ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 1, 2011

DOI: 10.1089/ars.2010.3611

FORUM REVIEW ARTICLE

Redox Regulation of Protein Tyrosine Phosphatases: Structural and Chemical Aspects

John J. Tanner, Zachary D. Parsons, Andrea H. Cummings, Haiying Zhou, and Kent S. Gates

Abstract

Protein tyrosine phosphatases (PTPs) are important targets of the H_2O_2 that is produced during mammalian signal transduction. H_2O_2-mediated inactivation of PTPs also may be important in various pathophysiological conditions involving oxidative stress. Here we review the chemical and structural biology of redox-regulated PTPs. Reactions of H_2O_2 with PTPs convert the catalytic cysteine thiol to a sulfenic acid. In PTPs, the initially generated sulfenic acid residues have the potential to undergo secondary reactions with a neighboring amide nitrogen or cysteine thiol residue to yield a sulfenyl amide or disulfide, respectively. The chemical mechanisms by which formation of sulfenyl amide and disulfide linkages can protect the catalytic cysteine residue against irreversible overoxidation to sulfinic and sulfonic oxidation states are described. Due to the propensity for backdoor and distal cysteine residues to engage with the active-site cysteine after oxidative inactivation, differences in the structures of the oxidatively inactivated PTPs may stem, to a large degree, from differences in the number and location of cysteine residues surrounding the active site of the enzymes. PTPs with key cysteine residues in structurally similar locations may be expected to share similar mechanisms of oxidative inactivation. *Antioxid. Redox Signal.* 15, 77–97.

The Role of Protein Tyrosine Phosphatases in Signal Transduction

REVERSIBLE PHOSPHORYLATION of tyrosine residues serves as a biochemical switch that alters the functional properties of proteins in many critical mammalian signal transduction pathways (66, 72, 113, 165, 167). For example, tyrosine phosphorylation plays a central regulatory role in cell metabolism, growth, proliferation, differentiation, immune response, motility, tissue homeostasis, and apoptosis (64, 66, 167). The phosphorylation status of tyrosine residues on proteins involved in these signal transduction pathways is controlled by the opposing actions of protein tyrosine kinases that catalyze the addition of phosphoryl groups and protein tyrosine phosphatases (PTPs) that catalyze their removal (Fig. 1) (64, 66, 167, 196).

The Catalytic Mechanism of PTPs

The catalytic mechanism by which PTPs remove the phosphoryl group from phosphotyrosine residues is well established. Here we describe PTP catalysis and active-site architecture using the residue numbering system of PTP1B, because this enzyme is considered an archetypal member of the classical PTP family (167). The catalytic core of classical PTPs features a central, highly twisted, mixed β-sheet flanked by helices on both sides (Fig. 2A). The active site is located at the middle of the C-terminal edge of the sheet. The PTP signature sequence motif, (I/V)HCXXGXXR(S/T), begins at the C-terminus of the final strand of the sheet (β8), and encompasses the loop connecting β8 to αC (known as the P-loop) and the first turn of αC (Fig. 2A, green) (2, 67, 195). The side chain of the catalytic Cys points into the P-loop and engages several hydrogen bond donors of the loop and the N-terminus of αC (Fig. 2B) (6, 124). These interactions induce a substantial depression of the pK_a of the catalytic Cys from the expected value of over 8.0 to below 6.0 (67, 100, 195). Thus, the cysteine thiol group at the active site of the classical PTPs exists almost exclusively in the nucleophilic, thiolate form (RS^-) at physiological pH. The imidazole side chain of His214 hydrogen bonds to the carbonyl residue of Cys215, and evidence gathered in the context of the *Yersinia* PTP (YopH) suggests that this residue plays an important role in maintaining the ability of the P-loop to stabilize the active-site thiolate residue (197).

Substrate phosphotyrosine residues bind at the active site *via* an array of interactions with backbone amide residues and the guanidinium side chain of Arg221 in the P-loop (Fig. 1) (6, 67, 70, 195). These interactions position the substrate's phosphoryl residue for nucleophilic attack by the thiolate residue of Cys215 (139, 144). In-line attack of the active-site thiolate residue on the phosphorus atom of the phosphotyrosine

Departments of Chemistry and Biochemistry, University of Missouri, Columbia, Missouri.

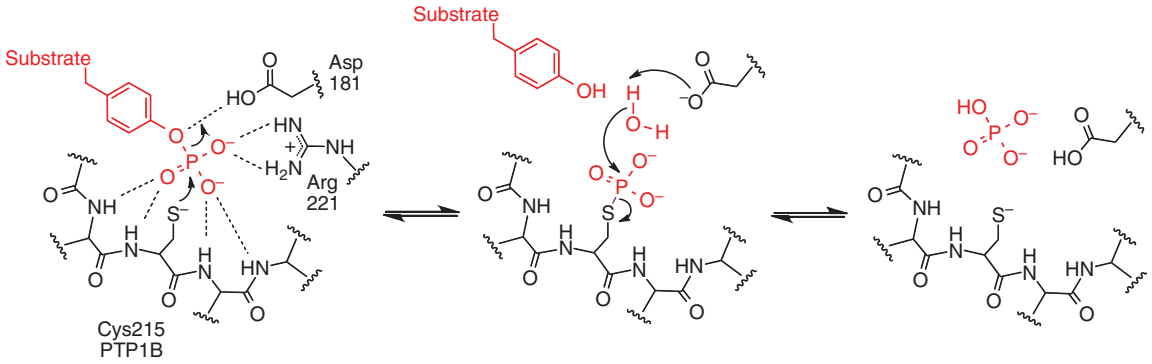

FIG. 1. Catalytic mechanism of protein tyrosine phosphatases (PTPs). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

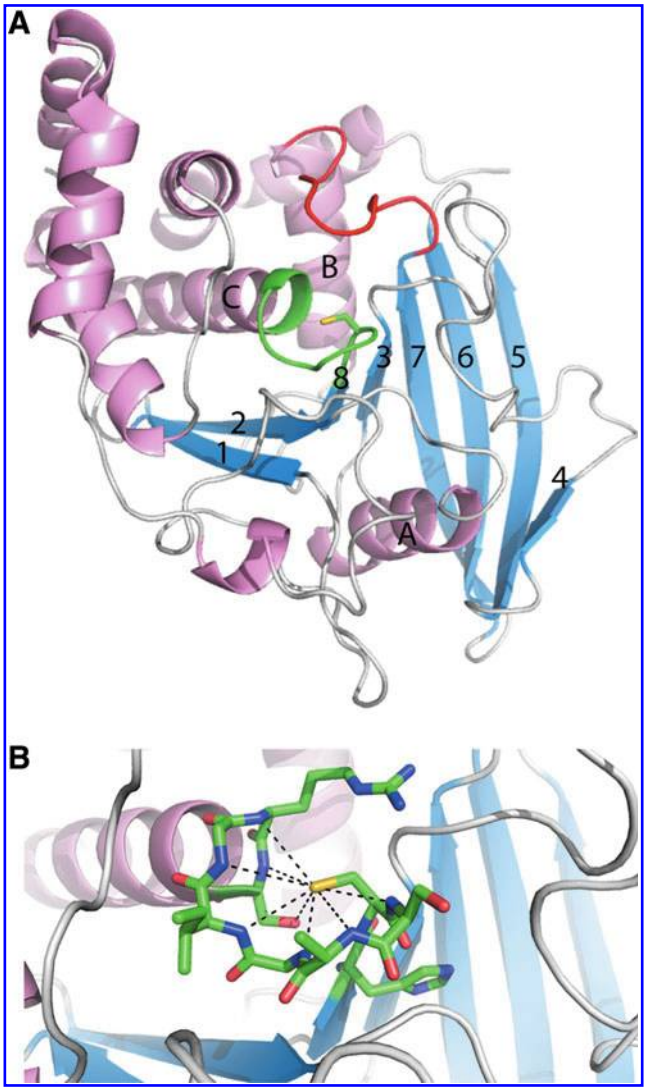

FIG. 2. Three-dimensional structure of classical PTPs. (A) The overall fold of PTPs as exemplified by PTP1B (PDB code 1SUG). Residues of the PTP signature sequence motif are green. The WPD loop is red. Strands are labeled with numbers. Helices A, B, and C are also labeled. **(B)** A close-up view of the P-loop of PTP1B. PTP, protein tyrosine phosphatase. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

substrate yields a phosphoryl cysteine intermediate (37, 122). In the *Yersinia* enzyme, this step proceeds *via* a dissociative mechanism (195) in which the metaphosphate group is stabilized in flight by the Arg of the P-loop (Arg221 in PTP1B and Arg409 in YopH), whereas an Asp residue (Asp 181 in PTP1B and Asp356 in YopH) serves as a general acid catalyst to protonate the departing hydroxyl group of the substrate tyrosine residue (100). In the final step of catalysis, Asp181 acts as a general base to catalyze attack of water on the phosphoryl cysteine intermediate leading to release of inorganic phosphate and regeneration of the native enzyme (100). The side chain of a Gln residue (Gln 262 in PTP1B, Gln 446 in YopH) in the so-called Q-loop of the enzyme, is thought to position a molecule of water for attack on the phosphorylcysteine intermediate in the final step of the catalytic cycle (199).

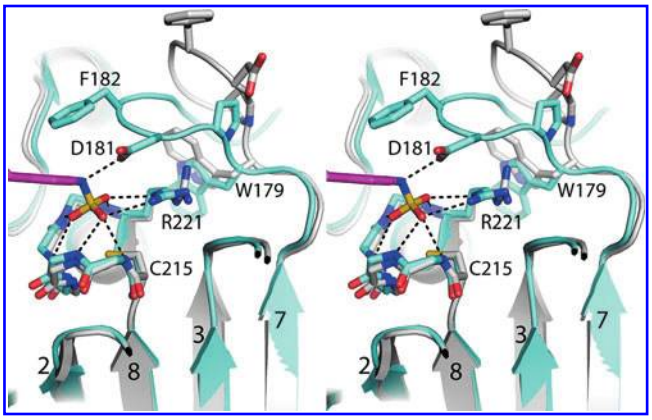

FIG. 3. Conformations of the WPD loop (stereographic view). The structure of PTP1B complexed with a sulfamic acid inhibitor (PDB code 2F71) is shown in cyan with the inhibitor in magenta. The structure of ligand-free PTP1B is shown in white (PDB code 2HNP). Note that the WPD loop closes upon the inhibitor, which brings Asp181 close to the inhibitor atom that represents the O atom of the scissile bond of the substrate. This configuration is consistent with Asp181 functioning as the acid that protonates the leaving group. Also, rotation of Arg221, which results in formation of electrostatic interactions with the substrate phosphoryl group, is coordinated with the closing of the WPD loop. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

The WPD loop connects $\beta 7$ to αB (Fig. 2A, red) and is important because it contains the Asp181 residue that functions as a general acid–base catalyst that protonates the phenolate leaving group and activates a water molecule for hydrolysis of the phosphoryl-enzyme intermediate. The WPD loop is flexible and exhibits at least two conformations corresponding to open and closed states (Fig. 3, open gray, closed cyan). The closed state is typically associated with the binding of substrate. Interactions of Phe182 with the aromatic ring of the substrate phosphoryltyrosine residue are important in driving closure of the WPD-loop (Fig. 3) (139, 144). A recent structure of PTP1B in the ligand-free state also has a closed WPD loop, suggesting that both open and closed conformations are likely accessed in solution in the absence of substrate (124). In classical PTPs, a tyrosine residue (Tyr46, in PTP1B) gives the active-site depth that confers specificity for hydrolysis of phosphoryltyrosine residues (139, 144). The phosphoryl group of phosphoryltyrosine residues can penetrate this relatively deep active-site cavity to access the enzyme's catalytic machinery, whereas phosphorylserine and threonine residues cannot.

Substrate Specificity and Regulation of PTPs

For many years, PTPs were viewed as housekeeping enzymes charged with the routine task of removing phosphoryl groups from tyrosine residues in a nonselective and unregulated manner. Research over the last 10–15 years has rendered this view obsolete. We now know that the human genome codes for about 100 different proteins in the PTP superfamily (3, 167). About 65 genes code for dual-specificity phosphatases (167). These enzymes possess rather shallow active sites that catalyze the hydrolysis of phosphoryl residues from phosphoserine, threonine, and tyrosine residues. Some members of this family process nonprotein substrates including phosphatidylinositols and RNA (167). There are 37 classical PTP genes and 35 of these encode catalytically active proteins that selectively hydrolyze phosphoryl groups from phosphotyrosine residues (167). The catalytic activities of these enzymes in cells are tightly regulated and they display distinct substrate specificities (36, 104, 167). Although the phosphotyrosine binding pocket containing the catalytic cysteine residue is structurally homologous in all PTPs, enzyme–substrate interactions beyond the catalytic pocket can lend substrate specificity to the catalytic domain of some PTPs (14, 51, 139, 144). Subcellular localization can also lead to specificity for selected substrates, as outlined in Dixon's Zip-Code model (105, 191). In some cases, noncovalent binding with noncatalytic domains of the PTP serves to bring substrates to the catalytic domain either directly or *via* adapter proteins (172).

The cellular activity of PTPs may be regulated at the level of gene expression, by protein–protein interactions mediated by Src homology 2 (SH2) domains within the enzyme, or phosphorylation of serine/threonine or tyrosine residues (36, 67, 104, 133, 146, 167). Receptor PTPs may be regulated by dimerization or the binding of various extracellular ligands (36, 104, 167). In recent years, oxidation–reduction (redox) reactions centered on the catalytic cysteine residue have emerged as an important mechanism for the regulation of cellular PTP activity (38, 58, 59, 95, 103, 107, 161, 162, 170). This review is focused on the redox regulation of classical PTP enzymes and is intended to update and complement several excellent reviews previously published on this subject (26, 35, 97, 141, 167, 170).

Redox regulation of PTPs in cells is mediated by hydrogen peroxide (H_2O_2) (38, 45, 47, 58, 59, 95, 103, 107, 128, 136, 159–162, 170, 178). For most of the 190 years since its discovery (166), H_2O_2 has been viewed as a toxic molecule (44, 151); however, more recently it has become clear that tightly controlled production of low concentrations of H_2O_2 plays an important role in regulating a number of important cell signaling pathways, including those that involved in cell proliferation, differentiation, metabolism, angiogenesis, and cell migration (4, 45, 47, 53, 69, 136, 159, 160, 178). Flavin-containing NADPH oxidase (Nox) and dual-cofactor flavin and heme NADPH oxidase-peroxidase enzymes are responsible for the regulated production of H_2O_2 in signal transduction (90, 91, 175). PTPs are an important target of H_2O_2 generated in signal transduction processes (15, 38, 58, 59, 75, 95, 103, 107, 136, 161, 162, 170). Reactive nitrogen species, including nitric oxide, peroxynitrite, and nitrosothiols, can also regulate the activity of PTPs (8, 21, 98, 164) though these processes will not be covered in this review.

The Chemistry of Thiol Oxidation

As a prelude to our discussion of the chemical and biochemical mechanisms underlying the redox regulation of PTPs, we provide here a brief overview of some fundamental sulfur redox chemistry that is central to these processes. The

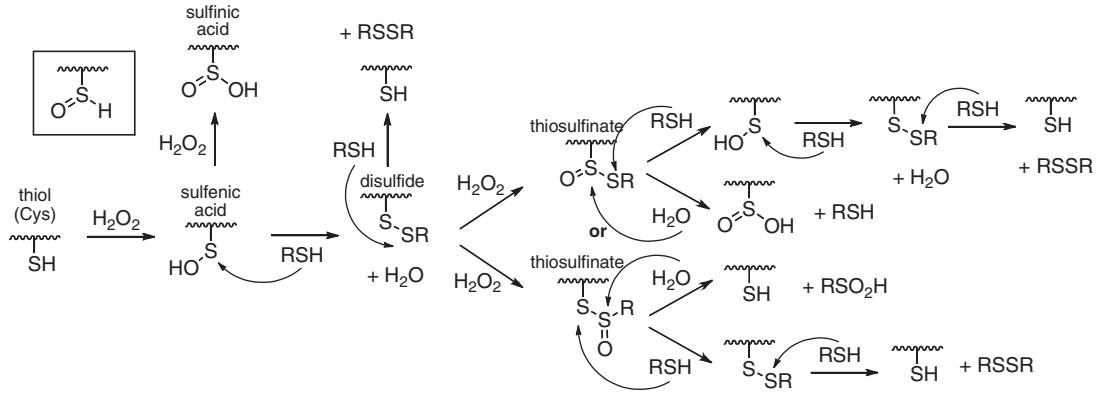

FIG. 4. Mechanisms of oxidative inactivation and thiol-mediated recovery of catalytic activity for an enzyme that contains a sulfenic acid redox switch.

Table 1. Rate Constants for Reactions That Are Relevant to Redox Regulation of Protein Tyrosine Phosphatases

Reaction	*Rate constant*	*Conditions*	*References*
$RSH + H_2O_2 \rightarrow RSOH$			
GSH (pK_a 8.8)	0.87 M^{-1} s^{-1}	37°C, pH 7.4	182
Cysteine (pK_a 8.3)	2.9 M^{-1} s^{-1}	37°C, pH 7.4	182
N-Acetylcysteine (pK_a 9.5)	0.16 M^{-1} s^{-1}	37°C, pH 7.4	182
PTP1B (pK_a 5.5)	9.1 M^{-1} s^{-1}	30°C, pH 7	9, 38
	42.8 M^{-1} s^{-1}	25°C, pH 7.0	
ΔSHP-1; ΔSHP-2 (catalytic domains)	9.4 M^{-1} s^{-1}, 8.8 M^{-1} s^{-1}	25°C, pH 7.5	24
MKP3	9.6 M^{-1} s^{-1}	20°C, pH 8.0	149
Cdc25B	164 M^{-1} s^{-1}	20°C, pH 7.0	155
	332 M^{-1} s^{-1}	37°C, pH 6.5	
Cdc25C	120 M^{-1} s^{-1}	20°C, pH 7.0	155
LMW-PTP (isoform 1)	13.1 M^{-1} s^{-1}	25°C, pH 7.5	22
Papain (Cys protease)	62 M^{-1} s^{-1}	23°C, pH 7.0	99
Human serum albumin	2.7 M^{-1} s^{-1}	37°C, pH 7.4	174
"RS^-" DTT monoanion	7 M^{-1} s^{-1}	20°C	193
"RS^-" GSH, cysteine or *N*-acetylcysteine	17–18 M^{-1} s^{-1}	25°C	135, 182
	19–26 M^{-1} s^{-1}	37°C	
Peroxiredoxin (*M. tuberculosis*) pK_a 5.2	8.2×10^4 M^{-1} s^{-1}	25°C, pH 7.4	65
NADH Peroxidase (H10Q mutant)	1.94×10^4 M^{-1} s^{-1}	5°C, pH 7.0	31
Peroxiredoxin 2 (pK_a 5–6)	1.3×10^7 M^{-1} s^{-1}	20°C, pH 7.4	126
$RSOH + H_2O_2 \rightarrow RSO_2H$			
Cdc25B-SOH (C426S mutant)	47 M^{-1} s^{-1}	20°C, pH 7.0	155
Cdc25C-SOH (C330S mutant)	110 M^{-1} s^{-1}	20°C, pH 7.0	155
MKP3-SOH (ΔC218S mutant)	101 M^{-1} s^{-1}	20°C, pH 8.0	149
Human serum albumin-SOH (HSA)	0.4 M^{-1} s^{-1}	37°C, pH 7.4	174
Peroxiredoxin-SOH (*M. tuberculosis*)	40 M^{-1} s^{-1}	25°C, pH 7.4	65
NADH Peroxidase-SOH (H10Q mutant)	1.8×10^4 M^{-1} s^{-1}	25°C, pH 7.0	31
$RSOH + R'SH \rightarrow RSSR'$			
HSA-SOH + cysteine	21.6 M^{-1} s^{-1}	25°C, pH 7.4	174
HSA-SOH + GSH	2.9 M^{-1} s^{-1}	25°C, pH 7.4	174
Cdc25B back-door cysteine	0.16 s^{-1}	20°C, pH 7.0	155
Cdc25C-SOH back-door cysteine	0.012 s^{-1}	20°C, pH 7.0	155
Cysteine-SOH + cysteine	$>1\times10^5$ M^{-1} s^{-1}	18°C, pH 7.0	115
Disulfide+H_2O_2			
$CH_3SSCH_3 + H_2O_2$	1×10^{-5} M^{-1} s^{-1}	0.1 *M* $H_2PO_4^-$	135
Thiosulfinate + RSH			
Cysteine thiosulfinate + Cys-SH	5×10^3 M^{-1} s^{-1}	18°C, pH 7.0	Figure 2 in 116
Phenyl thiosulfinate + BuS^-	2.9×10^6 M^{-1} s^{-1}	25°C (aqueous buffer	80
	~1 M^{-1} s^{-1}@ pH 7	cont. 60% dioxane)	
Thiosulfinate + H_2O			
Cysteine thiosulfinate + H_2O	5×10^{-4} s^{-1}	20°C, pH 7.0	Figure 5 in 114
Phenyl thiosulfinate + HO^-	170 M^{-1} s^{-1}	25°C (aqueous buffer	81
	1.7×10^{-5} s^{-1} @ pH 7	cont. 60% dioxane)	
Sulfinyl amide (model compound)			
Sulfinyl amide + 2-mercaptoethanol	5.5 M^{-1} s^{-1}	24°C, pH 7.0 (50% MeCN)	153
Sulfinyl amide + H_2O	4.5×10^{-4} s^{-1}	24°C, pH 7.0 (50% MeCN)	153
R'SH + RSSR			
GSSG + GSH (disulfide exchange)	0.41 M^{-1} s^{-1}	25°C, pH 7.0	78
PTP-S^- + GSSG → PTP-SSG	0.012 M^{-1} s^{-1}	30°C, pH 7.0	10
CysSSCys + GSH	10.2 M^{-1} s^{-1}	37°C, pH 7.4	71
GSSG + 2-mercaptoethanol	57 M^{-1} s^{-1}	30°C, pH 7.0	163
Reactivation of PTP_{ox}			
$MKP3_{ox}$ by thioredoxin/thioredoxin reductase (TR/TRR)	316 M^{-1} s^{-1}	20°C, pH 8.0	149
$MKP3_{ox}$ by GSH	1–2 M^{-1} s^{-1}	20°C, pH 8.0	149

(*continued*)

Table 1. (Continued)

Reaction	*Rate constant*	*Conditions*	*References*
$Cdc25B_{ox}$ by DTT	0.5 M^{-1} s^{-1}	20°C, pH 7.0	155
$Cdc25B_{ox}$ by TR/TRR	1007 M^{-1} s^{-1}	20°C, pH 7.0	155
$\Delta SHP\text{-}2_{ox}$ by TR/TRR	36.5 M^{-1} s^{-1}	25°C, pH 7.5	24
$\Delta SHP\text{-}1_{ox}$ by TR/TRR	Not reactivated	25°C, pH 7.5	24
$\Delta SHP\text{-}2_{ox}$ by GSH	0.07 M^{-1} s^{-1}	25°C, pH 7.5	24
$\Delta SHP\text{-}2_{ox}$ by DTT	0.84 M^{-1} s^{-1}	25°C, pH 7.5	24
PTP-SSG by glutaredoxin	483 M^{-1} s^{-1}	30°C, pH 7.0	10

DTT, dithiothreitol; GSH, glutathione; GSSG, glutathione disulfide; PTP, protein tyrosine phosphatase.

ability of cysteine residues to act as redox switches in PTPs (and other proteins) relies upon the unique capacity of the γ-sulfur atom in this amino acid to cycle reversibly between several different oxidation states under physiological conditions (18, 48, 123, 128, 130, 134). In this section, we will consider the fundamental chemical reactions that flow from the peroxide-mediated oxidation of cysteine residues.

Thiol oxidation proceeds *via* attack of equilibrium amounts of the thiolate anion on H_2O_2 (Fig. 4) (12, 135, 182). Accordingly, for biologically relevant alkyl thiols under physiological conditions, reaction rates with H_2O_2 are faster for more acidic, low pK_a thiols, which present a larger concentration of the anionic thiolate form at neutral pH (Table 1) (182). The reaction of the pure thiolate forms of cysteine, glutathione (GSH), and *N*-acetylcysteine with H_2O_2 at 25°C all occur with similar rate constants of 17–18 M^{-1} s^{-1} (135). The H_2O_2-mediated oxidation of cysteine residues in some proteins occurs at a much higher rate. For example, peroxiredoxins are oxidized by H_2O_2 with rate constants as high as 1×10^7 M^{-1} s^{-1} (65, 126). In these cases, the reaction likely is accelerated by binding of hydrogen peroxide to the enzyme active site or general acid catalysis that serves to protonate the hydroxide leaving group. Indeed, peroxide-mediated oxidations of sulfur atoms in small molecules are subject to acid catalysis (5, 12, 33).

Reaction of a thiol group with H_2O_2 yields a sulfenic acid residue (Fig. 4) (28, 54, 55, 79). Sulfenic acids are most commonly depicted in the *O*-protonated form (RS-OH). Indeed, microwave and infrared spectroscopic results suggest that the *O*-protonated form is favored for some sulfenic acids in the gas phase, in crystals, and in solution (19, 27, 117, 125). Nonetheless, there is evidence that, in some cases, the *O*-protonated form exists in equilibrium with the *S*-protonated form (inset, Fig. 4) in solution and in the neat liquid (34). The pK_a value for *t*-butylsulfenic acid has been reported as 10.5 (121). Other measurements place a substantially lower value on biologically relevant sulfenic acids. For example, a pK_a value of 7.6 has been reported for cysteine sulfenic acid (42). In the protein MtAhpE a value of 6.6 was reported for the active-site cysteine sulfenic acid (65) and in AhpC the pK_a of an active-site sulfenic acid was measured at 6.1 (124).

Low-molecular-weight sulfenic acids are typically quite unstable because they can decompose by a variety of pathways (79). Sulfenic acids have the potential to act as nucleophiles (23, 62, 76) and electrophiles (54, 77, 79, 110), or to undergo dimerization to the corresponding thiosulfinate (34, 79). On the other hand, only a few reactions are commonly available to cysteine sulfenic acid residues sequestered within the active site of proteins. First, protein sulfenic acids may undergo further peroxide-mediated oxidation to yield the sulfinic acid (Fig. 4) (43, 74, 174). The rate at which sulfenic acids react with H_2O_2 typically is comparable to that for the initial reaction of the corresponding thiol with H_2O_2 (Table 1). Except in the case of peroxiredoxins (68), oxidation of cysteine residues in proteins to the sulfinic acid form is biochemically irreversible and, therefore, constitutes destruction of a redox switch. Second, sulfenic acids are subject to nucleophilic attack by thiols (43, 54, 79, 174). Thus, either low-molecular-weight thiols (Fig. 4 and Table 1) such as GSH or neighboring cysteine residues in the protein (Fig. 5) can react with the cysteine sulfenic acid residue to yield a disulfide linkage. Finally, recent crystallographic studies of PTP1B revealed that the amide nitrogen on the carboxyl side of a cysteine residue can undergo intramolecular cyclization onto the sulfenic acid residue to yield an acyl sulfenamide (Fig. 6) (140, 177). The resulting structure is formally known as an isothiazolidin-3-one, but is often informally referred to as a sulfenyl amide (140, 177). Chemical model reactions helped clarify the

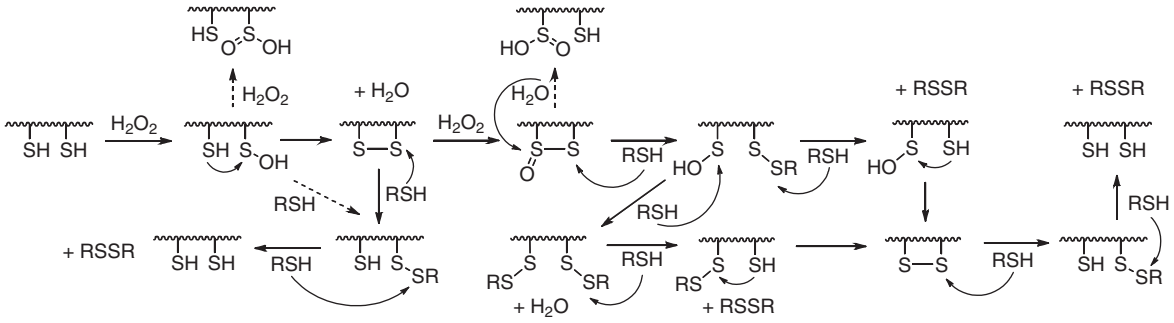

FIG. 5. Potential mechanisms of oxidative inactivation and thiol-mediated recovery of catalytic activity for a two-cysteine redox switch.

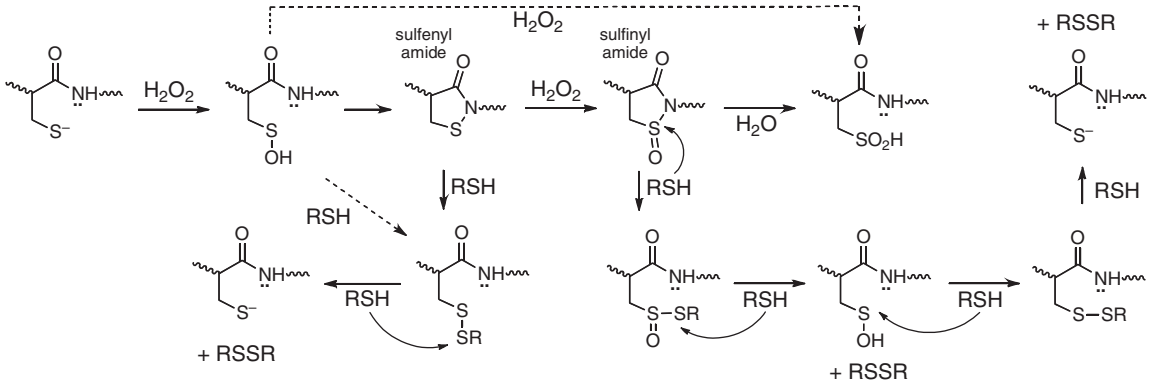

FIG. 6. Potential mechanisms of oxidative inactivation and thiol-mediated recovery of catalytic activity for sulfenyl amide redox switch.

mechanism by which the sulfenyl amide residue forms by showing that cyclization of an amide nitrogen atom onto a neighboring sulfenic acid residue is a facile reaction under physiological conditions (154). Thus, the sulfenyl amide formation first seen in the context of PTP1B may be a general mechanism for redox regulation of protein function. Both the disulfide and sulfenyl amide linkages allow for regeneration of the native cysteine residue *via* reactions with small molecule thiols such as GSH and dithiothreitol, or thiol-containing proteins such as thioredoxin, glutaredoxin, and peroxiredoxin (Figs. 4–6 and Table 1). These reactions may proceed *via* glutathionylated (protein-SSG) intermediates that can have significant lifetimes in the cell (9, 32, 137, 154). In the remainder of this review, the terms "irreversible oxidation" and "overoxidation" refer to the generation of an inactive enzyme whose activity cannot be regenerated by treatment with thiols.

Conversion of protein sulfenic acids to either disulfides or sulfenyl amides generally is thought to protect a cysteine redox switch against irreversible overoxidation to the sulfinic acid. The intramolecular nature of the reactions involved in the formation of sulfenyl amide and intraprotein disulfide residues affords a kinetic advantage that allows these processes to compete favorably against the bimolecular reaction of the sulfenic acid with low concentrations of H_2O_2. There are at least two possible mechanisms by which conversion of a sulfenic acid to a disulfide or sulfenyl amide might inhibit irreversible overoxidation. First, disulfides and sulfenyl amides may be resistant to further oxidation relative to the unprotected sulfenic acid residue. Although this assumption is likely correct, to our knowledge, there exists little quantitative data that speak to the issue. One-electron oxidation potentials show that the disulfide group ($E^\circ = 1.39$ V *vs.* NHE in water) is resistant to oxidation relative to a thiolate group ($E^\circ = 0.8$ V) (17, 198); however, the required comparison values for the sulfenic acid and sulfenyl amide functional groups are not available. The more relevant issue is the relative reactivity of thiols, sulfenic acids, disulfides, and sulfenyl amide residues with H_2O_2. Along these lines, Richardson and coworkers reported that H_2O_2-mediated oxidation of methyl disulfide occurs $\sim$1 million times more slowly than the corresponding reaction with thiol (135). However, again, kinetic benchmarks for the inherent susceptibility of the sulfenyl amide and sulfenic acid groups toward peroxide-mediated oxidation are not available.

Irrespective of the degree to which disulfides and sulfenyl amides resist further oxidation, there is a second mechanism by which conversion of a sulfenic acid to these functional groups can protect against overoxidation of a protein redox switch. Unlike an unprotected single-cysteine sulfenic acid redox switch, for which further oxidation to the sulfinic acid represents irreversible destruction, further oxidation of disulfide and sulfenyl amide residues yields thiosulfinate and sulfinyl amide groups that can be returned to the native thiol form by reactions with biological thiols (Figs. 4–6 and Table 1) (79, 80, 116, 153). These thiol-mediated resolving reactions are complex and proceed *via* a number of transient, obligate intermediates (Figs. 4–6).

Both the thiosulfinate and sulfinyl amide groups are susceptible to hydrolysis reactions that have the potential to cause irreversible destruction of the redox switch (Figs. 4–6 and Table 1) (79, 81, 114, 120, 153). However, in the context of small molecule model compounds, rate constants for the thiol reactions are much larger than those for hydrolysis, and at typical physiological concentrations if water and thiol enjoy equal access to the thiosulfinate or sulfinyl amide groups, hydrolytic destruction is expected to represent a minor pathway (Table 1) (79, 81, 114, 153). Thus, irreversible oxidative destruction of disulfide and sulfenyl amide redox switches likely requires conversion to the sulfonyl ($>SO_2$) derivatives that presumably requires relatively harsh oxidizing conditions. Overall, conversions of cysteine sulfenic acids to disulfides or sulfenyl amides provide failsafe mechanisms that protect redox-switched proteins against irreversible overoxidation.

As an aside, a survey of sulfenic acid reactions in the organic chemistry literature suggests that amino acid side chains other than cysteine have the potential to engage in reactions with protein sulfenic acids (Fig. 7). For example, alcohols and amines react with sulfenic acids under quite mild conditions to give sulfenate esters and sulfenamides, respectively (Fig. 7A, B) (1, 50, 54, 99). (Care should be taken to distinguish *sulfenamides*, derived from the reaction of aryl or alkylamines with sulfenic acids, from *sulfenyl amides*, derived from the reaction of an amide nitrogen with a sulfenic acid.) Reactions central to the action of the drug omeprazole (prilosec) demonstrate that a sulfenic acid intermediate can undergo intramolecular reaction with a benzimidazole nitrogen under physiological conditions to generate a sulfenamide (Fig. 7C) (83, 148). Together, these reactions suggest possible

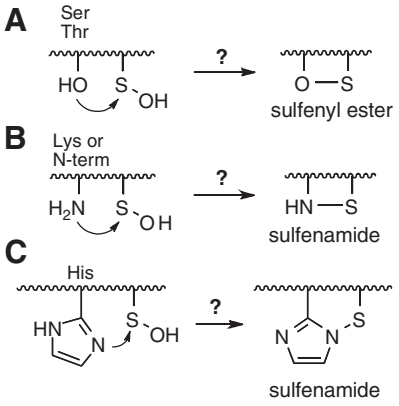

FIG. 7. Potential protein redox switches involving cys-ser/thr, cys-lys or cys-His pairs.

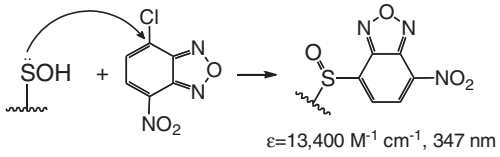

FIG. 8. Labeling of protein sulfenic acid residues by NBD-Cl.

roles for serine, lysine, or histidine side chains in redox regulation or oxidative degradation of protein function (Fig. 7). In the case of the potential histidine reaction, a hint of this type of reactivity has been seen in a recent crystal structure of an archael peroxiredoxin showing a histidine nitrogen coordinated to a sulfenic acid residue (118). Finally, cysteine residues are observed to form covalent crosslinks with tyrosine side chains in several proteins. The mechanism of these processes remains uncertain, but it is conceivable that in some cases, crosslinking involves direct reaction of a sulfenic acid with the tyrosine side chain (46).

Early Recognition That the Activity of PTPs Is Regulated by H_2O_2

In the early 1990s, several studies were published showing that H_2O_2 added exogenously or generated in response to the stimulation of cells with growth factors caused a decrease in cellular PTP activity and a corresponding increase in tyrosine phosphorylation (58, 59, 132, 161, 162, 200). In 1998, two reports detailed the *in vitro* inactivation of purified PTPs by H_2O_2 (38, 95). PTPs investigated in this early work included the dual specificity phosphatase VHR and the classical enzymes LAR and PTP1. H_2O_2 causes time-dependent inactivation of these PTPs, with rate constants ranging from 9 to 18 M^{-1} s^{-1} (38). A subsequent study measured the rate constant for inactivation of PTP1B by H_2O_2 at 42.8 M^{-1} s^{-1} (Table 1) (9). Time-dependent inactivation was consistent with a process involving covalent modification of the enzyme (152). Further experiments showing that substrates and competitive inhibitors slowed the inactivation reactions provided evidence that H_2O_2 reacts with a residue at the enzyme active site (38). Inactivation of PTP1B by H_2O_2 selectively blocked modification of the active-site peptide by iodoacetic acid, consistent with selective oxidation of the catalytic cysteine residue 215 (95). *t*-Butyl hydroperoxide and cumene hydroperoxide were incapable of inactivating the enzyme, presumably because steric hinderance prevented the peroxide groups from reaching the catalytic cysteine residue at the bottom of the catalytic pocket (38). More recent studies showed that a relatively unhindered organic hydroperoxide, 2-hydroperoxytetrahydrofuran, inactivated PTP1B with a rate constant comparable to H_2O_2 (20 M^{-1} s^{-1}) and more reactive aromatic acyl peroxides inactivated the enzyme very effectively (20,000 M^{-1} s^{-1}) (13). Importantly, in all of the peroxide-mediated inactivation reactions described above, PTP activity could be recovered by treatment with thiol (13, 38).

The structural nature of the oxidized enzymes in these seminal studies was not exhaustively characterized (38). In light of current knowledge, the results were consistent with oxidation of the catalytic cysteine residue to either sulfenic acid, disulfide, or sulfenyl amide intermediates. In one case, the sulfenic acid-labeling reagent 7-chloro-2-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl; Fig. 8) (41, 127) was used as a spectroscopic label to provide evidence for generation of a sulfenic acid residue on the dual specificity phosphatase VHR (38). It was later noted that the results of this labeling experiment were suggestive of a mixture of sulfenic acid and thiol adducts of NBD formed on the protein, rather than a pure sulfenic acid adduct (28). A 1999 study reported that treatment of oxidatively inactivated PTP1B with NBD-Cl, followed by MALDI mass spectrometry, led to detection of the active-site tryptic fragment bearing the NBD adduct at the catalytic Cys215 (Fig. 8) (9).

This early work showed that the catalytic cysteine of PTPs is susceptible to thiol-reversible oxidation. These results, combined with the backdrop of information that existed in the mid 1990s regarding other redox-regulated proteins (28), may have created a widespread notion that oxidatively inactivated PTPs exist with their catalytic cysteine residue in the sulfenic acid form. Although this could be true in some cases, the literature surveyed in this review suggests that the active-site sulfenic acid residue generated during oxidative inactivation of the PTPs may commonly undergo secondary reactions with cysteine residues to generate a disulfide linkage. Formation of a sulfenyl amide intermediate is also possible, although it remains unclear whether this is a common process.

Lessons Learned from Redox Regulation of Dual Specificity Phosphatases: Important Roles for Back-Door and Interdomain Cysteine Residues

Redox regulation has been examined for a number of dual specificity phosphatases. A common theme emerging from these studies is that the active-site cysteine residue becomes engaged as an intraprotein disulfide during oxidative inactivation. The enzyme Cdc25B provides a good example (20, 155). This dual-specificity phosphatase contains a so-called back-door cysteine residue located within the active-site cavity at a distance of 4.5 Å from the catalytic cysteine residue (measured C_β-to-C_β in the X-ray structure of the native enzyme; Fig. 9). Upon treatment of Cdc25B with H_2O_2, the back-door cysteine traps the initially generated sulfenic acid intermediate to yield a disulfide linkage as shown in Figures 5 and 9 (20, 155). Mutagenesis studies demonstrated that the presence of the disulfide-forming, back-door cysteine protects the catalytic cysteine residue against irreversible overoxidation

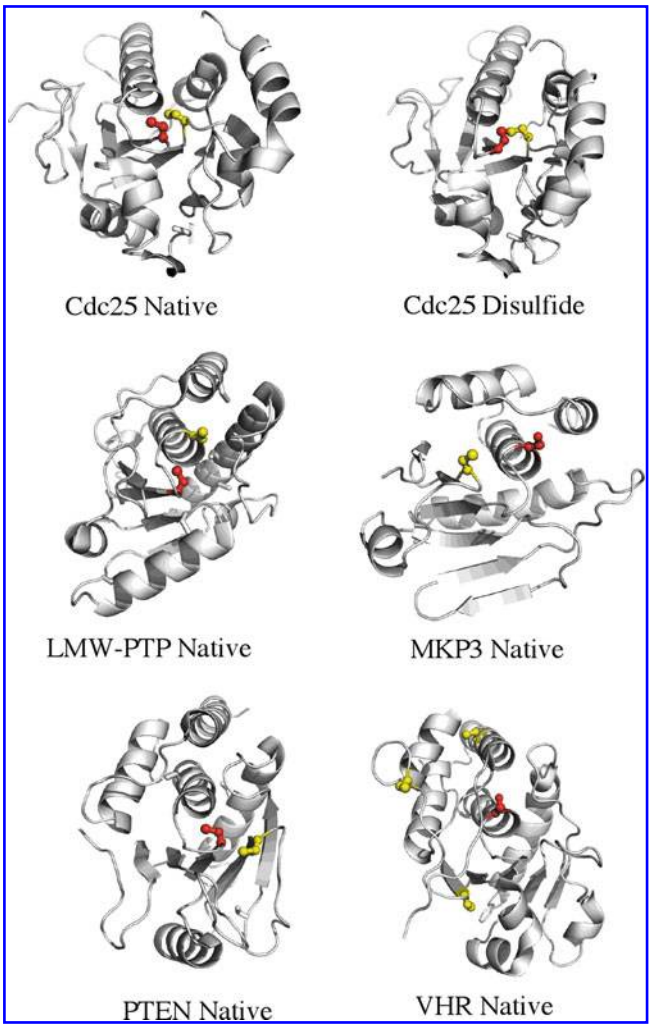

FIG. 9. Back-door cysteines in dual-specificity phosphatases. The catalytic cysteines are shown in red and back-door cysteines in yellow. VHR has no obvious back-door cysteines. Images were prepared using Pymol from the following crystal structures: 1YS0 (Cdc25 native), 1YMK (Cdc25 disulfide), 1XWW (LMW-PTP), 1MKP (MKP3), 1D54 (PTEN), and 1J4×(VHR). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

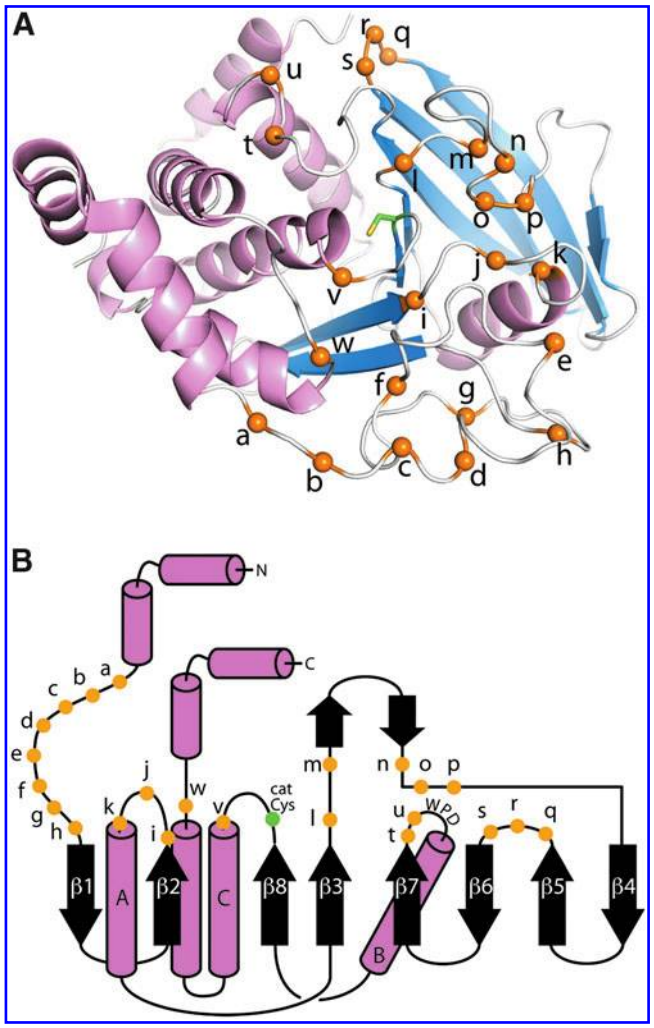

FIG. 10. Three-dimensional structural context of cysteine residues located on the active-site face of classic PTPs. (A) The locations of cysteine residues of 30 PTPs of known three-dimensional structure are mapped onto the structure of PTP1B. These residues are denoted by orange spheres and are labeled a–w. The catalytic cysteine is shown in green sticks. Residue numbers of the cysteine residues in the data set used for this analysis are listed in Table 2. **(B)** Cysteine residues from panel A are shown in a topology diagram representing the classic PTP fold, as exemplified by PTP1B. The orange circles represent the locations of cysteine residues. The green circle represents the catalytic cysteine. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

to the sulfinic acid. Indeed, the rate constants estimated for intramolecular disulfide formation ($0.16\,s^{-1}$) and H_2O_2-mediated conversion of the sulfenic acid to the sulfinic acid ($47\,M^{-1}\,s^{-1}$) allow us to calculate that the rate of irreversible overoxidation of Cdc25B will not match the rate of intramolecular disulfide formation until H_2O_2 reaches a concentration of 1 m*M* or above. These values, along with others shown in Table 1, provide a quantitative description of how disulfide formation protects a cysteine-based redox switch against irreversible overoxidation. The oxidatively inactivated form of Cdc25B does not bind substrate with high affinity and X-ray structural analysis suggests that disulfide formation distorts the P-loop of the enzyme in manner that precludes binding of phosphotyrosine residues (Fig. 9) (20, 155). Despite the facile formation of the active site disulfide in solution, it was possible to observe the intermediate sulfenic acid in crystallographic studies (20).

Other dual-specificity phosphatases, including KAP (156), PTEN (86, 94), MKP3 (149), Cdc25C (155), and LMW-PTP (22, 75), also contain back-door cysteine residues that engage with the catalytic cysteine residues to generate disulfides during oxidative inactivation of the enzymes. These back-door cysteines reside 5.7–10 Å away from the catalytic cysteines in the crystal structures of the native enzymes (Fig. 9). In the crystal structure of native MKP3 (158), the back-door cysteine-to-catalytic cysteine distance of 10 Å is clearly much farther than the expected C_β-to-C_β distance in a disulfide (about 4.0 Å). In this case, the back-door cysteine resides in a loop region that is evidently flexible enough to allow reaction with the sulfenic

acid residue generated on the active-site cysteine residue during oxidative inactivation of the enzyme. These findings highlight the fact that cysteine residues that are seemingly distant in the X-ray structure of the native enzyme may engage with an active-site sulfenic acid during oxidative inactivation to yield a disulfide linkage.

Many phosphatases are multidomain enzymes (167) and studies with the full length constructs of the dual-specificity phosphatases MKP3 and hYVH1/DUSP12 have provided evidence that cysteine residues in regions beyond the catalytic domain can engage with the active-site cysteine during oxidative inactivation to generate interdomain disulfides that protect the enzyme against oxidation and irreversible overoxidation (16, 149). Finally, in the case of VHR, one of the only phosphatases for which there is evidence supporting the existence of an unprotected sulfenic acid at the active site of the oxidized enzyme, it may be significant that no obvious backdoor cysteines can be identified in the X-ray crystal structure of the native enzyme (Fig. 9) (192). In this case, the nearest cysteine residue is ~11 Å distant from the catalytic cysteine and resides within a region of β-sheet secondary structure rather than in a flexible loop (Fig. 9).

Overall, the current picture of redox regulation of the dual specificity phosphatases suggests that engagement of backdoor and interdomain cysteines with the catalytic cysteine residue is a common occurrence during oxidative inactivation. Further, the data suggest that back-door cysteines can be rather distant from the catalytic cysteine if they reside in a region of flexible protein structure. Results in the context of the Cdc25 enzymes showed that disulfide formation protects the catalytic cysteine residue against irreversible overoxidation.

Redox Regulation of Classical PTPs

The cellular activity of a number of classical PTPs, including PTP1B (95, 101, 103, 106), TCPTP (101, 106), PEST (101, 183), LAR (15), SHP-1 (84), SHP-2 (15, 87, 107), HePTP (93), and PTPκ (185), is regulated by redox processes. It seems likely that the number of PTPs recognized to be redox regulated will continue to increase in coming years. To date, however, redox regulation has been well characterized for only a handful of classical PTPs. Here, we will discuss chemical and structural features surrounding the redox regulation of LYP, SHP-1/2, and PTP1B by H_2O_2. The dimerization and catalytic activity of some receptor PTPs can be regulated by redox reactions at a noncatalytic D2-domain (35, 176, 186); however, these systems are not discussed further in this review.

Redox Regulation of LYP

The PTP LYP (PTPN22) is expressed in cells of hematapoietic origin (29). Dephosphorylation of Lck and ZAP-70 kinases by LYP serves to downregulate T-cell signaling (29, 190). A burst of H_2O_2 accompanies T-cell signaling (85, 87)

Table 2A. Residue Numbers of Cysteines Located on the Active-Site Face of Classic Protein Tyrosine Phosphatases

PTP	*PDB code*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*
PTPN22 LYP	2P6X												129
SHP-1	1FPR										329		
SHP-2	2SHP										333		
PTP1B	2F71			32								92	
T-cell PTP	1L8K											94	
LAR	1LAR												
RPTPα (D2)	1P15												
RPTPμ	1RPM												
PTPL1, PTPN13	1WCH			2229						2282			
RPTPα (D1)	1YFO	242		248									
RPTP CD45	1YGR												
HePTP	1ZC0							116					
PTPRR	2A8B							433					
PTPN3	2B49											725	
PTPN5	2BV5							316					
PTPN14	2BZL											990	
RPTPκ	2C7S												
RPTPσ	2FH7												
PTPRO	2GJT					964							
RPTPγ	2H4V												
RPTPβ	2HC1			1719									
PTP IA-2	2I1Y			726									
PTPN4	2I75				673					726		734	
RPTPε	2JJD								186				
PTPRD	2NV5												
PTPRJ	2NZ6												
PTPN18	2OC3		46										131
PTPRT	2OOQ												
PTPN9	2PA5				321		338						
PTPRN2	2QEP												

Table 2B. Residue Numbers of Cysteines Located on the Active-Site Face of Classic Protein Tyrosine Phosphatases

PTP	*PDB code*	*m*	*n*	*o*	*p*	*q*	*r*	*s*	*t*	*u*	*v*	*w*
PTPN22 LYP	2P6X			139							231	
SHP-1	1FPR			363								
SHP-2	2SHP			367								
PTP1B	2F71			121								
T-cell PTP	1L8K			123								
LAR	1LAR			1434								
RPTPα (D2)	1P15			635		660						
RPTPμ	1RPM			1008	1009							
PTPL1, PTPN13	1WCH			2319								
RPTPα (D1)	1YFO		339	341								473
RPTP CD45	1YGR	729		737		764						868
HePTP	1ZC0			183		207						
PTPRR	2A8B			501		525						
PTPN3	2B49			754		779						
PTPN5	2BV5			384								
PTPN14	2BZL							1050	1082			
RPTPκ	2C7S			996								
RPTPσ	2FH7			1501								
PTPRO	2GJT	1039		1047								
RPTPγ	2H4V			961				988				
RPTPβ	2HC1	1804		1812								
PTP IA-2	2I1Y			820			846					
PTPN4	2I75			763								
RPTPε	2JJD			244								
PTPRD	2NV5			1336								
PTPRJ	2NZ6	1141		1149								
PTPN18	2OC3			141							233	
PTPRT	2OOQ			1019						1079		
PTPN9	2PA5			412								
PTPRN2	2QEP			856			882					

and it has been suggested that this may inactivate cellular LYP as part of these signal transduction processes (173). Redox regulation of LYP in cells has not yet been demonstrated. Regulation of cellular LYP activity is of interest because inhibitors of LYP may be medicinally useful for the treatment of autoimmune disorders caused by gain-of-function mutations that increase LYP activity (190).

X-ray crystallographic studies of LYP revealed a back-door cysteine residue engaged as a disulfide with the catalytic cysteine residue (back-door Cys denoted as "l" in Fig. 10 and Table 2). This transformation induces minimal conformational change in the polypeptide chain relative to the native enzyme (173). The back-door Cys of LYP (Cys129) is located on the β3-β4 loop, and the C_α-C_α distance between this residue and the catalytic Cys (Cys227) is 5.4 Å (Fig. 11B). The distance between S atoms of these two Cys residues is 4.9 Å. Comparison the structures of the native (PDB 2QCJ) and disulfide-bonded enzymes (PDB 3H2X) shows that the disulfide formation requires rotation of the catalytic Cys by about 110° around its C_α-C_β bond vector, which brings the two S atoms within covalent bonding distance (173, 190). Presumably, oxidation of the catalytic Cys to the sulfenic acid (Cys-SOH) disrupts the electrostatic network involving the P-loop shown in Figure 2, which enables the necessary dihedral rotation. The back-door disulfide bond of LYP is structurally analogous to that formed in dual-specificity PTPs such as PTEN and Cdc25 (86, 94, 155). Another cysteine (Cys231) is located in the active site of LYP 7.55 Å from the catalytic cysteine (Fig. 11A), but does not form a disulfide with the active-site cysteine in this crystal structure. A structurally analogous cysteine engages the catalytic thiol as a disulfide during oxidative inactivation of LMW-PTP (22, 75). Although the crystallographic studies did not reveal engagement of Cys231 with the catalytic cysteine of LYP, there are indications that mutation of either potential back-door cysteine in this enzyme (Cys129 or Cys231) can affect the yield of catalytic activity that is recovered from oxidized enzyme upon treatment with thiol (173); however, there has been no kinetic analysis of inactivation, overoxidation, or reactivation for this enzyme. Thus, the interplay of these two back-door cysteines with the catalytic thiol group remains uncertain.

Redox Regulation of SHP-1/2

There are two SH2 domain-containing PTPs known as SHP-1 and SHP-2 (119). These proteins contain two tandem N-terminal SH2 domains. The native forms of these enzymes display relatively low catalytic activity because a portion of the SH2 domains blocks access to the active site (61). Docking of the SH2 domain to appropriate phosophotyrosine peptides frees the active of the enzyme and enables catalytic activity (92). SHP-1 and −2 show very high sequence and structure homology but regulate distinct cell signaling pathways (119). For example, the action of SHP-2 downregulates signaling pathways stimulated by T-cell receptor activation, endothelin-1, and platelet-derived growth factor and the activity

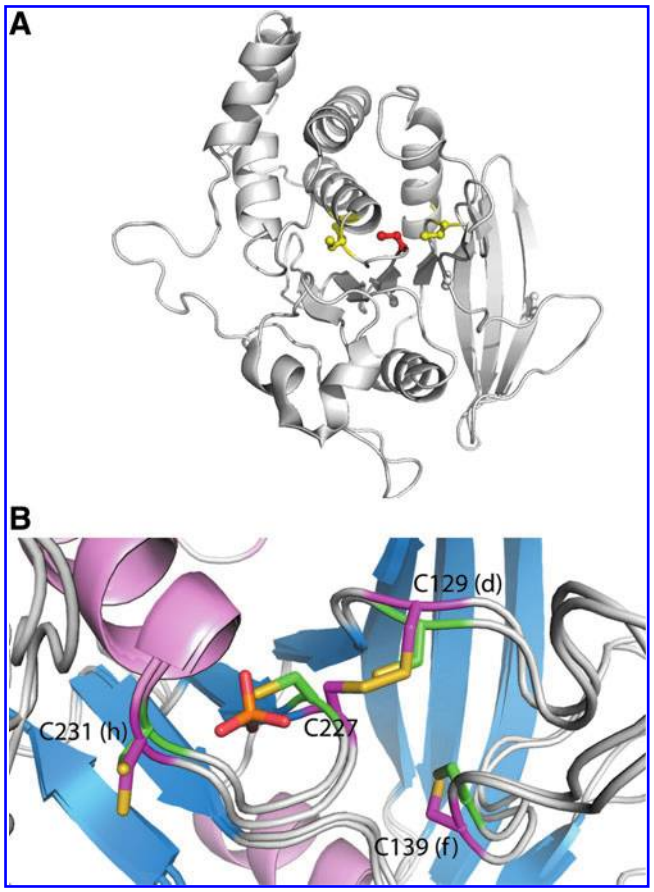

FIG. 11. Redox regulation of PTP-LYP. (A) Three-dimensional structure of LYP with the catalytic cysteine residue shown in red and the two potential backdoor cysteine residues shown in yellow (PDB code 2QCJ). Cysteine 129, which engages with the catalytic cysteine residue in the oxidized structure, is the yellow cysteine on the right. **(B)** Comparison of native (green side chains) and oxidatively inactivated (magenta side chain) LYP. The sulfate ion is bound to the oxidized enzyme (PDB codes 3H2X and 2QCJ). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

of the enzyme is regulated by redox processes in response to these stimuli (15, 87, 107, 108). On the other hand, the activity of SHP-1 is redox-regulated in B-cell receptor-stimulated signaling processes (84). SHP-2 is considered a potential medicinal target for the treatment of some leukemias and Noonan syndrome (189).

The catalytic domains of SHP-1 and SHP-2 are inactivated by H_2O_2 with second-order rate constants of 9.4 M^{-1} s^{-1} and 8.8 M^{-1} s^{-1}, respectively (24). Inactivation of the full-length enzymes containing the inhibitory SH2 domains is somewhat slower at 2.0 M^{-1} s^{-1} for SHP-1 and 2.4 M^{-1} s^{-1} for SHP-2 (24, 179). Catalytic activity of the oxidatively inactivated enzymes can be recovered by treatment with various thiols (24). Mass spectroscopic analysis revealed that the oxidatively inactivated enzymes possess a native cysteine thiol residue at the active site and a disulfide linkage between cysteines 329 and 363 in SHP-1 and 333 and 367 in SHP-2 (labeled j and o in Fig. 10 and Table 2) (24). This suggests that oxidation of the active-site cysteine residue initiates a disulfide relay (Fig. 12). The existence of this type of disulfide relay was foreshadowed by the results of previous thiol-titration experiments with SHP-1 (131). The structural environments of the cysteine residues involved in this disulfide relay are distinct from that of the back-door cysteines described earlier in this review. Prototypical back-door cysteines reside within the catalytic pocket of the enzyme. On the other hand, in crystal structures of native SHP-1/2, the cysteine residues involved in the disulfide relays reside at distal locations *outside* the catalytic pocket at distances of 6.9 and 9.4 Å (SHP-1, 369/329) and 8.0 and 11.6 Å (SHP-2 367/333) from the active-site cysteine residue (C_β-C_β; Fig. 13) (7, 187).

Studies in the context of SHP-1 show that oxidation of mutants lacking either of these distal cysteine residues leads to formation of a disulfide between the one remaining distal cysteine and the catalytic cysteine (shown as the central disulfide structures in Fig. 12) (24). The results suggest that both loops containing these distal cysteines in SHP-1/2 possess sufficient flexibility for engagement with the active-site sulfenic acid that is initially generated upon treatment of the enzyme with H_2O_2. A double-mutant lacking both of the distal cysteine residues is irreversibly oxidized even by relatively modest H_2O_2 concentrations, providing support for the notion that disulfide formation during the oxidative inactivation of these enzymes protects against irreversible overoxidation (24).

The redox regulation of SHP-1 and SHP-2 in cells was examined using a dimedone-based fluorescent probe that was designed to trap protein sulfenic acid residues (and *not* disulfides) (1, 96, 108, 129). The dimedone probes did, in fact, trap the SHP enzymes. This result is somewhat surprising in light of

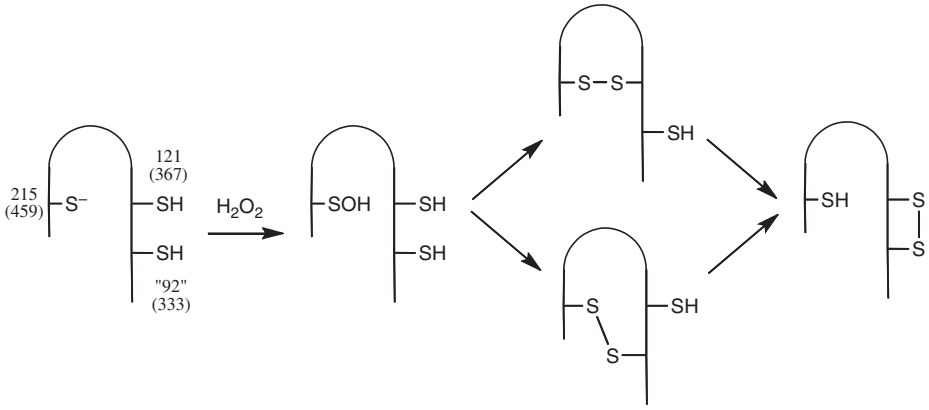

FIG. 12. Schematic mechanism for oxidative inactivation of SHP-1 and SHP-2. (The first number is PTP1B numbering and the second number is SHP-2 numbering.)

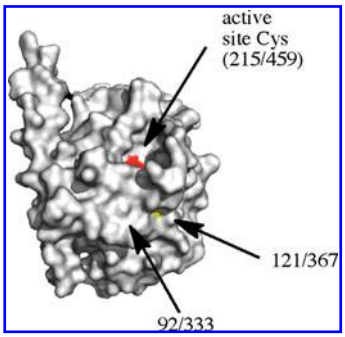

FIG. 13. Location of redox-active cysteines in SHP-2. The disulfide relay in SHP-2 involves distal cysteine residues that reside outside the active-site pocket. Cysteine 92/333 is buried in this surface view, but the general location is indicated by the arrow (PBD code 3B7O). The first number reflects PTP1B numbering and the second is SHP-2 numbering. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

the more recently elucidated disulfide relay described above. The results obtained with the dimedone probe may suggest that oxidatively inactivated SHP-1/2 exists in cells as a mixture of sulfenic acid and disulfide forms or that the probe can capture some of the sulfenic acid intermediate en route to the disulfide. Alternatively, the dimedone probes may react with a sulfenyl amide intermediate that is generated en route to the disulfides (150). Finally, a less likely possibility is that trapping of oxidized SHP-1/2 by these probes reflects an ability (11) of dimedone to react with disulfides in which one of the sulfur atoms is a good leaving group [*e.g.*, the intermediate disulfides in Fig. 12; for a chemical precedent for such a reaction in organic solvent in the presence of pyridine, see Expt 9 in Table II of ref. (11)].

The three-dimensional structure of oxidized SHP-1/2 has not been elucidated, and it will be interesting to observe whether the active site of the oxidized enzyme is largely intact and poised to bind substrates. In addition, structural characterization of the oxidized SHPs may help elucidate why the enzymes are inactive despite the fact that they possess a native cysteine thiol residue at the active site. One of the cysteines in this disulfide is located in the loop consisting of residues 119–130 (PTP1B numbering). We speculate that the consequent repositioning of Tyr124 (Tyr370 in SHP-2, and 364 in SHP-1) may, disrupt interactions of this residue with His216 (His458 in SHP-2 and 452 in SHP-1). Ultimately, repositioning of His216 may inactivate the enzyme, as this residue is known to play a crucial role in maintaining P-loop conformation and catalytic activity (197). For example, mutation of the analogous His residue in YopH to Ala or Asn raises the pK_a of the active-site cysteine residue from 4.67 to 7.35 and 5.99, respectively, resulting in substantial losses in catalytic activity (197). Consistent with the hypothesis that perturbation of the loop containing Cys121 (PTP1B numbering) can compromise catalytic activity, in the context of PTP1B, covalent modification of this residue by 4-(aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole causes an $\sim$85% loss in catalytic activity (57).

The observed mechanism for oxidative inactivation of SHP-1/2 may have some implications for drug design. The highly similar nature of PTP active sites and the polar nature of small molecules needed to efficiently associate with their phosphotyrosine binding pockets presents a serious impediment to the development of selective, cell permeable PTP inhibitors (30). This makes it desirable to seek regions outside the active site of the PTPs where noncovalent binding (or chemical reactions) of small molecules might modulate catalytic activity. Thus, the realization that SHP-1 and SHP-2 are inactivated by modification of residues well outside the catalytic pocket highlights the exciting possibility that small molecules acting at such allosteric sites can be used to inhibit or inactivate PTPs (57, 111, 180). Clearly, the region surrounding Cys121 is a site where small molecules might exert an inhibitory effect on the PTPs, though it remains to be determined whether selectivity between various PTP family members can be achieved with such an approach.

Redox-Regulation of PTP1B

PTP1B is a negative regulator of the insulin and leptin signaling pathways (169). The enzyme is a validated therapeutic target for the treatment of type 2 diabetes and a potential target for cancer therapy (63, 73, 171, 188, 194). The activity of PTP1B is redox-regulated as part of the insulin signaling cascade (53). Cellular PTP1B is rapidly inactivated by a burst of H_2O_2 that is produced upon binding of insulin to its cell-surface receptor (102, 103, 106). The second-order rate constants measured *in vitro* for the inactivation of PTP1B by H_2O_2 range from 9 to 42 M^{-1} s^{-1} (9, 38). Two studies reported that the active-site cysteine residue is selectively oxidized upon inactivation of the enzyme by H_2O_2 (95, 101), whereas another reported oxidation of the active-site cysteine occurs alongside collateral oxidation of other residues, including Cys32, Cys121, and a number of methionines during oxidative inactivation (9).

Peroxide-mediated inactivation of intracellular PTPs during insulin signaling occurs rather rapidly (5–15 min) (103, 106). In contrast, the *in vitro* inactivation of purified PTPs by H_2O_2 occurs with modest rate constants (10–40 M^{-1} s^{-1}), suggesting that the loss of enzyme activity would be rather sluggish ($t_{1/2} = 5$–200 h) at the peroxide concentrations thought to exist during cell signaling (0.1–1 μM) (159, 160). This apparent kinetic discrepancy may be resolved by colocalization or compartmentalization of target PTPs with the Nox enzymes that produce superoxide (and ultimately H_2O_2).[169] Colocalization has the potential to yield transient, high local concentrations of H_2O_2 that cause rapid enzyme inactivation (25, 175), although it is important to remember that colocalization by no means guarantees efficient channeling of reactive oxygen species from the Nox enzymes to the target PTP (109). [As an aside, colocalization or compartmentalization may also explain the observation that only a small subset of cellular PTPs are oxidized during ligand-stimulated cell signaling processes (15, 101, 107).] In addition, H_2O_2 may be converted spontaneously or enzymatically into third messengers such as peroxymonophosphate, acylperoxides, lipid peroxides, protein hydroperoxides, or peroxymonocarbonate that are more potent PTP inactivators (13, 52, 56, 88, 89, 147). H_2O_2 could also oxidize some as yet unidentified sensor protein that, in turn, oxidizes PTP1B (181). Oxidized GSH is a slow inactivator of PTP1B (Table 1) and is not likely to serve as a third messenger in the inactivation of this enzyme during insulin signaling (10). Further, at least one study has provided evidence that cellular GSH/glutathione disulfide ratios are not perturbed during signaling

processes involving receptor-mediated generation of H_2O_2 (40). However, there is one example in which a plant PTP is inactivated more rapidly by glutathione disulfide (559 M^{-1} s^{-1}) than by H_2O_2 (39).

In 2003, crystallographic studies revealed an unprecedented protein structure associated with the redox regulation of PTP1B. Two groups independently observed that the catalytic Cys215 in the enzyme cyclizes with the neighboring amide nitrogen to yield a sulfenyl amide residue (Fig. 6) (140, 177). In one case, the enzyme was treated with H_2O_2 either in the crystal or before crystallization (140). In the second case, the structure arose when crystallizations were conducted in the presence of various small molecules (177). None of the small molecules present in these mixtures were classical oxidizing agents. Thus, oxidation of the PTP1B in these mixtures may have resulted from spontaneous auto-oxidation of the catalytic cysteine during crystallization or data collection. Alternatively, the active-site cysteine may have been oxidized *via* by reactive oxygen species generated by auto-oxidation of the electron-rich small molecules in the crystallization solutions. The uncyclized sulfenic acid form of PTP1B was observed crystallographically under some crystal soaking conditions (177). These soaking mixtures were rather complex, and the identity of specific components that either stabilize the sulfenic acid or block the cyclization reaction remains uncertain. Along these lines it is interesting to recall that the sulfenic acid labeling reagent NBD-Cl (Fig. 8) was used previously in combination with mass spectroscopy to provide evidence for the presence of a sulfenic acid residue in oxidized PTP1B, though these experiments did not provide a quantitative estimate for the yield of this intermediate (9).

The mechanism of sulfenyl amide formation likely involves intramolecular attack of the adjacent amide nitrogen atom on the sulfur atom of the sulfenic acid residue (82, 143, 154). The cyclization appears to be a facile reaction under physiologically relevant conditions (154), although the rate of this reaction, and its potential to out-compete H_2O_2 or cellular GSH for reaction at the initially generated sulfenic acid, is not yet known. A clever isotopic labeling study provided evidence that the PTP1B sulfenyl amide residue formed cleanly in solution upon oxidation of the enzyme with low concentrations of H_2O_2 (140). In a time-course experiment involving soaking of PTP1B crystals with H_2O_2, the sulfenyl amide was generated rather rapidly (within 2 h or less), whereas extended incubation (up to 5 h) resulted in no further change in the electron density maps (140). This result provided qualitative evidence that, at least in the crystal, oxidation of the sulfenyl amide was significantly slower than the initial oxidation of the catalytic cysteine residue in the enzyme—an indication that sulfenyl amide formation protects against irreversible overoxidation. Despite these results, a recent study detected substantial amounts of the overoxidized sulfinic and sulfonic acid forms of PTP1B in cultured human cancer cell lines (101).

The P-loop in the sulfenyl amide form of PTP1B shows a collapsed structure that is reminiscent of those seen in substrate-trapping mutants (184) where the catalytic cysteine has been mutated to serine (140, 145, 177). The secondary phosphoryltyrosine binding pocket, including residues Arg24 and 254, appears largely unperturbed. These observations might initially suggest that the oxidized enzyme could retain some affinity for phosphotyrosine substrates or inhibitors of the native enzyme; however, there is evidence indicating that oxidation of PTP1B compromises binding of the enzyme to its normal insulin receptor kinase substrate (140). The structural reorganization associated with collapse of the P-loop increases the solvent exposure of Tyr 46 and renders this residue susceptible to phosphorylation by the insulin receptor kinase (140), although the functional significance of this observation remains unclear as phosphorylation of the enzyme was not observed in HepG3 or A431 human cancer cell lines (101). In surface views of oxidized PTP1B, the sulfur atom of the sulfenyl amide residue is well hidden (Fig. 14) (140), yet, as described in the following paragraph, thiols react readily with the oxidized enzyme to regenerate catalytic activity.

In cells, PTP1B activity recovers within 30–40 min after insulin stimulation. This likely involves reactions of the oxidized enzyme with biological thiols. Indeed, early studies showed that the catalytic activity of oxidatively inactivated PTP1B can be recovered by treatment with various thiols such as dithiothreitol, GSH, and the thioredoxin/thioredoxin reductase system, although rate constants for these reactions have not been measured (38, 95, 140). *In vitro* reactivation of the oxidized enzyme by the cellular thiol GSH appears qualitatively sluggish (38), compared to that by dithiothreitol and thioredoxin (38, 95). The ability of thiols to reactivate oxidized PTP1B is consistent with the notion that the oxidized enzyme exists in the sulfenyl amide form. For example, studies with small molecule model compounds indicate that

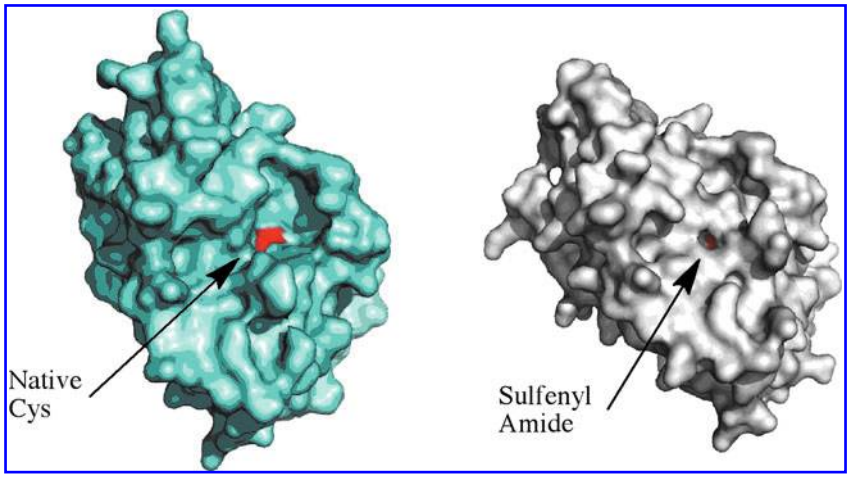

FIG. 14. The sulfenyl amide residue in oxidized PTP1B appears relatively inaccessible. In the left-hand image, the catalytic cysteine in native PTP1B (PDB code 2HNP) is colored red and indicated by the arrow. In the right-hand image, the sulfenyl amide residue of oxidized PTP1B (PDB code 1OEM) is colored red and indicated by the arrow. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

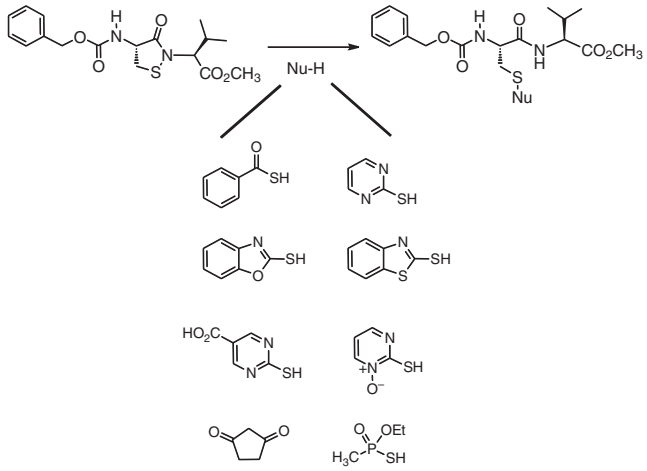

FIG. 15. Small molecules that capture a peptide sulfenyl amide.

the sulfenyl amide linkage is stable against hydrolysis, but inherently unstable in the presence of thiols, reacting rapidly to regenerate the native thiol residue (112, 154). In addition, treatment of the sulfenyl amide-containing enzyme with GSH in the crystal yields native PTP1B (140). Of course, the thiol-recoverable nature of oxidatively inactivated PTP1B also is consistent with involvement of either a sulfenic acid or a disulfide as the resting state of the oxidized enzyme.

Regardless of whether the oxidatively inactivated enzyme exists in solution as the sulfenic acid or the sulfenyl amide form, the glutathionylated enzyme (PTP-SSG) is an obligate intermediate in some reactivation processes (*e.g.*, see Figs. 4–6) (154). In fact, glutathionylated PTP1B has been detected in cells treated with H_2O_2 or stimulated with growth factors (9, 137). This suggests that cellular GSH can compete with enzymes such as thioredoxin for reaction with oxidized PTP1B. The result also suggests that attack of the first equivalent of GSH on the oxidized enzyme is faster than reaction of a second equivalent of GSH with the PTP1B-SSG intermediate. It remains unclear how to mesh the observation of glutathionylated PTP1B in cells with the *in vitro* finding that the glutathionylated intermediate PTP1B-SSG can be returned to native, active enzyme effectively by glutaredoxin (also known as thioltransferase; Table 1) (10). Overall, it is clear that the insulin signaling process transiently generates a cellular pool of reversibly oxidized PTP1B; however, the relative amounts of sulfenyl amide, sulfenic acid, and glutathionylated enzyme in this pool remain unclear. No methods for the selective detection of sulfenyl amide residues currently exist.

Researchers at Sunesis Pharmaceuticals identified a number of nucleophiles capable of selectively reacting with a peptide sulfenyl amide residue over a disulfide (Fig. 15) (150). The ability of these compounds to react with a sulfenic acid or sulfenic acid model compound (such as a sulfenate ester) was not examined and it is likely that some or all of these nucleophiles will display cross-reactivity with sulfenyl amide and sulfenic acid residues. Nonetheless, compounds that selectively trap the oxidized form of PTP1B could potentiate insulin signaling if the resulting covalent linkage is stable enough to slow or prevent return of the oxidized enzyme to the catalytically active form. The stability of the RS-Nu products (Fig. 15) against thiols was not examined in the Sunesis study. In principle, compounds of this type could display exquisite selectivity due to the relatively rare nature of the sulfenyl amide residue in the cell, but these agents must meet the challenge of competing effectively against thioredoxin and millimolar concentrations of GSH for reaction with the oxidized enzyme. This nucleophilic trapping approach was only examined in the context of peptide models of the sulfenyl amide and has not yet been tested in the context of the intact protein or in cells.

Cys121 of PTP1B is structurally equivalent to the distal cysteine residues (Cys363 and 367 in SHP-1 and −2, respectively; Cys o in Fig. 10 and Table 2). Indeed, almost all PTPs feature a cysteine in this position (Fig. 10 and Table 2). Cys92 of PTP1B (Cys k in Fig. 10 and Table 2) is located in the same region of the PTP fold as the other distal cysteines of the SHPs (329 and 333, Cys j in Fig. 10 and Table 2). However, the structural environment of Cys92 in PTP1B is distinct from the analogous residues in SHP-1/2. In the context of PTP1B, this residue (Cys k in Fig. 10) resides within a constrained region in the N-terminus of an α-helix (helix A in Fig. 10), whereas in SHP-1/2 this residue (Cys j in Fig. 10) is located several residues upstream from the start of α-A in a loop region of presumably increased flexibility. Crystallographic data indicates that Cys92 and 121 of PTP1B do not engage with the active-site cysteine during oxidative inactivation suggesting that the precise details of the environment around these cysteines and the local flexibility of the polypeptide chain dictate the mechanism of redox regulation in the PTPs.

Finally, it is intriguing to consider the possibility that some oxidized PTPs might serve as substrate traps. The potential importance of substrate binding by catalytically inactive PTPs is supported by the existence of pseudophosphatases encoded within the human genome (167, 168). Pseudophosphatases lack key residues required for catalytic activity but retain the ability to noncovalently bind phosphotyrosine peptides. There is growing evidence that pseudophosphatases can play important roles in signaling processes (168). As noted in a previous section of this review, the active site of oxidized LYP strongly resembles native enzyme (173), suggesting that the oxidized enzyme might retain the ability to bind phosphotyrosine-containing peptides. In addition, it has been noted that the irreversibly oxidized sulfinic and sulfonic acid forms of PTP1B closely resemble the native enzyme in three-dimensional structure (140, 177) and "may not be merely irrelevant products" because they might retain the ability to associate with phosphotyrosine-containing substrates (60, 101). This idea deserves further study, though it seems less likely to us that these proteins will bind substrate with high affinity because the oxygen atoms of the sulfinic and sulfonic acid groups effectively occupy the phosphoryl binding pocket created by the P-loop of the enzyme (140, 177).

Conclusions

Transient oxidative inactivation of PTPs can serve as an important part of the switching and timing mechanisms that determine the intensity and duration of the cellular responses to a given signaling stimulus. The oxidative inactivation of PTPs also may be important in various pathophysiological

conditions involving oxidative stress (142). At a fundamental biochemical level, it seems likely that the catalytic cysteine residue of all PTPs will be susceptible to oxidation. This is because the same nucleophilic properties that confer catalytic activity on the active-site cysteine residue of PTPs impart reactivity toward H_2O_2. As noted above, the selective inactivation of PTPs that is observed during signaling events (15, 101, 107) may involve colocalization or compartmentalization of specific PTP and Nox enzymes. Under conditions of general oxidative stress, where H_2O_2 likely diffuses freely throughout cells (157), it seems probable that the catalytic cysteine residue of many PTPs will be subject to oxidation. Thus, the differential inactivation of PTPs that is observed under conditions of general oxidative stress (138) may not reflect differential rates of enzyme inactivation but, rather, different susceptibilities toward irreversible over-oxidation or different rates in the recovery of catalytic activity.

The structures and reactivities of oxidized PTPs are as important as the rate at which the initial oxidation of these enzymes occurs. The structure of the oxidized enzyme likely determines susceptibility to irreversible overoxidation. For example, we have discussed in this review how conversion of oxidized PTPs to the disulfide or sulfenyl amide forms may protect against irreversible overoxidation of their catalytic cysteine residues. In addition, the structure of the oxidized enzyme undoubtedly influences the rate of spontaneous (GSH-mediated) and enzyme-catalyzed regeneration of the native PTP. Finally, it is conceivable that some oxidized PTPs acquire new properties. Along these lines, there is a striking example in which oxidation of a yeast PTP (Sdp1) *increases* catalytic activity by enabling substrate recognition (49). It is also possible that oxidation of some PTPs may confer new pseudophosphatase activity to the protein (168).

The PTPs can undergo structural rearrangements after oxidation of their catalytic cysteine residue and the examples surveyed in this review show that there is substantial structural diversity in the oxidized PTPs. Due to the propensity of back-door and distal cysteine residues to engage with the active-site cysteine after oxidative inactivation, differences in the structure of the oxidatively inactivated PTPs may stem to a large degree from differences in the number and location of cysteine residues surrounding the active site of the enzymes. For a number of crystallographically characterized classical PTPs, we have mapped the location of cysteine residues that have the potential to engage with the active-site cysteine during oxidative inactivation (Fig. 10). This map demarcates both known back-door cysteines (*e.g.*, cysteine l; Fig. 10 and Table 2) that reside within the catalytic pocket and distal cysteine residues (*e.g.*, cysteines j and o; Fig. 10) that reside outside the catalytic pocket in the structures of the native enzymes. Clearly, there is great diversity in the number and location of cysteine residues located on the active-site face of the PTP enzymes (Fig. 10). PTPs with key cysteine residues in structurally similar locations may be expected to share similar mechanisms of oxidative inactivation. In light of precedents from the dual-specificity phosphatases, it is also important to recognize that interdomain cysteine residues have the potential to participate in oxidative inactivation processes.

Full understanding of many mammalian signal transduction pathways requires characterization of the roles played by PTPs. Redox regulation of PTPs can be an important part of this picture. Continued study is required to understand mechanisms by which PTPs are oxidized in cells and to determine which PTPs are actually subject to redox control under physiological and pathophysiological conditions. It will also be important to characterize the chemical and structural features of the oxidized PTPs that govern the recovery of their catalytic activity in cells. Sustained efforts in these areas will yield improved fundamental understanding of signal transduction processes and have the potential to reveal new therapeutic targets for the treatment of human disease.

References

1. Allison WS. Formation and reactions of sulfenic acids in proteins. *Accounts Chem Res* 9: 293–299, 1976.
2. Almo SC, Bonanno JB, Sauder JM, Emtage S, Dilorenzo TP, Malashkevich V, Wasserman SR, Swaiminathan S, Eswaramoorthi S, Agarawal R, Kumaran D, Madegowda M, Ragumani S, Patskovsky Y, Alvarado J, Ramagopal UA, Faber-Barata J, Chance MR, Sali A, Fiser A, Zhang Z-Y, Lawrence DS, and Burley SK. Structural genomics of protein phosphatases. *J Struct Func Genomics* 8: 121–140, 2007.
3. Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Ostermann A, Godzik A, Hunter T, Dixon JE, and Mustelin T. Protein tyrosine phosphatases in the human genome. *Cell* 117: 699–711, 2004.
4. Artréaux BD and Toledano MB. ROS (reactive oxygen species as signalling molecules: mechanisms that generate specificity in ROS homeostatsis. *Nat Rev Mol Cell Biol* 8: 813–824, 2007.
5. Bach RD, Su M-D, and Schlegel HB. Oxidation of amines and sulfides with hydrogen peroxide and alkyl hydrogen peroxide. The nature of the oxygen transfer step. *J Am Chem Soc* 116: 5379–5391, 1994.
6. Barford D, Flint AJ, and Tonks NK. Crystal structure of human protein tyrosine phosphatase 1B. *Science* 263: 1397–1404, 1994.
7. Barr AJ, Ugochukwu E, Lee WH, King ON, Filippakopoulos P, Alfano I, Savitsky P, Burgess-Brown NA, Muller S, and Knapp S. Large-scale structural analysis of the classical human protein tyrosine phosphatome. *Cell* 136: 352–363, 2009.
8. Barrett DM, Black SM, Todor H, Schmidt-Ullrich RK, Dawson KS, and Mikkelsen RB. Inhibition of protein-tyrosine phosphatases by mild oxidative stresses is dependent on S-nitrosylation. *J Biol Chem* 280: 14453–14461, 2005.
9. Barrett WC, DeGnore JP, Keng Y-F, Zhang Z-Y, Yim MB, and Chock PB. Roles of superoxide radical anion in signal transduction mediated by reversible regulation of protein-tyrosine phosphatase 1B. *J Biol Chem* 274: 34543–34546, 1999.
10. Barrett WC, DeGnore JP, König S, Fales HM, Keng Y-F, Zhang Z-Y, Yim MB, and Chock PB. Regulation of PTP1B via glutathionylation of the active site cysteine 215. *Biochemistry* 38: 6699–6705, 1999.
11. Barton DHR, Hesse RH, O'Sullivan AC, and Pechet MM. A new procedure for the conversion of thiols into reactive sulfenylating agents. *J Org Chem* 56: 6697–6702, 1991.
12. Barton JP, Packer JE, and Sims RJ. Kinetics of the reaction of hydrogen peroxide with cysteine and cysteamine *J Chem Soc Perkin* 2: 1547–1549, 1973.
13. Bhattacharya S, LaButti JN, Seiner DR, and Gates KS. Oxidative inactivation of PTP1B by organic peroxides. *Bioorganic Med Chem Lett* 18: 5856–5859, 2008.

14. Black DS and Bliska JB. Identification of p130Cas as a substrate of Yersinia YopH (Yop51), a bacterial protein tyrosine phosphatase that translocates into mammalian cells and targets focal adhesions. *EMBO J* 16: 2730–2744, 1997.
15. Boivin B, Zhang S, Arbiser JL, Zhang Z-Y, and Tonks NK. A modified cysteinyl-labeling assay reveals reversible oxidation of protein tyrosine phosphatases in angiomyolipoma cells. *Proc Nat Acad Sci USA* 105: 9959–9964, 2008.
16. Bonham CA and Vacratsis PO. Redox regulation of the human dual specificity phosphatase YVH1 through disulfide bond formation. *J Biol Chem* 284: 22853–22864, 2009.
17. Bonifacic M and Asmus K-D. One-electron redox potentials of RSSR+•/RSSR couples from dimethyl disulphide and lipoic acid. *J Chem Soc Perkin* 2: 1805–1809, 1986.
18. Brandes N, Schmitt S, and Jakob U. Thiol-based redox switches in Eukaryotic proteins. *Antioxid Redox Signal* 11: 997–1014, 2008.
19. Bruice TA and Sayigh AB. Structure of anthraquinone 1-sulfenic acid (Fries acid) and related compounds. *J Am Chem Soc* 81: 3416–3420, 1959.
20. Buhrman G, Parker B, Sohn J, Rudolph J, and Mattos C. Structural mechanism of oxidative regulation of the phosphatase Cdc25B via an intramolecular disulfide bond. *Biochemistry* 44: 5307–5316, 2005.
21. Caselli A, Camici G, Manao G, Moneti G, Pazzagli L, Cappugi G, and Ramponi G. Nitric oxide causes inactivation of the low molecular weight phosphotyrosine protein phosphatase. *J Biol Chem* 269: 24878–24882, 1994.
22. Caselli A, Marzocchini R, Camici G, Manso G, Moneti G, Pieraccini G, and Ramponi G. The inactivation mechanism of low molecular weight phosphotyrosine-protein phosphatase by hydrogen peroxide. *J Biol Chem* 273: 32554–32560, 1998.
23. Chatterji T, Kizil M, Keerthi K, Chowdhury G, Posposil T, and Gates KS. Small molecules that mimic the thiol-triggered alkylating properties seen in the natural product leinamycin. *J Am Chem Soc* 125: 4996–4997, 2003.
24. Chen C-Y, Willard D, and Rudolph J. Redox regulation of SH2-domain-containing protein tyrosine phosphatases by two backdoor cysteines. *Biochemistry* 48: 1399–1409, 2009.
25. Chen K, Kirber MT, Yang Y, and Keaney JFJ. Regulation of ROS signal transduction by NADPH oxidase 4 localization. *J Cell Biol* 181: 1129–1139, 2008.
26. Chiarugi P and Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci* 28: 509–514, 2003.
27. Chou TS, Burgtorf JR, Ellis AL, and Lammert SR. Azetidinone sulfenic acids. Sulfenic acids from penicillin sulfoxides and a study of their reactivities. *J Am Chem Soc* 96: 1609–1610, 1974.
28. Claiborne A, Yeh JI, Mallet TC, Luba J, Crane EJ, Charrier V, and Parsonage D. Protein-sulfenic acids: diverse roles for an unlikely player in enzyme catalysis and redox regulation. *Biochemistry* 38: 15407–15416, 1999.
29. Cloutier JF and Veillette A. Cooperative inhibition of T-cell antigen receptor signaling by a complex between a kinase and a phosphatase. *J Exp Med* 189: 111–121, 1999.
30. Combs AP. Recent advances in the discovery of competitive protein tyrosine phosphatase 1B inhibitors for the treatment of diabetes, obesity, and cancer. *J Med Chem* 53: 2333–2344, 2010.
31. Crane EJ, Parsonage D, and Claiborne A. The active-site histidine-10 of enterococcal NADH peroxidase is not essential for catalytic activity. *Biochemistry* 35: 2380–2387, 1996.
32. Dalle-Donne I, Rossi R, Giustarini D, Colombo R, and Milzani A. S-Glutathionylation in protein redox regulation. *Free Rad Biol Med* 43: 883–898, 2007.
33. Dankleff MAP, Curci R, Edwards JO, and Pyun H-Y. The influence of solvent on the oxidation of thioxane by hydrogen peroxide and by t-butyl hydroperoxide. *J Am Chem Soc* 90: 3209–3218, 1968.
34. Davis FA and Billmers RL. Chemistry of sulfenic acids. 6. Structure of simple sulfenic acids generated by flash vacuum pyrolysis. *J Org Chem* 50: 2593–2595, 1985.
35. den Hertog J, Groen A, and van der Wijk T. Redoxi regulation of protein-tyrosine phosphatases. *Arch Biochem Biophys* 434: 11–15, 2005.
36. den Hertog J, Östman A, and Böhmer F-D. Protein tyrosine phosphatases: regulatory mechanisms. *FEBS J* 275: 831–847, 2008.
37. Denu JM, Lohse DL, Vijayalakshmi J, Saper MA, and Dixon JE. Visualization of intermediate and transition-state structures in protein tyrosine phosphatase catalysis. *Proc Nat Acad Sci* 93: 2493–2498, 1996.
38. Denu JM and Tanner KG. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry* 37: 5633–5642, 1998.
39. Dixon DP, Fordham-Skelton AP, and Edwards R. Redox regulation of a soybean tyrosine-specific protein phosphatase. *Biochemistry* 44: 7696–7703, 2005.
40. Dooley CT, Dore TM, Hanson GT, Jackson WC, Remington SJ, and Tsien RY. Imaging dynamic redox changes in mammalian cells with green fluorescent protein indicators. *J Biol Chem* 279: 22284–22293, 2004.
41. Ellis HR and Poole LB. Novel application of 7-chloro-nitrobenzo-2-oxa-1,3-diazole to identify cysteine sulfenic acid in the AhpC component of alkyl hydroperoxide reductase. *Biochemistry* 36: 15013–15018, 1997.
42. Enami S, Hoffmann MR, and Colussi AJ. Simultaneous detection of cysteine sulfenate, sulfinate, and sulfonate during cysteine interfacial ozonolysis. *J Phys Chem B* 113: 9356–9358, 2009.
43. Evans BJ, Doi JT, and Musker WK. Fluorine-19 NMR study of the reaction of p-fluorobenzenethiol and disulfide with periodate and other selected oxidizing agents. *J Org Chem* 55: 2337–2344, 1990.
44. Farber JL. Mechanisms of cell injury by activated oxygen species. *Env Health Perspect* 102: 17–24, 1994.
45. Finkel T. Oxidant signals and oxidative stress. *Curr Opin Cell Biol* 15: 247–254, 2003.
46. Firbank SJ, Rogers MS, Wilmot CM, Dooley DM, Halcrow MA, Knowles PF, McPherson MJ, and Phillips SEV. Crystal structure of the precursor of galactose oxidase: an unusual self-processing enzyme. *Proc Nat Acad Sci USA* 98: 12932–12937, 2001.
47. Forman HJ. Use and abuse of exogenous hydrogen peroxide in studies of signal transduction. *Free Rad Biol Med* 42: 926–932, 2007.
48. Forman HJ, Fukuto JM, and Torres M. Redox signaling: thiol chemistry defines which reactive oxygen and nitrogen species can act as second messengers. *Am J Cell Physiol* 287: C246–C256, 2004.

49. Fox GC, Shafiq M, Briggs DC, Knowles PP, Collister M, Didmon MJ, Makrantoni V, Dickinson RJ, Hanrahan S, Totty N, Stark MJR, Keyse SM, and McDonald NQ. Redox-mediated substrate recognition by Sdp1 defines a new group of tyrosine phosphatases. *Nature* 447: 487–492, 2007.
50. Fries K. Alpha-anthraquinonesulfenic acid. *Chem Ber* 45: 2965–2973, 1913.
51. Garton AJ, Flint AJ, and Tonks NK. Identification of p130cas as a substrate for the cytosolic protein tyrosine phosphatase PTP-PEST. *Mol Cell Biol* 16: 6408–6418, 1996.
52. Glascow WC, Hui R, Everhart AL, Jayawickreme SP, Angerman-Stewart J, Han B-B, and Eling TE. The linoleic acid metabolite, (13S)-hydroperoxiyoctadecadienoic acid, augments the epidermal growth factor receptor signaling pathway by attenuation of receptor dephosphorylation. *J Biol Chem* 272: 19269–19276, 1997.
53. Goldstein BJ, Mahadev K, and Wu X. Redox paradox: insulin action is facilitated by insulin-stimulated reactive oxygen species with multiple potential signaling targets. *Diabetes* 54: 311–321, 2005.
54. Goto K, Holler M, and Okazaki R. Synthesis, structure, and reactions of a sulfenic acid bearing a novel bowl-type substituent: the first synthesis of a stable sulfenic acid by direct oxidation of a thiol. *J Am Chem Soc* 119: 1460–1461, 1997.
55. Goto K, Tokitoh N, and Okazaki R. Synthesis of a stable arenesulfenic acid bearing a bowl-shaped macrobicyclic cyclophane skeleton. *Angew Chem Int Ed Eng* 34: 1124–1126, 1995.
56. Gracanin M and Davies MJ. Inhibition of protein tyrosine phosphatases by amino acid, peptide, and protein hydroperoxides: potential modulation of cell signaling by protein oxidation products. *Free Rad Biol Med* 42: 1543–1551, 2007.
57. Hansen SK, Cancilla MT, Shiau TP, Kung J, Chen T, and Erlanson DA. Allosteric inhibition of PTP1B activity by selective modification of a non-active site cysteine residue. *Biochemistry* 44: 7704–7712, 2005.
58. Hecht D and Zick Y. Selective inhibition of protein tyrosine phosphatase activities by H2O2 and vanadate *in vitro*. *Biochem Biophys Res Commun* 188: 773–779, 1992.
59. Heffetz D, Bushkin I, Dror R, and Zick Y. The insulinomimetic agents H2O2 and vanadate stimulate protein tyrosine phosphorylation in cells. *J Biol Chem* 265: 2896–2902, 1990.
60. Herrlich P and Böhmer FD. Redox regulation of signal transduction in mammalian cells. *Biochem Pharmacol* 59: 35–41, 2000.
61. Hof P, Pluskey S, Dhe-Paganon S, Eck MJ, and Shoelson SE. Crystal structure of the tyrosine phosphatase SHP-2. *Cell* 92, 1998.
62. Hogg DR and Robertson A. Sulphenate ions as ambident nucleophiles. *Tet Lett* 43: 3783–3784, 1974.
63. Hooft van Huijsduijnen R, Bombrun A, and Swinnen D. Selecting protein tyrosine phosphatases as drug targets. *Drug Disc Today* 7: 1013–1019, 2002.
64. Hubbard SR and Hill JH. Protein tyrosine kinase structure and function. *Ann Rev Biochem* 69: 373–398, 200.
65. Hugo M, Turell L, Manta B, Botti H, Monteiro G, Netto LES, Alvarez B, Radi R, and Trujillo M. Thiol and sulfenic acid oxidation of AhpE, the one-cysteine peroxiredoxin from mycobacterium tuberculosis: kinetics, acidity constants, and conformational dynamics. *Biochemistry* 48: 9416–9426, 2009.
66. Hunter T. Signaling −2000 and beyond. *Cell* 100: 113–127, 2000.
67. Jackson MD and Denu JM. Molecular reactions of protein phosphatases—insights from structure and chemistry. *Chem Rev* 101: 2313–2340, 2001.
68. Jacob C, Holme AL, and Fry FH. The sulfinic acid switch in proteins. *Org Biomol Chem* 2: 1953–1956, 2004.
69. Janssen-Heininger YMW, Mossman BT, Heintz NH, Forman HJ, Kalyanaraman B, Finkel T, Stamler JS, Rhee SG, and van der Vliet A. Redox-based regulation of signal transduction: principles, pitfalls, and promises. *Free Rad Biol Med* 45: 1–17, 2008.
70. Jia Z, Barford D, Flint AJ, and Tonks NK. Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B. *Science* 268: 1754–1758, 1995.
71. Jocelyn PC. The standard redox potential of cysteine-cystine from the thiol-disulphide exchange reaction with glutathione and lipoic acid. *Eur J Biochem* 2: 327–331, 1967.
72. Johnson LN and Lewis RJ. Structural basis for control by phosphorylation. *Chem Rev* 101: 2209–2242, 2001.
73. Johnson TO, Ermolieff J, and Jirousek MR. Protein tyrosine phosphatase 1B inhibitors for diabetes. *Nature Rev Drug Discov* 1: 696–709, 2002.
74. Kamiyama T, Enomoto S, and Inoue M. A novel synthesis oof aromatic sulfinic acids. *Chem Pharm Bull* 36: 2652–2653, 1988.
75. Kanda M, Ihara Y, Murata H, Urata Y, Kono T, Yodoi J, Seto S, Yano K, and Kondo T. Glutaredoxin modulates platelet-derived growth factor-dependent cell signaling by regulating the redox status of low molecular weight protein-tyrosine phosphatase. *J Biol Chem* 281: 28518–28528, 2006.
76. Keerthi K and Gates KS. Entering the leinamycin rearrangement via 2-(trimethylsilyl)ethyl sulfoxides. *Org Biomol Chem* 5: 1595–1600, 2007.
77. Keerthi K, Sivaramakrishnan S, and Gates KS. Evidence for a Morin type intramolecular cyclization of an alkene with a phenylsulfenic acid group in neutral aqueous solution. *Chem Res Toxicol* 21: 1368–1374, 2008.
78. Keire DA, Strauss E, Guo W, Noszál B, and Rabenstein DL. Kinetics and equilibriia of thiol/disulfide interchange reactions of selected biological thiols and related molecules with oxidized glutathione. *J Org Chem* 57: 123–127, 1992.
79. Kice JL. Mechanisms and reactivity in reactions of organic oxyacids of sulfur and their anhydrides. *Adv Phys Org Chem* 17: 65–181, 1980.
80. Kice JL and Rogers TE. A kinetic study of the reaction of mercaptans with phenyl benzenethiolsulfinate and benzenethiolsulfonate in aqueous dioxane. *J Am Chem Soc* 96: 8015–8019, 1974.
81. Kice JL and Rogers TE. Mechanism of the alkaline hydrolysis of aryl thiolsulfinates and thiolsulfonates. *J Am Chem Soc* 96: 8009–8015, 1974.
82. Kim W, Dannaldson J, and Gates KS. Reactions of 3H-benzodithiol-3-one 1-oxide with amines and anilines. *Tetrahedron Lett* 37: 5337–5340, 1996.
83. Krüger U, Senn-Bilfinger J, Sturm E, Figala V, Klemm K, Kohl B, Rainer G, Schafer H, Blake TJ, Darkin DW, Ife RJ, Leach CA, Mitchell RC, Pepper ES, Salter CJ, and Viney NJ. (H+-K+)-ATPase inhibiting 2-[(2-pyridylmethyl)sulfinyl] benzimidazoles. 3. Evidence for the involvement of a sulfenic acid in their reactions. *J Org Chem* 55: 4163–4168, 1990.
84. Kumar D, Dhiraj K, Siddiqui Z, Basu SK, Kumar V, and Rao KVS. The strength of receptor signaling is centrally

controlled through a cooperative loop between Ca2+and an oxidant signal p281. *Cell* 121: 281–293, 2005.
85. Kwon J, Devadas S, and Williams MS. T-cell receptor-stimulated generation of hydrogen peroxide inhibits MEK-ERK activation and lck serine phosphorylation. *Free Rad Biol Med* 35: 406–417, 2003.
86. Kwon J, Lee S-R, Yang K-S, Ahn Y, Kim YJ, Stadtman ER, and Rhee SG. Reversible oxidation and inactivation of the tumor suppessor PTEN in cells stimulated with peptide growth factors. *Proc Nat Acad Sci USA* 101: 16419–16424, 2004.
87. Kwon J, Qu C-K, Maeng J-S, Falahati R, Lee C, and Williams MS. Receptor-stimulated oxidation of SHP-2 promotes T-cell adhesion through SLP-76–ADAP. *EMBO J* 24: 2331–2341, 2005.
88. LaButti JN, Chowdhury G, Reilly TJ, and Gates KS. Redox regulation of protein tyrosine phosphatase 1B by peroxymonophosphate. *J Am Chem Soc* 129: 5320–5321, 2007.
89. LaButti JN and Gates KS. Biologically relevant properties of peroxymonophosphate (=O3POOH). *Bioorg Med Chem Lett* 19: 218–221, 2009.
90. Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nature Rev Immunol* 4: 181–189, 2004.
91. Lambeth JD, Kawahara T, and Diebold B. Regulation of Nox and Duox enzymatic activity and expression. *Free Rad Biol Med* 43: 319–331, 2007.
92. Lechleider RJ, Sugimoto S, Bennett AM, Kashishian AS, Cooper JA, Shoelson SE, Walsh CT, and Neel BG. Activation of the SH2-containing phosphotyrosine phosphatase SH-PTP2 by its binding site, phosphotyrosine 1009, on the human platelet-derived growth factor receptor. *J Biol Chem* 268: 21478–21481, 1993.
93. Lee K and Esselman WJ. Inhibition of PTPs by hydrogen peroxide regulates the activation of distinct MAPK pathways. *Free Rad Biol Med* 33: 1121–1132, 2002.
94. Lee S-R, Yang K-S, Kwon J, Lee C, Jeong W, and Rhee SG. Reversible inactivation of the tumor supressor PTEN by H2O2. *J Biol Chem* 277: 20336–20342, 2002.
95. Lee SR, Kwon KS, Kim SR, and Rhee SG. Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. *J Biol Chem* 273: 15366–15372, 1998.
96. Leonard SE, Reddie KG, and Carroll KS. Mining the thiol proteome for sulfenic acid modifications reveals new targets for oxidation in cells. *ACS Chem Biol* 4: 783–799, 2009.
97. Leslie NR, Lindsay Y, Ross SH, and Downes CP. Redox regulation of phosphatase function. *Biochem Soc Trans* 32: 1018–1020, 2004.
98. Li S and Whorton R. Regulation of protein tyrosine phosphorylation 1B in intact cells by S-nitrosothiols. *Arch Biochem Biophys* 410: 269–279, 2003.
99. Lin WS, Armstrong DA, and Gaucher GM. Formation and repair of papain sulfenic acid. *Can J Biochem* 53: 298–307, 1975.
100. Lohse DL, Denu JM, Santoro N, and Dixon JE. Roles of aspartic acid-181 and serine-222 in intermediate formation and hydrolysis of the mammalian protein-tyrosine-phosphatase PTP1. *Biochemistry* 36: 4568–4575, 1997.
101. Lou Y-W, Chen Y-Y, Hsu S-F, Chen R-K, Lee C-L, Khoo K-H, Tonks NK, and Meng T-C. Redox regulation of the protein tyrosine phosphatase PTP1B in cancer cells. *FEBS J* 275: 69–88, 2008.
102. Mahedev K, Motoshima H, Wu X, Ruddy JM, Arnold RS, Cheng G, Lambeth JD, and Goldstein BJ. The NAD(P)H oxidase homolog Nox4 modulates insulin-stimulated generation of H_2O_2 and plays an integral role in insulin signal transduction. *Mol Cell Biol* 24: 1844–1854, 2004.
103. Mahedev K, Zilbering A, Zhu L, and Goldstein BJ. Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1B *in vivo* and enhances the early insulin action cascade. *J Biol Chem* 276: 21938–21942, 2001.
104. Majeti R and Weiss A. Regulatory mechanisms for receptor protein tyrosine phosphatases. *Chem Rev* 101: 2441–2448, 2001.
105. Mauro LJ and Dixon JE. "Zip codes" direct intracellular protein tyrosine phosphatases to the correct "address." *Trends Biochem Sci* 19: 151–155, 1994.
106. Meng T-C, Buckley DA, Galic S, Tiganis T, and Tonks NK. Regulation of insulin signaling through reversible oxidation of the protein tyrosine phosphatases TC45 and PTP1B. *J Biol Chem* 279: 37716–37725, 2004.
107. Meng T-C, Fukada T, and Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases *in vivo*. *Mol Cell* 9: 387–399, 2002.
108. Michalek RD, Nelson KJ, Holbrook BC, Yi JS, Stridiron D, Daniel LW, Fetrow JS, King SB, Poole LB, and Grayson JM. The requirement of reversible cysteine sulfenic acid formation for T-cell activation and function. *J Immunol* 179: 6456–6467 2007.
109. Miles EW, Rhee SG, and Davies DR. The molecular basis of substrate channeling. *J Biol Chem* 274: 12193–12196, 1999.
110. Mitra K, Barnes CL, and Gates KS. Crystal structure of methyl trans-3-[(2-(methoxycarbonyl)phenyl)sulfinyl]acrylate: a product resulting from trapping of a sulfenic acid by methyl propiolate. *J Chem Crystallog* 29: 1133–1136, 1999.
111. Molina G, Vogt A, Bakan A, Dai X, Queiroz de Oliveira P, Znosko W, Smithgall TE, Bahar I, Lazo JS, Day BW, and Tsang M. Zebrafish chemical screening reveals an inhibitor of Dusp6 that expands cardiac cell lineages. *Nat Chem Biol* 5: 680–687, 2009.
112. Morin RB and Gordon EM. Chemistry of dehydropeptides. A rearrangement of an isothiazolone. *Tet Lett* 24: 2159–2162, 1973.
113. Muratore KE and Cole PA. A lock on phosphotyrosine signaling. *ACS Chem Biol* 2: 454–456, 2007.
114. Nagy P and Ashby MT. Reactive sulfur species: kinetics and mechanism of the hydrolysis of cysteine thiosulfinate ester. *Chem Res Toxicol* 20: 1364–1372, 2007.
115. Nagy P and Ashby MT. Reactive sulfur species: kinetics and mechanisms of the oxidation of cysteine by hypohalous acid to give cysteine sulfenic acid. *J Am Chem Soc* 129: 14082–14091, 2007.
116. Nagy P, Lemma K, and Ashby MT. Reactive sulfur species: kinetics and mechanisms of reaction of cysteine thiosulfinate ester with cysteine to give cysteine sulfenic acid. *J Org Chem* 72: 8838–8846, 2007.
117. Nakamura N. A stable sulfenic acid, 9-triptycenesulfenic acid: its isolation and characterization. *J Am Chem Soc* 105: 7172–7173, 1983.
118. Nakamura T, Yamamoto T, Abe M, Matsumura H, Hagihara Y, Goto T, Yamaguchi T, and Inoue T. Oxidation of archaeal peroxiredoxin involves a hypervalent sulfur intermediate. *Proc Nat Acad Sci USA* 105: 6238–6242, 2008.
119. Neel BG, Gu H, and Pao L. The "Shp"ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. *Trends Biochem Sci* 28: 284–293, 2003.

120. Oae S, Takata T, and Kim YH. Alkaline hydrolysis of unsymmetrical thiolsulfinates: evidence for selective attacking of hydroxide ion on sulfinyl sulfur atom. *Tetrahedron Lett* 18: 4219–4222, 1977.
121. Okuyama T, Miyake K, Fueno T, Yoshimura T, Soga S, and Tsukurimichi E. Equilibrium and kinetic studies of reactions of 2-methyl-2-propanesulfenic acid. *Heteroatom Chem* 3: 577–583, 1992.
122. Pannifer ADB, Flint AJ, Tonks NK, and Barford D. Visualization of the cysteinyl-phosphate intermediate of a protein-tyrosine phosphatase by X-ray crystallography. *J Biol Chem* 273: 10454–10462, 1998.
123. Paulson CE and Carroll KS. Orchestrating redox signaling networks through regulatory cysteine switches. *ACS Chem Biol* 5: 47–62, 2010.
124. Pedersen AK, Peters GH, Moller KB, Iverson LF, and Kastrup JS. Water-molecule network and active-site flexibility of apo protein tyrosine phosphatase 1B. *Acta Crystallogr D Biol Crystallogr* 60: 1527–1534, 2004.
125. Penn RE, Block E, and Revelle LK. Flash vacuum pyrolysis studies. 5. Methanesulfenic acid. *J Am Chem Soc* 100: 3622–3623, 1978.
126. Peskin AV, Low FM, Paton LN, Maghazai GJ, Hampton MB, and Winterbourne CC. The high reactivity of peroxiredoxin 2 with hydrogen peroxide is not reflected in its reaction with other oxidants and thiol reagents. *J Biol Chem* 282: 11885–11892, 2007.
127. Poole LB and Ellis HR. Identification of cysteine sulfenic acid in AhpC of alkyl hydroperoxide reductase. *Methods Enzymol* 348: 122–136, 2002.
128. Poole LB, Karplus PA, and Claiborne A. Protein sulfenic acids in redox signaling. *Ann Rev Pharm Tox* 44: 3325–3347, 2004.
129. Poole LB, Klomsiri C, Knaggs SA, Furdui CM, Nelson KJ, Thomas MJ, Fetrow JS, Daniel LW, and King SB. Fluorescent and affinity-based tools to detect cysteine sulfenic acid formation in proteins. *Bioconjugate Chem* 18: 2004–2017, 2007.
130. Poole LB and Nelson KJ. Discovering mechanisms of signaling-mediated cysteine oxidation. *Curr Opin Chem Biol* 12: 18–24, 2008.
131. Pregel MJ and Storer AC. Active site titration of tyrosine phosphatases SHP-1 and PTP1B using aromatic disulfides. *J Biol Chem* 272: 23552–23558, 1997.
132. Qin S, Inazu T, Yamamura H, Qin S, Inazu T, and Yamamura H. Activation and tyrosine phosphorylation of p72syk as well as calcium mobilization after hydrogen peroxide stimulation in peripheral blood lymphocytes. *Biochem J* 308: 347–352, 1995.
133. Ravichandran LV, Chen H, Li Y, and Quon MJ. Phosphorylation of PTP1B at Ser(50) by Akt impairs its ability to dephosphorylate the insulin receptor. *Mol Endocrinol* 15: 1768–1780, 2001.
134. Reddie KG and Carroll KS. Expanding the functional diversity of proteins through cysteine oxidation. *Curr Opin Chem Biol* 12: 746–754, 2008.
135. Regino CAS and Richardson DE. Bicarbonate-catalyzed hydrogen peroxide oxidation of cysteine and related thiols. *Inorg Chimica Acta* 360: 3971–3977, 2007.
136. Rhee SG. H_2O_2, a necessary evil for cell signaling. *Science* 312: 1882–1883, 2006.
137. Rinna A, Torres M, and Forman HJ. Stimulation of the alveolar macrophage respiratory burst by ADP causes selective glutathionylation of protein tyrosine phosphatase 1B *Free Rad Biol Med* 41: 86–91, 2006.
138. Ross SH, Lindsay Y, Safrany ST, Lorenzo O, Villa F, Toth R, Clague MJ, Downes CP, and Leslie NR. Differential redox regulation within the PTP superfamily. *Cell Signal* 19: 1521–1530, 2007.
139. Salmeen A, Andersen JN, Myers MP, Tonks NK, and Barford D. Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. *Mol Cell* 6: 1401–1412, 2000.
140. Salmeen A, Anderson JN, Myers MP, Meng T-C, Hinks JA, Tonks NK, and Barford D. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. *Nature* 423: 769–773, 2003.
141. Salmeen A and Barford D. Functions and mechanisms of redox regulation of cyteine-based phosphatases. *Antioxid Redox Signal* 7: 560–577, 2005.
142. Samet JM and Tal TL. Toxicological disruption of signaling homeostasis: tyrosine phosphatases as targets. *Annu Rev Pharmacol Toxicol* 50: 215–235, 2010.
143. Sarma BK and Mugesh G. Redox regulation of protein tyrosine phosphatase 1B (PTP1B): a biomimetic study on the unexpected formation of a sulfenyl amide intermediate. *J Am Chem Soc* 129: 8872–8881, 2007.
144. Sarmiento M, Zhao Y, Gordon SJ, and Zhang Z-Y. Molecular basis for substrate specificity of protein-tyrosine phosphatase 1B. *J Biol Chem* 273: 26368–26374, 1998.
145. Scapin G, Patel S, Patel V, Kennedy B, and Asante-Appiah E. The structure of apo protein-tyrosine phosphatase 1B C215S mutant: more than just an S—>O change. *Protein Sci* 10: 1596–1605, 2001.
146. Schwarzer D, Zhang Z, Zheng W, and Cole PA. Negative regulation of a protein tyrosine phosphatase by tyrosine phosphorylation. *J Am Chem Soc* 128: 4192–4193, 2006.
147. Seiner DR and Gates KS. Kinetics and mechanism of protein tyrosine phosphatase 1B inactivation by acrolein. *Chem Res Toxicol* 20: 1315–1320, 2007.
148. Senn-Bilfinger J, Krüger U, Sturm E, Figala V, Klemm K, Kohl B, Rainer G, Schafer H, Blake TJ, Darkin DW, Ife RJ, Leach CA, Mitchell RC, Pepper ES, Salter CJ, Huttner J, and Zaolnai L. (H+-K+)-ATPase inhibiting 2-[(2-pyridylmethyl) sulfinyl]benzimidazoles. 2. The reaction cascade induced by treatment with acids. Formation of 5H-pyrido[1′,2′:4,5][1,2,4] thiadiazino[2,3-a]benzimidazol-13-ium salts and their reactions with thiols. *J Org Chem* 52, 1987.
149. Seth D and Rudolph J. Redox regulation of MAP kinase phosphatase 3. *Biochemistry* 45: 8476–8487, 2006.
150. Shiau TP, Erlanson DA, and Gordon EM. Selective reduction of peptide isothiazolidin-3-ones. *Org Lett* 8: 5697–5699, 2006.
151. Sies H. Biochemistry of oxidative stress. *Angew Chem Int Ed Eng* 25: 1058–1071, 1986.
152. Silverman RB. *The Organic Chemistry of Enzyme-Catalyzed Reactions*. San Diego, CA: Academic Press, 2000.
153. Sivaramakrishnan S, Cummings AH, and Gates KS. Protection of a single-cysteine redox switch from oxidative destruction: on the functional role of sulfenyl amide formation in the redox-regulated enzyme PTP1B. *Bioorg Med Chem Lett* 20: 444–447, 2010.
154. Sivaramakrishnan S, Keerthi K, and Gates KS. A chemical model for the redox regulation of protein tyrosine phosphatase 1B (PTP1B). *J Am Chem Soc* 127: 10830–10831, 2005.

155. Sohn J and Rudolph J. Catalytic and chemical competance of regulation of Cdc25 phosphatase by oxidation/reduction. *Biochemistry* 42: 10060–10070, 2003.
156. Song H, Hanlon N, Brown NR, Noble MEM, Johnson LN, and Barford D. Phosphoprotein-protein interactions revealed by the crystal structure of kinase-associated phosphatase in complex with phospho-Cdk2. *Mol Cell* 7: 615–626, 2001.
157. Srikun D, Albers AE, Nam CI, Iavarone AT, and Chang CJ. Organelle-targetable fluorescent probes for imaging hydrogen peroxide in living cells via SNAP-tag protein labeling. *J Am Chem Soc* 132: 4455–4465, 2010.
158. Stewart AE, Dowd S, Keyse SM, and McDonald NQ. Crystal structure of the MAPK phosphatase Pyst1 catalytic domain and implications for regulated activation. *Nat Struct Biol* 6: 174–181, 1999.
159. Stone JR. An assessment of proposed mechanisms for sensing hydrogen peroxide in mammalian systems. *Arch Biochem Biophys* 422: 119–124, 2004.
160. Stone JR. Hydrogen peroxide: a signaling messenger. *Antioxid Redox Signal* 8: 243–270, 2006.
161. Sullivan SG, Chiu DTY, Errasfa M, Wang JM, Qi JS, and Stern A. Effects of H2O2 on protein tyrosine phosphatase activity in HER14 cells. *Free Rad Biol Med* 16: 399–403, 1994.
162. Sundaresan M, Yu Z-X, Ferrans VJ, Irani K, and Finkel T. Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. *Science* 270: 296–299, 1995.
163. Szajewski RP and Whitesides GM. Rate constants and equilibrium constants for thiol-disulfide interchange reactions involving oxidized glutathione. *J Am Chem Soc* 102: 2011–2026, 1980.
164. Takakura K, Beckman JS, MacMillan-Crow LA, and Crow JP. Rapid and irreversible inactivation of protein tyrosine phosphatases PTP1B, CD45, and LAR by peroxynitrite. *Arch Biochem Biophys* 369: 197–207, 1999.
165. Tarrant MK and Cole PA. The chemical biology of protein phosphorylation. *Ann Rev Biochem* 78: 797–825, 2009.
166. Thenard LJ. Hydrogen peroxide. *Ann Chim Phys* 8: 306–313, 1818.
167. Tonks NK. Protein tyrosine phosphatases: from genes, to function, to disease. *Nature Rev Cell Biol* 7: 833–846, 2006.
168. Tonks NK. Pseudophosphatases: grab and hold on. *Cell* 139: 464–465, 2009.
169. Tonks NK. PTP1B: From the sidelines to the frontlines! *FEBS Lett* 246: 140–148, 2003.
170. Tonks NK. Redox redux: revisiting PTPs and the control of cell signaling. *Cell* 121: 667–670, 2005.
171. Tonks NK and Muthuswamy SK. A brake becomes an accelerator: PTP1B—A new therapeutic target for breast cancer. *Cancer Cell* 11: 214–215, 2007.
172. Tonks NK and Neel BG. Combinatorial control of the specificity of protein tyrosine phosphatases. *Curr Opin Cell Biol* 13: 182–195, 2001.
173. Tsai SJ, Sen U, Zhao L, Greenleaf WB, Dasgupta J, Fiorillo E, Orrú V, Bottini N, and Chen XS. Crystal structure of the human lymphoid tyrosine phosphatase catalytic domain: insights into redox regulation. *Biochemistry* 48: 4838–4845, 2009.
174. Turell L, Botti H, Carballal S, Ferrer-Sueta G, Souza JM, Durán R, Freeman BA, Radi R, and Alvarez B. Reactivity of sulfenic acid in human serum albumin. *Biochemistry* 47: 358–367, 2008.
175. Ushio-Fukai M. Localizing NADPH oxidase-derived ROS. *Science STKE* 349: 1–6, 2006.
176. van der Wijk T, Overvoorde J, and den Hertog J. Hydrogen peroxide induced intermolecular disulfide bond formation between receptor protein-tyrosine phosphatases. *J Biol Chem* 279: 44355–44361, 2004.
177. van Montfort RLM, Congreeve M, Tisi D, Carr R, and Jhoti H. Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. *Nature* 423: 773–777, 2003.
178. Veal EA, Day AM, and Morgan BA. Hydrogen peroxide sensing and signaling. *Mol Cell* 26: 1–14, 2007.
179. Weibrecht I, Boehmer S-A, Dagnell M, Kappert K, Oestman A, and Boehmer F-D. Oxidatiion sensitivity of the catalytic cysteine of the protein tyrosine phosphatases SHP-1 and SHP-2. *Free Rad Biol Med* 43: 100–110, 2007.
180. Wiesmann C, Barr KJ, Kung J, Zhu J, Erlanson DA, Shen W, Fahr BJ, Zhong M, Taylor L, Randal M, McDowell RS, and Hansen SK. Allosteric inhibition of protein tyrosine phosphatase 1B. *Nat Struct Mol Biol* 11: 730–737, 2004.
181. Winterbourn CC. Reconciling the chemistry and biology of reactive oxygen species. *Nat Chem Biol* 4: 278–286, 2008.
182. Winterbourn CC and Metodiewa D. Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. *Free Rad Biol Med* 27: 322–328, 1999.
183. Wu RF, Xu YC, Ma Z, Nwariaku FE, Sarosi GAJ, and Terada LS. Subcellular targeting of oxidants during endothelial cell migration. *J Cell Biol* 171: 893–904, 2005.
184. Xie L, Zhang Y-L, and Zhang Z-Y. Design and characterization of an improved protein tyrosine phosphatase substrate-trapping mutant. *Biochemistry* 41: 4032–4039, 2002.
185. Xu Y, Shao Y, Voorhees JJ, and Fisher GJ. Oxidative inhibition of receptor-type protein-tyrosine phosphatase kappa by ultraviolet irradiation activates epidermal growth factor receptor in human keratinocytes. *J Biol Chem* 281: 27389–27397, 2006.
186. Yang J, Groen A, Lemeer S, Jans A, Slijper M, Roe SM, den Hertog J, and Barford D. Reversible oxidation of the membrane distal domain of receptor PTPalpha is mediated by a cyclic sulfenamide. *Biochemistry* 46: 709–719, 2007.
187. Yang J, Liu L, He D, Song X, Liang X, Zhao ZJ, and Zhou GW. Crystal structrure of the human protein-tyrosine phosphatase SHP-1. *J Biol Chem* 278: 6516–6520, 2003.
188. Yip S-C, Saha S, and Chernoff J. PTP1B: a double agent in metabolism and oncogenesis. *Trends Biochem Sci* 35: 442–449, 2010.
189. Yu W-M, Guvench O, MacKerell ADJ, and Qu C-K. Identification of small molecular weight inhibitors of Src homology 2 domain-containing tyrosine phosphatase 2 (SHP-2) via *in silico* database screening combined with experimental assay. *J Med Chem* 51: 7396–7404, 2008.
190. Yu X, Sun J-P, He Y, Guo X, Liu S, Zhou B, Hudmon A, and Zhang Z-Y. Structure, inhibitor, and regulatory mechanism of Lyp, a lymphoid-specific tyrosine phosphatase implicated in autoimmune diseases. *Proc Nat Acad Sci USA* 104: 19767–19772, 2007.
191. Yudushkin IA, Schleifenbaum A, Kinkhadwala A, Neel BG, Schultz C, and Bastiaens PIH. Live-cell imaging of enzyme substrate interaction reveals spatial regulation of PTP1B. *Science* 315: 115–119, 2007.

192. Yuvaniyama J, Denu JM, Dixon JE, and Saper MA. Crystal structure of the dual specificity protein phosphatase VHR. *Science* 272: 1328–1331, 1996.
193. Zhang N, Schuchmann H-P, and von Sonntag C. The reactions of superoxide radical anion with dithiothreitol: a chain process. *J Phys Chem* 95: 4718–4722, 1991.
194. Zhang S and Zhang Z-Y. PTP1B as a drug target: recent developments in PTP1B inhibitor discovery. *Drug Disc Today* 12: 373–381, 2007.
195. Zhang Z-Y. Chemical and mechanistic approaches to the study of protein tyrosine phosphatases. *Acc Chem Res* 36: 385–392, 2003.
196. Zhang Z-Y. Protein tyrosine phosphatases: structure and function, substrate specificity, and inhibitor development. *Ann Rev Pharmacol Toxicol* 42: 209–234, 2002.
197. Zhang Z-Y and Dixon JE. Active site labeling of the Yersinia protein tyrosine phosphatase: the determination of the pKa of the active site cysteine and the function of the conserved histidine 402. *Biochemistry* 32: 9340–9345, 1993.
198. Zhao R, Lind J, Merényi G, and Eriksen TE. One-electron redox potential of thiobenzoic acid. Kinetic characteristics of benzoylthiyl radical β-fragmentation. *J Am Chem Soc* 120: 2811–2816, 1998.
199. Zhao Y, Wu L, Noh SJ, Guan K-L, and Zhang Z-Y. Altering the nucleophile specificity of a protein-tyrosine phosphatase-catalyzed reaction. *J Biol Chem* 273: 5484–5492, 1998.
200. Zick Y and Sagi-Eisenberg R. A combination of hydrogen peroxide and vanadate concomitantly stimulates protein tyrosine phosphorylation and polyphosphoinositide breakdown in different cell lines. *Biochemistry* 29: 10240–10245, 1990.

Address correspondence to:
Prof. Kent S. Gates
Departments of Chemistry and Biochemistry
University of Missouri
125 Chemistry
Columbia, MO 65211

E-mail: gatesk@missouri.edu

Prof. John J. Tanner
Departments of Chemistry and Biochemistry
University of Missouri
125 Chemistry
Columbia, MO 65211

E-mail: tannerjj@missouri.edu

Date of first submission to ARS Central, September 17, 2010; date of acceptance, October 2, 2010.

Abbreviations Used

DTT = dithiothreitol
GSH = glutathione
GSSG = glutathione disulfide
HSA = human serum albumin
NBD-Cl = 7-chloro-2-nitrobenzo-2-oxa-1,3-diazole
Nox = NADPH oxidase
PTP = protein tyrosine phosphatase
SH2 = Src homology 2
VHR = vaccinia-H1-related PTP
WPD loop = Trp-Pro-Asp loop
YopH = *Yersinia* PTP