J. Biochem. 2011;150(4):345–356 doi:10.1093/jb/mvr104

JB THE JOURNAL OF BIOCHEMISTRY

JB Review

Regulation of protein tyrosine phosphatases by reversible oxidation

Received July 1, 2011; accepted August 16, 2011; published online August 19, 2011

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Oxidation of the catalytic cysteine of protein–tyrosine phosphatases (PTP), which leads to their reversible inactivation, has emerged as an important regulatory mechanism linking cellular tyrosine phosphorylation and signalling by reactive-oxygen or -nitrogen species (ROS, RNS). This review focuses on recent findings about the involved pathways, enzymes and biochemical mechanisms. Both the general cellular redox state and extracellular ligand-stimulated ROS production can cause PTP oxidation. Members of the PTP family differ in their intrinsic susceptibility to oxidation, and different types of oxidative modification of the PTP catalytic cysteine can occur. The role of PTP oxidation for physiological signalling processes as well as in different pathologies is described on the basis of well-investigated examples. Criteria to establish the causal involvement of PTP oxidation in a given process are proposed. A better understanding of mechanisms leading to selective PTP oxidation in a cellular context, and finding ways to pharmacologically modulate these pathways are important topics for future research.

Keywords: Protein-tyrosine phosphatase/signal transduction/reversible oxidation/oxidant/reactive oxygen species/redox state.

Abbreviations: COX, cyclooxygenase; DSP, dual-specificity phosphatase; DTT, dithiothreitol; Gpx, glutathione peroxidise; LOX, lipoxygenase; MKP, MAP kinase phosphatase; MRM, multiple reaction monitoring; MS, mass spectrometry; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NEM, *N*-ethylmaleimide; NOX, NADPH oxidase; nPTP, non-receptor-like PTPs; PDGF, platelet-derived growth factor; PIP3, phosphatidylinositol (3,4,5)-triphosphate; PI3K, PI-3 kinase; Prx, peroxiredoxin; PTP, protein tyrosine phosphatases; RNS, reactive nitrogen species; ROS, reactive oxygen species; rPTPs, receptor-like PTPs; RTK, receptor tyrosine kinase; UV, ultraviolet; VEGF, vascular endothelial growth factor.

Tyrosine phosphorylation is controlled by the balanced and specific action of tyrosine kinases and protein tyrosine phosphatases (PTPs). The regulation of PTPs is thus of major importance for cell signalling.

The members of the PTP superfamily are encoded by about 100 genes. They fall into two main categories: 38 'classical PTPs', which specifically dephosphorylate protein–phosphotyrosine. These are further subdivided in receptor-like transmembrane PTPs (rPTPs) and non-transmembrane PTPs (nPTPs). There are also 61 dual-specificity phosphatases (DSPs), which can often dephosphorylate protein–phosphoserine/threonine residues, in addition to phosphotyrosine. One subgroup of this family comprises the MAP kinase phosphatases (MKPs). The well-known tumour suppressor PTEN (Phosphatase and Tensin homolog), which dephosphorylates phosphatidylinositol (3,4,5)-triphosphate (PIP3), is also structurally related to PTPs. Classical PTPs, DSPs and PTEN all catalyse dephosphorylation by a cysteine-based mechanism, and the presence of a conserved $HC(X_5)R$ motif in the catalytic site is a hallmark of the PTP family (*1–3*).

Initial studies on PTP regulation demonstrated that PTPs are subjected to regulatory phosphorylation, which could activate or inactivate PTPs (*4–8*). In the case of the rPTPs, studies from the last decade have provided genetic and biochemical evidence for the presence of both agonistic and antagonistic ligands for some of the rPTPs, including DEP-1, LAR and PTP-σ (*9–11*). A series of studies have also indicated that some rPTPs, including RPTPα, are inhibited by regulated dimerization (*12–15*).

However, the regulatory mechanism, which has attracted most interest during recent years, is the inhibitory reversible oxidation of the active-site cysteine of PTPs (*1*, *16–18*). An intriguing implication of these findings is that PTPs act as key mediators of a previously neglected cross-talk between, on one hand, redox status and reactive oxygen species (ROS) and reactive nitrogen species (RNS) levels of cells and, on the other hand, growth factor-induced tyrosine kinase-dependent cell signalling (Fig. 1).

The review will focus on the oxidation of classical PTPs but will use some examples from studies of the DSPs and PTEN, which are also regulated by reversible

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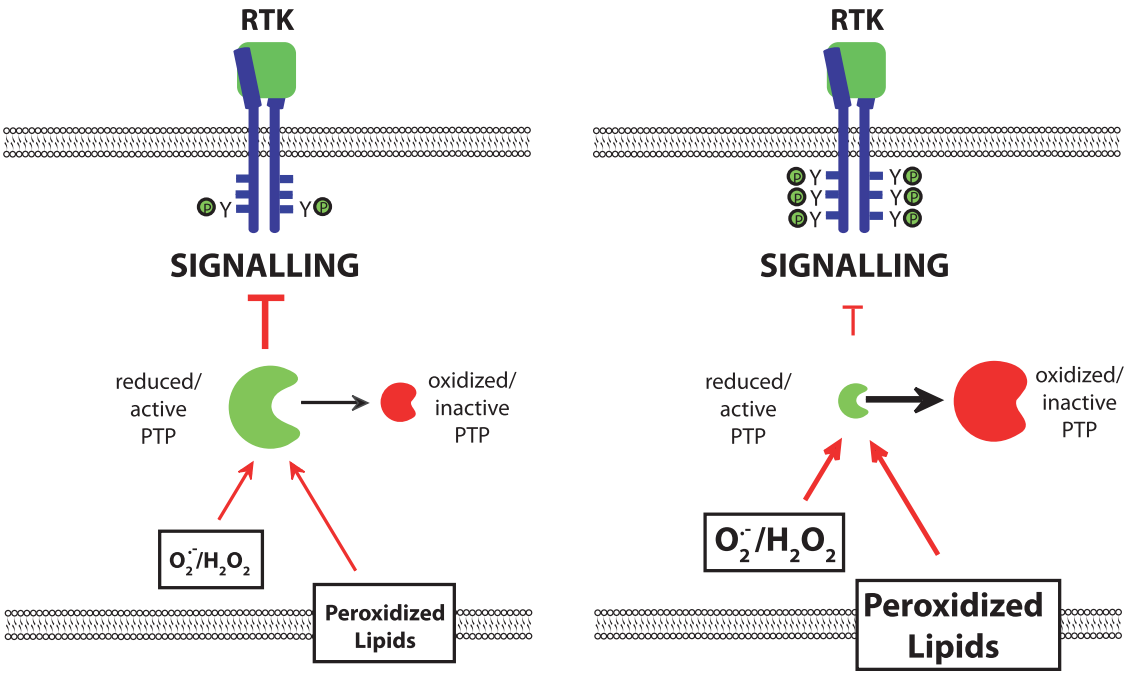

Fig. 1 Crosstalk between RTK signalling and redox signalling can occur at the level of PTP oxidation. A high reducing environment in the cell (left), through reduction in activity/presence of ROS producers and/or elevated activity/presence of ROS scavengers, leads to decreased PTP oxidation and attenuated RTK signalling. A high oxidizing environment in the cell (right), through elevated activity/presence of ROS producers and/or low activity/presence of ROS scavengers, leads to increased PTP oxidation and enhanced RTK signalling. The figure indicates direct oxidation of PTPs by $O_2^{\cdot-}$ and H_2O_2. However, as noted in the text, also indirect oxidation effects of H_2O_2 have been suggested.

oxidation of the active site cysteine. The review emphasizes ROS-mediated mechanisms, although RNS have also been implied in PTP regulation. The latter topic is comprehensively covered in (*19*). PTPs will be referred to by their common names. A list of all mentioned PTPs, including also their systematic designation, is provided as Supplementary Table S1.

For more general information on PTPs, the reader is referred to a series of thorough recent reviews, which have discussed PTPs from different perspectives, including their substrate specificity, regulation, cell biological roles, *in vivo* function, involvement in disease and potential as drug targets (*1–2*, *16*, *20–22*).

Biochemistry and specificity of PTP oxidation

The catalytic site of the classical PTPs contains a cysteine whose SH-group exists in the thiolate state (S^-) to enable catalysis. The pKa values for the catalytic cysteine have experimentally or by calculations been determined to be in the range of 4–6 for different members of the PTP family (*23*). This makes these sites much more susceptible to oxidation than most other cysteines that have pKa values of 8–9 (*24*).

Inhibitory oxidation of PTPs *in vitro* has been shown to be induced by a wide range of oxidants. A list of oxidants that have been shown to interact with PTP active sites is given in Table I. They range from different ROS, over RNS, to toxic constituents of 'ambient particular matter' (PM), such as quinones and aldehydes (*25*). However, not all oxidants affect PTPs. For example, *t*-butyl hydroperoxide and cumene hydroperoxide, two commonly used oxidants in cell biology, did not oxidize PTP1B or LAR PTP *in vitro* (*26*).

Many studies of PTP oxidation have been performed with hydrogen peroxide (H_2O_2). This was also motivated by the assumption that H_2O_2, being produced in cells downstream of many surface receptors (*27*, *28*), is a physiologically relevant PTP oxidant. Recent findings have challenged this concept, based on the relatively slow reaction of H_2O_2 with the thiolate cysteines in PTPs, which has been postulated to be unlikely to match the very rapid H_2O_2-consuming processes in cells (*29*, *30*). PTP oxidation has therefore been suggested to be highly compartmentalized to areas of decreased H_2O_2 clearance or to occur through oxidation by other oxidants produced downstream of H_2O_2.

Reaction with H_2O_2 oxidizes PTP active-site thiolates initially to sulphenic acid (SOH), whereas high concentrations of oxidant can cause formation of sulphinic (SO_2H) or sulphonic acid (SO_3H) derivatives. The initially formed sulphenic acid product can rapidly react further with amino acid residues in the proximity. Different secondary reaction products have been observed *in vitro*, including sulphenylamides (SN) by reaction with a neighbouring peptide backbone residue (*31*, *32*), and intramolecular disulphides by reaction with proximal-free cysteines (Table II). Crystal structures of oxidized PTPs have revealed both types of secondary reaction products. These secondary reactions have in common that they are reversible by

Table I. Agents which can oxidize PTPs.

Oxidant	Examples of PTP	Comments	References
Hydrogen peroxide (H_2O_2)	PTP1B, VHR, RPTPα, Cdc25, PTEN, SHP1, SHP2, MKP3	Commonly used for analysis of PTP oxidation *in vitro*, considered as signalling intermediate leading to PTP oxidation	(*26*, *33*, *36*, *37*, *46*)
Singlet oxygen ($^1O_2$)	PTP1B, CD45	Singlet oxygen is one of the ROS produced upon UV irradiation	(*32*)
Lipid peroxides 15-hydoperoxy-eicosatetraenoic acid	SHP1, TC-PTP, PTP-H1	Produced in cells lacking Gpx 4, more potent than H_2O_2	(*59*)
Oxidized glutathione (GSSG)	PTEN, PTP-L1, PTP1B, SHP1, MKP3	GSSG/GSH mixtures used to titre relative oxidant sensitivity	(*39*)
S-nitrosothiols	PTP1B, PTEN	Thiol modification protects from further oxidation by H_2O_2	Summarized in (*19*, *75*)
Polyaromatic quinones	PTP1B, CD45	Environmental pollutants	(*25*, *76*)
Amino acid, peptide and protein hydroperoxides	PTP1B	More effective than H_2O_2	(*77*)
Peroxymonophosphate	PTP1B	7000 times more active than H_2O_2 in inactivating PTPs	(*78*)
Hypothiocyanous acid (HOSCN)	PTP1B	Product of myeloperoxidase in the context of inflammatory reactions, HOSCN is more potent than H_2O_2	(*79*)
As_2O_3	PTEN	Causes increased Ca^{2+} currents in cardiac muscle cells leading to arrhythmia	(*72*)

Table II. Oxidative modification of PTP catalytic sites.

PTP	Oxidation products	Methods complementing enzymatic measurements	Comments	References
Cdc25, LMW-PTP, PTEN	Intramolecular disulphide bond	Crystal structures, mutagenesis, MS	Catalytic cysteine with proximal 'backdoor' cysteine	(*45*, *80–84*)
PTP1B	Intramolecular sulphenylamide structure	Crystal structure, mutagenesis	Profound conformational changes of catalytic centre	(*31*, *85*)
RPTPα, second catalytic domain	Intramolecular sulphenylamide structure	Crystal structure, mutagenesis	Conformational changes of catalytic centre, may translate in structural alteration of RPTPα and trigger dimer formation	(*12*, *86*)
PTP1B	Sulphinic and sulphonic acid derivatives	MS	Common products of H_2O_2 exposure, however, have also been observed in intact cells for PTP1B	(*26*, *61*)
MKP3	Intramolecular disulphide bonds	MS, mutagenesis	Multiple cysteines can serve as partner for the catalytic cysteine	(*46*)
hYVH1 (DUSP12)	Intramolecular disulphide bonds	MS, mutagenesis	Catalytic cysteine forms disulphide with 'backdoor' cysteine only at high oxidant concentrations since C-terminal Zn-coordinating domain is protective and oxidized first	(*40*)
SHP1, SHP2	Intramolecular disulphide bonds	MS, mutagenesis	Two 'backdoor' cysteines form a disulphide, catalytic cysteine is preserved as thiol but presumed to loose thiolate character	(*38*)
Lyp/PTPN22	Intramolecular disulphide bonds	Crystal structure, mutagenesis	Another proximal cysteine, which is not engaged in disulphide, regulates activity	(*87*)

reduction, and are stabilizing from further oxidation. Moreover, associated with the oxidation, conformational changes of the PTP active site occur. For example, in case of PTP1B, sulphenylamide formation strongly affects the conformation of the PTP loop, and the pTyr loop, two conserved structural motifs of PTP catalytic sites. Also, surface exposure of the sulphenylamide structure occurs (*31*). The structural changes in oxidized PTPs are likely to enable new protein–protein interactions. For example, enhanced binding of oxidized PTP1B with calpain has been linked to facilitated proteolytic degradation of this PTP. The cell cycle regulator Cdc25C can also readily undergo oxidation, leading to destabilization of the protein in intact cells. Moreover, formation of an intramolecular disulphide bond between the catalytic cysteine 377 and cysteine 330 has been linked to enhanced binding of 14.3.3 protein to Cdc25C, associated with retention of the PTP in the cytoplasm (*33*, *34*). Structural changes of PTP catalytic sites

upon oxidation, such as formation of the exposed sulphenylamide structure, may also facilitate access for reducing agents or enzymes.

Biochemical studies revealed strong differences in the susceptibility of different PTPs to oxidation *in vitro*. Also, the two catalytic domains found in many rPTPs (designated D1 for the membrane-proximal domain and D2 for the membrane-distal domain) differ in susceptibility at least in some PTPs. For example, studies using an antibody against terminally oxidized catalytic cysteine (*35*) (see 'PTP oxidation in a cell signalling context and methods for its monitoring' section) revealed a high sensitivity of the D2 domain of RPTPα as opposed to the D1 domain. These studies were extended to other PTPs, showing that PTP1B, and PTP LAR D1 and D2 domains were also effectively oxidized, whereas the RPTPμ D1 domain was not (*36*). The relative resistance of the RPTPα and RPTPμ D1 domains to oxidation was attributed to the particular orientation of a conserved Arg residue in the PTP loop of the two D1 domains. Using the same methodology, the SH2-domain PTPs SHP1 and SHP2 were found to be similarly sensitive to oxidation, but less sensitive than the RPTPα D2 domain (*37*). The sensitivity of SHP1 and SHP2 to oxidation has also been recently analysed using other techniques (*38*). Furthermore, *in vitro* studies comparing efficiency of PTP oxidation based on activity assays revealed similar sensitivities of PTP1B, SHP1, PTEN, MKP3 and PTP-L1, whereas the structurally somewhat distinct lipid phosphatases of the myotubularin class were nearly resistant to oxidative inactivation (*39*).

There is thus some evidence for intrinsic differences between various PTP domains with regard to oxidation sensitivity. Additionally, it has become clear that regulatory domains in PTPs contribute to the sensitivity to oxidation. In SHP1, deletion of the SH2 domain facilitates oxidation, suggesting a protective role of the 'closed conformation' of SH2 domain PTPs (*37*). Protection from oxidation *in vitro* has also been described for the C-terminal Zn-binding domains in hYVH1 (*40*). In contrast, the C-terminal domain of PRL-1 (phosphatase of regenerating liver-1) seems to facilitate oxidation of the catalytic domain (*41*). Very likely, intra- and intermolecular protein–protein interactions of PTPs will also affect their redox regulation in a cellular context.

Pronounced selectivity for oxidation of some specific PTPs has already been observed in cells. For example, while H_2O_2 treatment of fibroblasts caused oxidation of multiple PTPs, stimulation of the same cells with platelet derived growth factor (PDGF) led to selective oxidation of SHP2. This was dependent on the recruitment of SHP2 to the activated PDGF receptors and may have been caused by local exposure of SHP2 to high concentrations of oxidant (*42*). In contrast, insulin stimulation preferentially oxidized PTP1B and TC-PTP, two PTPs which are relevant for negative regulation of insulin receptors (*43*). In T cells, activation of T-cell receptor quite selectively led to the oxidation of SHP2, but not SHP1 despite the similar susceptibility of both PTPs to oxidation *in vitro* (see above). Conversely, in H_2O_2-treated EOL-1 cells, SHP1 was more prone to oxidation than SHP2 (*37*).

As indicated above, *in vitro* treatment of reversibly oxidized PTPs with reducing agents fully restores their activity. An early study on PTP1B oxidation showed that the thioredoxin (thioredoxin/thioredoxin reductase/NADPH) system was more effective than the glutaredoxin system in reactivating the enzyme (*44*). Effective reduction with thioredoxin was also shown for Cdc25B (*45*) and MKP3 (*46*). In the latter case, the enzymatic reduction *in vitro* with thioredoxin was more efficient than reduction with DTT, but still slower than that of Cdc25B by thioredoxin. It thus appears that PTPs differ with regard to their sensitivity to different reducing agents.

PTP oxidation in a cell signalling context and methods for its monitoring

Some major parts of the redox environment relevant for PTP oxidation are introduced in Fig. 2. Key components are the NADPH oxidase (NOX) enzymes and the respiratory chain of the mitochondria, which are well-characterized cellular sources of ROS, and the lipoxygenase (LOX) and cyclooxygenase (COX) enzymes, which generate peroxidized lipids. Another major class of regulators are the H_2O_2-reducing enzymes, e.g. catalase, peroxiredoxins (Prx) and glutathione peroxidases (Gpx), of which some are also capable of reducing peroxidized lipids. Many of these redox enzymes, e.g. NOX, Prx and Gpx enzymes exist as

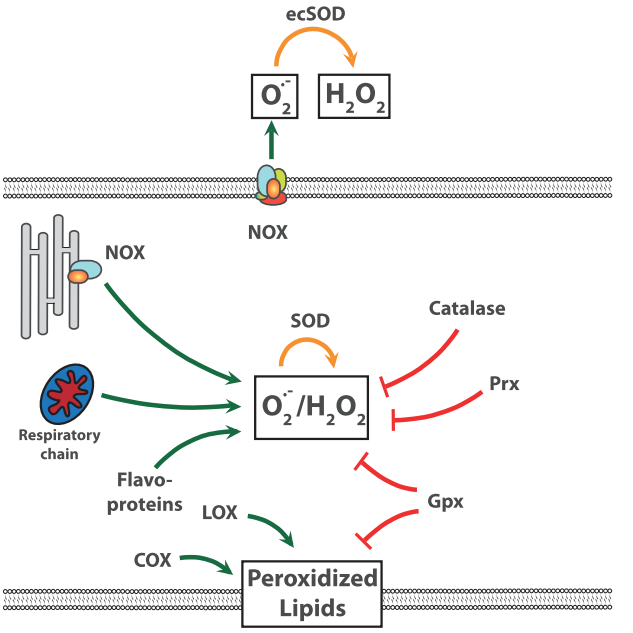

Fig. 2 Overview of the major mammalian redox enzymes that balance the levels of ROS. Isoforms exist for most of the enzymes presented, with the exception of catalase. These isoforms can be differentially localized within cellular compartments or across tissues, which is not represented in this figure. NOX, NADPH oxidase; SOD, superoxide dismutase; XO, xanthine oxidase; LOX, lipoxygenase; COX, cyclooxygenase; Prx, Peroxiredoxin; Gpx, Glutathione peroxidase.

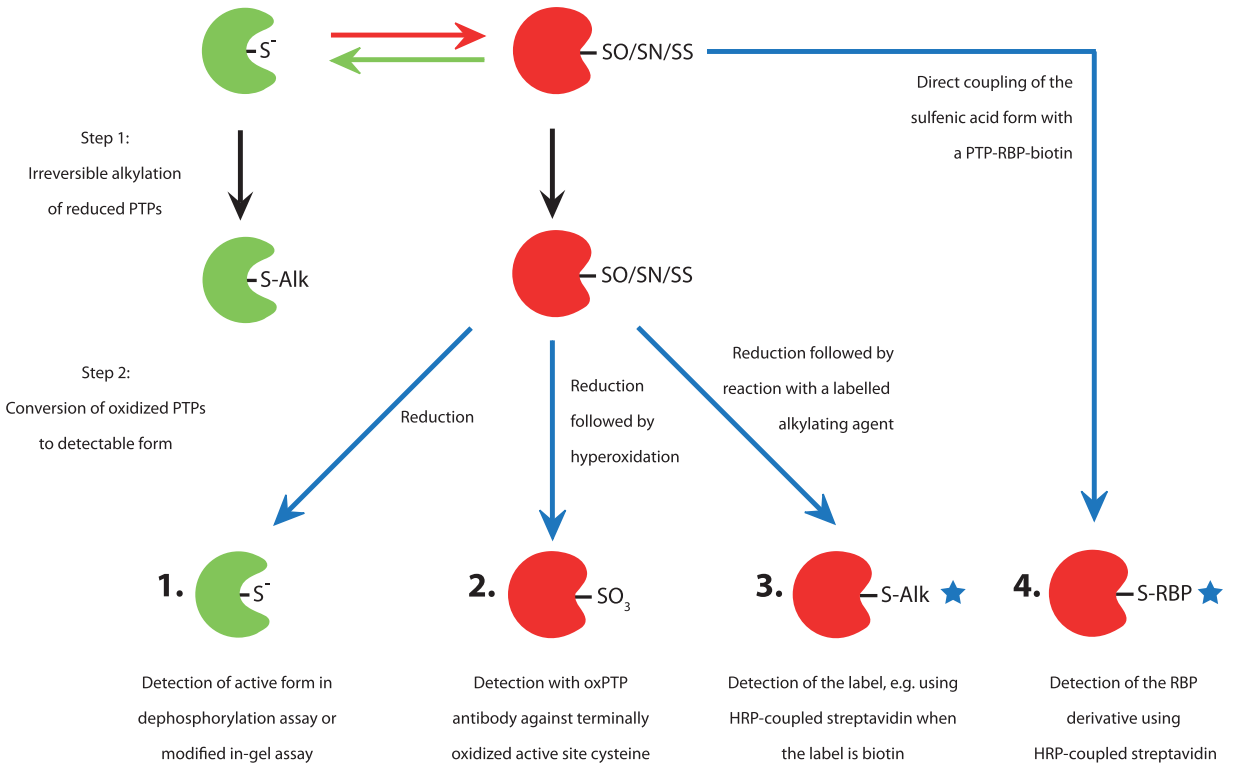

Fig. 3 Methods to monitor PTP oxidation. Irreversible alkylation (Alk) of the reduced form of PTPs is a common step for most PTP oxidation analyses, followed by reduction of the reversibly oxidized PTPs. Method 1 is based on activity measurements like dephosphorylation of radioactively labelled peptides *in vitro* or in the modified in-gel assay (*53*, *110*). Method 2 converts the originally oxidized PTPs to the sulphonic acid form, which can be detected with a specific antibody (*35*). The third method applies another alkylation step with a labelled alkylating agent which can be subsequently detected directly or used to enrich this pool of PTPs (*111*). In Method 4, sulphenic acids are directly reacted with a dimedone-derivative coupled to biotin that shows selectivity for PTPs, termed a PTP redox-based probe (PTP–RBP) which can subsequently be detected with streptavidin–HRP (*112*).

isoforms, which show differential expression, cellular localization and enzymatic activity. Finally, the activities and levels of molecules that reduce oxidized PTPs, such as thioredoxin, will also impact on net PTP oxidation.

Elucidation of how PTP oxidation is involved in cell signalling obviously requires methods to monitor the individual and global PTP oxidation status. Some features of selected methods which have been used are illustrated in Fig. 3. Recently, some mass spectrometry (MS)-based methods for measurement of PTP oxidation have also emerged. Held *et al.* (*47*) have presented an 'oxoMRM (multiple reaction monitoring)' method where non-oxidized cysteines are differentiated from oxidized cysteines through labelling with two different forms of *N*-ethylmalemide (NEM). This assay design is such that the oxidation of any cysteine residue can be monitored, although the protein of interest will need to be enriched for. A second mass spectrometry-based method for analyses of PTP oxidation uses an antibody to isolate, in two different set-ups, either the oxidized active-site PTP peptides or all active site PTP peptides. The relative oxidation of all different PTPs can thereby be determined. This approach has already revealed interesting differences between the relative oxidation of different PTPs in tumour cells from different organs, as well as in different $HER2^{+}$ breast cancer cell lines (*48*).

Concerning the role of PTP oxidation in cell signalling two related, but also distinct, general concepts are emerging:

- first, the growth factor-independent 'redox status' of cells will impact on tyrosine kinase signalling by regulating the activity of PTPs (Fig. 1) and
- secondly, tyrosine kinase-regulated production of ROS, and concomitant selective PTP oxidation, is an intrinsic part of cell signalling from many classes of cell surface receptors (Fig. 4).

A key study illustrating the link between cellular 'redox status', PTP oxidation and growth factor signalling reported on links between PrxII and PDGF receptor signalling. PrxII knockdown enhanced PDGF receptor signalling in tissue culture and *in vivo* through increased ROS levels and reduced PTP activity (*49*). Additional support has now been presented for the general notion that receptor tyrosine kinase (RTK) signalling, through PTP oxidation, is affected by the expression levels or activity of enzymes involved in ROS production or scavenging (Fig. 1 and Table III). ROS regulators analysed in these studies include, in addition to the already mentioned reducing Prx, different members of the reducing Gpx enzymes, as well as various NOX family members involved in ROS production.

Mitochondrial respiration is an important source of ROS production (*50*). The levels of

mitochondria-derived ROS can vary dependent on cell metabolism. Also, signalling from cell surface receptors, such as integrins, can elevate mitochondrial ROS production with impact on PTP oxidation (*51*). Recent studies now also indicate that conditions affecting the levels of mitochondria-derived ROS will affect PTP oxidation and growth factor signalling. One such example is the recent study showing that hypoxia/reoxygenation, a condition leading to transient mitochondria-derived ROS increase (*52*), is also associated with increased PTP oxidation and a concomitant increase in cellular responses to PDGF stimulation (*53*).

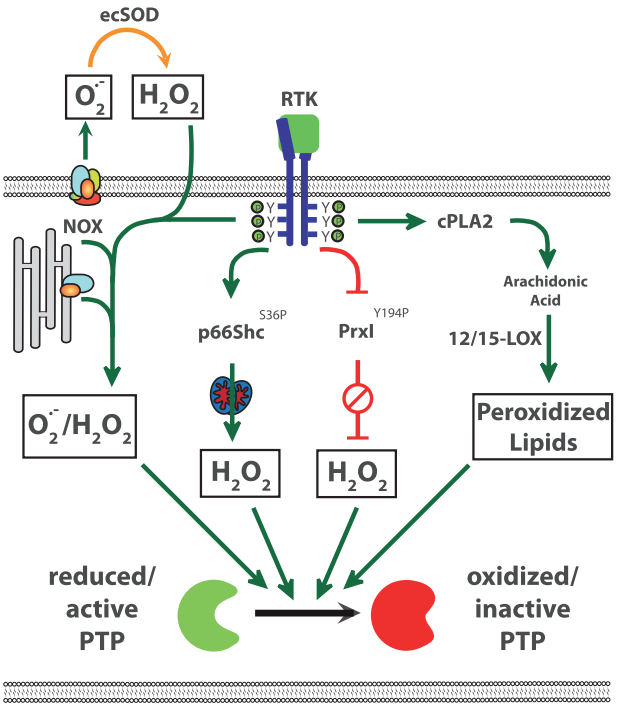

Fig. 4 Four potentially general mechanisms for growth factor-induced PTP oxidation. Growth factor stimulation can cause the activation of NOX complexes, either through increased NOX expression or accumulation of NOX complex cofactors, leading to superoxide and H_2O_2 production. A second pathway is the activation through serine-36 phosphorylation of p66Shc, leading to mitochondrial H_2O_2 production. A third mechanism involves the inhibitory phosphorylation of PrxI on tyrosine-194, leading to locally elevated levels of H_2O_2. A fourth mechanism is the activation of cPLA2, which produces arachidonic acid that in turn serves as a substrate for 12/15-LOX to produce peroxidized lipids. Like in Fig. 1, this scheme suggests direct oxidation of PTPs by $O_2^{\cdot-}$ and H_2O_2, while as is noted in the text, also indirect oxidation effects have been suggested for H_2O_2.

Many of the details related to growth factor-induced PTP oxidation (Fig. 4) have been covered in recent reviews (*1*, *16*, *27*). A selection of examples of cell surface-triggered signalling, which involves PTP oxidation is provided in Table IV, and includes signalling from e.g. RTKs, integrins, B- and T-cell receptors and G-protein-coupled receptors.

The mechanisms whereby activation of the different cell surface receptor activation induce a controlled and spatially restricted production of ROS are still poorly characterized, but some potentially general mechanisms are emerging (Fig. 4).

A series of studies have outlined a pathway connecting RTK activation with increased NOX activity through PI-3 kinase (PI3K) activation and Rac translocation to NOX enzymes (*54*). Analyses of PDGF receptor signalling have also demonstrated that deletion of p66Shc, which produces mitochondrial ROS, is associated with a decreased PTP oxidation and a concomitant attenuation of the biochemical and cellular responses to PDGF stimulation (J. Frijhoff, manuscript in preperation). p66Shc also promotes insulin signalling through oxidation of PTEN (*55*). Another potentially general mechanism was also recently presented which emphasizes an RTK-mediated inhibitory tyrosine phosphorylation of PrxI leading to a spatially restricted increase in H_2O_2 in the vicinity of activated RTKs (*56*). Furthermore, a less characterized pathway involves growth factor-induced arachidonic acid release by cytosolic phospholipase A_2. These lipids can affect PTP oxidation by effects on NOX enzymes (*57*, *58*), or as precursors of peroxidized lipids, which recently were shown to act as potent PTP oxidants (*59*). The mechanisms whereby peroxidized lipids induce PTP oxidation should be better clarified; however, existing data from *in vitro* studies suggest that these oxidants have the capacity to act as direct oxidants of PTPs (*59*).

In many of the studies above, PTP oxidation has been correlated with alterations in signalling and cellular response. However, causal relationships between

Table III. Redox enzymes affect RTK signalling through PTP oxidation.

Redox enzyme	Role in PTP oxidation	Reference
ROS-producing enzymes		
NOX	Promotes oxidation of PTP-PEST in focal contacts to induce migration	(*88*)
NOX4	Promotes oxidation of PTP1B at the ER in EGF signalling	(*89*)
NOX1 and NOX5L	Promote PTP1B oxidation in IL-4 signalling	(*90*)
SOD1	Promotes PTP oxidation in growth factor signalling	(*91*)
DUOX1	Promotes SHP2 oxidation for enhanced and sustained TCR signalling	(*92*)
p66Shc	Produces mitochondrial ROS to promote both insulin signalling through PTEN oxidation and PDGF signalling through PTP oxidation	(*55*) J. Frijhoff *et al.*, manuscript in preparation
ROS-scavenging enzymes		
Gpx 1	Scavenges H_2O_2 to protect PTEN from oxidation, desensitizing insulin signalling	(*68*)
Prx II	Protects PTPs from oxidation in PDGF signalling	(*49*)
Gpx 4	Protects PTPs from oxidation by lipid peroxides in PDGF signalling	(*59*)

Table IV. Stimulation of a wide variety of cell surface receptors leads to PTP oxidation.

Cell-surface receptors	Target PTP	Reference
EGFR	PTP1B, PTEN	(*44*, *63*, *89*, *91*)
InsulinR	PTP1B, TC-PTP, PTEN	(*43*, *55*, *63*, *68*, *93*)
PDGFR	SHP2, LMW-PTP, PTEN	(*35*, *42*, *63*, *94*)
VEGFR2	DEP-1, PTP1B	(*95*)
TCR	SHP2, SHP1	(*92*, *96*, *97*)
BCR	'BCR-associated PTP', SHP1	(*98*, *99*)
Integrins	LMW-PTP, SHP2	(*51*, *100*)
Endothelin 1 receptor	SHP2	(*101*)
Angiotensin II receptor (AT1A)	SHP2	(*102*)
Sphingosine 1-phosphate receptor [S1P(2)]	PTP1B	(*103*)
Urotensin II	SHP2	(*71*)
Interleukin-4 receptor	PTP1B	(*90*)
Adiponectin	PTP1B	(*104*)
TNFR1	MKP1, MKP3, MKP5, MKP7	(*105*)

the PTP oxidation and the alterations in cell signalling and responses have not always been established.

A set of criteria can be proposed to be fulfilled for establishing a causal relationship between oxidation of a given PTP and a physiological or pathological phenotype:

- oxidation of the particular PTP should be shown by activity loss and techniques to detect active site modification;
- PTP oxidation should be linked to hyper-phosphorylation of physiological substrate(s), and have similar effects as loss of the PTP (by siRNA or by gene inactivation);
- interference with the physiological/pathological oxidant production should rescue PTP activity and attenuate the phenotype; and
- the system should be refractory to interference with oxidant production when the PTP of interest is not present.

Disease-associated signalling involving PTP oxidation

Many disease states, notably chronic diseases such as cancer, diabetes, atherosclerosis and chronic inflammation have been associated with elevated levels of ROS and/or RNS in the affected tissues. This can partly be attributed to deregulation of metabolism, altered gene expression of enzymes involved in ROS/RNS production or decomposition (*60*), or to invasion of inflammatory cells. Furthermore, adverse environmental factors, such as ultraviolet (UV) or ionizing radiation can lead to ROS production in affected tissues. Only relatively few studies have addressed the potential role of PTP oxidation for the pathology of these conditions (Table V).

Several investigators have recently investigated PTP oxidation in cancer cells. It can be speculated that PTP inactivation may contribute to pathology by removing regulatory constraints from mitogenic, motogenic and anti-apoptotic pathways. Indeed, high ROS levels in cancer cells appear capable of driving PTPs into both reversibly and irreversibly oxidized states, as shown for PTP1B in A431 carcinoma cells (*61*).

An interesting case has been made for PTEN, an established tumour suppressor for multiple cancers. Oxidation may be one of the different non-genetic mechanisms leading to loss of its activity (*62*). It has previously been shown that a fraction of PTEN is oxidized by mitogenic cell stimulation (*63*). Mitochondria-derived ROS was later shown to inhibit PTEN in cancer cell lines, leading to activation of AKT (*64*). In acute T-cell leukaemia (T-ALL) PTEN protein is mostly present, even often at elevated level, but frequently inactivated by either phosphorylation (through casein kinase 2) or reversible oxidation. Reversal of oxidation by reductant treatment rescued PTEN function and down-regulated AKT activity. Cells with genetic PTEN loss were refractory to this treatment (*65*). The mediators of PTEN oxidation and the type of oxidative modification have not yet been explored in this system. In pancreatic cancer cell lines, evidence for a role of arachidonic acid metabolites in PTEN oxidation has been obtained, which may lead to an oxidized form in absence of an intramolecular disulphide bridge (see above) (*66*). Finally, in an animal model of Ras-or ErbB2-induced breast cancer, loss of PrxI aggravated the phenotype by enhancing PTEN oxidation. Interestingly, PrxI formed complexes with PTEN, which are possibly important for the protective function (*67*). In contrast, in the context of insulin, signalling oxidation of PTEN plays a beneficial role. Mice deficient in Gpx1, one of the ROS-lowering enzymes (see above), were protected from high-fat diet-induced insulin resistance. This could be linked to an elevated oxidation state of PTEN, associated with enhanced PI3K–AKT signalling (*68*).

In cardiovascular diseases, PTP oxidation has been implicated in restenosis (*49*, *69*), resistance of monocytes in diabetic individuals to vascular endothelial growth factor (VEGF) stimulation (*70*), cardiac hypertrophy (*71*) and in hypoxia–reoxygenation in the context of ischaemia (*53*). Very recently, PTP oxidation has been implicated in cardiac side effects of a therapeutic agent for acute promyelocytic leukaemia, arsenic trioxide (As_2O_3). Oxidation of PTEN in cardiac muscle cells as a consequence of As_2O_3 treatment causes enhanced formation of phosphatidylinositol-3-phosphate, and in turn elevated Ca^{2+} currents and arrhythmia (*72*).

Table V. Implication of PTP oxidation in pathologies.

Pathologic condition	Oxidized PTP(s)	Comments	References
Cancer	PTP1B	Irreversible oxidation states were demonstrated in cancer cell lines	(*61*)
Cancer	PTEN	Loss of the tumour suppressor PTEN leads to activation of AKT signalling, important role of PTEN oxidation shown in T-ALL, role demonstrated also in mouse models of breast cancer	(*62*, *64*, *65*, *67*)
Restenosis	PTPs negatively regulating PDGFR signalling	Elevated PDGFR signalling through PTP oxidation contributes to restenosis in a mouse model of PrxII deficiency, and in a rat carotid balloon-injury model	(*49*, *69*)
Hypoxia–reoxygenation	SHP2, DEP1	A condition associated with ischaemia and organ transplantation, which leads to high ROS levels	(*53*)
Cardiac hypertrophy	SHP2	Urotensin II causes cardiomyocyte hypertrophy via SHP2 inactivation and EGF receptor transactivation in cultured primary rat cardiomyocytes	(*71*)
Cardiac arrhythmia	PTEN	Oxidized as side effect of treating promyelocytic leukaemia with As_2O_3	(*72*)
Diabetes	PTP1B	PTP oxidation may underlie resistance of monocytes to VEGFA stimulation, which is linked to failed arteriogenesis	(*70*)
Insulin resistance	PTEN	Oxidation of PTEN in Gpx1-deficient mice protects from high-fat diet-induced insulin resistance.	(*112*)
Selenium depletion	PTP1B	Condition affects activity and expression of Se-dependent peroxidases, depletion caused elevated PTP1B-glutathionylation in rats	(*106*)
UV irradiation	PTP1B, RPTPκ	UV (UVA, UVB) leads to EGFR activation through inactivation of negatively regulating PTPs in A431 cells and keratinocytes. RPTPκ inactivation by UVA/B was shown in human buttock skin *in vivo*.	(*107*, *108*)
Glutamate-induced neurotoxicity	Erk1/2 directed PTPs, possibly MKP3	Sustained Erk1/2 activation presumably mediated by PTP oxidation causes neuronal cell death	(*109*)

In many cases, the existing studies correlate PTP oxidation with the pathology, but do not rigorously test a causal involvement.

Future perspectives

Studies on PTP oxidation are now maturing. A number of research areas are predicted to yield significant results in the upcoming years.

Concerning the biochemistry of PTP oxidation, one area of major importance is the issue of specificity of the process. Novel tools for monitoring of PTP oxidation are likely to help obtaining a more detailed description of the oxidation status of different PTPs under various conditions. Development of improved methods for the spatio-temporal aspects of PTP oxidation is also likely to yield new and important information.

As noted above, the re-activation of PTPs remains poorly characterized. It will be interesting to see to what extent this also represents an important regulatory mechanism. The recent suggestion that other oxidants than H_2O_2, such as peroxidized lipids, are involved in PTP oxidation will most likely be followed-up by new studies. In this context, it is noteworthy that oxidized peroxidases have been shown to mediate oxidation of transcription factors and other proteins (*73*, *74*). If these, or related enzymes, also mediate PTP oxidation appears as a relevant topic for future explorative studies.

As noted on the discussion of the roles of PTP oxidation in patho-physiology, the studies on PTP oxidation in tissue settings are still relatively few. Continued efforts in this area will potentially uncover novel settings where PTP oxidation contributes to disease through impacts on growth factor signalling. An exciting implication of such findings would be the possibility to use correction of aberrant PTP oxidation as a novel therapeutic strategy. Obviously, such efforts will also depend on the emergence of novel ways to specifically affect the oxidation of individual PTPs. Development of such approaches should appear as a stimulating challenge for future studies.

Supplementary Data

Supplementary Data are available at *JB* online.

Acknowledgements

Elias Arner contributed to the review with critical and constructive comments. Markus Dagnell and other members of the Östman group are acknowledged for support and encouragement throughout the work with the review.

Funding

Work in the authors' laboratories has been supported by grants from the European Community (''PTP-NET'' Marie Curie Network MRTN-CT-2006-035830; F.D.B. and A.Ö.), the Deutsche Forschungsgemeinschaft (BO1043/6-3; F.D.B.), and from the Deutsche Krebshilfe (Collaborative grant 108401, TP2; F.D.B.) and Swedish Research Council (A.Ö.).

Conflict of interest
None declared.

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