosphatase activity against both phosphotyrosine and phosphoserine/phosphothreonine phosphopeptides [166] as well as against di- or mono-phosphorylated peptides corresponding to the active site of ERK, JNK and p38 [165]. The crystal structure of DUSP18 displays a catalytic fold similar to other DUSPs;

however, it also contains a unique C-terminal region which probably contributes to a distinct substrate specificity [167]. Initially, it was reported that Myc-tagged DUSP18 could not dephosphorylate HA (haemagglutinin)-tagged ERK, JNK or p38 transiently expressed in COS cells, as determined by phosphorylation of their respective substrates Elk-1, c-Jun and ATF-2 (activating transcription factor 2) [165]. However an independent study demonstrated activity of DUSP18 towards JNK. Using phospho-specific antibodies and Western blotting, purified HA-tagged JNK, but not ERK or p38, was dephosphorylated directly by purified recombinant GST-tagged DUSP18 *in vitro* [70]. Moreover, JNK phosphorylation was reduced in cells co-transfected with DUSP18 and JNK, and HA-tagged JNK could be co-immunoprecipitated with both GST-tagged and Myc-tagged DUSP18. This specificity for JNK was confirmed by luciferase reporter assays [70]. However, JNK does not co-localize with DUSP18 in the mitochondria [168], which indicates that DUSP18 may have additional substrates in this organelle or that mitochondrial localization represents a mechanism to spatially regulate DUSP18 activity. Clearly, further work is required to determine the physiological role of DUSP18.

### DUSP19

DUSP19 localizes to the cytoplasm in NIH 3T3 cells, and *DUSP19* mRNA is widely distributed in mouse tissues [169]. Interestingly, DUSP19 exhibits a variation of the conserved DUSP catalytic domain sequence motif, with the last serine residue replaced by alanine. Site-directed mutagenesis of this alanine residue to serine does not increase the catalytic activity of DUSP19 towards pNPP; however, GST-tagged DUSP19 has 10-fold less activity against pNPP compared with GST-tagged DUSP22 and GST-tagged DUSP14 [169].

DUSP19 appears to play a role in the regulation of JNK signalling; however, the precise mechanism by which it regulates this pathway remains controversial. An initial study demonstrated that the activity of JNK was reduced when co-expressed with DUSP19 in COS7 cells and this was most marked when JNK was activated by TNF$\alpha$ (tumour necrosis factor $\alpha$) and thapsigargin compared with UV light or anisomycin, suggesting that DUSP19 regulates JNK in a context-specific manner. However, although DUSP19 could directly dephosphorylate JNK in an *in vitro* phosphatase assay in which HA-tagged active MAPKs ERK, JNK and p38 were immunoprecipitated from COS7 cells and incubated with purified GST-tagged DUSP19, JNK did not bind directly to GST-tagged DUSP19 in an *in vitro* binding assay [169].

Like other atypical DUSPs discussed later in this review, there is some evidence that DUSP19 regulates MAPK signalling pathways via interactions with upstream MKKs (MAPK kinases); however, the precise mechanism by which this occurs has yet to be elucidated. DUSP19 has been shown to bind directly to MKK7, a JNK kinase activator [169] and it has been suggested that, by binding to both JNK and DUSP19, MKK7 facilitates the DUSP19-mediated dephosphorylation of JNK. However, a subsequent set of studies demonstrated that DUSP19 can play a scaffold role for the JNK signalling pathway and that the activity of JNK is biphasically regulated by DUSP19 [170]. At low to moderate levels of DUSP19, MKK7 activity and that of the JNK-activated transcription factor ATF-2 was inhibited. At high levels, however, the activity of MKK7 and ATF-2 was enhanced. Interestingly, the inhibition of MKK7 at low to moderate levels of DUSP19 was not dependent on a catalytically active DUSP domain. It has been reported that DUSP19 also binds directly to ASK1 (apoptosis signal-regulating kinase 1), an activating kinase upstream of MKK7 [170] and that expression of DUSP19 enhances the interaction of ASK1 and MKK7, consistent with a role for DUSP19 as a scaffold protein within the JNK activation cascade. Consequently, one explanation for the biphasic regulation of the JNK cascade by DUSP19 is that depending on DUSP19 expression levels, DUSP19 could either sequester the upstream kinase ASK1 and result in a reduction of downstream MKK7–JNK signalling activity, or, alternatively, DUSP19 could act to facilitate binding of ASK1 and MKK7 and result in enhanced downstream JNK signalling. DUSP19 was the first DUSP identified to have a potential role as a scaffold protein within a MAPK signalling cascade, which could have numerous physiological consequences which have yet to be explored.

### DUSP21

The characterization of DUSP21 is limited, with only two reported studies [165,168]. *DUSP21* mRNA expression appears to be restricted to testis [165]. Exogenous DUSP21 localizes to both the cytoplasm and the nucleus in COSM6 cells [165]; however, like DUSP18, endogenous DUSP21 appears to localize to the mitochondria in COS7 cells, and this localization is dependent on the presence of amino acids 43–128 [168]. Purified DUSP21 has activity against mono- and di-phosphorylated synthetic MAPK peptides, with a preference for phosphotyrosine residues. However, DUSP21 has no effect on MAPK activity when tested in an *in vivo* cell-based assay [165].

### DUSP22

DUSP22 has been cloned and characterized by four independent research groups [171–174]. *DUSP22* mRNA appears to be expressed widely in human tissues [172,173]; however, an independent study demonstrated testis-specific expression of *DUSP22* mRNA [171]. These conflicting data may be due to differential expression of two mRNA variants of DUSP22. Expression of a 3.0 kb mRNA transcript has been demonstrated in multiple tissues, whereas the expression of a small 1.3 kb mRNA transcript is restricted to testis and liver [174]. DUSP22 preferentially dephosphorylates phosphotyrosine residues over phosphoserine/phosphothreonine residues [172], which is similar to many other DUSPs, but it is still not clear whether this indicates weaker intrinsic serine/threonine phosphatase activity *in vivo*. There have been a number of conflicting reports focusing on the substrate specificity of DUSP22. One of the first reports showed that DUSP22 can dephosphorylate ERK *in vitro* and that it has the capacity to suppress T-cell antigen receptor-induced activation of ERK2 in Jurkat T-cells [173]. Another early report showed that DUSP22 could preferentially dephosphorylate p38 and JNK, but not ERK, when co-transfected in COS7 cells [171], and a clear reduction in JNK and p38, but not ERK, phosphorylation was demonstrated following incubation of immunoprecipitated active HA-tagged ERK, JNK and p38 with purified recombinant DUSP22. Furthermore, DUSP22 caused a dual (phosphotyrosine and phosphothreonine) loss of p38 phosphorylation, but only phosphotyrosine loss on JNK [171]. However, two additional and independent studies determined that DUSP22 enhances the phosphorylation and activation of JNK [172,174]. Mouse embryonic stem cells deficient in DUSP22 ($JKAP^{-/-}$ ES) were used to demonstrate that DUSP22 is required for full activation of JNK in response to cytokines [174]. Also, phospho-specific antibodies against JNK and its substrate c-Jun were used to demonstrate that JNK phosphorylation was enhanced when this kinase was co-expressed with DUSP22 in HEK-293 cells [174] and COS cells [172]. Interestingly, DUSP22 did not bind and activate JNK directly [174], but

instead increased the activation of the upstream JNK kinases MKK4 [172] and MKK7 [174]. DUSP22 was shown to bind directly to MKK7 only [174]. On the basis of these studies, DUSP22 represents a potential target for therapeutic intervention in various inflammatory and proliferative disorders associated with dysfunctional JNK signalling, and preliminary work has identified potential small molecule inhibitors [175–177].

More recent work has identified alternative DUSP22 substrates. First, a reduction in STAT3 luciferase reporter activity was demonstrated in cells transfected with DUSP22 and conversely, the phosphorylation and activation of STAT3 was enhanced in cells treated with *DUSP22* siRNA (short interfering RNA) [178]. Also, a role for DUSP22 was identified in the inhibition of IL-6-induced STAT3 activation by oestradiol [179]. Secondly, DUSP22 was found to directly bind to and dephosphorylate ER$\alpha$ at Ser$^{118}$ in HEK-293T cells and conversely, the oestradiol-induced phosphorylation of ER$\alpha$ at Ser$^{118}$ was enhanced in cells treated with *DUSP22* siRNA [179]. Consequently, DUSP22 may have more than one physiological substrate and the regulation of specific signalling cascades by this enzyme may be cell-type- and context-specific. Given the diverse roles described to DUSP22, it will be important to dissect these further with more physiologically relevant systems, such as specific cell types derived from *DUSP22*-knockout mice.

## DUSP23

The smallest of the catalytically active PTPs, DUSP23 was cloned and characterized by two independent research groups. *DUSP23* mRNA is expressed in most human fetal tissues, but only in adult testis and colon [180]. In the mouse, however, *DUSP23* mRNA is expressed widely, with highest expression in testis, spleen, liver and heart [181]. Recently, the crystal structure of DUSP23 has been determined, and the orientation of the catalytic domain more closely resembles that of the protein tyrosine phosphatase PTP1b than the prototypic DUSP, DUSP3 [182], which may explain a preference for phosphotyrosine- rather than phosphoserine/phosphothreonine-phosphorylated peptides [181].

The MAPK substrate specificity of DUSP23 remains to be clarified as two independent investigations report conflicting data. In the first study, active MAPKs ERK, JNK and p38 were immunoprecipitated from HEK-293 cells and incubated with GST-tagged DUSP23. This was followed by immunoblotting with phospho-specific antibodies showing a reduction in ERK phosphorylation by DUSP23, and no effect on JNK or p38 phosphorylation [180]. In the second study, DUSP23 was shown to play a similar role to that of DUSP19 and DUSP22 in the activation of MAPK cascades [181]. FLAG-tagged DUSP23 was co-transfected with HA-tagged ERK, JNK or p38 in COS7 cells, which led to an increase in sorbitol-induced activation of JNK and p38, effects which did not result from cross-talk with ERK, which was unaffected [181]. Enhanced phosphorylation of the upstream kinases MKK4 and MKK6 was also observed and, interestingly, the enhanced activation of the upstream kinases and also that of JNK and p38 was not dependent on the catalytic activity of DUSP23 [181]. Consequently, the *in vitro* effect of DUSP23 on ERK may not be physiologically relevant, and *in vivo* DUSP23 may act like DUSP19 as a scaffold to facilitate MKK binding to JNK or p38, or, alternatively, mediate inhibition of an as yet unidentified negative regulator of the JNK/p38 MAPK pathways.

## DUSP26

DUSP26 has been cloned and characterized by five independent research groups [183–187]. *DUSP26* mRNA has highest expression in human and mouse brain [183,184,186,187], heart [184,187], adrenal gland [184] and skeletal muscle [183,184,187]. DUSP26 is absent from most cancer cell lines, although it is expressed in some neuroblastoma cell lines [187] and in primary anaplastic thyroid carcinoma tissue [185].

The current literature is inconsistent regarding the MAPK substrate specificity of DUSP26. In an initial study using HEK-293T cells transfected with DUSP26, a reduction in the phosphorylation of p38 MAPK and its substrate ATF-2 was observed, whereas there was no effect on the phosphorylation or activation of JNK or ERK [187]. Similar results were shown in an independent study using anaplastic thyroid carcinoma cells stably overexpressing DUSP26 which were assessed for MAPK activation. Activation of p38, and, to a lesser extent, ERK, was impaired in cells transfected with wild-type DUSP26, but there was no effect on JNK activation. Conversely, an enhancement of p38 phosphorylation was seen in cells treated with *DUSP26* siRNA. Furthermore, purified p38 was dephosphorylated by purified recombinant DUSP26 as demonstrated by an *in vitro* phosphatase assay, and endogenous p38 was immunoprecipitated as a complex with DUSP26 from KTA3 cells, suggesting that the effect on p38 is direct [185].

However, in an independent study using similar techniques, but using PC12 cells, DUSP26 had no effect on the activation of any of the MAPKs *in vitro* or *in vivo* [184]. Another independent study demonstrated that DUSP26 had no effect on the phosphorylation of ERK, but instead expression of DUSP26 in COS7 cells resulted in an increase in the basal phosphorylation of JNK and p38 [186]. Interestingly, this was an effect seen only in the basal state, as the phosphorylation of JNK and p38 was not enhanced in cells stimulated with sorbitol [186]. A fifth study demonstrated a reduction in both JNK and ERK activity in cells expressing DUSP26. Although DUSP26 did not bind to and directly dephosphorylate JNK and ERK, DUSP26 could bind directly to the heat-shock factor Hsf4b, a substrate of ERK [183]. It was suggested that Hsf4b may facilitate the interaction between ERK and DUSP26 and allow dephosphorylation of ERK by DUSP26 [183]. It is clear that the effect of DUSP26 on MAPKs is variable and probably cell-type- and context-specific and it is possible that DUSP26 has multiple substrates. Alternative DUSP26 substrates were investigated by one group and analysis of phospho-Akt in NGF (nerve growth factor)-activated PC12 cells revealed a reduction in Akt phosphorylation on Ser$^{473}$. Conversely, an increase in Akt phosphorylation on Ser$^{473}$ and Thr$^{308}$ was observed in cells treated with *DUSP26* siRNA. However, this effect was shown to be indirect [184].

The physiological role of DUSP26 remains to be confirmed. One suggested role for DUSP26 is in stress signals in the brain [186]. Also, in PC12 cells, *DUSP26* mRNA expression increases upon differentiation [184]. It is therefore possible that DUSP26 plays a role in neurite outgrowth or differentiation. Finally, preliminary results suggest a role in carcinogenesis. In anaplastic thyroid cancer, there is amplification of chromosomal region 8p12 which contains DUSP26. It has been shown that DUSP26 may play an oncogenic role in these cells, with overexpression of DUSP26 resulting in an increase in colony formation, whereas knockdown of *DUSP26* using siRNA results in a decreased proliferation rate [185].

## DUSP27

Initially, the computationally predicted open reading frame of DUSP27 was termed DUPD1 (DUSP and pro-isomerase domain-containing 1). However, DUSP27 does not contain the pro-isomerase domain as originally annotated. Purified recombinant DUSP27 can dephosphorylate synthetic phosphoserine/phosphothreonine peptides, but has much greater affinity

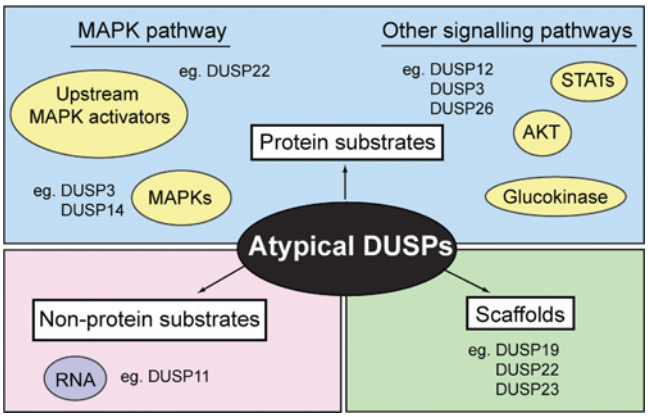


**Figure 5 Function and substrate specificity of atypical DUSPs**

Atypical DUSPs have the most varied substrate specificity of all the PTPs. In addition to MAPK substrates ERK, JNK and p38 common to particular typical DUSPs, atypical DUSPs have been shown to target other protein substrates and RNA, although, for some, alternative protein substrates, e.g. Akt, a direct interaction has not been shown. Also, atypical DUSPs (e.g. DUSP19, DUSP22 and DUSP23) may increase the activity of specific MAPK cascades. In some cases (e.g. DUSP19 and DUSP23), the activity of atypical DUSPs is not dependent on the catalytic activity, and it is thought that, in these cases, the DUSPs act as scaffold proteins to facilitate interaction of signalling proteins.

for phosphotyrosine [188]. Analysis of its three-dimensional structure identified a catalytic site with distinct differences from DUSP3. It has been proposed that a dual-phosphorylated substrate would fit well into the catalytic groove provided the phosphorylated residues were separated by two amino acids rather than one as in the MAPK TXY domain. These observations suggest that the substrate(s) of DUSP27 are distinct from those of DUSP3 and that they are not likely to be MAPKs [188]. DUSP27 is expressed in adult fat, skeletal muscle and liver. This restricted tissue distribution suggests that DUSP27 may play a role in the regulation of cellular metabolism; however, further studies are required to determine its substrate specificity.

## CONCLUSIONS

Despite the growing interest and research focus on DUSPs, including the atypical subgroup in the last 10 years, the field is still crowded with confusing and conflicting reports. The experimental discrepancies are especially pronounced when identifying substrate specificity, which is a notoriously difficult task for all PTPs [189]. Although some inconsistencies can be explained, e.g. by exclusion of specific controls, more often the independent studies are complete and the results are convincing, albeit completely contradictory to other published reports. It is clear that *in vitro* substrate identification assays do not necessarily reflect the situation *in vivo*, since many atypical DUSPs only target their substrate(s) in specific cell types and/or in response to specific stimuli. In addition, it is possible that they require additional binding partners *in vivo* or that they act as scaffolds within specific signalling cascades. These functions are highly context-specific and this probably contributes to the discordance in results obtained in independent studies for particular atypical DUSPs. Unlike the typical DUSPs, atypical DUSPs have a variety of targets including non-protein substrates (Figure 5); however, it appears that few generalizations can be made. Hence implementing substrate-trapping techniques that can also identify targets beyond MAPKs will be crucial. Atypical DUSPs have been implicated in the regulation of normal cellular processes such as cellular proliferation, metabolism and differentiation as well as in pathological states such as inflammation, diabetes and cancer. However, it is clear that accurate determination of their functional roles will depend on the utilization of physiologically relevant systems, such as knockout cells that can be reconstituted with wild-type proteins or catalytically inactive DUSP mutants at appropriate expression levels.

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