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Protein tyrosine phosphatase structure–function relationships in regulation and pathogenesis

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Protein phosphorylation on tyrosine residues is tightly controlled by protein tyrosine phosphatases (PTPs) at multiple levels: spatio-temporal expression, subcellular localization and post-translational modification. Structural and functional analysis of the PTP domains has provided insight into catalysis and regulatory mechanisms that control the enzymatic activity. Understanding the molecular basis of PTP regulation is of fundamental importance to dissect the pleiotropic effect of these enzymes in both health and disease. Here, we review recent insights into the regulation of receptor-like PTPs by extracellular ligands and into regulation by reversible oxidation that impairs catalysis directly. The physiological roles of PTPs are essential in homeostasis in eukaryotic cells and pertubation of their functional attributes causes different disease states. As an example, we discuss recent findings indicating how inappropriate oxidation of PTPs in cancer cells may contribute to cell transformation. On the other hand, PTPs from many pathogens are key virulence factors and manipulate signalling pathways in the host cells to promote invasion and survival of the microorganisms. This research area has received relatively little attention but has advanced remarkably. We review the structural features of pathogenic PTPs, their similarities and differences with eukaryotic PTPs, and the possible exploitation of this knowledge for therapeutic intervention.

Structures of PTPs

Analysis of protein tyrosine phosphatase (PTP) structures is not only important for understanding their function and regulation but also for identifying strategies for pharmacological modulation of PTP activity. Given the progress in establishing PTP deregulation in different pathologies such as cancer, PTPs deserve much attention as candidate drug targets. We focus on Cys-based PTPs [1] and discuss novel insights in PTP structure which may be important for pharmacological modulation, and emphasize novel findings and controversial issues for transmembrane (receptor-like) PTPs (RPTPs).

Abbreviations
AML, acute myeloid leukaemia; DUSP, dual specificity PTP; FLT3, *Fms*-like tyrosine kinase 3; FLT3/ITD, internal tandem duplication of FLT3; LMWPTP, low molecular weight PTP; PDGF, platelet-defined growth factor; Prx, peroxiredoxin; PTP, protein tyrosine phosphatase; PTPLP, PTP-like phytase; ROS, reactive oxygen species; RPTP, transmembrane PTP; RTK, receptor tyrosine kinase; YopH, *Yersinia* outer protein H.

PTP structures: similar at the core, yet many variations beyond that

PTP1B was the first PTP for which the crystal structure was solved [2]. In addition, the crystal structure of a PTP1B–substrate complex [3] provided structural insight into the molecular basis of PTP-mediated dephosphorylation for the first time. The crystal structure of the first dual specificity PTP (DUSP) demonstrated why DUSPs can dephosphorylate pTyr as well as pSer and pThr, because the catalytic site is shallower in the DUSPs than in pTyr-specific PTPs. The shorter side-chains of pSer and pThr can reach the catalytic site cysteine in DUSPs but not in PTPs [4]. The crystal structure of the membrane-proximal PTP domain of RPTPα shows a similar fold to PTP1B, and by now it is evident that the active sites of all classical PTPs have highly conserved core structures [5,6]. In fact, the core structure of PTPs from microorganisms is similar to eukaryotic PTPs as well (see below).

Small molecule PTP inhibitors

The design of active-site-directed small molecules with high specificity to a given PTP is severely hampered by the similarity in structure. Nevertheless, high throughput *in silico* screens for inhibitors of Shp2, known to be a *bona fide* oncogene involved in several types of leukaemia, identified phenylhydrazonopyrazolone sulfonate as a potent and cell-permeable inhibitor displaying high specificity for Shp2 over the related tyrosine phosphatases Shp1 and PTP1B [7]. Highly potent and selective inhibitors were also found for PTP1B. One of the most efficient is a difluoromethylphosphonate derivative, proved to be competitive and tight-binding (K_i = 2.4 nM) with over 10-fold selectivity over the most closely related PTP, TCPTP, and more than 600-fold selectivity over other PTPs [8]. Another recently reported inhibitor of PTP1B is trodusquemine, a natural product isolated from dogfish shark which showed non-competitive inhibition kinetics on PTP1B and, remarkably, over 200-fold preference against TCPTP [9,10].

In the context of a highly conserved active site, identification of allosteric binding sites is of particular interest. Recently an allosteric site was identified in the vicinity of the catalytic WPD loop of RPTPγ [11]. It is a small hydrophobic pocket and several specific inhibitors bound to this site, thus perturbing the WPD loop and inducing a novel 'superopen' conformation. Earlier reports mentioned an 'atypical' open state of the catalytic WPD loop in three PTPs, STEP, LYP and GLEPP1, corresponding to an inactive conformation [5,12]. The WPD aspartic acid is positioned far out of the active site, which may facilitate the design of potent inhibitors. Bidentate inhibitors binding two different sites on the same PTP molecule have much higher specificities and lower binding constants. Efficient bidentate modulators were obtained for Lyp, a potential target for many autoimmune disorders [13], SHP-1, with an important role in hematopoietic cell functions [14], and YopH, a virulence factor of *Yersinia pestis*, the causative agent of the plague [15,16]. Good insight into the structural features of PTPs facilitates the design of specific PTP inhibitors.

Dimerization of RPTPs

RPTPα-D1 formed dimers in the crystal structure, in a manner that suggested dimer-induced inactivation of RPTPα because a helix–loop–helix wedge-like structure to the N-terminal side of one of the protomers occluded the catalytic site and *vice versa* [17]. It is broadly accepted that protein tyrosine kinases are activated by oligomerization in the presence or absence of an activating ligand [18]. By analogy, it is an attractive idea that RPTPs are also regulated by oligomerization [17,19]. There are numerous reports supporting or contradicting this hypothesis [20]. Barr *et al.* [5] have recently reported, based on structural and biophysical analysis, that single and tandem PTP domains do not form dimers in physiological buffers. They also showed that the reciprocal orientation of the catalytic D1 domain and the non-catalytic D2 domain is highly conserved and does not fit with the inhibitory wedge model. On the other hand, there is a multitude of data supporting that many RPTPs, like RPTPα, GLEPP1, RPTPσ, Sap-1, CD45 and RPTPε dimerize in living cells and that dimerization regulates their activity [21–27]. Moreover, dimer formation of GLEPP1 and RPTPε in living cells involves their intracellular regions [23,27]. Dimer formation may involve both homotypic D1–D1, D2–D2 interactions and heterotypic D1–D2 interactions, in a specific manner for each dimer. In the case of RPTPε dimer formation the D1–D1 interactions are weak while the D2–D2 interactions are strong and have a major contribution to dimerization [27]. The contribution to dimerization is not limited to D1 and D2. Other regions of PTPs contribute to dimerization too. For instance, RPTPα dimerizes constitutively in living cells, which is mediated by multiple domains [22,28]. The apparent contradiction with Barr *et al.* [5] may be explained if one assumes that the flexible linker between the two intracellular domains allows reorientation of D1 and D2. However, the crystal structures of the D1–D2 domains

of PTP-LAR and CD45 indicate extensive interdomain interactions, suggesting limited flexibility of the relative orientation of D1–D2 in solution.

As indicated above, not only the cytoplasmic domain but also the transmembrane region and/or the proximal, hydrophobic region frequently seem to play a role in dimer formation, as in the case of PTPBR7, PTP-SL [29], GLEPP1 [23] and Sap-1 [24]. There are a couple of poorly conserved elements in the transmembrane region which may have a function. Alignment of the membrane-spanning sequences of 20 human RPTPs shows that two hydrophobic amino acids, isoleucine and valine, are by far the most frequent residues (Fig. 1). Notably, in seven of the 20 aligned sequences the Sternberg–Gullick motif GxxxG [30] was observed and in one sequence the glycine zipper, GxxxGxxxG [31]. Both these motifs are known to mediate helix–helix interactions and stabilize formation of homo-oligomers within a membrane. The transmembrane helices may have an essential role in dimer formation through one or more of four different types of motions within the lipid bilayer (translational, piston, pivot and rotational movement [32,33]).

The extracellular regions of RPTPs were found in many cases to be involved in dimerization, e.g. in Sap1 [24], DEP-1 [34], LAR and RPTPμ [35]. At least for these RPTPs there is a strong case that dimerization of the whole molecule is triggered by dimerization of the extracellular region. It is interesting to note that dimerization of the intracellular domain may change the ternary structure of the ectodomains on the outside of the cells, thereby suggesting that RPTPs have the potential to signal across the membrane from the inside out [36].

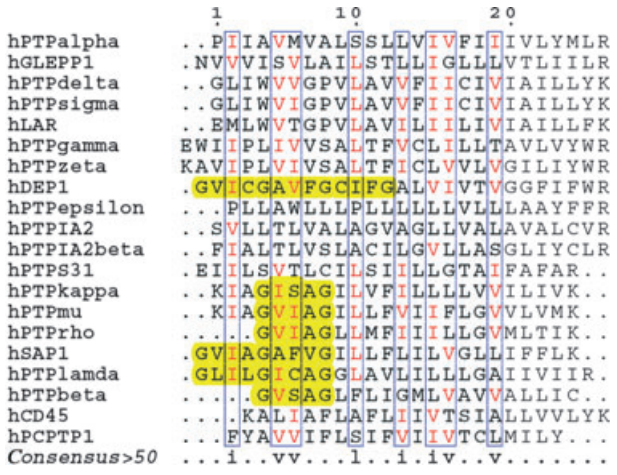

Fig. 1. Sequence alignment of the transmembrane domain of RPTPs. Transmembrane domain sequences including an extra N-terminal and C-terminal amino acid of 20 human RPTPs were aligned with MULTALIN and represented with ESPRIPT.

Much progress has been made in the structural analysis of the ectodomains of RPTPs. For instance, the RPTPμ ectodomains interact in a homophilic manner when expressed on opposing cells and this interaction actually determines the distance between the two adjacent cells [37]. Moreover, the structures of the proteoglycan binding site in the ectodomain of RPTPσ provided insight into the proteoglycan-induced molecular switch for RPTPσ clustering [38].

The functional consequences of RPTP dimerization vary from RPTP to RPTP. For instance, various alternatively spliced isoforms of CD45 have been shown to differentially homodimerize in T cells. The smallest isoform, CD45RO, forms homodimers with the highest efficiency and dimer formation led to decreased T-cell receptor signalling [39]. Another example is GLEPP1. GLEPP1 dimers were found in living cells and were associated with decreased activity toward the putative substrate TrkC [23]. Interestingly, dimerization of DEP-1, which belongs to the same RPTP class R3 as GLEPP1, has the opposite effect, increasing DEP-1 catalytic activity [34]. This suggests that the correlation between dimerization and regulation of catalytic activity should be established specifically for each RPTP and cannot be extrapolated even to members of the same sub-type of RPTPs.

Ligands of RPTP extracellular domains

A broad variety of PTP ligands have been identified to bind to the extracellular domains of RPTPs so far, including growth factors (e.g. pleiotrophin), components of extracellular matrix (e.g. laminin–nidogen complex), cell surface proteins (e.g. nucleolin), neuronal cell adhesion molecules (e.g. contactins) and synaptic proteins (e.g. syndecan and dallylike). Ligand binding to a PTP molecule does not necessarily lead to modulation of its enzymatic activity [20]. For example, numerous ligands of RPTPβ/ζ have been identified; nevertheless only binding of pleiotrophin or VacA cytotoxin were found to inhibit its enzymatic activity (see below). On the other hand, for three other ligands of RPTPβ/ζ, contactin, tenascin and TAG-1/Axonin-1, there are no reported effects on activity [20]. Here, we primarily focus on ligands that have been reported to modulate the activity of PTPs.

The ligands that bind the extracellular region of RPTPβ/ζ have been intensively studied. Two of them, the heparin-binding growth factor pleiotrophin and the cytotoxin VacA, which is produced by *Helicobacter pylori*, were found to induce inhibition of

intracellular enzymatic activity, as reflected by an increase in tyrosine phosphorylation of the RPTPβ/ζ substrates, β-catenin, GIT1 and Fyn [40–42]. It has been broadly accepted that ligand binding induces dimerization of the intracellular region and hence inactivation of RPTPβ/ζ. A couple of elements plead for this model: (a) oligomerization of the intracellular region of RPTPβ/ζ by an artificial dimerizer or polyclonal antibodies against its extracellular region resulted in decreased enzymatic activity [40]; (b) as VacA tends to form hexamers [43] it can be assumed that binding of VacA induces inactivation of RPTPβ/ζ through oligomerization; (c) the recently reported structure of the intracellular region of RPTPγ – a member of the same RPTP class R5 and having a high sequence identity to RPTPβ/ζ – revealed a 'head-to-toe' dimeric structure in which the active site of the D1 domain is occluded by the D2 domain thus preventing the access of substrate into the active site [5]. On the other hand, given that RPTPβ/ζ in brain is mainly synthesized as proteoglycans [44], the presence of highly sulfated sugar residues in the extracellular region makes dimer formation unlikely. Therefore, a mechanism cannot be ruled out in which ligand (pleiotrophin or VacA) binding induces a conformational modification of the extracellular region of RPTPβ/ζ leading to a conformation of the intracellular region which favours dimer formation. Thus, following ligand binding, the monomer–dimer equilibrium of the cytoplasmic region of RPTPγ [5] may be shifted to the inactivated dimer form. Further studies regarding the mechanism of RPTPβ/ζ inactivation through ligand binding may be particularly important due to the potential therapeutic applications of this interaction. Given that pleiotrophin and RPTPβ/ζ substrates β-catenin, Fyn and β-adducin were found to promote survival of neuronal stem cells and their differentiation to dopaminergic neurons, a new therapeutic strategy was proposed for treatment of Parkinson's disease, based on blocking of RPTPβ/ζ activity [45].

A remarkable example in which ligand binding to the extracellular region of an RPTP activates an intracellular inside-out signalling pathway that is dependent on the intracellular PTP activity was recently published. Cell surface receptor syndecan-2 – a heparin sulfate proteoglycan – has been reported to be a ligand of DEP-1 [46]. Interaction between the C-terminal regulatory region of syndecan-2 and DEP-1 ectodomain promotes a transmembrane signalling pathway. This signalling process involves the intracellular PTP activity of DEP-1, Src-kinase activity and dephosphorylation of the p85 subunit of PI-3 kinase. Thus, syndecan binding to DEP-1 eventually leads to β1 integrin mediated adhesion and cytoskeletal organization. Recently, thrombospondin-1 was reported to be a ligand of DEP-1 as well, and thrombospondin-1 binding to DEP-1 leads to increased dephosphorylation of DEP-1 substrates and attenuated downstream signalling. Moreover, DEP-1 contributes to thrombospondin-1 mediated inhibition of endothelial cell growth [47].

Taken together, regulation of RPTPs by dimer formation and the subsequent activity modulation probably differ for each particular RPTP; it is likely that dimer formation is triggered by any combination of (a) ligand binding to the extracellular region, (b) dimerization of the extracellular region, (c) dimerization of the intracellular region and (d) dimerization and dimer stabilization by the transmembrane region. Subsequently, the (re)positioning of the intracellular PTP domains in the dimer results in an increase, decrease or no modification of the enzymatic activity. In principle, a 'head-to-toe' orientation of the protomers in the dimer may lead to inactivation of the catalytic activity. Environmental interference, like oxidative stress or binding of other intracellular ligands to the dimer, may contribute to conformational changes of the dimer and consequently to modulation of the enzymatic activity. Crystal structures of the entire transmembrane RPTP proteins would greatly enhance our understanding of the regulation of RPTPs and how dimerization or multimerization/clustering might affect their activities.

Regulation of PTPs by reversible oxidation

Reversible oxidation of PTPs has emerged as an important general regulatory mechanism for members of this enzyme family. We briefly summarize some important features of the biochemistry of this process and its integration in cell regulation. For a more detailed review of these topics, the reader is referred to a recent comprehensive review [48].

Biochemistry of PTP catalysis and reversible oxidation

The catalytic site of PTPs contains a cysteine whose SH group exists in the thiolate state (S−) making it highly susceptible to oxidation. Many *in vitro* studies have shown that a wide range of oxidants can induce PTP oxidation. However, the large majority of studies have analysed the effects of hydrogen peroxide (H_2O_2) since this oxidant is produced upon activation of many cell surface receptors [49,50]. Interestingly, recent findings have challenged the concept that H_2O_2 is the most relevant oxidant. This has been based on the argument

that the relatively slow reaction of H_2O_2 with the thiolate cysteines in PTPs should be unable to match the efficient H_2O_2-consuming processes in cells [51–53]. PTP oxidation may therefore occur through oxidation by oxidants other than H_2O_2 as well. One such class of alternative oxidants is peroxidized lipids which recently were shown to be highly active as PTP oxidants [54]. It has also been suggested that PTP oxidation is highly compartmentalized and occurs where H_2O_2 clearance is decreased, e.g. by inhibitory phosphorylation of peroxiredoxins (Prx) [55].

Reaction with H_2O_2 oxidizes PTP active-site thiolates to sulfenic acid (SOH). Different secondary reaction products include sulfenylamides (SN) by reaction with a neighbouring peptide backbone residue [56–58] and intramolecular disulfides by reaction with proximal-free cysteines [59–63]. Sulfhydration of the active site of PTP1B represents an additional type of inhibitory active-site modification recently described in the case of PTP1B [64]. In addition to blockade of the catalytic activity, these modifications are accompanied by structural changes [65]. In the case of the SN form of PTP1B these changes include alterations in the conformation of the PTP and pTyr recognition loops [56,57]. These alterations are also accompanied by an exposure of the Sγ atom of the oxidized cysteine making it more accessible for reduction [56,57]. These unique structural properties of the oxidized form of PTP1B were recently exploited by the development of an 'intra-body' specifically detecting this modified form of PTP1B [66]. The susceptibility of different PTPs to oxidation *in vitro* shows large variations that are determined either by intrinsic properties of the PTP domains or by regulatory domains outside the PTP domain [67–69]. Pronounced selectivity for oxidation of some specific PTPs have also been observed in cells. For example, T-cell receptor activation quite selectively led to the oxidation of SHP2 but not SHP1 [70] despite the similar susceptibility of both PTPs to oxidation *in vitro* [71,72] A few early studies also indicate that oxidized PTPs differ with regard to their sensitivity to different reducing agents [63]. The structural variations and different types of oxidized forms of PTPs (see above) are likely to contribute to the selectivity in reduction susceptibility.

PTP oxidation as an intrinsic part of growth factor signalling

PTP oxidation is now a well-established intrinsic component of cell signalling triggered by many classes of cell surface receptors, including receptor tyrosine kinases, integrins, cytokine receptors, G-protein-coupled receptors and T- and B-cell receptors. The mechanisms coupling cell surface receptor activation and PTP oxidation are being uncovered. The best characterized pathway involves increased NOX activity, through PI-3 kinase activation and Rac translocation to NOX enzymes, following activation of receptor tyrosine kinases (RTKs) [73]. Analyses of platelet-defined growth factor (PDGF) receptor signalling have also demonstrated that deletion of p66Shc, which increases mitochondrial reactive oxygen species (ROS), is associated with a decreased PTP oxidation and a concomitant attenuation of the biochemical and cellular responses to PDGF stimulation (J. Frijhoff and A. Östman, submitted for publication). p66Shc also promotes insulin signalling through oxidation of PTEN [74]. Another potentially general mechanism was recently presented which emphasizes an RTK-mediated inhibitory tyrosine phosphorylation of Prx1 leading to a spatially restricted increase in H_2O_2 in the vicinity of activated RTKs [55]. The general pattern that is emerging from these analyses of growth-factor-induced PTP oxidation is that oxidation does not affect all PTPs equally and that oxidation occurs transiently and in a spatially restricted manner.

ROS pathways impacting on PTP oxidation

The overall cellular redox environment is controlled by the concerted action of enzymes involved in production and scavenging of ROS. Key ROS-producing enzymes include the NOX and DUOX enzymes, oxygenases, flavoproteins and enzymes of the mitochondrial respiratory chain. The major reducing ROS scavengers include enzymes of the catalase, Prx and glutathione peroxidase families. Recent studies have clearly demonstrated that the expression levels and specific activities of these enzymes, independent of their regulation by cell surface receptors, control PTP oxidation and thereby modulate growth factor signalling (reviewed in [48]).

A key study illustrating the link between cellular 'redox status', PTP oxidation and growth factor signalling showed that Prx2 knockdown enhanced PDGF receptor signalling in tissue culture and *in vivo* through increased ROS levels and reduced PTP activity [75]. Similar results have now been reported when growth factor signalling and PTP oxidation have been analysed in cells with manipulated expression of other redox-regulating enzymes [48]. For example, NOX4 and DUOX1 have been linked to oxidation states of PTP1B and SHP2, respectively [76,77]. Together, these studies suggest that PTP oxidation is a key component of a preliminarily characterized, but potentially very general, cross-talk between cellular redox status and response to growth factor signalling.

PTP oxidation in cancer cells

Pertubation of PTP functions has been implicated in several pathologies including inflammation, atherosclerosis and cancer. One mechanism that is receiving increased attention is the potentially pathogenic oxidation of PTPs, which occurs in the context of several of these pathologies and may even contribute to their development. We would like to emphasize here some remarkable recent progress in the understanding of the role of PTP oxidation in cancer cells. Cancer cells frequently exhibit comparatively higher ROS levels than their normal counterparts (reviewed in 78). It is generally assumed that these are contributing to oncogenesis by fuelling mitogenic, motogenic and survival signalling. Moreover, elevated ROS in conjunction with deregulated mechanisms for maintenance of genetic stability have been shown to enhance mutagenesis [79,80], thereby presumably contributing to tumour-promoting lesions. Still, ROS production in cancer cells is regulated and, by adaptive responses, balanced to levels which are compatible with cell survival (reviewed in [81]). Elevated ROS in cancer cells have prompted therapeutic considerations. On the one hand, quenching of ROS by small molecules may be beneficial and aid conventional cancer therapies [82]. On the other hand, elevated ROS levels may make cancer cells prone to selective toxic effects of agents that increase ROS levels further, beyond a threshold causing cytotoxicity [81–83].

For some cancer types it has been possible to link elevated ROS production to specific oncogenic lesions, such as oncogenic mutations causing activation of the small GTPase RAS in multiple cancers [62,84,85] or gene rearrangements leading to constitutively active protein tyrosine kinases such as BCR-ABL in chronic myeloid leukaemia [86], constitutively active *Fms*-like tyrosine kinase 3 (FLT3) internal tandem duplication of FLT3 (FLT3ITD) [80] in acute myeloid leukaemia (AML), or mutant Janus kinase 2 (JAK2V617F) in myeloproliferative disease [87]. The causes of elevated ROS production were identified to be either elevated activity of membrane-associated NADPH oxidases or enhanced mitochondrial activity [84,85,87–89].

PTPs present one class of possible targets of elevated ROS in cancer cells. Their inactivation may eliminate negative regulation of mitogenic, anti-apoptotic and motogenic pathways (Fig. 2). Indeed, PTP oxidation in

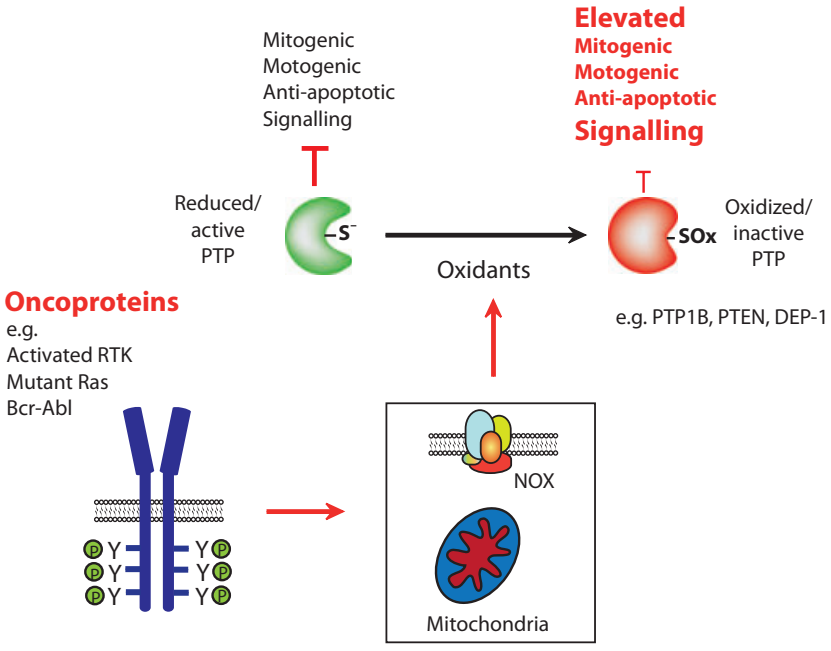

Fig. 2. PTP oxidation in cancer cells may contribute to cell transformation. Oncoproteins such as activated Ras or activated tyrosine kinases can drive ROS formation in cancer cells through mechanisms involving activation of NOX enzymes, elevated activity of mitochondria or further pathways. ROS appear to oxidize selected PTPs, leading to inactivation by modification of the catalytic cysteine (shown in the thiolate state) to different possible oxidation products (denoted SO-x). The exact identity of oxidants and molecular reasons leading to a pronounced selectivity of this process are not yet known. Some PTPs whose oxidation has been demonstrated in cancer cells are indicated. Oxidized PTPs can no longer control mitogenic, motogenic and anti-apoptotic signalling processes, which are therefore enhanced. It is assumed that this process contributes to oncogenesis, which has been shown recently in some examples.

cancer cells has now been convincingly demonstrated in several studies. Global inactivation of PTP activity in cell lysates was reported early on, e.g. in BCR-ABL expressing cells [86]. In pioneering research, the oxidation of PTP1B in several cancer cell lines has been demonstrated by mass spectrometric analysis. A significant fraction of PTP1B in A431 carcinoma cells was even shown to be irreversibly inactivated [90]. A recent comprehensive analysis has now revealed that oxidation of PTPs is indeed a common phenomenon in cancer cells [91]. Different cancer cell lines exhibited specific PTP oxidation profiles. Frequently, multiple PTPs were found to be oxidized. For example, A431 cells harboured not only oxidized PTP1B (PTPN1) but also oxidized PTPN4, PTPN21, PTPN23, PTPRA, PTPRE, PTPRJ, PTPRK and PTPRS [91].

Importantly, recent studies have also shown that PTP oxidation in cancer cells is not only a secondary effect but appears to causally contribute to cell transformation. One example is the tumour suppressor PTEN. Loss or reduction of PTEN function by mutations or deletions occurs frequently in solid tumours. As a consequence, higher levels of the PTEN substrate phosphatidylinositol 3,4,5-trisphosphate lead to activation of multiple anti-apoptotic, pro-mitogenic and metabolic pathways, partially mediated by activation of the AKT/PKB pathway (reviewed in [92]). PTEN is highly susceptible to inactivation by reversible oxidation [69] and this appears to be another mechanism for partial inactivation of PTEN in certain cancer types. In human T-cell acute lymphoblastic leukaemia (T-ALL), AKT/PKB is frequently hyperactivated. In a fraction of these cases, PTEN is inactivated or lost by mutation. However, in another fraction of cases AKT/PKB activation is partially linked to PTEN inactivation by reversible oxidation. Notably, in PTEN-positive T-ALL lines, AKT/PKB activation is diminished by treatment of cells with reducing agents, whereas this is not possible in cells that have lost PTEN expression [93]. Other studies have suggested a role for PTEN oxidation in pancreatic cancer [94] and more recently in RAS- and ERB2-driven tumours [95].

Work from two of our laboratories has recently provided evidence for a role of oxidation of DEP-1 in cell transformation [89]. DEP-1 is known to negatively regulate different RTKs and is also considered a tumour suppressor [96,97]. Recently, the hematopoietic RTK FLT3 has been identified as another substrate of DEP-1 [98]. As indicated above, AML cells harbouring a mutant form of FLT3, FLT3ITD, exhibit elevated ROS levels. In such cells, DEP-1 is partially inactivated, but activity is restored by cell treatment with FLT3ITD inhibitors which diminish ROS formation. DEP-1 is inactivated in primary AML blasts if they express FLT3ITD but not if they harbour wild-type FLT3. Different means to interrupt ROS production, such as overexpressing the ROS scavenger enzyme Prx1, also reactivate DEP-1. Importantly, abrogation of ROS formation attenuates transformation *in vitro* and *in vivo* in a DEP-1 dependent manner. These results suggest that inactivation of DEP-1, a negative regulator of FLT3, contributes to FLT3ITD-driven transformation.

Pathogenic PTPs

In recent years, a significant amount of structural data has been published on PTPs from several pathogens that explain their physiological functions and the molecular basis for substrate binding (Table 1). This has provided a platform for design and development of drug candidates. However, few structures are available from pathogenic microorganisms in comparison with human PTPs. We discuss here the progress made in uncovering these structures and the relevance of PTP molecules for pathogen biology.

Identification of PTPs in the genomes of pathogens

The available genome data of many microorganisms facilitated the prediction of their protein phosphatase complements or 'phosphatome' and the comparative analysis with those of the host [99–107], showing significant differences to the human [1,108]. The automatic classification of genome data into the various families of phosphatases has populated the phosphatase resource PhosphaBase that features over 11 000 entries (http://www.bioinf.manchester.ac.uk/phosphabase/ [109]). A much slower progress has followed on the biological and functional analysis of phosphatases from microorganisms. However, the emerging picture is that phosphatases, mainly PTPs, play very significant roles in controlling their physiology and pathogenicity. Bacterial pathogens have developed a number of sophisticated strategies to efficiently infect and proliferate in the host cells. These involve manipulation of the host signalling pathways by bacterial effectors to facilitate invasion and survival. Many of these effectors are phosphatases that alter the phosphorylation of proteins and phosphoinositides (reviewed in [110–112]). In protozoan parasites PTPs are also critical in infection and transmission of the parasites. The role of PTPs in viruses is less clear.

Given the role of PTPs in the virulence and viability of pathogens, PTPs represent an important source of

Table 1. Pathogenic phosphatases. Pathogen of origin, phosphatase name, PDB identification code, catalytic activity and function are listed here. See text for details. AcNP, *Autographa californica* nucleopolyhedrovirus.

Organism	Protein	PDB ID	Ligand	Activity	Function	Reference
Vaccinia virus	VH1	3CM3 2RF6	Phosphate Sulfate	DSP	Essential for viability in host cells, prevents antiviral response	113
Variola virus	VH1	2P4D		DSP	Essential for viral particle production	114
Baculovirus (AcNP)	BVP	1YN9	Phosphate	mRNA phosphatase	mRNA capping, not essential for replication	115
Salmonella typhimurium	SptP	1G4U 1G4W	Rac1	PTP	Responsible for cellular recovery after infection/internalization	118
Salmonella typhimurium	SptP	1JYO	SicP	PTP		119
Yersinia enterocolitica	YopH	1YPT	Tungstate	PTP	Essential for virulence, disrupting signalling pathways necessary for phagocytosis	125
Yersinia enterocolitica	YopH	1YTS	Sulfate	PTP		126
Yersinia enterocolitica	YopH	1YTN	Nitrate	PTP		127
Yersinia enterocolitica	YopH	3F9B	Divanadate	PTP		128
Yersinia pestis	YopH	1QZ0	Ac-DADE-F2Pmp-L-NH2	PTP		129
Yersinia enterocolitica	YopH	1XXP	SKAP-HOM P-peptide	PTP		130
Yersinia enterocolitica	YopH	1PA9	*N*,4-dihydroxy-*N*-oxo-3-(sulfooxy) benzenaminium	PTP		131
Yersinia enterocolitica	YopH	3BLT, 3BLU, 3BM8	Phenyl ethenesulfonate	PTP		162
Yersinia pestis	YopH	2Y2F	[4-[3-(Difluoro-phosphono-methyl) phenyl]phenyl]methoxyazanium	PTP		133
Escherichia coli	Wzb	2WJA	Ni(II) ion	LMWPTP	Export of colanic acid	137
Campylobacter jejuni	Cj1258	2GI4		LMWPTP	Unknown	139
Mycobacterium tuberculosis	MptpA	1U2P	Chloride	LMWPTP	Essential for virulence	163
Mycobacterium tuberculosis	MptpB	1YWF	Phosphate	Polyphosphatase	Essential for virulence	147
Mycobacterium tuberculosis	MptpB	2OZ5	OMTS inhibitor	Polyphosphatase	Essential for survival in infected host	148
Staphylococcus aureus	PtpA	3ROF	Phosphate	LMWPTP	Unknown	146
Neisseria meningitidis	NMA1982	2F46	Chloride	Putative phosphatase	Unknown	151
Streptococcus pneumoniae	CpsB	2WJD	Mn(II) ion	Metal-dependent PTP	Regulation of capsular polysaccharide biosynthesis, critical for virulence	137
Bacillus subtilis	YwqE	3QY6	Fe(III) ion	Metal-dependent PTP	Regulation of polysaccharide biosynthesis	152
Selenomonas ruminantium	PTPLP	1U24 2B4O 2PSZ	Chloride, glycerol	Inositol polyphosphatases	Unknown	154, 155,156
Mitsuokella multacida	PTPLP	3F41	1,2-Ethanediol	Inositol polyphosphatases	Unknown	157
Leishmania major	LmAcr2	2J6P	Sulfate	Arsenate reductase and phosphatase	Arsenic detoxification	158
Trypanosoma brucei	TbPTP1	3M4U	Phosphate	PTP	Functional switch in lifecycle control	161

new targets for chemical intervention in the treatment of infections. In order to develop potent compounds as drug candidates, structural information of the target is critically important.

Structures of viral PTPs

Only five structures of viral phosphatases are reported, three of them in the PTP superfamily: two DUSPs, VH1 from *Vaccinia* virus [113] and VH1 from *Variola* virus (causing agent of smallpox [114]), and one mRNA capping triphosphatase (BVP from *Baculovirus* [115]). VH1 is highly conserved among poxviruses and essential for viability of *Vaccinia* virus in tissue cultures. The *Variola* VH1 is important in blocking host antiviral response via interferon γ signalling and dephosphorylation of the host STAT1. This VH1 is essential for the production of mature virus particles and therefore an attractive drug target. Viral VH1 phosphatases share a similar fold with mammalian DSPs and the human VHR. The electrostatic surface charge distribution is very similar between the two viral phosphatases but clearly different from human VHR, which shows a more electronegative surface (Fig. 3). Other differences include the N-terminal helix orientation and deletions at the loop connecting the last strand in the β sheet (Fig. 3). Two insertion loops flanking the active site in VHR form high walls with a Tyr and Phe at the rim, but in the viral VH1s there is a shallow pocket adjacent to the active-site cleft. All these differences could be exploited in the design of specific inhibitors. Initial efforts show promising results with inhibitors of *Variola* VH1 with IC_{50} values in the low micromolar range [114].

Structures of bacterial PTPs

In bacteria, two PTP subfamilies have been identified, the low molecular weight PTPs (LMWPTPs) and the classic type I Cys-based PTPs. The 3D structures for a number of them have been determined, showing a very similar fold to their mammalian orthologues, with the main differences being due to sequence variations and electrostatic surface potential.

Type I PTPs

Salmonella enterica uses a number of virulence factors that induce cytoskeletal rearrangements in the host cell, facilitate bacterial internalization and reverse the morphological changes afterwards to restore normal cellular functions [116]. SptP is a multidomain effector protein responsible for cellular recovery after internalization [117]. It contains an N-terminal region that binds the chaperone SicP, essential for translocation of SptP, a GAP domain that binds to activated Rac1 and Cdc42, and a C-terminal PTPase domain. The structure of the PTP domain [118] reveals a classic fold, featuring a conserved P-loop signature motif and a WPD loop (containing the catalytic Asp) as found in eukaryotic PTPs (Fig. 3). Despite similarities there are interesting differences: a larger opening of the active site (double pocket), an acidic pocket on top of the active site, not present in other PTPs, and the absence of a PTP1B-like basic secondary pocket (P2 in Fig. 3). The N-terminal domain shows conservation of the 3D binding interface and critical activating residues with the host GAP [119].

Yersinia species are the causing agents of bubonic plague (*Y. pestis*), gastrointestinal disorders (*Y. pseudotuberculosis*, *Y. enterocolitica*) and potential biological warfare agents. *Yersinia* uses the type III secretion system to inject up to six effectors into the host cells. One of these effectors is *Yersinia* outer protein H (YopH), a two-domain protein with an N-terminal docking region linked by a Pro-rich region to a PTP domain. YopH is essential for virulence in mice [120] disrupting signalling pathways necessary for phagocytosis. The N-terminal domain is required for binding to the specific chaperone SycH that drives translocation of YopH, and for binding to phosphorylated target proteins in the host [121]. NMR and X-ray structures of both domains are now available in the apo forms as well as in complex with small molecules and peptide ligands [122–124]. The X-ray crystal structure of the catalytic domain of YopH was one of the first PTP structures to be solved [125] as well as complexes with phosphate, tungstate, nitrate and vanadate [126–128]. The 3D fold is similar to classic mammalian PTPs, such as PTP1B. In the structure of YopH in complex with a peptide substrate analogue (Ac-DADE-F2Pmp-L-NH2) [129], two molecules of peptide were found bound to the PTP domain. One molecule binds in the active site with the pTyr mimic towards the P loop, while the second molecule binds to a non-catalytic remote site, on the opposite side of the molecule (Fig. 3). Further mutagenesis and functional studies in cells and animal infection models demonstrated that the phospho-binding site 1 (at the N-terminal domain) and site 2 (PTP domain secondary site) cooperate to promote phospho-substrate targeting *in vivo*, and this activity is important for *Yersinia* virulence [130].

Structures of YopH with potent inhibitors are also available, including *p*-nitrocatechol sulfate [131], aryl vinyl sulfonates that form covalent adducts with the catalytic Cys [132] and the aminooxy-containing

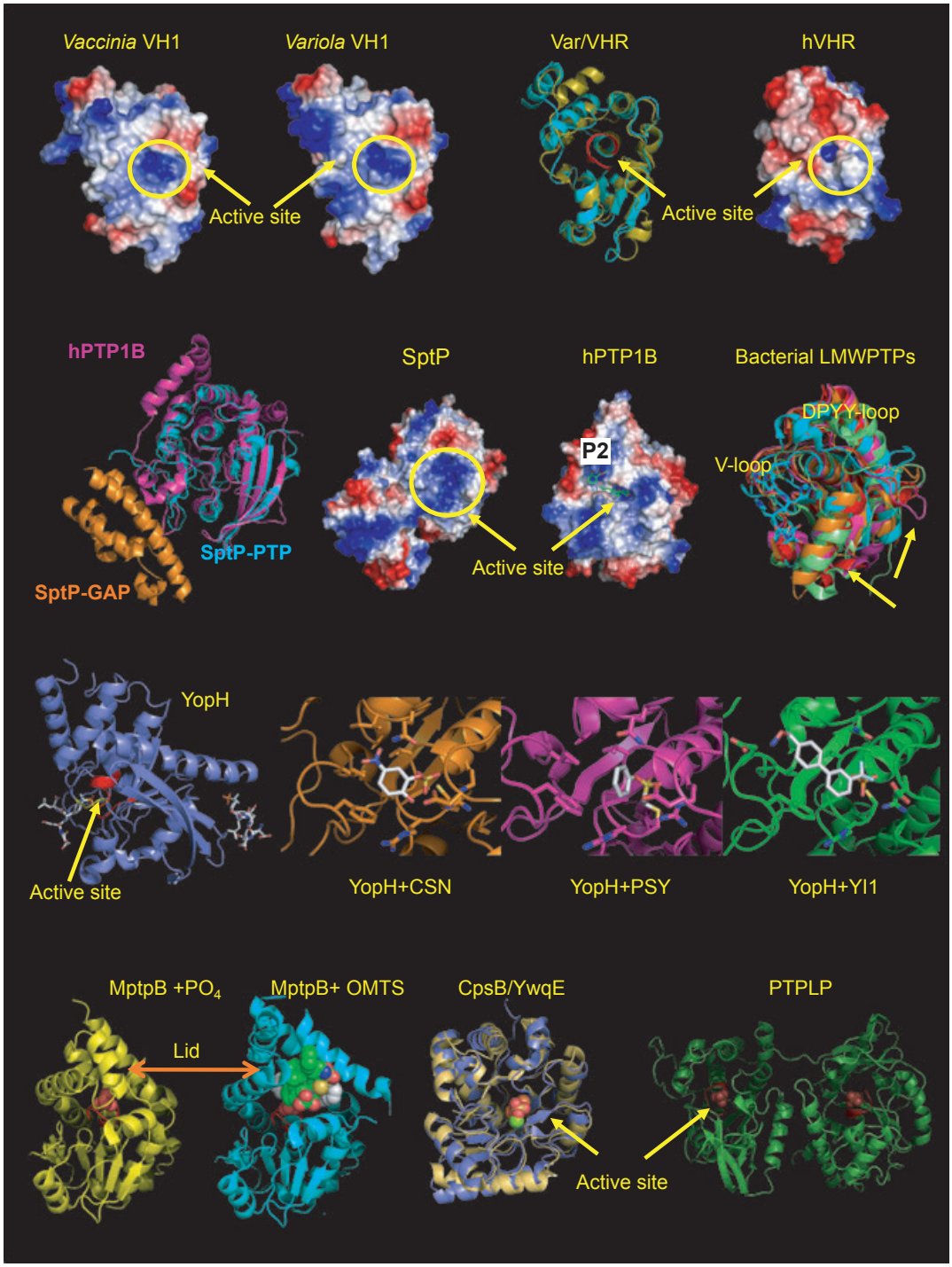

Fig. 3. Structural comparison of phosphatases from pathogens. Ribbon diagrams are provided for the molecular structures and in some cases the electrostatic surface is shown, with the electronegative charged regions in red and electropositive in blue. When relevant, the active site is indicated by an arrow (or circled). Ligands are shown in a ball-and-stick representation or in CPK colour scheme. The protein names are used in the figure, and the PDB ID coordinates used are the following: *Vaccinia* VH1 (2RF6), *Variola* VH1 (2P4D), human VHR (1VHR), *Salmonella* SptP (1G4W), human PTP1B (1XBO), bacterial LMWPTPs (2WJA, 2GI4, 1U2P, 3ROF), human LMWPTPB (1XWW), *Yersinia* YopH (1XXP, 1PA9, 3BLT, 2Y2F), *M. tuberculosis* MptpB (1YWF, 2OZ5), *S. pneumoniae* CpsB (2WJD), *B. subtilis* YwqE (3QY6), *Mitsuokella multiacida* PTPLP (3F41). CNS is *N*,4-dihydroxy-*N*-oxo-3-(sulfooxy)benzenaminium, PSY is phenyl ethenesulfonate, and YI1 is [4-[3-(difluoro-phosphono-methyl)phenyl]phenyl]methoxyazanium.

inhibitor [133] that led to the design of furanyl-based oxime derivatives. The best compound of this series (IC_{50} = 190 nM) shows specific inhibition of intracellular bacterial growth in human macrophages at 10 μM with no significant toxicity.

Type II PTPs (LMWPTPs)

LMWPTPs are found in all bacterial species, playing important roles in different aspects of polysaccharide transport and virulence [111]. All reported structures of prokaryotic LMWPTPs share the same overall a/b fold typical of the eukaryotic LMWPTPs, with a central four-stranded parallel β sheet flanked by five α helices and the conserved P loop at the bottom of the active-site cleft. Main differences are observed primarily at two positions: connections α4–β4 and β4–α5, where there is poor conservation across species (arrows in Fig. 3).

In *Escherichia coli* the LMWPTP Wzb participates in the export of colanic acid [134] and dephosphorylates the Wzc kinase [135]. Homologous phosphatase/kinase pairs have been identified in other Gram-negative bacteria (*Acinetobacter johnsonii*, *Erwinia amylovora*, *Pseudomonas solanacearum*, *Klebsiella pneumoniae* [110,135]) and *Mycobacterium tuberculosis* [136]. The structure of Wzb has been determined by NMR spectroscopy [137] and shows differences in the gatekeeper residue position at one side of the active site. Instead of the aromatic W or Y found in the mammalian LMWPTP, Wzb has a hydrophobic Leu residue and also lacks the second Y in the DPYY loop. The importance of these residues in substrate binding may explain the lower activity observed for this phosphatase. *Campylobacter jejuni*, which causes food-borne bacterial enteritis, contains a single Tyr phosphatase Cj1258 [138] of unknown physiological role. The NMR structure [139] revealed a typical LMWPTP fold confirming its assignment as a phosphatase and it shows flexibility in loop regions that surround the active site (V loop, DPYY loop) [139] in response to ligand binding.

M. tuberculosis contains two secreted PTPs, MptpA and MptpB, both required for intracellular survival in macrophages and playing a critical role in establishing infection and sustaining survival of *M. tuberculosis* in the host cells [140–142]. For this reason they constitute important targets against tuberculosis [143]. The X-ray structure of MptpA shows the conserved LMWPTP fold, with significant differences at the V loop and in the molecular surface electrostatics [143], responding to their different interacting partners. MptpA binds to the bacterial PtKA [136], while the mammalian counterpart targets and dephosphorylates a number of receptor Tyr kinases, not present in bacteria (reviewed in [144]). Several inhibitors for MptpA have been reported (reviewed in [143]) with a family of chalcones showing an effect on *M. tuberculosis* survival in macrophages [145]. Chalcones are intermediates in flavonoid biosynthesis in plants and are competitive active site binding inhibitors of MptpA [145]. However, the high homology to the human orthologue still poses serious selectivity issues before these drugs can be used for tuberculosis treatment.

Staphylococcus aureus contains two LMWPTPs (PtpA and PtpB) but their biological roles have not yet been assigned. The main differences in the structure of PtpA [146] locate to the V-loop position and the Trp44 analogue of the human gatekeeper Trp/Tyr 49 (B/A isoform). The reason for the conformational changes is explained by the binding of a fragment from the His tag of the recombinant protein near the V loop (shown in cyan and sticks in Fig. 3) and underscores the importance of the V loop.

Unusual PTP structures

MptpB shows an unusual fold, resembling the human myotubularin MTMR, that deviates from the classic PTP fold. The structure contains a flexible lid that partially covers the active site in the PO_4 bound form [147] but is open in the complex with inhibitor [148] (Fig. 3). This phosphatase displays a very large active site, unlike the narrow and deep PTP cleft, which is consistent with its phosphoinositide activity [149]. The P-loop signature contains an extra basic Arg residue like other lipid phosphatases such as PTEN and MTMs. Interestingly MptpB contains the catalytic Asp in the P loop rather than in the distant WPD loop of classic PTPs. This feature is shared with MTMs and it is the trademark of a large family of Mptp-like atypical lipid phosphatases in microorganisms [150].

Neisseria meningitidis causes meningitis and septicaemia. Only one structure is available on a protein of unknown function NMA1982 [151], with a molecular weight of 17 461 Da. The overall fold is reminiscent of the LMWPTP but contains a more extended β sheet and extra helices and lacks other regions such as the V loop, Tyr-binding loop and the DPYY loop. The signature CX_5R motif of classical PTPs is replaced by a CX_4R motif in NMA1982. The active site is shallow and contains an additional basic residue (Arg). No obvious acid/base catalyst is present around the active site, questioning the activity of this putative PTP and its catalytic mechanism.

The most peculiar structures are those of two unique metal-dependent PTPs reported in *Bacillus subtilis* and *Streptococcus pneumoniae* [137,152], belonging to the

polymerase and histidinol phosphatase (PHP) family. Although unrelated, both PHPs and LMWPTPs have analogous biological roles regulating synthesis and export of capsular polysaccharides, critical for virulence [153]. The X-ray structures of CpsB [137] and YwqE [152] have been determined in the presence of different ligands (phosphate, sulfate and metal ions), providing insight into mechanistic details of catalysis. Their folds are clearly distinct from LMWPTPs or other PTPs, and exhibit a central β barrel surrounded by α helices (Fig. 3). The C-terminal edge of the barrel contains the active site and metal binding sites. Despite the different fold, the binding mode for the PO_4 in the active site shares structural similarities with CpsB and its functional analogue Wzb.

Another interesting family contains the PTP-like phytases (PTPLPs). These are inositol polyphosphatases found in a wide range of plant and human pathogens that catalyse sequential dephosphorylation of InsP6 to generate lower inositol phosphates. The structures of several PTPLPs have been reported from gastrointestinal colonizing bacteria *Selenomonas ruminantium* [154–156] and *Mitsuokella multacida* [157]. PTPLPs have a core PTP domain and an IPP domain unique to this phytase family. The PTP domain contains the CX_5R signature with the catalytic Cys, Arg and Asp in analogous positions of the classic PTPs suggesting a similar mechanism of catalysis. The *Mitsuokella* PTPLP has a tandem repeat with two PTP domains (D1, D2) (Fig. 3), similar to that found in mammalian receptor PTPs. The P-loop sequence varies between domains with D1: HCYAGMGRT and D2: HCQAGAGRT. These differences may be related to differences in catalytic activity and specificity, with D1 favouring dephosphorylation of less phosphorylated forms of myoinositol and D2 favouring the highly phosphorylated forms.

Structures of protozoan PTPs

PTPs are a small family in protozoa compared with serine/threonine phosphatases [102]. The structures of two of them have been reported. LmACR2 from *Leishmania major* is an intriguing enzyme that shows both arsenate reductase and phosphatase activity *in vitro*, and displays sequence and structural similarity with Cdc25 which belongs to the rhodanese/Cdc25 family [158]. *Trypanosoma brucei* Tyr-specific phosphatase TbPTP1 is a critical switch in regulation of the lifecycle of the parasite [159]. It is also involved in the glycosomal signalling pathway and another STP phosphatase, TbPIP39, was identified as its substrate [160]. The structure of TbPTP1 [161] shows a classic PTP fold but lacks the allosteric inhibitor BZ3 binding site. Yet, this inhibitor impairs TbPTP1 activity [159], both *in vitro* and in the parasite. Thus the mode of binding of this inhibitor remains unknown.

Conclusions

Structure–function analyses of different PTP subfamilies shed light on the specific features of their catalytic mechanisms. Recent structural and functional data concerning intracellular and extracellular domains as well as their ligands bring an increasing body of evidence that activities of RPTPs are rigorously modulated by dimerization and/or binding of specific ligands. The crystal structure of an entire transmembrane PTP with and without ligand bound to the ectodomain is expected to provide essential insights into the regulation of RPTPs. Oxidation of PTPs by now is established as an important regulatory mechanism for PTPs and future work will focus on the questions why PTPs are differentially oxidized and what the oxidizing agents of PTPs are. This will contribute to understanding the role of oxidation-mediated inactivation of PTPs in cancer. Structure–function analysis of PTPs from pathogens revealed both high similarities with mammalian PTPs as well as remarkable differences in some instances. PTPs from pathogens are often essential virulence or survival factors and the structural data are imperative for rational drug design of new antibiotics, vaccines and anti-infectives.

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