Phosphatases are a diverse family of enzymes, comprising at least 10 distinct protein folds. Like most other enzyme families, many have sequence variations that predict an impairment or loss of catalytic activity classifying them as pseudophosphatases. Research on pseudoenzymes is an emerging area of interest, with new biological functions repurposed from catalytically active relatives. Here, we provide an overview of the pseudophosphatases identified to date in all major phosphatase families. We will highlight the degeneration of the various catalytic sequence motifs and discuss the challenges associated with the experimental determination of catalytic inactivity. We will also summarize the role of pseudophosphatases in various diseases and discuss the major challenges and future directions in this field.

Introduction

One of the most notable analogies used in cellular signaling is the 'writer–reader–eraser' toolkit to describe the network of kinases, adaptors, and phosphatases [1]. The designation of phosphatases as 'erasers' has led to the widespread misconception that this protein family is only responsible for turning off signaling pathways. This is far from being true, and phosphatases are now recognized as being active regulators of signaling networks in their own right, playing a critical role in physiological and pathological conditions, making them promising targets for therapeutic intervention. Over 20 enzyme families are known to

Abbreviations

DUSP, dual-specificity phosphatase; PTP, protein tyrosine phosphatase; RPTP, receptor-linked protein tyrosine phosphatase; SCF complex, Skp, Cullin, F-box containing complex; STYX, serine/threonine/tyrosine-interacting protein.
contain pseudoenzyme members [2,3] and approximately 14% of all phosphatases are pseudophosphatases (26 out of 189). A pseudophosphatase is a protein that belongs to one of the phosphatase families and contains a mutation that predicts an impairment or loss of its catalytic activity. Thus, the designation of a protein (or protein subdomain) as pseudophosphatase is done purely bioinformatically. Whether this protein is enzymatically active or not is irrelevant to its designation as a pseudoenzyme. As will be shown below, there are enzymatically active and inactive pseudophosphatases. A general introduction into pseudoenzymes has been provided recently explaining the general definition and providing a global overview of pseudoenzymes across all major enzyme families [3].

Using the eraser analogy, then pseudophosphatases would be pseudoerasers, raising the question of what biological roles they might have. We have previously defined general modes of action of any pseudoenzyme [4] as follows (Fig. 1): (a) substrate binding competitors that modulate the biologic effects of active enzymes, (b) allosteric modulators of active enzymes, (c) spatial anchors or substrate traps, and (d) signal integrators. Moreover, moonlighting enzymes, which have acquired additional functions in evolution, can teach us lessons about the biology of pseudoenzymes. For instance, the bacterial pseudokinase SidJ and the ubiquitous pseudokinase SelO were shown to catalyze protein polyglutamylation and AMPylation, respectively [5,6]. In this review, we will provide an overview of pseudoenzymes in major phosphatase families and discuss their biologic functions referring to the four basic modes of action illustrated above.

Classification and origin of pseudophosphatases

Understanding the origin of pseudophosphatases is more complicated than of pseudokinases [7]. The catalytic domains of protein kinases consist mostly of a single protein structural fold implying that they originated from an ancestral kinase [8]. On the contrary, protein phosphatases exhibit different folds and catalytic mechanisms [9,10], often exhibiting other hydrolase activities such as phosphonatases, dehalogenases, and sugar phosphomutases. Two systematic attempts have been made to classify phosphatases based on sequence and structure. The classification by the Köhn group was based on 254 human phosphatase domains and grouped the phosphatome into 7 CATH classes [11–13]. A more recent analysis by the Manning group used 1425 phosphatases from nine organisms to classify phosphatases into 10 folds, 21 families and 178 subfamilies, of which 101 subfamilies are present in humans [9]. This led to identification of 189 known or predicted human protein phosphatases. The 10 major phosphatase folds are as follows: cysteine-based class 1-3 (CC1-3), PPP-like, histidine phosphatases, alkaline phosphatases, metal-dependent protein phosphatases (PPM), haloacid dehalogenase-like (HAD), protein histidine phosphatase (PHP), and regulator of transcription 1 (RTR1). The majority of human protein phosphatases belong to the CC1 fold, which has the largest share of pseudophosphatase domains in humans (Fig. 2). Pseudophosphatases have been so far identified in all families except in the PPP-like family. A possible reason for this is that this family of phosphatases is characterized by binding to a wide range of regulatory units that regulate targeting and biologic activity of these phosphatases, which has limited the selective advantage of pseudophosphatases that might adopt such functions. As outlined above, the definition of a pseudophosphatase is currently based on sequence and does not require the demonstration of catalytic impairment. As the number of studies increases, contradictory reports of pseudophosphatase catalytic activity are appearing, adding to the challenge of understanding these proteins. Several pseudophosphatases have been reported to be catalytically active, such as PTPN23 (HD-PTP) [14] and PHLPP1/2 [9,15], but as stated above, catalytic inactivity is not a criterion to define pseudoenzymes. We will provide a systematic summary of the current literature for each pseudophosphatase (Table 1).

Pseudoenzymes within the CC1-fold phosphatase family

The CC1-fold class is the largest group of phosphatases comprising 106 members, including 20 pseudophosphatases [9]. Furthermore, additional fourteen pseudophosphatase domains are present in proteins with another phosphatase domain (twelve receptor protein tyrosine phosphatases and two CDC14s). The smaller CC2 and CC3 classes have only 2 and 3 members, respectively, and no pseudophosphatases identified. CC1-fold phosphatases contain several core catalytic motifs, such as the HCX₃R motif, that are mutated in several pseudophosphatases of this class. The HCX₃R motif is found in the phosphate-binding loop (P loop), where the cysteine is essential for the formation of a cysteinyl-phosphate intermediate with the phosphorylated substrate [16,17]. The conserved arginine participates in the generation of an environment with a low pKa around the catalytic cysteine. This allows the cysteine to be present as a thiolate...
anion at physiological pH. The unprotonated cysteine acts as a nucleophile and forms a thiol phosphate intermediate. Based on this, any phosphatase that lacks the cysteine or the arginine, or both, ought to lose its catalytic activity. In many but not all protein tyrosine phosphatases (PTPs) an aspartate present in the conserved WPD loop assists in this step, serving as a general acid by donating a proton to the tyrosyl-leaving group of the substrate [18,19]. Subsequently, the aspartate acts as a general base to facilitate the hydrolysis of the cysteinyl-phosphate intermediate. As will be discussed below, these core motifs are degenerated in many members of the CC1 class. The CC1 group contains the PTPs, dual-specificity phosphatases (DUSPs), myotubularins, and PTENs (phosphatase and tensin homolog). Aspartate-containing PTPs of the EyA (eyes absent) family belong to the HAD fold and will be discussed later. A graphic representation of all pseudophosphatases within the CC1 family is shown in Fig. 2.

**Pseudophosphatases within the DUSP family**

**STYX (serine/threonine/tyrosine-interacting protein)**

The archetypal pseudophosphatase, STYX, was originally discovered in a search for new protein tyrosine phosphatases by screening expressed sequence tags from mouse testis [20]. STYX is an inactive atypical DUSP (Fig. 2). A mutation of the catalytically essential cysteine to a glycine (HGX3R) renders it inactive. Back mutation of the glycine to a cysteine restores its catalytic activity shown by the ability to hydrolyze the generic phosphatase substrate para-nitrophenylphosphate (pNPP) and recombinant phospho-ERK [20,21]. The ability of the back-mutated STYX to dephos-
phorylate ERK suggested a role for this pseudophosphatase in MAPK signaling. There are several possibilities for how a pseudophosphatase could modulate kinase activity. The most obvious would be competition with an active phosphatase. Due to its similarity to DUSPs, one would expect competition with one of the active DUSPs that dephosphorylate members of the MAPK family. This was experimentally verified by showing that STYX competes with DUSP4 for binding to ERK [21]. Therefore, depletion of STYX should increase the access of active phosphatase, resulting in more dephosphorylated ERK. Surprisingly, the opposite was observed as knockdown of STYX increased ERK phosphorylation [21]. The key to understanding this counterintuitive result came through a combination of computational modeling and experimental data, and through the observation that STYX largely localizes to the nucleus. STYX acts as a nuclear anchor for ERK1/2 and competes with DUSP4 for ERK, thereby changing the nucleocytoplasmic cycling kinetics of ERK1/2. Depletion of STYX results in more rapid cycling of ERK1/2 between the nucleus and the cytosol, allowing faster phosphorylation and reactivation by the cytosolic upstream kinases MEK1/2. Typically, active phosphatases bind their substrates transiently, as the interaction is lost due to

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**Fig 2.** Phylogenetic tree of human phosphatases with CC1 fold. Pseudophosphatases are colored in red. The tree was created using the phosphatome visualization tool CORALP, http://phanstiel-lab.med.unc.edu/coralp.
dephosphorylation. STYX has retained the binding capability of its catalytically active relatives, but the interaction is prolonged due to the absence of hydrolysis meaning STYX is a putative substrate trap.

The catalytic inactivity of many pseudophosphatases often provokes the question of whether they have evolved to regulate biological processes beyond phosphorylation-based signaling. An example for a function for STYX outside the paradigm of kinase–phosphatase signaling came from the suggestion that it regulates spermatogenesis by binding to the mRNA binding phosphoprotein Carhsp24 [22]. Whether this interaction indeed regulates spermatogenesis and whether it does not involve kinase signaling remain to be demonstrated.

In order to identify additional functions for STYX, its interactome was mapped, which led to the identification of numerous F-Box proteins [23,24]. F-Box proteins are parts of the SCF-family of Cullin1 RING ubiquitin ligases [25]. They bind to their substrates (often upon phosphorylation of a so-called phosphodegron) and bring them into the SCF complex using the F-Box domain to interact with SKP1, which then binds to Cullin1. Among the F-box proteins that interacted with STYX was FBXW7, an important tumor suppressor that is altered in over 2.5% of all malignancies [26], and higher among specific cancers such as colorectal cancer (over 13%). Strikingly, STYX interacts with FBXW7 via the F-Box domain, thereby competing with binding to SKP1. This prevents incorporation of FBXW7 into the SCF complex and therefore inhibits degradation of FBXW7 substrates. Several of these substrates are proteins that control cell growth and survival such as c-myc, cyclin E1, and MCL1 [24]. Accordingly, STYX overexpression protected cells from chemotherapy-induced apoptosis by counteracting FBXW7 function [24]. Furthermore, we found that breast cancer patient survival inversely correlated with low FBXW7 and high

Table 1. List of pseudophosphatases, the families they belong, and their biologic functions

| Fold and Subfamily | Name | Function and mode of action |
|--------------------|------|----------------------------|
| CC1/ DUSP          | STYX | Nuclear anchor for ERK1/2 and modulator of F-box proteins |
| CC1/ DUSP          | MK-STYX | Stress granule formation. Interaction with and modulation of the activity of the mitochondrial phosphatase PTPMT1 |
| CC1/ DUSP          | DUSP27 | Unknown |
| CC1/PTP            | PTPRU D1 | Competes for substrates with its active paralogs: PTPRK, PTPRM, and PTPRT |
| CC1/PTP            | D2 domains of PTPRC, PTPRD, PTPRF, PTPRS, PTPRK, PTPRM, PTPRT, PTPRU, PTPRA, PTPRE, PTPRG, and PTPRZ | Redox sensing, substrate recognition, and modulation of the activity of the catalytically intact D1 domains. |
| CC1/PTP            | PTPRN | Regulation of exocytosis of secretory granules. Modulates activity of PTPRA. Regulation of glucose tolerance |
| CC1/PTP            | PTPRN2 | Regulation of glucose tolerance. Regulation of fertility in female mice |
| CC1/PTP            | PTPN23 | Regulation of FYN, Src, and FAK possibly by dephosphorylation. Regulation of ESCRT-mediated endosomal sorting |
| CC1/PTP            | PTPN14 | Regulation of YAP, β-catenin, p130Cas, and Roquin2 possibly by dephosphorylation |
| CC1/PTP            | PTPN21 | Regulation of KIF1C |
| CC1/PTEN           | GAK | Uncoating of clathrin by binding to phospholipids |
| CC1/PTEN           | Tensin-1 | Localizes to focal adhesions and regulates cell migration |
| CC1/PTEN           | Tensin-2 | Dephosphorylation of IRS1 and regulation of insulin receptor signaling |
| CC1/MTM            | MTMR5, MTMR9, MTMR10, MTMR11, MTMR12, and MTMR13 | Bind to active MTMRs and modulate their activity. |
| PPM                | TAB1 | Allosteric activator of TAK1 and regulates MAPK signaling |
| PPM                | PP2D1 | Unknown |

Table 1. (Continued).

| Fold and Subfamily | Name | Function and mode of action |
|--------------------|------|----------------------------|
| PPM                | PHLPP1&2 | Regulation of Akt signaling |
| HP                 | PIP5K1&2 | Regulation of inositol pyrophosphate metabolism |
| HAD                | TIM50 | Translocation through the inner mitochondrial membrane |
The relevance of the STYX-FBXW7 crosstalk in cancer has been recently validated by an independent group for colon cancer [27]. Besides the biomedical implications of this finding, it demonstrated for the first time that the assembly of an SCF complex is regulated by a direct protein–protein interaction. Several other F-box proteins were found to interact with STYX [23,24] suggesting a more general function for STYX in the regulation of SCF complexes, which has to be explored in future studies. When analyzing the evolution of STYX, it appears that it has always been a pseudophosphatase, even in evolutionarily distant species [28]. The notable exception is fungi, where the STYX homologs appear to retain the catalytic cysteine in the CX₅R motif. Why this is the case is currently unclear, and we can only speculate that this is related to the lack of canonical tyrosine kinases in fungi [29,30]. It is possible that the STYX ancestor has acquired an inactivating mutation that allowed it to adopt a new cellular role. In support of this hypothesis is the observation that around 50% of all human phosphatases interact with components of the SCF complex [23]. Altogether, the pseudophosphatase STYX appears to have adopted more than one mode of action of pseudoenzymes, namely mode 1 (substrate competition) and mode 3 (spatial anchor). STYX might also potentially fit to mode 4 (signal integrator) as it has the potential to link kinase/phosphatase signaling to ubiquitin.

**MK-STYX/STYXL1/DUSP24**

The inactive MK-STYX (also known as STYXL1) was originally discovered by a database search for additional STYX family members [31]; however, despite the similar name it is more related to DUSPs, or MAPK phosphatases (MKP), than it is to STYX. It has a longer catalytic domain compared to STYX and also lacks the cysteine and preceding histidine in the HCX₅R motif. Back mutation of these residues reactivated MK-STYX, which was able to dephosphorylate a phosphorylated poly(Glu-Tyr) substrate [32]. Although MK-STYX shows high similarity to MKPs, there is no evidence to date that it functions in the MAPK signaling pathways, a phenomenon that remains poorly understood. In contrast to STYX, which is largely nuclear, MK-STYX localizes to mitochondria and the cytosol. The best understood interaction partner of MK-STYX is the RNA binding protein G3BP-1 that plays a role in stress granule formation [33]. Overexpression of MK-STYX reduced the formation of G3BP-positive stress granules in arsenite-treated cells in a manner independent on the phosphorylation status of G3BP [32].

Another cellular role of MK-STYX is the sensitization toward apoptosis-inducing agents [34,35]. Cells without MK-STYX are unable to release cytochrome c from mitochondria and are thus more resistant to apoptosis induced by chemotherapeutics [34]. A subsequent proteomic screen identified the mitochondrial phosphatase PTPMT1 as an interaction partner for MK-STYX [36]. PTPMT1 seems to be mediator of the chemoresistance acquired by MK-STYX knockdown. Double knockdown of PTPM1 and MK-STYX restored cytochrome c release upon chemotherapeutic treatment and strongly reduced cell viability upon paclitaxel treatment. MK-STYX reduces PTPMT1 phosphatase activity in vitro, but the mechanism behind this is not yet clear. This might occur either by allosteric modulation or by preventing PTPMT1 from binding to its substrate [36]. Based on this, MK-STYX would adopt either mode 1 or mode 2 of the aforementioned modes of action of pseudoenzymes. High MK-STYX levels appear to be a favorable condition for tumor cells. In accordance with this, the chimeric protein EWS-FLI1 in Ewing’s sarcoma family tumors was shown to drive MK-STYX expression (37). It still needs to be determined whether this phenomenon is a driver in Ewing sarcoma.

Beside its role in tumors, MK-STYX was recently also found to be associated with cognitive disorders and epilepsy in a consanguineous Asian family bearing a homozygous missense mutation [38]. This suggests a role of MK-STYX in neural development. Interestingly overexpression of MK-STYX was sufficient to induce neurite outgrowth in rat pheochromocytoma PC12 cells [39] and in primary hippocampal neurons [40]. Whether and how this is linked to neural development remains to be determined.

**DUSP27**

DUSP27 belongs to the group of atypical DUSPs, and it lacks the cysteine of the CX₅R motif that is replaced by a serine (as in MK-STYX). There is some confusion about DUSP27 in the literature as there are two proteins called DUSP27: the pseudophosphatase DUSP27 (Q5VZP5, Uniprot) and the active phosphatase DUPD1/DUSP27 (Q68J44, Uniprot) [41]. This unfortunate situation has made it difficult to assess whether the pseudophosphatase DUSP27 lacks catalytic activity. To the best of our knowledge, this has so far not been tested thoroughly. Very little is known about this pseudoenzyme. DUSP27 was found to be upregulated in the tonsils of patients suffering from...
immunoglobulin A nephropathy (IgAN) [42], but it is not clear what functional relevance this overexpression bears. An intron variant of DUSP27 was associated with heroin addiction in a cohort of African American people, but not in a cohort of Caucasian people [43]. It is difficult to deduce the biologic function of DUSP27 based on the available literature, and it remains to be determined in future which of the four basic modes of action for pseudoenzymes DUSP27 adopts.

**Pseudophosphatases within the PTP family**

Members of the PTP family (Fig. 2) possess four critical catalytic motifs that can be degenerated in pseudophosphatases, including a more constrained version of the HCX₅R motif, which is typically HCSAGXGR. The KNRY loop is a defining feature as it forms a deep pocket giving selectivity toward phosphotyrosine. The WPD motif aspartic acid acts as a general acid/base catalyst during dephosphorylation. Finally, the Q loop coordinates the water molecule required for hydrolysis [44]. Numerous sequence variants are present in these otherwise highly conserved motifs and define the pseudophosphatases within the PTP family.

**D2 domains of receptor-linked protein tyrosine phosphatases (RPTPs)**

In humans, twelve RPTPs contain tandem phosphatase domains with one membrane proximal D1 catalytically active domain and a distal D2 pseudophosphatase domain (Fig. 3A). This arrangement was proposed to derive from a single common ancestor first seen in the unicellular choanoflagellate Monosiga [9], which is consistent with the phylogenetic clustering of D1 domains away from D2 domains (Fig. 2) [9,11]. Paralogous receptors are grouped based on features of their extracellular domains. R1 refers to PTPRC/CD45; R2A to PTPRD, PTPRF, and PTPRS; R2B to PTPRK, PTPRM, PTPRT, and PTPRU; R4 to PTPRA and PTPRE; and R5 to PTPRG and PTPRZ. The D1 domains of these receptors are almost all catalytically active, with the recently reported exception of PTPRU (see below), while the D2 domains are pseudophosphatases. Curiously, all D2 domains retain a HCX₅R motif except PTPRC, PTPRG, and PTPRZ (Figs 3 and 4). Otherwise, they are designated as pseudophosphatases because of variants in their WPD, KNRY, or Q-loop motifs that are common to all PTPs (Fig. 3B). Interestingly, the intact HCX₅R motif appears to have been maintained throughout animal evolution with a few exceptions, for example, in sponges where lineage-specific duplications can be seen (Fig. 4).

This intriguing arrangement of active and inactive domains is reminiscent of the Janus kinase family, where a pseudokinase domain plays a negative regulatory role toward an active kinase domain [45,46]. However, a common role for these RPTP pseudophosphatase domains remains elusive. To date, RPTP D2 domains have been implicated in redox sensing, substrate recognition, and regulation of D1 catalytic activity. It is entirely possible that context- or family-dependent functions exist. For example, the PTPRG and PTPRZ domains are unlikely to play a role in redox sensing as they have lost the D2 ‘catalytic’ cysteine.

Part of the challenge in understanding RPTP function has been the absence of a clear-cut ligand-mediated signaling mechanism, akin to the receptor tyrosine kinases [47]. However, ligands for the R5 RPTP family members PTPRG and PTPRZ have been described, including contactin [48,49] and pleiotrophin [50]. Structural studies on the intracellular domains of these receptors have revealed a head-to-toe dimer arrangement [51,52], and further work in vitro and in cells suggests that, at least for PTPRZ, activity is regulated by pleiotrophin ligand-dependent dimerization and occlusion of the D1 domain active site by an opposing D2 domain [52]. A similar mechanism has been suggested for the human R2A family and the *Drosophila* ortholog LAR, whereby interactions with heparan sulfate proteoglycans regulate monomer–dimer conformations [53,54]. Furthermore, it has been suggested that R2A family D2 domains can inhibit R2A D1 domains heterotypically [55]. However, unlike PTPRG and PTPRZ, the crystal structures of R2A intracellular domains did not reveal a blocked dimer conformation [56], so further work is required to understand how extracellular interactions regulate R2A phosphatase activity. Homo- and heterodimerization of PTPTA has also been proposed [57], and although inactive homodimers have been observed in cells [58], the signaling consequences remain to be determined. Finally, various reports have suggested both positive and negative effects of the presence of the D2 domain on D1 catalytic activity, most likely reflecting conformational and stability changes caused by interdomain interactions that are evident in several RPTP intracellular domain structures [56,59,60].

In addition to a regulatory role, the D2 domains of several RPTPs have been implicated in substrate recognition. The D2 domain of the hematopoietic receptor CD45 recognizes several proposed substrates including the T-cell receptor zeta chain [61], Lck [62],
and JAK2 [63]. More recently, the PTPRK and PTPRM D2 domains were shown to be required for the selective dephosphorylation of PARD3 and PKP3 [64]. Furthermore, chimeric recombinant proteins revealed that the PTPRK D2, but not PTPRM D2, is necessary and sufficient for the recognition of a further substrate Afadin, in a phosphorylation-independent manner [64]. Interestingly, a pocket formed between the D1 and D2 domains of CD45 has been identified as a predicted allosteric site to inhibit receptor activity and targeted with a small molecule [65]. Thus, identifying the features of D1 and D2 binding sites involved in substrate recognition is an important future question.

It remains to be determined why these receptors have evolved D2 pseudophosphatases for functional regulation and substrate recognition and why most have a conserved and apparently redundant catalytic cysteine. Some insight into the latter comes from studies that have demonstrated a redox sensing function of certain RPTP D2 domains, particularly PTPRA, through this cysteine. It is now widely appreciated that reactive oxygen species (ROS) function beyond oxidative stress as highly selective signaling molecules that are sensed by specific cysteine residues on certain proteins [66]. The classical PTPs are well-established cellular targets of ROS, and multiple studies have demonstrated varying levels of oxidation of their catalytic domain cysteines and D2 domain cysteines [67,68]. Previous structural studies on active PTP domains have identified several reversible oxidative modifications of catalytic cysteines including a sulfenic acid in the PTPRS D1 domain [59], a sulfenamide in PTP1B (PTPN1) [69], and a disulfide in SHP2 (PTPN11) [70]. The major impact of PTP oxidation in the cell is reversible catalytic inactivation, which in turn promotes kinase signaling [71,72]. Therefore, the redox sensing capability of the catalytically inactive D2 domains opens up new functional possibilities in redox signaling. The best studied example is PTPRA, where a structural study showed reversible oxidation of the D2 domain cysteine through formation of a sulfenamide, between the cysteiny1 sulfur and peptide backbone amide [73]. Interestingly, the PTPRA D1
and D2 domains show differential sensitivity to oxidation in vitro [74]. The formation of intermolecular disulfide bonds between receptors has been reported as another potential regulatory mechanism [75]. Limited proteolysis and fluorescence resonance energy transfer (FRET) experiments have demonstrated oxidation-dependent conformational changes in several RPTPs, including PTPRA and LAR, that were linked to the D2 catalytic cysteines [76]. Moreover, redox-dependent conformational changes in PTPRA have been suggested to promote rotational coupling, transmitting changes across the cell membrane to the extracellular domain, as measured by epitope tag masking, indicating a potential ‘inside-out’ signaling mechanism [77]. In combination, there is clearly compelling evidence for a redox sensing function of RPTP D2 domains; however, many outstanding questions remain including identification of the physiological function of redox sensing and whether the observed differential sensitivity of D1 and D2 domains in vitro reflects distinct modes of regulation in cells.

The D2 pseudophosphatase domains are critical for the function of these twelve RPTPs; however, further studies are required to understand their mechanism(s) of action in the context of receptor signaling. The data so far support two modes of pseudoenzyme action, serving as allosteric modulators (mode 2) and as mediators of substrate recognition (mode 4).

PTPRU (PTPλ/φ, PCP-2)
PTPRU, otherwise known as PCP-2, PTPλ, or PTPφ, is part of the R2B homophilic receptor family. It was first identified from a human pancreatic adenocarcinoma cDNA library and shown to localize to cell–cell contacts [78]. It has been implicated as both a tumor suppressor [79] and oncogene [80], and plays a role in development [81–84]. However, unlike its paralogs it was recently determined to be unique among the RPTP family in possessing two pseudophosphatase domains [85]. Although it has an intact HCX5R motif, it shows sequence variation in the KNRY loop,
usually involved in recognition of phosphotyrosine, and possesses a WPE rather than WPD motif (Fig. 3B). Recombinant PTPRU was found to be inactive against several model substrates including pNPP. Structural studies revealed an unusually disordered KNRY-loop region compared with all previous PTP structures, and occlusion of the active site by a kink in the PTP loop bringing a unique threonine in the PTP loop into proximity of the catalytic cysteine (HCSAGTGR). A similar PTP-loop kink has previously been observed in the oxidized crystal structure of PTP1B [69]. Indeed, PTPRU spontaneously oxidized revealing a disulfide bond between the catalytic cysteine and a ‘backdoor’ cysteine. Thus, in addition to the PTPRA D2 domain, PTPRU is another example of a redox-sensitive pseudophosphatase. A model is proposed where PTPRU competes for substrates with its active paralogs, PTPRK, PTPRM, and PTPRT [85] and, thus, adopts mode 1 of the general modes of action of pseudophosphatases.

PTPRN (IA-2) and PTPRN2 (Phogrin)

PTPRN, which is also known as IA-2 (islet cell autoantigen 2), belongs to the RPTP family, but it does not have a tandem phosphatase domain, instead having a single phosphatase domain [86] that appears to be a pseudophosphatase and clusters phylogenetically with the RPTP D2 domains (Fig. 2). In mammals and in most examined species, both the KNRY and WPD are altered. The same is true for PTPRN2 (also known as phogrin) (Fig. 3B). So far, to the best of our knowledge, no substrate (protein or nonprotein) has been identified for PTPRN. As for PTPRN2, it was proposed that it might act as a phosphoinositide phosphatase [87].

PTPRN was originally discovered by screening for genes expressed in pancreatic islets [88,89]. PTPRN is also expressed in the central nervous system and neuroendocrine cells, where it associates with neurosecretory granules [90]. It was shown to be a major autoantigen in type 1 diabetes [91,92]. The WPD loop in PTPRN is mutated to WPA, but back mutation of the alanine to aspartate had only a very minor catalytic activity. PTPRN contains an aspartate residue close to the cysteine (H908DGAGR915), in a position that would normally be alanine in the canonical classical PTP loop. The case of PTPRN shows that a positively charged amino acid appears to disrupt the function of this motif. Mutation of the aspartate in PTPRN to alanine (D911A) restores catalytic activity toward pNPP and tyrosine-phosphorylated MBP significantly [93].

The biological roles of PTPRN have been well investigated and provide another strong case for how pseudophosphatases have adopted new cellular roles beyond dephosphorylation. Targeted disruption of the PTPRN gene in mice resulted in impaired glucose-mediated insulin secretion [94], whereas overexpression of PTPRN in an insulinoma cell line resulted in increased insulin secretion [95]. After synthesis and transport to the Golgi, PTPRN is cleaved by a furin-like convertase resulting in a mature transmembrane fragment (TMF) that localizes to secretory granules [90,96]. Upon stimulation with insulin, PTPRN-TMF is delivered to the plasma membrane where it undergoes proteolytic cleavage by calpain-1 [90,97], which releases a cytosolic fragment (PTPRN-CCF) that translocates to the nucleus to regulate transcription of insulin [97]. The CCF binds to STAT5 in the nucleus and prevents its tyrosine dephosphorylation, by competing with nuclear phosphatases (mode 1 of pseudoenzymes modes of action) [98]. The cytoplasmic domain of PTPRN-TMF interacts with β1V-spectrin [99] and β2-syntrophin [100], implying that PTPRN might regulate the coupling of secretory granules to the actin cytoskeleton and therefore the exocytosis of their content [101,102]. Furthermore, knockout of PTPRN in mouse islet cells showed a downregulation of villin, another actin binding protein [103]. It remains to be shown whether PTPRN really associates with actin filaments and whether this is important for exocytosis of secretory vesicles.

PTPRN was also shown to interact with PTPRA and to reduce its activity toward pNPP by 20% [104]. However, it is not clear whether this is due to an allosteric effect of PTPRN on PTPRA or whether PTPRN binds to the substrate and protects it from dephosphorylation by the phosphatase domain of PTPRA. Thus, PTPRN adopts mode 1 of the general modes of action of pseudophosphatases as it competes with active phosphatases for substrate binding. In addition, it adopts mode 2 by acting as an allosteric modulator and it has new roles that are beyond the kinase-phosphatase signaling paradigm.

PTPRN2 (phogrin) is very closely related to PTPRN, sharing 74% sequence similarity to the intracellular domain [105]. PTPRN2 is expressed in pancreas β-cells, in the brain, and in neuroendocrine cells. PTPRN2 was discovered by several groups almost at the same time. It was identified as another autoantigen in type 1 diabetes mellitus [105,106], as a new component of dense core granules [107] in a screen for new phosphatases [108], and as a new putative signaling protein in rat olfactory cells [109].

The sequence alterations in PTPRN2 are similar to those of PTPRN with the presence of an aspartate
close to the cysteine in the HCX₃R motif. In addition, PTPRN2 has a mutation in the WPD loop (Fig. 3B). Cui et al. [108] display some catalytic activity of phosphatase toward pNPP, but it was lower than what is normally seen with active phosphatases. In addition, this activity was observed only at a pH of 4.5, which raises questions about its functional relevance. Mutation of the aspartate in the HCX₃R motif to alanine increases catalytic activity but only at pH 5.6 [109]. It is very unlikely that the cytosolic portion of PTPRN2 encounters such pH conditions in vivo, making these findings physiologically questionable.

PTprn2 knockout mice show a very similar phenotype to Ptprn knockout mice. They also exhibit impaired glucose tolerance and impaired glucose-induced insulin secretion. However, knockout of PTPRN2 alone was not sufficient to prevent the development of diabetes [110]. The sequence similarity between PTPRN and PTPRN2 implies some level of redundancy. Effects on the neuroendocrine system were thus observed only in double knockout mice. Female mice show infertility due to problems in secretion of the pituitary hormones LH and FSH [111]. It is unclear why this effect is only observed in female mice, whereas male knockout mice showed normal LH and FSH secretion and also normal fertility.

Pseudophosphatases within the nonreceptor PTPs

The human genome encodes 17 nonreceptor PTPs, three of which contain a single PTP domain that not only have an intact HCX₃R motif but also contain mutations in the KNRY and WPD loops. PTPN14, PTPN21, and HD-PTP/PTPN23 thus diverge from the amino acid consensus observed among catalytically active PTPs and are thus bona fide pseudophosphatases. The purified catalytic domain of each of these proteins was indeed shown to be inactive in vitro toward the synthetic substrate DiFMUP and against a panel of 38 different phosphopeptides [51,112]. However, a growing body of evidence suggests that PTPN14, PTPN21, and HD-PTP/PTPN23 might not be totally inactive, but would perhaps display a weak activity for some specific substrates.

HD-PTP/PTPN23

The HD-PTP/PTPN23 protein is found throughout the Holozoa clade, and although its members contain a highly conserved HCX₃R motif, they exhibit at least one substitution in the KNRY or WPD loops. The human PTPN23 also contains a catalytic domain that differs from the consensus of active PTPs. The tyrosine located within the KNRY motif is indeed replaced by a histidine, and the aspartic acid in the WPD loop is replaced with a glutamic acid. Even though PTPN23 harbors an intact HCX₃R motif, it contains a unique serine located near the catalytic cysteine (HCSSGVGR), at a position generally occupied by an alanine in active tyrosine phosphatases [9]. This single residue was found to strongly inhibit the catalytic activity of PTPN23. The substitution of this serine into an alanine was sufficient to confer to PTPN23 a weak catalytic activity toward the synthetic substrate DiFMUP [112].

Some studies have shown that PTPN23 is involved in epithelial cell migration via its interaction with FAK and Src kinases [113,114]. Whether or not PTPN23 is a direct phosphatase for these proteins has been debated [113,115]. It is clear however that the depletion of PTPN23 exerts an effect on cell motility and favors epithelial–mesenchymal transition (EMT) by affecting the phosphorylation status of Src, E-cadherin, and β-catenin [115]. Similarly, the overexpression of PTPN23 was found to decrease the phosphorylation of these proteins and receptors and signaling effectors such as EGFR, c-MET, and ERK1/2 [116]. The phosphatase activity of PTPN23 was demonstrated in vitro toward the kinase FYN and was abolished by the mutation of the catalytic cysteine. On the other hand, residues from the catalytic domain of PTPN23 can be mutated to match the consensus sequence of active phosphatases, thereby significantly increasing the dephosphorylation of Fyn. These data indicate that PTPN23 might exert a weak phosphatase activity toward Fyn. Substrate-trapping experiments further showed that this activity could be specific to Fyn, as closely related kinases such as Src and Yes were unable to bind PTPN23 in the same conditions [14]. The mechanistic details of how PTPN23 evolved to be Fyn-specific remain to be elucidated.

In addition to its role in cell signaling, PTPN23 binds several ESCRT (endosomal sorting complexes required for transport) proteins and participates in the endosomal sorting of receptors and the formation of multivesicular bodies (MVB) prior to their lysosomal delivery [117]. The depletion of PTPN23 in murine fibroblasts was shown to affect the sorting and degradation of PDGFRβ. Ligand-induced PDGFRβ accumulated into large aberrant intracellular compartments upon silencing of PTPN23 [118]. Moreover, the depletion of PTPN23 inhibited the degradation of ligand-stimulated α5β1 integrin receptors in HeLa cells. Silencing PTPN23 therefore increased recycling and the number of receptors present at the cell surface. As a result, the depletion of PTPN23 was shown to impact integrin signaling and to promote a pro-
migratory and pro-invasive phenotype [119]. Similarly, PTPN23 was shown to be crucial for the efficient endosomal sorting and degradation of EGFR. Interestingly, the catalytic activity of PTPN23 is dispensable for this function, as the expression of a truncated PTPN23 lacking its phosphatase domain was sufficient to restore the trafficking of activated EGFRs [120,121].

In agreement with an important role in receptor trafficking and signaling, PTPN23 was further shown to be an essential gene. The homozygous knockout of the Ptpn23 gene in mice led to embryonic lethality [122]. Consistent with its crucial function, no homozygous deletions were observed in humans. However, some patients were shown to have alterations in the PTPN23 gene, resulting in a similar phenotype characterized by neurodevelopmental delays and brain abnormalities [123]. PTPN23 also acts as a haploinsufficient tumor suppressor. The heterozygous loss of Ptpn23 predisposes mice to sporadic lung adenoma and B-cell lymphoma, and promotes Myc-driven lymphomagenesis. Interestingly, the heterozygous deletion of PTPN23 is observed in several types of human cancers and often correlates with poor survival [124]. Further support for a tumor suppressor function of PTPN23 has been obtained in various tumors such as breast cancer, colorectal carcinoma, and hepatocellular carcinoma [14,116]. It is difficult to link PTPN23 to a mode of action for pseudoenzymes. Its combined effect on Src-family kinases and its role within the ESCRT complex would fit to mode 4 (signal integrator). However, to definitively draw this conclusion, we would need to determine whether different pools of PTPN23 contribute to the various effects, or whether it is the same pool.

PTPN14

Human nonreceptor PTPN14 possesses a phosphatase domain with an intact PTP HCX5R motif and WPD loop. However, it harbors a RSRI motif instead of the classical KNRY sequence [9]. This divergence suggested that PTPN14 is catalytically inactive, which was supported by the absence of in vitro activity toward a synthetic substrate and against a panel of phosphopeptides [51]. However, a few substrates have since been identified including YAP, β-catenin, p130Cas, and Roquin2 [125–128].

PTPN14 is best known for its role in the hippo signaling pathway via the negative regulation of YAP [127]. Although it was proposed that PTPN14 acts as a phosphatase of YAP, we consider the evidence for its phosphatase activity as very weak. In fact, others have reported that the phosphatase domain of PTPN14 is not required for the negative effect on YAP activity [129]. Thus, while there is agreement that PTPN14 is a negative regulator of YAP, the mechanism of action remains unclear. PTPN14 was further shown to be part of a tumor-suppressive p53-PTPN14-YAP axis involved in pancreatic cancer. PTPN14 is thus required for p53 tumor suppressor activity by negatively controlling the activity of YAP and its impact on cell survival, proliferation, and migration [130]. PTPN14 was also found to control the phosphorylation of YAP in gastric cancer [131–133]. In addition, PTPN14 was found to regulate the phosphorylation of p130Cas in colorectal cancer [126]. The depletion of PTPN14 also induced the growth and metastasis of breast cancer xenografts. Interestingly, breast cancer cells expressing a catalytically inactive PTPN14 secreted more growth factors and cytokines and also displayed elevated levels of cell-surface EGFRs [134]. In light of the uncertainty around the mechanistic mode of action of PTPN14, it is difficult to assign this pseudophosphatase to any of the general modes of action for pseudoenzymes.

PTPN21

The phosphatase domain of the human PTPN21 contains a canonical PTP HCX5R motif and overall resembles PTPN14. PTPN21 has a RNRF motif instead of the conserved KNRY sequence for active PTPs. The aspartic acid residue from the WPD loop is also replaced with a glutamic acid for PTPN21 [9]. Thus, PTPN21 is a pseudophosphatase and these sequence alterations were considered to abolish its catalytic activity. However, HD-PTP displays the same WPE loop and might retain a weak phosphatase activity [14,51,131].

PTPN21 is localized at adhesion sites, along with actin filaments. Together with Src and FAK, PTPN21 was shown to promote cell adhesion and motility. An intact catalytic domain was required for PTPN21-mediated stabilization of actin filaments and its impact on cell migration [135]. Interestingly, PTPN21 is highly expressed in tumor samples from patients diagnosed with bladder carcinoma. Although PTPN21 did not interact directly with EGFR, it was shown to colocalize with the receptors along the endocytic pathway and to affect their stability. The expression of a catalytically inactive mutant in PTPN21-depleted cells was also shown to inhibit the growth and migration of bladder cancer cells [136]. On the other hand, PTPN21 was shown to regulate podosome formation and neuronal cargo transport via activation of KIF1C,
independent of its catalytic activity [137]. Recently, a Ptpn21 knockout mouse model demonstrated its role in hematopoietic stem cell (HSC) homeostasis. The loss of PTPN21 was found to decrease the stiffness and increase the motility of HSCs. This mechanism specifically involves the catalytic activity of PTPN21 and the dephosphorylation of the cytoskeleton-associated protein Septin1, although PTPN21-mediated dephosphorylation has not been demonstrated directly [138].

**Pseudophosphatases in the PTEN family**

In humans, two auxilin family members exist, GAK and DNAJC6, both of which are considered pseudophosphatases. GAK (cyclin-G-associated kinase) is a kinase that is associated with cyclin G and CDK5 and that has (adjacent to its kinase domain) a tensin-type phosphatase domain. The phosphatase domain of GAK is likely to be catalytically inactive due to the lack of the arginine in the HCX5R motif that is replaced by an alanine (HCMDGRAA). In the case of tensins, tensin-1 lacks the cysteine (HNNKGNRGR), while tensin-2 lacks the arginine (YCKGNKKGK). Whether these phosphatase domains are ‘dead’ remains to be rigorously tested, as, for instance, the depletion of GAK was shown to increase phosphorylation of Rab10 [139]. Generally, the pseudophosphatase domain is thought to retain the ability to bind to phospholipids, without hydrolyzing them [140]. A purified version of the pseudophosphatase domain of GAK was found to bind to phosphatidylinositol monophosphates (PI(3)P, PI(4)P, and PI(5)P) and to lesser extent to PI(4,5)P2 [141]. This was suggested to mediate recruitment of GAK to clathrin-coated pits where it is required for uncoating [141]. Thus, GAK fits to the fourth general mode of action (signal integration) of pseudoenzymes as it uses its pseudophosphatase domain to bring other parts of the protein into action. The pseudophosphatase domain of tensin-1 was reported to interact with PP1α (PP1CA) and to therefore mediate localization to focal adhesions and regulate cell migration [142,143]. Thus, tensin-1 adopts mode 3 of action of pseudoenzymes by acting as a spatial anchor. The absence of the cysteine in the HCX5R motif makes it very unlikely that tensin-1 is active, but clear demonstration of its inactivity is missing to the best of our knowledge. Tensin-2 retains the cysteine residue, but lost the histidine and arginine residues. There is evidence that tensin-2 might be catalytically active, as it was shown to bind to PI(3,4,5)P3, which was shown to increase its ability to dephosphorylate IRS1 and to regulate insulin receptor signaling [144,145]. Thus, tensin-2 is potentially a catalytically active pseudophosphatase.

**Pseudophosphatases within the Myotubularin family**

Myotubularins comprise a family of phosphoinositide phosphatases that were shown to dephosphorylate the D-3 position on phosphatidylinositol 3-phosphate (PI3P) and PI(3,5)P2 and therefore produce PI and PI5P [146]. Because phosphoinositides provide landmarks for binding of the membrane trafficking machinery, myotubularins play an important role in the regulation of various endomembrane processes, but are also involved in a lot of other processes such as cell proliferation, differentiation, survival, or cytoskeleton [146,147]. The myotubularin family comprises 14 members in humans, six of which are considered pseudophosphatases, which are MTMR5, MTMR9, MTMR10, MTMR11, MTMR12, and MTMR13. As shown in Fig. 3A, the HCX5R motif in these proteins is completely degenerated, lacking both the cysteine and arginine residues. Myotubularins are different in that they do not have a WPD loop, but instead, the catalytic aspartate residue is located within the HCX5R loop. It is notable that this aspartic acid residue is also remarkably conserved in the pseudophosphatase members of the myotubularin family (Fig. 5A). Nevertheless, in the absence of a cysteine that can form a nucleophilic attack on the D-3 phosphate, the myotubularin family pseudophosphatases cannot act as lipid phosphatases.

A common feature of all myotubularin family members is the formation of hetero- and homodimers. Although MTMR11 (a pseudophosphatase) has not been shown directly to form dimers, a recent systematic approach mapping the interactome of all phosphatases found that MTMR11 interacts with MTM1 and MTMR2 [23]. In Fig. 5B, we have extracted from databases (BioGRID and STRING) and from the literature [147] the interactomes of the myotubularin family and show that the pseudophosphatase members act as heterodimeric partners for the active members of the family. Interestingly, a phylogenetic analysis (Fig. 5C) shows that active and inactive Myotubularin-like proteins coexisted before the origin of Metazoans (note Capsaspora and Salpingoeoca, the unicellular eukaryotes, related to common ancestors of metazoans and fungi). Also, plants and fungi appear to have only active Myotubularin-like proteins. In general, the inactive myotubularins allosterically increase allosterically the activity of active members [147–149]. Thus, these pseudophosphatases fit to the second
MTMR9 was shown to localize to the intermediate compartment and to the Golgi and to recruit MTMR7 and MTMR8 to these sites to regulate trafficking from the endoplasmic reticulum to the Golgi and the structural integrity of the Golgi apparatus [150]. Thus, these pseudophosphatases might also fit to the third general mode of action (spatial anchoring) proposed for pseudoenzymes.

**Pseudophosphatases within the PPM fold family**

PPM phosphatases are Ser/Thr phosphatases that require Mg$^{2+}$ or Mn$^{2+}$ for their catalytic activity. Their catalytic core is composed of the following sequence elements: DGH---DG---DN/D, which all play a role in metal binding and catalysis of dephosphorylation [9,151]. These elements are not fully conserved in all PPM family members, making it difficult to define which members of this family are pseudophosphatases. According to the general definition of pseudoenzymes [2–4], any enzyme family member with alteration of conserved sequence motifs is a pseudoenzyme regardless of its catalytic activity. Based on this, we consider PHLPP1 and PHLPP2 as pseudophosphatases because their DGH motif is mutated to a DGD and the DG motif is completely degenerated. However, PHLPP1&2 have been shown to dephosphorylate Akt, which has led to some confusion about their status as pseudoenzymes, because of the misconception that pseudoenzymes ought to be catalytically inactive. Catalytically active pseudoenzymes are not without precedent such as the abovementioned tensin-2 or the pseudokinase WNK1, for which we understood the basis for its catalytic activity only after having solved its structure [152]. A crystal structure for PHLPP1&2 is not available, but homology modeling of PHLPP2 based on PP2Cα suggests that the intactness of the two aspartic acid residues in the DGD and the DS motif is...
sufficient to ensure coordination of the metal ion [153]. Thus, PHLPP1&2 are catalytically active pseudophosphatases. PHLPP1&2 have been reported to be deleted in cancer, which led to their designation as a potential tumor suppressors, which is mainly attributed to their ability to regulate Akt signaling and therefore cell survival [153,154]. Whether PHLPP1&2 adopt any of the general modes of action of pseudophosphatases has not been directly tested and remains to be determined.

TAB1 is accepted to be a pseudophosphatase, and it has been experimentally demonstrated to be catalytically inactive [155]. This is mainly attributed to the fact that its DGH motif is totally degenerated (Fig. 6). TAB1 is one of the regulatory subunits of TAK1, a protein kinase that is upstream of several pro-inflammatory kinase cascades, namely the p38 MAPK, the JNK1/2, and the NFkB pathway. TAB1 activates TAK1 kinase activity and, by binding to other cellular proteins, serves not only as a regulator of TAK1, but also as a signal integrator (mode 2 and mode 4).

The other predicted pseudophosphatase is PP2D1 (Fig. 6), but we do not have experimental evidence whether it truly lacks catalytic activity. Very little is known about this pseudophosphatase, and its only known interaction partner based on the BioGRID (https://thebiogrid.org) database is amyloid protein Aβ.

**Pseudophosphatases within the histidine phosphatase (HP) family**

HP family phosphatases are a group of enzymes that are characterized by a key histidine residue that becomes transiently phosphorylated during dephosphorylation. Among proteins with HP domains, only three have been identified to be pseudophosphatases, namely diphosphoinositol pentakisphosphate kinase (PPIP5K)1&2 and phosphofructokinase 3 (PFKFB3).

The three catalytic signature motifs of the HP family are as follows: RHGXRXP, R581, and HD. Human PPIP5K1 and PPIP5K2 have an altered HD motif (replaced by HV) and are thus pseudophosphatases and predicted to be inactive. In fact, purified PPIP5K1 (from bacterial cells) was shown to lack catalytic activity against several phosphoinositides (Ins(1,3,4,5,6)P5, InsP6, PP-InsP5, nor (PP)2-InsP4) and is thus a bona fide catalytically dead pseudophosphatase [156]. Similarly, PPIP5K2 was also experimentally demonstrated to be inactive [157]. One report claimed that human PPIP5K1 tagged with BioEase and purified using biotin pulldown displays some catalytic activity [158]. However, it is possible that this is due to a contaminant, although mutating the arginine of the RHGXRXP motif abolished phosphatase activity. Thus, whether PPIP5K1&2 are inactive or active pseudophosphatases remains to be conclusively demonstrated. The biological significance of the catalytically dead phosphatase domain remains to be determined, but is likely to be involved in inositol pyrophosphate metabolism [159] or to mediate binding to InsP6 [157]. Thus, these pseudophosphatases fit to mode 1 (substrate competition) of the general modes of action of pseudoenzymes.

PFKFB3 exhibits a loss of the R motif (mutated to S), which is expected to alter its fructose-2,6-bisphosphatase activity. However, there is experimental evidence that PFKFB3 has at least some enzymatic activity [157] and might thus still act in glucose/fructose metabolism.

**Pseudophosphatases within the HAD family**

The DXDXT motif is important for the catalytic activity of HAD family phosphatases. So far, the only
Conclusions and outlook

A decade ago, pseudoenzymes were a largely ignored area of research, and since then, the number of papers focusing on pseudokinases, pseudophosphatases, or pseudoenzymes increased by almost fivefold. Assuming that this trend will continue, we may expect transformative changes in this field that will be fueled by an increasing use of bioinformatic tools to compare pseudoenzymes across diverse species. The increased interest in this research area is reflected by two international meetings on pseudoenzymes organized in the past three years. This gave researchers an arena to discuss the biology of pseudoenzymes and their roles in diseases. As far as pseudophosphatases are concerned, we believe that some of the most transformative changes in the field of pseudophosphatases will be made here. Contrary to many other enzyme families, phosphatases do not possess a single fold and the catalytic motifs are highly divergent. This makes the analysis of the evolution and degeneration of catalytic motifs more complex and increases the chance that pseudophosphatases have evolved other functions. The elucidation of these new roles of pseudophosphatases will require the combination of multi-omics approaches and computational tools.

As discussed above, while some pseudophosphatases show no detectable catalytic activity, others display some residual activity or are fully active. This challenge of defining catalytic inactivity is reflected by the designation of pseudoenzymes with terms such as ‘zombie enzymes’. What is clearly needed in the field of pseudophosphatases is a gold standard assay, but there is at the moment no consensus on this. Activity against artificial substrates is a low bar for phosphatase activity but might be a poor proxy for biological activity. Certainly, demonstration of direct dephosphorylation is a compelling proof that a pseudophosphatase is not ‘dead’. However, caution should be used when interpreting such assays without appropriate controls, such as a mutation that removes the essential catalytic cysteine residue where it is conserved in CC1 phosphatases, in order to exclude contaminating phosphatase activity. As a follow-up, important insights to provide an explanation for catalytic activity will be gained from structural studies. Understanding the structure–function relationship of pseudophosphatases could help tackle another important challenge in this field, namely the development of strategies to pharmacologically target pseudophosphatases. This challenge is further complicated by the fact that active phosphatases themselves are hard to target using drugs [162]. This is partially due to the presence of positive charge around the active site, which resulted in screening efforts mainly identifying negatively charged molecules that do not cross the plasma membrane. However, pseudophosphatases have often lost these charged residues, thus providing new opportunities for pharmacologic modulation. In addition, many pseudophosphatases act as allosteric modulators, and thus, they could be targeted not via the active site, but by molecules that target their ability to bind active phosphatases. Another possibility is to make use of the PROTAC technology to target overexpressed pseudophosphatases for proteasomal degradation [163]. Alternatively, linker compounds could be developed to link pseudophosphatases to LC3 and therefore target them for lysosomal destruction. Our comprehensive summary highlights the need for increased molecular understanding of pseudophosphatase modes of action in order to fully determine their roles in physiology and disease, and to potentially exploit them therapeutically.

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Author contributions

All authors discussed the concept and structure of the review and wrote the review together. KP performed the phylogenetic tree analysis.

Conflict of interest

The authors declare no conflict of interest.

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