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9. Atypical DUSPs: 19 phosphatases in search of a role

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Abstract. Atypical Dual Specificity Phosphatases (A-DUSPs) are a group of 19 phosphatases poorly characterized. They are included among the Class I Cys-based PTPs and contain the active site motif HCXXGXXR conserved in the Class I PTPs. These enzymes present a phosphatase domain similar to MKPs, but lack any substrate targeting domain similar to the CH2 present in this group. Although most of these phosphatases have no more than 250 amino acids, their size ranges from the 150 residues of the smallest A-DUSP, VHZ/DUSP23, to the 1158 residues of the putative PTP DUSP27. The substrates of this family include MAPK, but, in general terms, it does not look that MAPK are the general substrates for the whole group. In fact, other substrates have been described for some of these phosphatases, like the 5'CAP structure of mRNA, glycogen, or STATs and still the substrates of many A-DUSPs have not been identified. In addition to the PTP domain, most of these enzymes present no additional recognizable domains in their sequence, with the exception of CBM-20 in laforin, GTase in HCE1 and a Zn binding domain in DUSP12. Although some of these enzymes have been now studied for years there is a great lack of data about the true physiological role of this group of phosphatases.

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Introduction

Protein tyrosine phosphorylation is a post-translational modification that, in a reversible manner, regulates many cellular functions such as proliferation, differentiation or cytokinesis to cite a few. This process is controlled by two groups of enzymes, protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). PTKs constitute a family of proteins with the same evolutionary origin [1], whereas PTPs share a similar enzymatic activity but show a diverse evolutionary origin [2]. Thus, PTPs have been classified, according to the key aminoacid involved in the catalysis, on Cys-based PTPs or Asp-based PTPs [2]. The Cys-based PTPs share a canonical motif (P-loop) in the phosphatase domain, CX₅R, that contains the Cys required to form the thiol-phosphate intermediate and can be further subdivided based on the characteristics of the catalytic domain in 3 classes. Class I is the more populated with 99 proteins and contains 2 groups, the well-known classical PTPs with 38 sequences [3] and the VH1-like PTPs with 61 members, a quite diverse group with an ample range of substrates, from the 5'CAP structure of mRNA to inositol phospholipids, including phospho-Ser, phospho-Thr and phospho-Tyr. Class-I consensus sequence for the P-loop can be extended to HCX₂GX₂R(S/T). In addition, these phosphatases present an Asp, approximately 30 amino acids upstream of the catalytic Cys, which acts in the catalysis as a general acid, protonating the leaving group. The phosphatase domain of Class I PTPs presents a characteristic structure, different from the topology that shows the phosphatase domain of the other two classes of Cys-PTPs. LMW-PTP, the only member of Class II, presents a distinctive fold and CDC25 phosphatases, which constitute the class III, contain a phosphatase domain that displays a rhodanese fold. The Asp, N-terminal to the catalytic Cys in Class I PTPs, is located in the C-terminus of LMW-PTP and is absent in Class II CDC25 enzymes. Although CDC25 phosphatases have been included frequently in a group of dual-specificity phosphatases along with VH1 phosphatases, they belong to a different group that shows an exquisite specificity for their substrates, cyclin-dependent kinases. In addition to the different phosphatase domain topology, CDC25 proteins lack any sequence similarity to other DSPs, apart from the CX₅R motif common to all Cys-based hydrolases.

There is a fourth class of PTPs that belong to the family of Haloacid Dehalogenases (HAD) and use Asp as the nucleophile in the catalysis. The activity of these phosphatases depends on the presence of divalent cations. The first proteins of this group characterized as phosphatases were the transcription factors Eyes absent (EYA) [4]. While EYA proteins seem to

dephosphorylate phosphor-Tyr [5], other members of this group dephosphorylate substrates in Ser/Thr: Chronophin, which regulates the cytoskeleton by targeting Ser-3 in cofilin [6]; FCP1, which dephosphorylates the C-terminal domain of RNA polymerase II, regulating transcription [7]; and dullard, which is implicated in nuclear membrane biogenesis through dephosphorylation of the phosphatidic acid phosphatase lipin, functioning in a phosphatase cascade [8].

Table 1. A-DUSPs present in the human genome. The table includes their names and their synonyms as well as the uniprot accession numbers to identify these phosphatases. Under the column of substrates, proteins that have been shown to be dephosphorylated have been included, along with those that have been tested but are not targets of these phosphatases.

PROTEIN	SYNONIMS	AA	UNIPROT	SUBSTRATES
VHR	DUSP3	185	P51452	ERK, JNK, p38, STAT5
MDSP	BEDP, DUSP13a	188	Q6B811	Unknown
TMDP	SKRP4, TS-DSP6, DUSP13b	198	Q9UII6	Unknown and Negative on ERK, JNK, p38 and DYRk β
DUSP14	MKP-6, MKP-L	198	O95147	ERK, JNK
DUSP15	VHY	295	Q9H1R2	Unknown
DUSP18	LMW-DSP20	188	Q8NEJ0	JNK
DUSP19	DUSP17, SKRP1, TS- DSP1, LMW-DSP3, LDP-2	217	Q8WTR2	JNK
DUSP21	LMW-DUSP21	190	Q9H596	Unknown and negative on ERK, JNK, p38
DUSP22	JSP-1, MKP-X, JKAP, LMW-DSP2, VHX	184	Q9NRW4	ERK, JNK (upstream protein), p38, ER α , STAT3
DUSP23	LDP3, VHZ	150	Q9BVJ7	ERK, JNK (upstream protein), p38
DUSP26	DUSP24, LDP4, MKP8, NATA1, SKRP3, NEAP	211	Q9BV47	ERK, JNK, p38, Akt, Kap3
DUPD1	DUSP27; FMDSP	220	Q68J44	Unknown
STYX		223	Q8WUJ0	Inactive
PTPM1	MOSP, PLIP, PNAS- 129	201	Q8WUK0	PI(5)P
Laforin		331	O95278	Glycogen, GSK3 β
DUSP11	PIR1	330	O75319	5'CAP
HCE1	CAP1A, HCAP1A	597	O60942	5'CAP
DUSP12	HYVH1, T-DSP4, GKAP	340	Q9UNI6	GKAP Negative on ERK, JNK, p38
DUSP27	STYXL2	1158	Q5VZP5	Inactive

In this chapter, we will discuss the group of VH1-like phosphatases known as A-DUSPs. These enzymes are included in the VH1-like group of Class I PTPs and contain 19 proteins (see table 1) that will be discussed in detail, focused on the physiological role played by these phosphatases. The viral protein VH1 (Vaccinia virus H1 open reading frame) was the first DUSP identified by Dixon and colleagues in 1991 [9]. The next year, VHR (VH1-related PTP) was isolated and cloned [10]. Efforts to identify the PTPs present in the human genome and other genomes have advanced in parallel to the sequencing of the human genome. Thus, some sequences were isolated at the end of the 90s, but most the A-DUSPs sequences were identified in the new millenium. DUPD1 is the most recently characterized phosphatase [11] and it still remains in the databases, DUSP27, an inactive A-DUSP whose mRNA has been detected but no study has been published yet on this protein.

Substrates of these enzymes are diverse, as they target not only proteins phosphorylated on Ser, Thr, and Tyr but also the mRNA capping structure or glycogen. A great effort has been dedicated to show that these enzymes dephosphorylate MAPKs (mitogen activated protein kinases) with negative results in some cases and controversial data in others. In general terms, data available on many of these phosphatases is still limited, given the recent identification of many of them.

VHR /DUSP3

VHR is a phosphatase of 185 amino acids that was cloned in 1992 with an expression cloning strategy [10]. In all these years, VHR has been widely used as a model to research general aspects of Class I PTPs, as the kinetic mechanism of PTPs [12-14] or to design inhibitors for these enzymes [15]. However, research about the physiological role has not been very productive, although VHR has started to reveal its role in cell cycle regulation [16] and in the activation of several signalling pathways related with the immune response [17, 18]. VHR was the first A-DUSP to be crystallized [19] and its three dimensional structure showed that VHR presents a catalytic cleft 6 Å deep, able to accommodate phospho-Ser and phospho-Tyr, in contrast to the deeper pocket (9 Å) of classic PTPs, like PTP1B [20]. This difference would explain the substrate preference of classic PTPs toward phospho-Tyr. VHR can be found in the cytosol as well as in the nucleus, and the localization might have important implications for its pathological function associated with proliferation [16]. Expression of VHR is ubiquitous [21] and is regulated during the cell cycle [16].

Similarity between VHR and MKPs catalytic domain suggested that VHR could dephosphorylate MAPKs in a similar way to MKPs. However, it

took a few years to demonstrate this point. In fact, it was not until 1999 when VHR was shown to dephosphorylate ERK (extracellular regulated kinase) [22], and a few years later when VHR was proven to dephosphorylate JNK (c-jun N-terminal kinase) [21, 23]. In addition, some studies have claimed dephosphorylation of p38 by VHR [24, 25]. In experiments *in vitro* using synthetic diphosphorylated peptides, corresponding to the activation loop of ERK and JNK, it was determined that VHR preferentially hydrolyze the phospho-Tyr residue in that sequence, thereby VHR would dephosphorylate phospho-Tyr followed by slow dephosphorylation of phospho-Thr [26]. However, a later study using phosphorylated recombinant ERK as substrate demonstrated that, *in vitro*, ERK is a poor substrate for VHR [27], indicating that, *in vivo*, it must exist some mechanism that allows the dephosphorylation of ERK and JNK by VHR. One possibility is the existence of a scaffold that would bring together this phosphatase and its substrate, function that could be developed by VRK3 [28] (see below). Reduction in VHR expression using siRNA interference has also shown an increase in the phosphorylation of ERK and JNK [16]. Although the capacity of A-DUSPs, including VHR, to dephosphorylate MAPK remains controversial, at least in this case, the available data suggest that VHR is a true MAPK phosphatase.

Regulation of VHR function is accomplished by Tyr phosphorylation. In T cells, VHR is phosphorylated by the tyrosine kinase Zap70 [17], which is critical for antigen signalling through the TCR (T cell receptor), in Tyr-138, phosphorylation that is required for VHR to inhibit ERK2; conversely, the mutation of Tyr-138 by Phe in VHR increases TCR-induced ERK2 kinase activity as well as activation of interleukin 2 (IL-2) promoter [17]. In a different situation, it has been described that VHR dephosphorylates STAT5 in cells stimulated by IFN- α and IFN- β [18]. Again, phosphorylation of VHR at Tyr-138 was required for its phosphatase activity toward STAT5. The tyrosine kinase Tyk2, which phosphorylates STAT5, is also responsible for the phosphorylation of VHR at Tyr-138. Thereby, these two reports suggest that phosphorylation of VHR in Tyr-138 links kinases that participate in the activation of signalling pathways to its ulterior down-regulation of the pathway by returning the components to the basal state.

The biological function of VHR has started to be uncovered in recent years. In this sense, it has been shown, using siRNA interference that cells with a reduced expression of VHR arrest at the G1-S and G2-M transitions of the cell cycle and exhibit senescence [16]. In agreement with this notion, cells lacking VHR were found to upregulate the cyclin dependent kinase inhibitor p21 (Cip-Waf1), and to reduce the expression of genes for cell-cycle regulators, DNA replication, transcription and mRNA processing. Loss of VHR also caused, after serum-induced activation, a several-fold increase of

its substrates, the MAPKs ERK and JNK. VHR-induced cell-cycle arrest was dependent on this hyperactivation of JNK and ERK, and was reversed by ERK and JNK inhibition or knockdown by RNA interference. These data supports the involvement of VHR in cell-cycle progression, role that is linked to its regulation of MAPK activation in the cell-cycle.

A few reports have implicated VHR in different types of cancer [29-31]. This role of VHR seems to be related to its role as a MAPK phosphatase. In the prostate cancer cell line LNCaP androgens protect from TPA (12-O-tetradecanoylphorbol-13-acetate) or thapsigargin-induced apoptosis via down-regulation of JNK activation. This effect could be explained by an increased in VHR expression induced by androgens [29]. Transfection of wild-type VHR, but not a catalytically inactive mutant, interfered with TPA and TG-induced apoptosis. Consistently, siRNA-mediated knockdown of endogenous VHR increased apoptosis in response to TPA or TG in the presence of androgens. In this work, it is also found that VHR expression is augmented in prostate cancer cells compared with normal prostate cells. Overexpression of BRCA1-IRIS, a product of the BRCA1 gene, in breast cancer, augments Cyclin D1 expression and increases cell proliferation. Increase in Cyclin D1 is explained by a reduction in VHR expression regulated by BRCA1-IRIS by an unknown mechanism [30]. Furthermore, overexpression of Cyclin D1 could be blocked by transfection of VHR in those cells. An additional study in cervix cancer has reported an increase in the expression of VHR and a change in its location [31]. Thus, in invasive cervix cancer, in addition to be highly expressed, VHR changes its localization to the nucleus, while in normal tissue VHR is always in the cytoplasm. In addition, cervix cancer cell lines such as HeLa, SiHa, CaSki, C33 and HT3 also present a higher expression of VHR, compared to primary keratinocytes. Altogether, the role that plays VHR in cell cycle progression along with VHR implications in several cancer types, suggest that this phosphatase could be a therapeutical target in this disease.

DUSP26

Several independent groups initiated the characterization of this phosphatase and published their studies between 2005 and 2007. The results obtained by these researches were as diverse as to show one effect, for example inhibition of p38 [32, 33] and JNK [33, 34], and the opposite, stimulation of p38 and JNK [35]. It has also been reported inhibition of ERK [34], in this case mediated by the interaction of DUSP26 with the protein Hsf4 (heat shock transcription factors 4), a substrate of MAPKs. Furthermore, an independent study in PC12 cells found no effect in any of

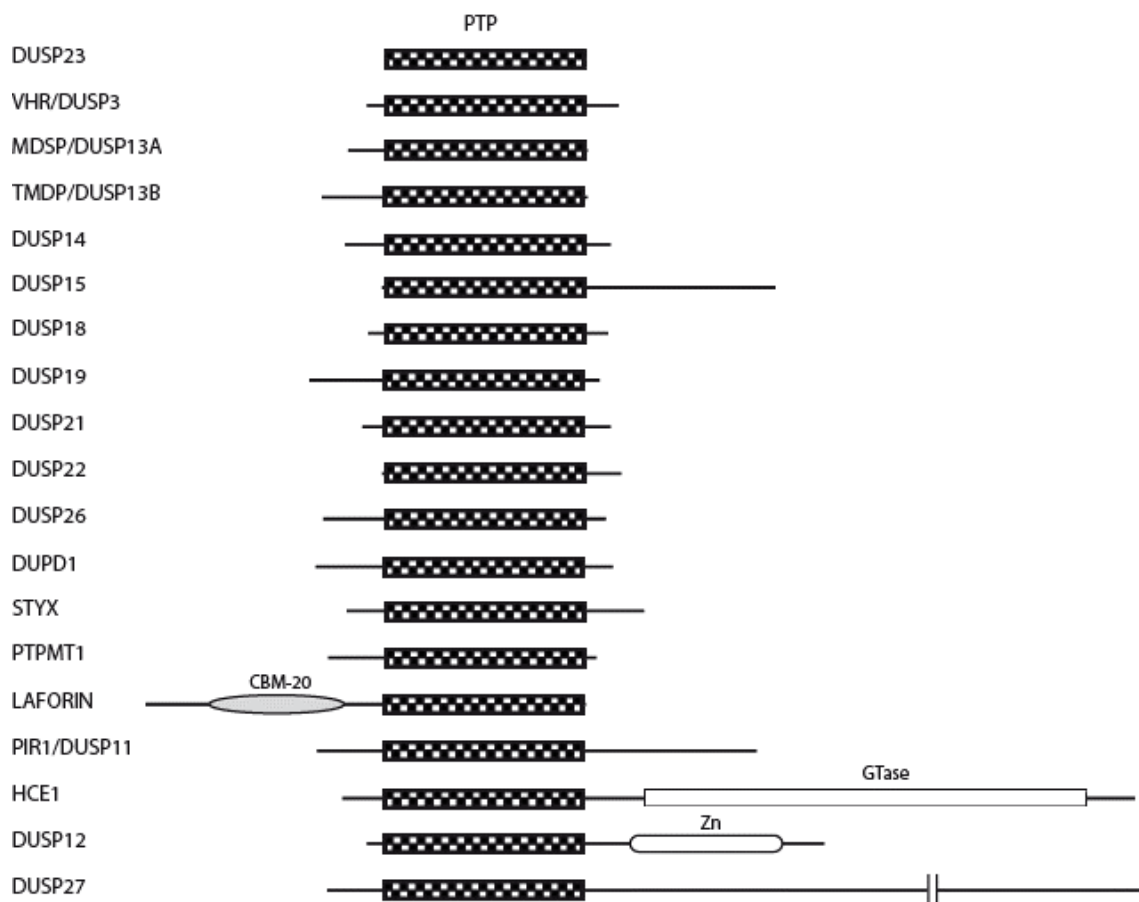


Figure 1. Domains present in the A-DUSPs. Apart of the characteristic PTP domain (150 aminoacids) presented by these proteins, only three of these phosphatases present an additional domain: CBM-20 (carbohydrate binding module-family 20) in Laforin, a GTase (guanylyltransferase) domain in HCE1, the mRNA capping enzyme and a Zn binding domain (Zn) in DUSP12. All the phosphatases have drawn to scale except DUSP27 that is the longest (1158 aminoacids).

the main MAPKs, ERK, JNK or p38 [36]. Apart of MAPKs, an additional substrate has been described for DUSP26 in PC12 cells, Akt, since overexpression of DUSP26 reduced Akt phosphorylation on Ser-473 [37].

DUSP26 is localized mainly in the nucleus [32, 35], although in PC12 cells is found in the cytosol [36]; and its expression is enriched in the brain [34, 35], particularly in neurons [34]. In addition, this phosphatase has been detected in several cancer cell lines, retinoblastoma, neuroepithelioma, and neuroblastoma [38]. In this sense, 8p12, the chromosomal region where DUSP26 gene is present, has been found to be amplified in anaplastic thyroid cancer (ATC) and, consequently, DUSP26 expression was increased in primary ATC tumors and ATC cell lines. In three ATC cell lines, cell growth was inhibited and apoptosis was increased by DUSP26 siRNA. In this study,

DUSP26 was detected in a complex with p38, which was dephosphorylated by this phosphatase. Therefore, in that report the authors propose that this phosphatase acts as an oncogen by promoting cell survival of ATC cells through inhibition of p38 induced apoptosis.

In PC12 cells, DUSP26 expression is up-regulated by nerve growth factor (NGF) and suppression of DUSP26 expression by siRNA enhanced NGF-induced neurite outgrowth and Akt activation. Moreover, DUSP26 impaired cell growth in response to EGF (epidermal growth factor) by reducing EGFR (EGF receptor) mRNA and protein, through down-regulation of the Akt pathway and Wilms' tumor gene product (WT1). Taken together, these studies suggest that DUSP26 could be involved in PC12 differentiation in response to NGF and EGF via regulation of the PI3K/Akt signalling pathway.

Recently, DUSP26 has also been involved in intracellular transport and cell adhesion [38], since it was found that DUSP26 binds KIF3, a microtubule-directed protein motor involved in transport of β -catenin/N-Cadherin to the plasma membrane. DUSP26 would be recruited to the KIF3 complex through interaction with one subunit of this complex, Kif3a, to dephosphorylate Kap3, which should be critical for transport of β -catenin/N-Cadherin. In this sense, over-expression of DUSP26 increases cell-cell contacts and adhesion. These authors also described a down-regulation of DUSP26 in gliomas, suggesting a tumor-suppressive role of this phosphatase.

DUSP12

DUSP12 was identified based on similarity with the yeast VH1 (YVH1) phosphatase, and then it was named human orthologue of YVH1 (hYVH1) phosphatase [39]. Several studies support a role for this phosphatase in cell growth [40], and meiosis and sporulation [41] in yeast. Independently, the rat orthologue was cloned from a liver cDNA library in a yeast-two hybrid screen with glucokinase and, thus, named glucokinase-associated phosphatase (GKAP) [42]. This protein contains an N-terminal DUSP domain and a cysteine-rich C-terminal domain capable of binding 2 mol of zinc/mol of protein, defining it as a novel zinc finger domain, which is essential for *in vivo* function [39]. DUSP12 in human cells is expressed in all tissues analyzed [39] and similar results have been obtained for GKAP. Expression of GKAP was also verified by RT-PCR in β -pancreatic cells [42].

DUSP12, *in vitro*, dephosphorylated phospho-Tyr, but not the artificial substrate phosphoserine kemptide, although it could hydrolyze phosphate from Ser phosphorylated glucokinase [42]. GKAP fused with GFP (green fluorescent protein) was found in the cytosol, where glucokinase

phosphorylates glucose. Furthermore, addition of DUSP12 enhanced glucokinase activity *in vitro* in a dose-dependent manner, suggesting that this association is of functional significance.

Implications of DUSP12 in glucose metabolism have received further support from genetic studies that have shown that the chromosomal region 1q21-23, where the *DUSP12* gene resides, is linked to type 2 diabetes (T2D) [43]. Furthermore, it has been found that several polymorphisms in DUSP12 are associated with T2D [44]. In addition, this chromosomal region is amplified in human liposarcomas and DUSP12 gene showed the highest level of amplification within that region [45].

A recent work has reported the interaction of Hsp70 (heat-shock protein 70) with DUSP12 [46]. The Zn-binding domain is required for this interaction, along with the ATPase domain of Hsp70. DUSP12 phosphatase activity towards an exogenous substrate under non-reducing conditions was increased by Hsp70. This study also supports a role of this phosphatase in cell survival, since overexpression of DUSP12 reduced the number of apoptotic cells in response to various apoptotic inducers, such as heat shock, H₂O₂ and Fas receptor activation. Activity of DUSP12 on ERK, JNK or p38 was tested under the stress conditions analysed, finding that this phosphatase does not dephosphorylate any of these kinases [46]. Furthermore, purified DUSP12 was unable to dephosphorylate purified activated MAPKs *in vitro* in the absence or presence of Hsp70. Therefore, substrates for DUSP12 remained to be identified. The role of this phosphatase on cell survival is further supported by data from a large-scale siRNA screening conducted by Blenis and co-workers that showed that knockdown of DUSP12 with siRNA caused significant apoptosis [47]. However, overexpression of DUSP12 could not rescue the cells exposed to cisplatin, which initiates apoptosis via the DNA damage response pathway, and suggest that hYVH1's effects are selective to specific cytotoxic stimuli, just mentioned above, which involve redox-sensitive signalling pathways. Both phosphatase and Zn-binding domains are required for the anti-apoptotic effects caused by this phosphatase. In an independent study, looking for genes that increase resistance to Fas-induced apoptosis, the expression of DUSP12 was found to be down regulated [48], confirming the role of this phosphatase in resistance to apoptosis and cell survival.

LAFORIN

Mutations in this DUSP cause the Lafora disease (LD) (OMIM254780) [49, 50], an autosomal recessive neurodegenerative disorder that results in progressive myoclonus epilepsy with onset in adolescence and death within 10 years of appearance. This disease was described at the beginning of last

century, in 1911, by the neurologist Gonzalo Rodríguez Lafora [51], a disciple of Santiago Ramón y Cajal. The hallmark of the disease is the formation of Lafora bodies, which contain insoluble, poorly branched glycogen-like polysaccharide or “polyglucosan”. These bodies develop in different tissues: liver, muscle, heart, skin, and neurons that normally accumulate little glycogen. Approximately 60% of the cases of LD result from mutations in the gene that encodes laforin, *EPM2A*. This phosphatase contains a glycogen binding domain in the N-terminus (GBM-20), followed by the phosphatase domain. Another gene has been identified, *EPM2B* (*NHLRC1*), whose mutation causes 20–30% of the cases of Lafora disease [52, 53]. *EPM2B* codes for a protein called malin that is an E3 ubiquitin ligase, which promotes ubiquitination and proteasomal degradation of laforin [54, 55], among other proteins related to glycogen metabolism.

The first protein identified to interact with laforin was PTG (protein targeting to glycogen) or R5 [56], a protein that targets the Ser/Thr phosphatase PP1 to glycogen [57]. PP1 regulates glycogen synthesis by dephosphorylating key enzymes involved in glycogen metabolism. Thus, PP1 activates glycogen synthetase (GS) and inactivates the glycogen degradation enzymes phosphorylase (Ph) and phosphorylase kinase (PhK). Overexpression of PTG/R5 markedly increases glycogen accumulation, and reduction of PTG expression diminishes glycogen stores. In contrast to PP1 phosphatase, several kinases such as AMPK (AMP-activated protein kinase), PKA, CKI and GSK3 could inhibit glycogen synthesis by acting on the same proteins than PP1. Later, it was shown that laforin and malin form a complex that promotes the ubiquitination and proteasome dependent degradation of PTG/R5, and, accordingly, inhibit glycogen accumulation in neurons [58]. In these cells, which do not express the enzymes involved in glycogen hydrolysis Ph and PhK, it was demonstrated that the malin-laforin complex caused proteasome-dependent degradation of MGS (muscle glycogen synthase) [58], thus mutant laforin and malin proteins would impair the capacity to degrade MGS with the consequence of increasing the amount of glycogen in neurons. Furthermore, Solaz-Fuster *et al.* [59] demonstrated, in FTO2B hepatoma cells, that malin, in concert with laforin, decreases PTG/R5 levels and glycogen stores without affecting glycogen synthase. Degradation of PTG/R5 by this complex could explain how the mutations that impair the physiological function of laforin or malin increase PTG levels and glycogen synthesis in the cells. In laforin-malin complex, laforin seems to behave as a substrate adaptor, recruiting proteins present in glycogen particles for ubiquitination by malin and subsequent degradation by the proteasome. Additional glycogen metabolism-related proteins have been reported to be degraded by this complex, as the glycogen debranching enzyme

(GDE)/amylo-1, 6-glucosidase,4-alpha-glucanotransferase (AGL)[60]. It has been also shown that malin promotes ubiquitination of laforin, which would mean that the same E3 ubiquitin ligase complex regulates its own quantity by controlling the amount of one of its components, the substrate adaptor laforin.

Interaction between laforin and malin is regulated by phosphorylation of laforin by AMPK, which binds directly to laforin [59]. Moreover, AMPK also phosphorylates PTG/R5 on Ser-8 and Ser-268, which accelerates its ubiquitination by the laforin-malin complex [61], following the classical mechanism of tagging proteins for ubiquitination by phosphorylation.

Interaction of laforin with other proteins has been described, although the functional significance of these interactions is unknown. Among these proteins are: EPM2AIP1 [62], a protein of unknown function; HIRIP5, a cytosolic protein that contains a NifU-like domain that could be involved in iron homeostasis [63]; GSK3 β , which was shown to be dephosphorylated by laforin [55, 64], although this remains controversial. In addition, it has been described that laforin forms homodimers [56, 64], which are critical for its phosphatase activity.

The complex malin-laforin could develop additional functions related to the clearing of misfolded proteins through the ubiquitin–proteasome system [65]. In this sense, it has been observed that malin and laforin co-localize in the endoplasmic reticulum (ER) and form aggregates when neurons are treated with proteasomal inhibitors [66]. In the same direction, knockdown of laforin by siRNA treatment in HEK293 and SH-SY5Y cells increased the sensitivity to agents that triggers ER-stress, impairing the ubiquitin-proteasomal pathway and augmenting apoptosis [67]. Therefore, the regulation of glycogen synthesis by proteasomal degradation of key proteins required for this process could be a particular case in the general function that laforin and malin develop in the cell, which could be the control of the accumulation of misfolded proteins under stress conditions. It is well known that protein aggregates in neurons lead to several fatal neurodegenerative disorders.

In addition to its function in protein degradation it has been proposed that laforin presents phosphatase activity against glycogen, dephosphorylation that is required for the maintenance of normal cellular glycogen [68]. Glycogen, a branched polymer of glucose, contains a small amount of covalently linked phosphate that can be release by Laforin *in vitro*. Moreover, glycogen from laforin-deficient mice is increased in several tissues, and this glycogen presents an increase in the covalent phosphate content [69]. These authors proposed that an increase in glycogen phosphorylation leads to aberrant branching and Lafora body formation. Thus, the physiological role of laforin would be to prevent excessive glycogen phosphorylation.

DUSP14

DUSP14 was initially identified as a CD28 interacting protein, called MKP6, in a yeast two-hybrid assay [70]. The potential substrates of DUSP14 can be ERK, JNK and p38, as GST-DUSP14 dephosphorylates them *in vitro*. Nevertheless, the expression of a catalytically inactive DUSP14 mutant, Cys-111 to Ser, in T-cells produces only an increase in ERK and JNK phosphorylation, whereas p38 phosphorylation remains invariable. This augmented phosphorylation in ERK and JNK is accompanied by an enhanced interleukin-2 production. DUSP14 seems to negatively regulate co-stimulatory signalling in T-cells, possibly by interacting directly with CD28. This would localize DUSP14 close to the cell membrane where the phosphatase could interact with the MAPKs activated early after stimulation. In fact, DUSP14 may function as an early response gene, since DUSP14 mRNA and protein expression are rapidly induced after co-stimulation of peripheral blood T lymphocytes through the TCR and CD28 receptors [48].

Apart of T cells, DUSP14 has been associated with different functions in other cell types. A recent study has suggested that DUSP14 could be the non-specific suppressor factor that regulates some types of hypersensitivity, such as delayed-type hypersensitivity or contact hypersensitivity [71]. There is also recent evidence that involves DUSP14 in the proliferation of pancreatic β -cells, possibly acting through modulation of ERK activity [72]. In this cell type, DUSP14 expression is augmented in response to Glucagon-like peptide-1 (GLP-1), which is a growth and differentiation factor for β -cells. However, GLP-1 has very limited proliferative skills. Thus, finding mechanisms that increase this proliferative ability could be of great interest in the treatment of diabetes. In this sense, blocking DUSP14 expression increases the proliferative effect of GLP-1 on β -cells. Another study, in a different context, has shown that DUSP14 expression is induced by the transcription factor Klf4 and by corticosteroids in the development of the epidermis in mice [73], suggesting a possible role of this phosphatase in the development of this tissue. This gene is also up-regulated in mice dorsal striatum by acute administration of MDMA (3-4-methylenedioxymethamphetamine), active compound of the widely abused drug ecstasy [74], along with two other DUSPs, DUSP1 and DUSP5. Induction of DUSP14 is partially due to activation of dopamine D1 receptors and independent of dopamine D2 receptors. Overall, it appears that DUSP14 is highly regulated at the transcriptional level in the cells studied.

An interesting fact reported by Nakano [71] is the possible existence of DUSP14 as a dimer in non-reducing conditions, suggesting that this

phosphatase might be functional in cells as a dimer. This is in agreement with a recent work that reports the crystal structure of VH1, which adopts a dimeric quaternary structure mediated by an N-terminal α -helix that protrudes from the PTP domain [75].

STYX: An inactive A-DUSP

STYX (phospho-Ser or Thr or Tyr interaction protein) is an inactive phosphatase that presents a Gly instead of the catalytic Cys in the P-loop of its phosphatase domain. The rest of the features of A-DUSPs are conserved in this protein. In fact, mutation of Gly to Cys recovers the phosphatase activity of this protein to dephosphorylate p-NPP and phospho-Tyr and phospho-Thr residues from peptide sequences of MAPK [76]. The PTP domain of this protein has been crystallized showing the same overall topology of the PTP fold [77]. Expression of STYX is found in all tissues studied with higher abundance in skeletal muscle, testis, and heart [76].

The generation of a knockout mouse that does not express this protein has shown that STYX is essential for normal spermiogenesis [78]. These mice present a great reduction in spermatozoa production with the result of male infertility. In addition, this study has described the interaction of STYX with an RNA-binding protein, CRHSP-24 (Ca_2^+ -regulated heat-stable protein of 24 kDa), which is phosphorylated in Ser and also targeted by the Ser/Thr phosphatase calcineurin (PP2B). It has been speculated that inactive phosphatases like STYX used the inactive domain to bind phosphorylated proteins in a similar way to SH2 domains and this way may regulate their function, for example, by protecting them from dephosphorylation by active phosphatases. In any case, as judging from the results obtain with the STYX knockout mouse, this inactive phosphatase does develop a key function in the cell that is required for spermiogenesis, although the mechanism is unknown.

PIR1 and HCE (RNGTT): RNA phosphatases

Two RNA phosphatases have been found in the human genome, PIR1 (Phosphatase that interacts with RNA/RNP complex 1)/DUSP11 [79, 80] and HCE1 [81]. Both possess an N-terminus phosphatase domain that contains the P-loop consensus motif for Cys-PTPs HCX2GX2R(S/T), although they lack the Asp required for the catalysis and present in most of the Cys-based PTPs. These two RNA phosphatases belong to different subgroups: HCE1 is included among the bifunctional metazoan mRNA capping enzymes, where the triphosphatase domain exclusively hydrolyzes the γ -phosphate of RNA; and PIR1 is included in monofunctional enzymes that hydrolyze RNA 5'

triphosphate to 5'-diphosphate and 5'-monophosphate [82]. In this last group, it is also included the baculovirus phosphatase BVP, the first enzyme shown to have RNA phosphatase activity, isolated from the *Autographa californica* nuclear polyhedrosis virus [83, 84]. Bifunctional metazoan capping enzymes as HCE possess two domains, the previously mentioned N-terminal RNA 5'-triphosphatase domain and a C-terminal guanylyltransferase domain [82]. It is this second domain that is absent in monofunctional enzymes such as PIR1 and BVP. HCE1 RNA triphosphatase domain removes the γ -phosphate from the 5' end of nascent mRNA to leave a diphosphate terminus and the guanylyltransferase domain catalyzes the subsequent transfer of a guanylyl group from GTP to produce the unmethylated 5' cap structure [82]. This modification facilitates transcript translation and later processing of the mRNA. Then, PIR1/DUSP11 lacks the guanylyltransferase domain, and although shows greater activity for RNA than for proteins, its function is unclear.

To our knowledge a function for PIR1 has not been established in eukaryotic cells and since PIR1 dephosphorylates RNA 5' triphosphate to 5'-monophosphate, its function as a capping enzyme seems unlikely because it needs a 5'diphosphate in order to form the CAP structure [80]. Consistent with this, BVP, the other monofunctional domain RNA phosphatase, is not required for the formation of a 5'CAP structure on late viral mRNAs [85] and is not essential for viral replication [86]. Although its function is not fully understood, a recent report has shown that could be related to a change in the insect host behaviour that favours dispersion of the viruses by increasing the locomotory pattern during the larval stage [87]. In addition, *A. californica* nuclear polyhedrosis virus contains a proper bifunctional capping enzyme with RNA triphosphatase and guanylyltransferase activities, LEF-4.

BVP and the mouse orthologue of HCE1 phosphatase (PDB 2C46) domains have been crystallized and both show the typical fold of the PTP domain of Class I phosphatases [88].

DUSP13: One gene, two phosphatases

The gene DUSP13 is located in human chromosome 10q22.2, 50kb downstream of the gene of another A-DUSP, DUPD1, which suggests that the two genes might have originated by gene duplication. DUSP13 and DUPD1 have been also found in mammals and in chicken, then the gene duplication that gave rise to the DUPD1/DUSP13 pair occurred prior to the divergence of mammals from birds [11]. The mRNA transcribed from DUSP13 gene is translated in two different phosphatases using alternative open reading frames [89]. These two proteins are encoded from different exons, but their transcripts share the same polyadenylation signal in exon 9.

These phosphatases are TMDP (testis and skeletal muscle-specific dual specificity phosphatase) also called DUSP13b, and MDSP (muscle-restricted dual specificity phosphatase) or DUSP13a. The finding that a gene is transcribed into an mRNA that produces two proteins from alternative open reading frames that belong to the same family is unique among eukaryotes. Expression of these two proteins is quite restricted; while TMDP is mainly expressed in testis, MDSP is found in skeletal muscle. Expression of both proteins is increased with tissue maturation [89]. TMDP has been found by *in situ* hybridization in spermatocytes and round spermatids [90]; and this has been taken as an indication of the role of this phosphatase in spermatogenesis.

TMDP dephosphorylates phospho-Tyr and phospho-Ser in artificial substrates as MBP (myelin basic protein) [90], but no physiological substrates have been identified yet. MDSP has been tested in co-transfection experiments for its ability to dephosphorylate several MAPkinases (ERK2, JNK1, p38 α and β) producing a negative result [89]. Given the restricted expression of this phosphatase to the skeletal muscle, other kinases were tested, in particular dual specificity tyrosine-regulated kinases (DYRKs) because of the similarity of the activation loop with MAPK, in addition to the role of DYRK1B in skeletal muscle development [89]. However, MDSP was also unable to dephosphorylate this kinase in cotransfection experiments.

The structure of the complete TMDP has been solved at a resolution of 2.4 Å [91]. Although the crystal showed an asymmetric unit with four molecules in two potential dimers, however, the dimeric contacts were not significant enough to hold the two molecules in solution. Furthermore, additional experiments such as gel filtration chromatography and dynamic light scattering showed that TMDP exists as a monomer in solution. TMDP structure is most similar to VHR. The main differences between the structures of these PTPs are found in the loop α 1– β 1, involved in substrate recognition, and in the loop β 3– α 4, part of so-called variable insert, which is shorter in TMDP. Unlike VHR, TMDP contains a wide and shallow pocket that seems to be adequate for accommodation of both phospho-Tyr and phospho-Thr and could explain its preference for dually phosphorylated substrates.

DUSP19 and DUSP23: Putative scaffold A-DUSPs

DUSP19

DUSP19 was initially described as SAPK (stress activated protein kinase) pathway-regulating phosphatase 1 (SKRP1) [92], LDP-2 (low-

molecular-mass DSP-2) [25] and LMW-DSP3 (low molecular weight dual specificity phosphatase 3) [93]. The mRNA of this phosphatase has been found in all tissues examined [25, 92]. DUSP19 sequence presents an Ala instead of a Ser after the Arg155 in the P-loop, HCXXGXXRS, which is conserved in most of the DUSPs. Endogenous DUSP19 is mainly detected in the cytoplasm, where it co-localizes with JNK. Recombinant DUSP19 protein showed extremely low phosphatase activity towards p-nitrophenyl phosphate (pNPP) and mutation of Ala-156 to a Ser increased this activity. However, DUSP19 and the Ala-156 Ser mutant exhibited similar activity to both phospho-Ser/Thr and phospho-Tyr residues of myelin basic protein.

DUSP19 was found to be specific for JNK [25, 92]. This phosphatase showed a greater inhibition when JNK was activated by TNF- α (tumor necrosis factor alpha) or thapsigargin, in contrast to UV light or anisomycin [92]. DUSP19 formed a complex with the JNK upstream activating kinase MKK7, but not with MKK4, and did not bind directly to JNK. Thereby, DUSP19 targeted its substrate, JNK, through MKK7 interaction. In addition, it has been reported that DUSP19 co-precipitated MKK7-activating MAPKK kinase (MAPKKKs) ASK1 (apoptosis signal-regulating kinase 1), but not MEKK1 (MAP kinase kinase kinase 1) [94]. In agreement with this, DUSP19 overexpression increased ASK1-MKK7 complexes and enhanced the activation of MKK7 by ASK1. The authors of this work proposed that this phosphatase acts as a scaffold in the JNK activation pathway, although the mechanism remains unknown and deserves further study.

DUSP23: The smallest A-DUSP

At the end of 2004 several studies reported the initial characterization of this phosphatase as VHZ [95], LDP-3 (low-molecular-mass dual-specificity phosphatase-3) [24] and DUSP23 [96]. This A-DUSP is the smallest active phosphatase with 150 aminoacids (16 kDa), whereas the catalytic domain of classical PTPs presents 280 aa [3]. Expression of this phosphatase has been detected as mRNA in every tissue tested, in mouse [24] and human [95]. As a protein, DUSP23 has been detected in peripheral blood lymphocytes (PBL), and in different cell lines of hematopoietic origin: Jurkat T cells, RAMOS B cells, HL-60 myeloid cells as well as the tumor cell lines M45 and HT29 [95]. Endogenous DUSP23 is detected in the cytosol [95] and overexpression of this protein produced the same results [24, 95, 96], although in one study some cells presented nuclear staining, in some cases in the nucleolus [95]. The structure of VHZ/DUSP23 reported recently [97] is particularly interesting since two aminoacids from another molecule in the crystal, Thr-135–Tyr-136, bind in the catalytic pocket with a malate ion, mimicking the

TXY motif found in the activation loop of MAPK, in a similar way to an enzyme-substrate complex. The fold of VHZ/DUSP23 is closer to VHR, but binding of the substrate peptide to the active site resembles more closely to PTP1B due to the presence of similar residues surrounding the active pocket, which provide a similar orientation to the substrate.

DUSP23 is an active phosphatase against pNPP and also dephosphorylates Tyr and Ser/Thr phosphorylated peptides [24, 96]. Its ability to dephosphorylate MAPK has been tested by different laboratories obtaining different results. In one study, recombinant GST-DUSP23 dephosphorylated ERK2 *in vitro* without affecting JNK or p38 [96]. In a different report, overexpression of DUSP23 produced an increase in JNK and p38 activity by sorbitol [24]. However, DUSP23 did not affect ERK activation by EGF. Osmotic stress stimuli other than sorbitol, such as mannitol, glucose or glycine, also increased the activation of the stress kinases JNK and p38, but other kinds of stresses, oxidative stress, heat shock, or UV irradiation, did not induce that effect. Interestingly, it was also observed a sorbitol-induced activation of upstream kinases of JNK and p38, MKK4 and MKK6. Surprisingly, the effect observed on JNK and p38 is independent of its phosphatase activity since Cys to Ser or Asp to Ala inactive mutants produced the same increase in sorbitol-induced activation of these kinases [24]. One explanation for these results would be that DUSP23 works as a scaffold in these pathways, although no interaction with proteins in this pathway has been reported. An alternative hypothesis would be that this PTP dephosphorylates a negative regulatory site on a protein acting on the initial steps of this signalling pathways.

PTPMT1, DUSP18 and DUSP21: Mitochondrial atypical-DSPs

PTPMT1

PTPMT1 (Protein-tyrosine phosphatase mitochondrial 1) is the first A-DUSP that has been demonstrated to be localized in the mitochondria [98]. Afterwards, another two DUSPs were also found in the mitochondria, DUSP18 and DUSP21 [99]. PTPMT1 was referred previously as PLIP (PTEN-like phosphatase) [100] and MOSP [2]. Initially this PTP was cloned from *Dictiostelium* after a database search for phosphatases similar to PTEN [101]. In fact, it presents two basic aminoacids in the P-loop, Arg-133 and Lys-136, in equivalent positions to PTEN Lys-125 and Lys-128, aminoacids that give PTEN specificity for PI(3,4,5)P3. Initial characterization of the substrate specificity of this phosphatase using several phosphoinositides

showed as the preferred substrate PI(5)P [100]. PTPMT1 is targeted to the mitochondria by an N-terminal signal sequence and it is found anchored to the matrix face of the inner membrane (IM) in this organelle, where it colocalizes with members of the respiratory chain. Expression of this phosphatase, although is highly enriched in testis tissue, has been shown in every tissue tested by PCR. This PTP presents orthologues in all phylogenetic kingdoms, including eubacteria.

Disruption of PTPMT1 expression by siRNA in the pancreatic β cell line INS-1 832/13 caused an increase in ATP production and markedly enhanced insulin secretion under both basal- and glucose-stimulated conditions [98]. At the same time, knockdown of this phosphatase produced an increase in Ser and Thr phosphorylation of some proteins, whereas no change was observed in protein tyrosine phosphorylation. However, a reduced expression of PTPMT1 did not affect the PI(5)P levels in the mitochondria, despite the *in vitro* observed activity of PTPMT1 [100]. These data support the idea of PTPMT1 playing a critical role in mitochondrial function by regulating the status of phosphorylation in Ser or Thr of mitochondrial proteins.

DUSP18

This PTP was characterized by two different groups as LMW-DSP20 [102] and DUSP18 [103]. This phosphatase is conserved in the different groups of vertebrates, is expressed in all tissues studied, and is localized in the mitochondria [99]. Unlike, PTPMT1 that presents an N-terminal mitochondrial localization signal, DUSP18 contains an internal mitochondrial localization signal that allows its internalization in the mitochondria. Inside the mitochondria, DUSP18 is localized in the IM, looking to the IMS (intermembrane space) compartment. Although it has been shown to be present in the cytosol and the nucleus, these results could be likely due to overexpression of these PTPs, in addition to the masking effect of N-terminal tags over the mitochondrial localization signals [102]. Kinetic analysis of DUSP18 with pNPP as a substrate showed that DUSP18 was an active PTP and was more efficient using pNPP than the other mitochondrial DUSPs, PTPMT1 and DUSP21, but not as efficiently as VHR [99]. DUSP18 dephosphorylates *in vitro* diphosphorylated synthetic MAPK peptides, with preference for the phospho-Tyr and diphosphorylated forms over phospho-Thr. However, *in vivo*, overexpression of DUSP18 was unable to dephosphorylate MAPKs in this study [99]. In contrast, there is other report showing that DUSP18 targets JNK *in vivo* [104]. However, in a different report has not been possible to detect the presence of JNK in mitochondria [99], suggesting that JNK is not a substrate for DUSP18.

Induction of apoptosis, a process in which mitochondria plays a critical role, resulted in release of DUSP18 from the mitochondria to the cytosol, in a similar way to cytochrome c, a key mediator of apoptosis. Treatment of cells with known inducers of apoptosis, such as starurosporine, an inespecific kinase inhibitor, or etoposide, a topoisomerase II inhibitor, showed cytosolic localization of DUSP18 after the mitochondrial outer membrane permeabilization produced during the apoptotic process [99].

DUSP21

Expression of DUSP21 mRNA has been detected exclusively in testes [102]. DUSP21 presents 69% sequence identity with DUSP18, and, as DUSP18, it also contains an internal mitochondrial signal sequence that, in this case, localizes the phosphatase to the matrix compartment of this organelle. Interestingly, despite this great similarity, these two DUSPs localize to different sides of the inner mitochondrial membrane. DUSP21 dephosphorylates synthetic MAPK peptides *in vitro* with preference for phospho-Tyr or dual-phosphorylated peptides over phospho-Thr. Co-transfection of this PTP with MAPK resulted in no activity against this family of kinases [102].

DUSP15 and DUSP22: Myristoilated A-DUSPs

DUSP15

DUSP15 was first described as VHY (VH1-like member Y) because its closest relative was VHX (VH1-like member X), currently known as DUSP22 [105]. DUSP15 has a very narrow tissue expression pattern. Thus, its mRNA was detected only in testes and very weakly in brain, spinal cord and thyroid. Protein was also detected in testes by Western Blot and histochemistry showed the presence of this phosphatase in spermatocytes [105]. The N-terminus of DUSP15 presents high similarity to the consensus myristoylation sequence of SFK (Src family kinases) and, as well as them, DUSP15 is post-transcriptionally modified by the addition of myristic acid to Gly in position 2, as well as DUSP22 [105]. This myristoylation is essential for DUSP15 recruitment to the plasma membrane. DUSP15 is an active phosphatase as it can dephosphorylate pNPP, but there is no information about its physiological substrates. The crystal structure of DUSP15 has been reported and shows several particular surface properties, which indicate that DUSP15 may have a unique substrate specificity [106].

DUSP22

DUSP22 presents a broad expression and was characterized under four names by different groups: LMW-DSP2, VHX, JSP-1 (JNK Stimulatory Phosphatase-1) and JKAP (JNK pathway-associated phosphatase). Results obtained in these initial reports were also diverse in relation with MAPK activation. Two reports showed that DUSP22 activated JNK [107, 108], other showed inhibition of ERK2 and NF-AT by this phosphatase [109]; and the fourth showed that DUSP22 bound to and inhibited p38 [110]. Chen et al. showed that targeted gene disruption of DUSP22 in embryonic stem cells abolished JNK activation by TNF- α and TGF- β , but not by ultraviolet-C irradiation [107]. DUSP22, in this report, was also shown to interact *in vivo* with JNK and MKK7, but not with SEK1. However, *in vitro*, JNK did not bind to DUSP22, suggesting that targeting of JNK is mediated by binding to other protein. Other group showed that DUSP22 acts as a positive regulator of JNK pathway, and its overexpression led to the activation of MKK4 [108], another upstream MAPKK. Altogether, the data presented by these two papers suggests that DUSP22 would exert its effect on JNK in an indirect manner, acting on a protein upstream of this kinase. The fact that DUSP22 is myristylated in the N-terminus to target it to the plasma membrane [105] should be taken into consideration when interpreting the previous data, which has been obtained mainly with overexpressed N-terminal tagged phosphatase.

In addition to its role in MAPK signalling pathways, DUSP22 has also been implicated in the regulation of the ER α (estrogen receptor-alpha) and STAT3. DUSP22 regulates ER α -mediated transcriptional activation by dephosphorylation of this receptor on Ser-118 [111]. The function of DUSP22 on STAT3 dephosphorylation has been investigated in the context of IL-6 stimulation [112]. Interaction between these two proteins has been observed *in vivo*. Despite what this data might suggest, disruption of DUSP22 in mice to generate knockout animals does not produce any observable phenotype in these animals.

The structure of this phosphatase has been solved for the peptide that contains the phosphatase domain, aminoacids 1-163 [113]. The topology resembles that of other DUSPs, but with a shallower catalytic pocket, 4.5 Å of depth, smaller than that of VHR, 6 Å, and half the pocket of PTP1b, 10 Å.

DUPD1 and DUSP27: A little of confusion

DUPD1 (DUSP and pro-isomerase domain-containing 1) was initially called DUSP27 [2], but in the HUGO (Human Genome Organisation) database appears as DUPD1 with DUSP27 as alias. The name DUSP27 is

used to refer to another open reading frame that codes a phosphatase with 1158 amino acids that corresponds to Swiss-Prot accession number Q5VZP5, which is an inactive phosphatase that has been detected as mRNA but no report has been published yet about this PTP. First attempts to characterize DUPD1 (Swiss-Prot accession number Q68J44) predicted an open reading frame that contained a dual specific phosphatase and a pro-isomerase domains [2]. Subsequent studies showed that the pro-isomerase domain was not included in the sequence [11]. DUPD1 is able to dephosphorylate synthetic phospho-Ser and phospho-Thr peptides but it acts more efficiently on phospho-Tyr peptides. The catalytic groove of DUSP27 can accommodate a dual-phosphorylated substrate where the two phosphorylated residues are separated by two amino acids instead of one as in the case of MAPKs. This finding indicates that DUPD1 substrates are not MAPKs. Although further studies are needed to identify DUPD1 substrates, its specific tissue distribution, restricted to adult fat, skeletal muscle and liver [11], suggest that DUSP27 may participate in the regulation of energy metabolism.

Conclusions and futures perspectives

A-DUSPs are a heterogeneous group of enzymes according to the range of substrates targeted. The controversial results obtained regarding substrates and the rich synonymia used to name these proteins have produced some confusion over this group of proteins. Nomenclature has been fixed, adopting the name DUSP followed by a number, and new substrates identified lately for these phosphatases have widen the repertoire of this family outside the MAPK world. The search for substrates has been done under the influence of sequence similarity between these proteins and MKPs. Therefore, most of the studies tried to show the effect of these phosphatases on MAPK. As we have shown along this review, results were negative in many cases and controversial in most of the rest. Nevertheless, some A-DUSPs have been shown to be true MKPs, for example VHR. Even in these cases, the absence of a targeting domain similar to the rhodanese in MKPs raises the question about the mechanism by which these phosphatases target MAPKs. In this sense, in at least two cases, VHR and DUSP26, two proteins have been found that may work as adaptors that facilitate phosphatase access to their substrates, VRK3 [28] and Hsf4 [34], respectively. At the same time, it has become evident that other substrates could exist for these phosphatases, as, for example, STAT transcription factors.

Although the information about these phosphatases is still scarce and the physiological role for most of them is unknown, some studies have suggested the implication of these phosphatases in some cellular functions, such as

apoptosis, DUSP12, or cell-cycle regulation, VHR; and in some pathologies, such as cancer, VHR and DUSP26, or diabetes, DUSP12. In this sense, the lack of knockout mice for A-DUSPs has been an obstacle in the determination of the physiological role of these phosphatases. So far, out of 19 A-DUSPs, only 3 have been disrupted in mice, STYX [78], DUSP22 [107] and laforin [114]. STYX deficient mice showed that this phosphatase is involved in spermatogenesis.

It seems likely that the next few years will see a critical advance in our understanding of A-DSPs. This will be thanks to approaches that manipulate gene expression, such as siRNA interference or generation of knockout mice, as well as proteomics and mass spectrometry technologies that will provide new data to dissect the substrate repertoire of A-DUSPs and the physiological function of these phosphatases.

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