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Minireview

Redox regulation of PTEN and protein tyrosine phosphatases in H_2O_2-mediated cell signaling

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Abstract Protein tyrosine phosphatase (PTP) is a family of enzymes important for regulating cellular phosphorylation state. The oxidation and consequent inactivation of several PTPs by H_2O_2 are well demonstrated. It is also shown that recovery of enzymatic activity depends on the availability of cellular reductants. Among these redox-regulated PTPs, PTEN, Cdc25 and low molecular weight PTP are known to form a disulfide bond between two cysteines, one in the active site and the other nearby, during oxidation by H_2O_2. The disulfide bond likely confers efficiency in the redox regulation of the PTPs and protects cysteine-sulfenic acid of PTPs from further oxidation. In this review, through a comparative analysis of the oxidation process of Yap1 and PTPs, we propose the mechanism of disulfide bond formation in the PTPs.

Key words: Protein tyrosine phosphatase; PTEN; Cdc25; Low molecular weight protein tyrosine phosphatase; Disulfide bond; Redox regulation; Hydrogen peroxide

1. Introduction

Protein tyrosine kinases and protein tyrosine phosphatases (PTPs) are involved in important cellular processes, such as cell growth, proliferation, and differentiation [1]. The cellular phosphorylation state is important in these processes and therefore these two groups of enzymes must be regulated precisely. The PTP superfamily is composed of almost 70 enzymes that, despite rather limited sequence similarity, share a common CX_5R (where X is any amino acid) active site motif and an identical catalytic mechanism. Based on their functions, structures, and sequences, PTPs can be classified into four main families: (1) tyrosine-specific phosphatases, (2) VH1-like dual specificity PTPs, (3) Cdc25, and (4) low molecular weight (LMW) phosphatases [2].

Ligands such as platelet-derived growth factor (PDGF) [3] and epidermal growth factor (EGF) [4] can stimulate the rapid and transient production of reactive oxygen species [5]. This production of reactive oxygen species is required for growth factor-induced tyrosine phosphorylation. Consistently, the addition of H_2O_2 instead of growth factor augments intracellular tyrosine phosphorylation levels and induces cellular proliferation [6,7].

All PTPs contain an essential cysteine residue (pK_a 4.7–5.4) in the signature active site motif, CX_5R, which exists as thiolate anion at neutral pH [8]. This thiolate anion contributes to the formation of a thiol-phosphate intermediate in the catalytic mechanism of PTPs. The active site cysteine is also the target of oxidation by H_2O_2 which can be reversed by small thiol compounds such as dithiothreitol and glutathione or by thioredoxin. The ability of intracellular H_2O_2 to inhibit PTP activity has been demonstrated by the observation that stimulation of A431 cells with EGF [9] or Rat-1 cells with PDGF [10] results in a selective reduction in the extent of chemical modification of active site cysteine. It has also been shown that treatment of various PTPs with H_2O_2 in vitro leads to oxidation of the active site cysteine to cysteine-sulfenic acid (Cys-SOH) and results in inhibition of activity [11].

According to recent studies, the active site cysteine of some PTPs (PTEN [12], Cdc25 [13], and LMW-PTP [14]) could form a disulfide bond with a neighboring cysteine when oxidized by H_2O_2. In this review, we compare biochemical, structural, and biological characteristics between PTPs, or between PTPs and non-PTPs that can form a disulfide bond. In addition, based on recent reports manifesting redox regulation mechanisms of PTPs and non-PTPs that are subject to redox regulation, a brief perspective will be presented.

2. PTEN

PTEN (phosphatase and tensin homolog) plays an essential role in regulating signaling pathways involved in cell growth and apoptosis. Mutations in the PTEN gene are now known to cause tumorigenesis in a number of human tissues [15]. Unlike most members of the PTP superfamily, PTEN utilizes the phosphoinositide second messenger, phosphatidylinositol 3,4,5-trisphosphate (PIP_3), as its physiological substrate

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Abbreviations: PTP, protein tyrosine phosphatase; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; PIP_3, phosphatidylinositol 3,4,5-trisphosphate

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[15,16]. PTEN reverses the action of phosphoinositide 3-kinase by catalyzing the removal of phosphate attached to the 3′-hydroxyl group of the phosphoinositide inositol ring. PIP_3 works as an important regulator of cell growth and survival signaling through a Ser/Thr protein kinase, Akt. By negatively modulating the phosphoinositide 3-kinase-Akt signaling pathway, PTEN functions as a key signal regulator and tumor suppressor [17,18].

PTEN consists of an N-terminal phosphatase domain, a lipid binding C2 domain, and a 50-amino acid C-terminal tail containing a PDZ binding sequence. Phosphorylation of three residues (Ser^{380}, Thr^{382}, and Thr^{383}) of the PTEN tail negatively regulates PTEN activity and also suppresses the activity by controlling the recruitment of PTEN into the PTEN-associated complex [19,20].

A recent work demonstrated that exposure of cells to H_2O_2 resulted in the oxidation of PTEN in a time-dependent and H_2O_2 concentration-dependent manner and its subsequent reduction [13]. By the analysis of cysteine mutants with mass spectrometry, it was revealed that essential Cys^{124} in the active site and Cys^{71} could form a disulfide bond (Fig. 1A). The in vivo redox states of PTEN were determined by acid trap and chemical modification of cysteine thiols followed by the comparison in non-reducing and reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). That reduction of H_2O_2-oxidized PTEN in cells might be dependent on thioredoxin was suggested by the analysis of immunoprecipitation with anti-PTEN antibody and inhibitor assay.

As mentioned above, mutations in the PTEN gene cause tumorigenesis by the constitutive Akt activation through an increased concentration of PIP_3. Recently, Leslie and coworkers reported that oxidative inactivation of PTEN in cells, by exogenously and endogenously produced H_2O_2, caused an increased concentration of PIP_3 and activated downstream signaling, such as cell proliferation through the Akt activation, while the signal was reversed by antioxidants [21]. This is significant in that the formation of H_2O_2 during normal cellular processes, for example, ligand binding, also results in the oxidation of PTEN and subsequent modulation of downstream signaling. It was proposed in the same report [21] that the decreased oxidative inactivation of PTEN could result in the forkhead activation and a subsequent activation of antioxidant enzyme such as catalase through a decreased Akt activation in *p66Shc*-null cells which had a reduced concentration of oxidants [22]. Therefore, *p66Shc*-null mice have increased resistance to paraquat and a 30% increase in life span [23].

3. Cdc25 phosphatases

Cdc25 family members consist of Cdc25A, which regulates the G1/S transition, and Cdc25B and Cdc25C which are involved in G2/M progression. The Cdc25 dual-specificity phosphatases control cell cycle progression by regulating cyclin-dependent kinases [24]. Thr^{14} and Tyr^{15}, located within the cyclin-dependent kinases, are major targets for Cdc25. Once these two residues are dephosphorylated, the proper cell cycle progression and subsequent association with cyclins follow [24].

Cdc25A is activated by phosphorylation and degraded by the ubiquitin-proteasome pathway in response to DNA damage [25]. Cdc25C is also regulated by phosphorylation on Ser^{216}, which forms a consensus binding site for a 14-3-3 protein [26]. Numerous lines of evidence suggest that binding of Cdc25C with a 14-3-3 protein leads to cytoplasmic localization [27].

Based on crystal structures of two Cdc25 family members, the active site cysteine could readily form an intramolecular disulfide bond with another conserved cysteine in the molecule [28,29]. Savitsky and Finkel showed that in vitro H_2O_2 stimulation caused an intramolecular disulfide bond between the active site Cys^{377} and another invariant, Cys^{330} (Fig. 1B). They also demonstrated that the exposure to H_2O_2 affected Cdc25C but not Cdc25A levels [14]. The in vivo stability of Cdc25C is reduced by mutation of either one of these two cysteines. In contrast, a double mutant of both cysteines results in a protein that is more stable than wild-type protein. Generally, it is accepted that Cys-SOH is unstable and prone to further irreversible oxidation. If the active site cysteine is oxidized to Cys-SOH, subsequent disulfide bond formation with Cys^{330} can rescue the protein and further oxidation is prevented. Mutants that lack either Cys^{330} or Cys^{377} do not have this protective mechanism. In the study of Savitsky and Finkel, the recovery of oxidized Cdc25C by cellular reductants was not revealed by PAGE analysis although a decreased interaction with 14-3-3 was indirect evidence of reduction [14].

The important role of Cdc25A and Cdc25B in cell cycle progression is apparent as many studies show their increased expression in various cancers [30–32] while some studies have demonstrated that oxidative stress induces cell cycle arrest in normal cells [33,34]. In addition, an increased concentration of H_2O_2 by inhibiting peroxiredoxins through Cdc2 activation in HeLa cells was proposed to result in the oxidation of Cdc25C and subsequent cell cycle arrest [35]. In some reports, the role of antioxidants such as thioredoxin [36] and glutathione [34,37] was addressed in terms of rescue of mitotic arrest and phase-specific modulation. These reports and suggestions indicate that redox regulation of Cdc25 phosphatase activity may be important for cell cycle regulation in both normal and cancer cells.

4. LMW-PTPs

LMW-PTP is a family of 18-kDa enzymes involved in cell growth regulation by dephosphorylating many growth factor receptors, such as PDGF receptor (PDGF-R), insulin receptor, and ephrin receptor. They act on growth factor-induced mitosis through dephosphorylation of activated PDGF-R [38], and on cytoskeleton rearrangement through dephosphorylation of p190RhoGAP and subsequent regulation of the small GTPase Rho [39].

LMW-PTP is activated by phosphorylation on two specific tyrosine residues, Tyr^{131} and Tyr^{132} [40,41]. Phosphorylation depends on localization of the enzyme since it is not confined to the cytoplasm but is also localized in the cytoskeleton- and plasma membrane-associated fractions [38]. Therefore, it seems that enzymatic activity depends on its localization: phosphorylated LMW-PTP can be detected only in the cytoskeleton-associated fraction.

Ramponi and coworkers demonstrated that in vitro LMW-PTP was oxidized by either H_2O_2 [12] or nitric oxide [42] to form a disulfide bond between the active site Cys^{12} and the neighboring Cys^{17}, and oxidized enzyme was reactivated by a reducing agent (Fig. 1C). In another report, they also showed

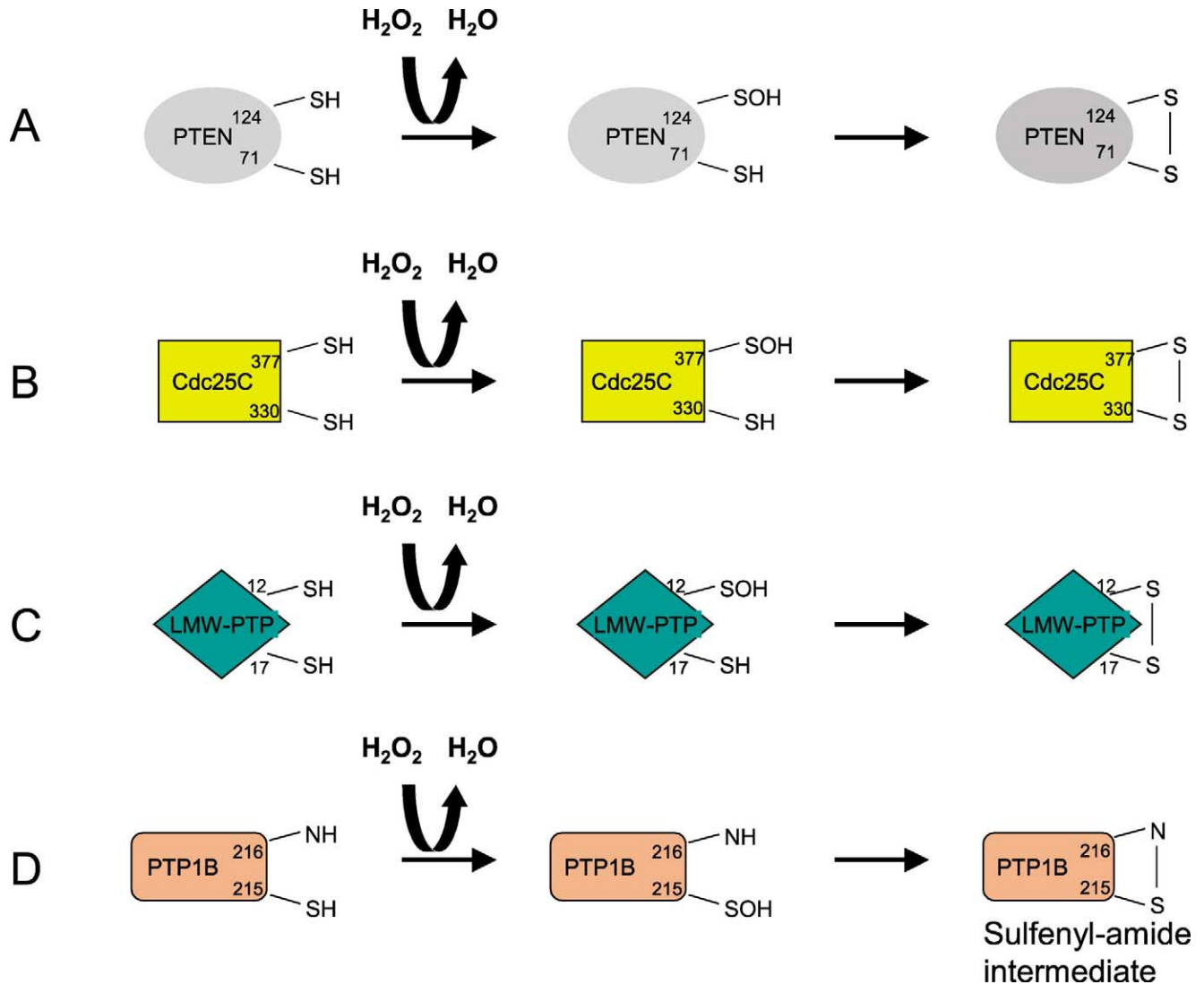

Fig. 1. Schemes for the oxidation of PTPs by H_2O_2. PTEN (A), Cdc25C (B), and LMW-PTP (C) are oxidized to form a disulfide bond via Cys-SOH as an intermediate. D: Reactive cysteine of PTP1B is oxidized to Cys-SOH and then converted into sulfenic-amide intermediate.

the in vivo inactivation of LMW-PTP by either exogenous or endogenous H_2O_2 [43]. After oxidative inactivation of LMW-PTP upon PDGF stimulation, the catalytic activity was recovered by reduction. However, in the above studies, the in vivo formation of the intramolecular disulfide bond in LMW-PTP was not manifested upon exposure to H_2O_2 extracellularly or intracellularly. Based on a dramatic decrease in rescuing enzymatic activity of a C17A mutant upon reduction, they suggested that the oxidation by H_2O_2 leads to intramolecular disulfide bond formation in wild-type enzyme. They showed that LMW-PTP oxidized in vitro was restored by LMW thiols. Likewise, they suggested that the restoration of LMW-PTP in vivo was glutathione-dependent based on observations using buthionine sulfoxide, an inhibitor of the γ-glutamylcysteine synthetase [43].

5. Structural comparison of PTEN, Cdc25, and LMW family proteins

PTP domains of high-resolution three-dimensional structures of the aforementioned three PTPs (PTEN [44], Cdc25 [28], and LMW-PTP [45]) were compared with each other on an atomic basis. Topological and structural comparison among PTP domains of the three proteins does not imply any evolutionary relationship among them (Fig. 2). However, in the case of PTEN and Cdc25, the local structures of the P loop encompassing the active site are strikingly similar to each other. An essential cysteine residue (Cys^{124} and Cys^{430} in PTEN and Cdc25A, respectively) in the P loop is protruded into the active site pocket and is in conformation to participate in catalysis as well as redox-mediated disulfide bond formation. This reactive cysteine residue and the cysteine partner (Cys^{71} and Cys^{384} in PTEN and Cdc25A, respectively) of the disulfide bond are closely located spatially although they are about ~50 residues apart in the primary sequence. Distances between α carbon atoms of the two cysteine residues are 5.08 Å and 4.76 Å in PTEN and Cdc25A, respectively, which is in the range (4.4–6.8 Å long) of the normal Cα–Cα distance of disulfide bonds [46]. This indicates that formation of the redox-mediated disulfide bond in these two proteins is a ready process and can occur without any major conformational change. It is believed that this spatial arrangement of

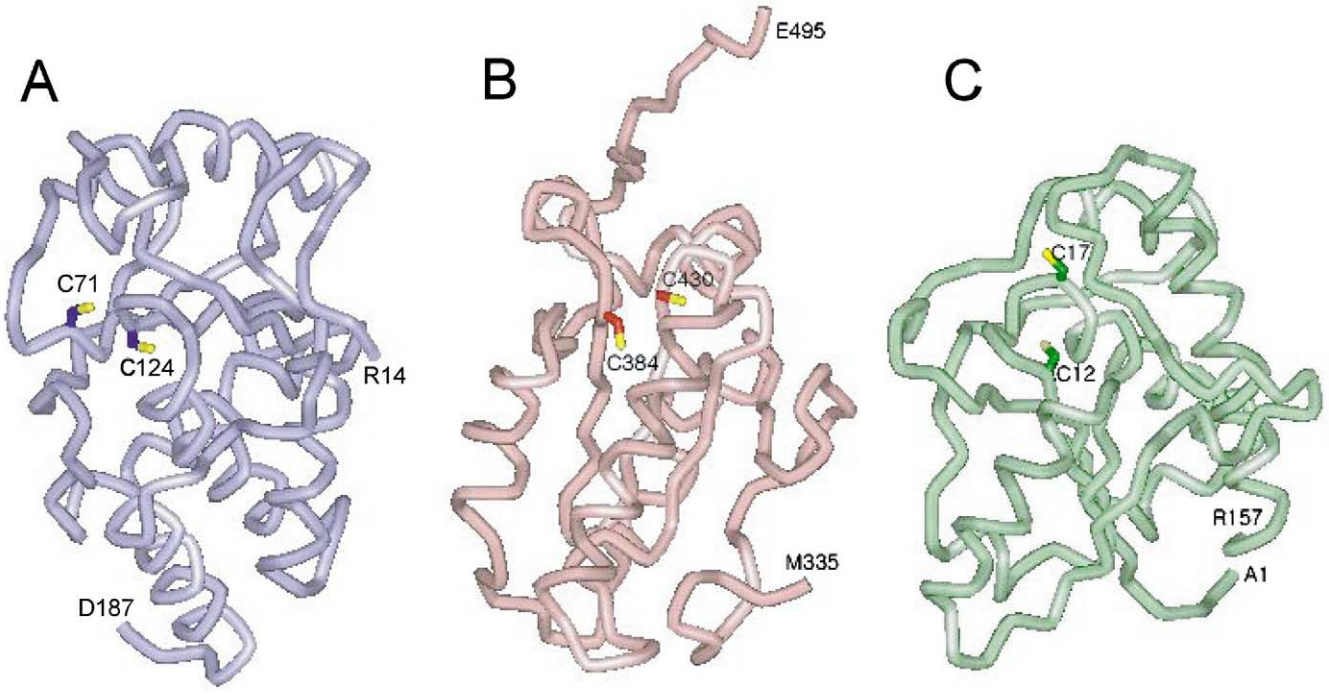

Fig. 2. Structural comparison of PTEN, Cdc25A, and LMW-PTP. Depicted are (A) the N-terminal phosphatase domain of PTEN (PDB entry 1D5R [44]), (B) Cdc25A (PDB entry 1C25 [28]), and (C) LMW-PTP (PDB entry 1DG9 [45]). Cys^{430} and Cys^{384} in Cdc25A are equivalent to Cys^{377} and Cys^{330} in Cdc25C, respectively. Indicated in each protein are the two cysteine residues responsible for H_2O_2-mediated disulfide bond formation as well as N- and C-termini. Distances between α carbon atoms of the two cysteine residues are 5.08 Å, 4.76 Å, and 8.08 Å in PTEN, Cdc25A, and LMW-PTP, respectively. The diagrams were created with WebLab ViewerPro 4.0 (Molecular Simulations).

two cysteine residues (one in the P loop and the other in its proximity) is a structural signature motif for the special class of PTP that forms the redox-mediated disulfide bond. A manual search with this structural motif through the structural database yielded Shp-2 as a novel candidate, but further intensive analyses should be made before reaching the conclusion that Shp-2 belongs to the special class of PTP.

Compared to PTP domains of PTEN and Cdc25, LMW-PTP has a different configuration of the cysteine pair. The two residues are located in the same loop and their α carbons are separated about 8.08 Å, so that the protein needs to undergo a substantial conformational change in order to form a disulfide bond. However, considering the flexible nature of the loop structure, disulfide bond formation between the two cysteines is considered to be a plausible process. Yet, it is not certain whether this unique feature of the cysteine pair is related to any special function of LMW-PTP or whether it accounts for conflicts in observing in vivo formation of the disulfide bond in LMW-PTP.

6. Benefit of the disulfide bond

It has been shown that treatment of various PTPs with H_2O_2 in vitro leads to oxidation of the active site cysteine to Cys-SOH and results in the inhibition of activity. It can be reversibly converted to the reduced form, Cys-SH. During redox regulation of PTP1B, glutathionylation of active site cysteine is observed. This process is proposed to protect Cys-SOH from further oxidation which can lead to the formation of the irreversible cysteine-sulfinic acid (Cys-SO_2H) and cysteine-sulfonic acid (Cys-SO_3H) [47]. Cys-SOH produced by H_2O_2 can undergo another type of modification. It is rapidly converted to a previously unknown sulfenylamide intermediate, in which the sulfur atom of the catalytic cysteine is covalently linked to the main chain nitrogen of an adjacent residue (Fig. 1D) [48,49]. Its function is also proposed to be protection of the active site cysteine residue of PTP1B from the irreversible further oxidation.

Unlike PTP1B and other PTPs, in the aforementioned three PTPs, a reversible disulfide bond is formed between the active site cysteine and the neighboring cysteine. Importantly, redox regulation through disulfide bond formation is another mechanism for regulating the activity of PTPs. The presence of the disulfide bond partner cysteine of the active site cysteine, which may be located either near to or distant from active site cysteine in primary sequence, may confer certain efficiency in the redox regulation of PTPs as well as protection of Cys-SOH from further oxidation. Although the modes and efficiency of redox regulation are different among PTPs, it seems likely that the redox regulation in PTPs has the same purpose of protecting Cys-SOH from further oxidation.

7. Comparison of non-PTP proteins regulated by disulfide bond

The formation and functional role of Cys-SOH have recently been revealed in several proteins: enzymatic activity in PTP and glutathione reductase; essential redox center in NADH peroxidase, NADH oxidase, and the peroxiredoxins; both iron coordination and nitric oxide binding in nitrile hydratase; sensor for cellular redox status and DNA binding activity in Fos, Jun, and bovine papillomavirus-1 E2 protein [50].

Likewise, the formation and functional role of disulfide bond have been studied extensively in redox-sensitive tran-

scription factors such as *Escherichia coli* OxyR and *Saccharomyces cerevisiae* Yap1 as well as bacterial chaperone Hsp33 [51]. Storz and coworkers showed that one mechanism responsible for the activation of OxyR upon exposure to H_2O_2 involves the formation of a disulfide bond [52]. The formation of the OxyR (Cys^{199}–Cys^{208}) disulfide bond results in major structural rearrangement, which in turn results in a change in DNA binding specificity and recruitment of RNA polymerase [53]. Toledano and coworkers reported that Yap1, a functional homolog of OxyR in yeast, also formed a disulfide bond between Cys^{598} and Cys^{303} upon exposure to H_2O_2 which in turn resulted in the accumulation of Yap1 in the nucleus and activation of transcription of stress response genes [54]. Under normal unstressed conditions, the critical four cysteines of Hsp33 serve as ligands for a zinc atom [55]. Upon exposure to oxidative stress, the cysteines are oxidized to form pairs of disulfide bonds. Like OxyR and Yap1, it is only the oxidized form of Hsp33 that exhibits chaperone activity. These three oxidized molecules are returned to their inactive forms through reduction of the disulfide bond by the thioredoxin or glutaredoxin systems. By contrast with the aforementioned three PTPs, these three molecules have 'activity' when they are disulfide-bonded.

8. Perspectives

Modulation of protein activity by disulfide bond formation is found not only in PTPs but also in very diverse proteins. It might be a universal and common mechanism of protein redox regulation, adopting a very fast and efficient turn-on/off mechanism of cellular signals.

In LMW-PTP, direct evidence of in vivo disulfide bond formation has yet to be presented. The gel mobility shift assay might be a good tool for the separation of disulfide-bonded species from reduced ones [13,14]. It will also be helpful to adopt the method of Watson and Jones, in which oxidized and reduced thioredoxins can be resolved in native PAGE after modification of cysteines with iodoacetic acid [56] since LMW-PTP has the CX_4C motif and its mass is 18 kDa, similar to thioredoxin.

Contrary to LMW-PTP and PTEN, Cdc25C is known to be considerably down-regulated in the protein concentration upon oxidative stress. Although the formation of the disulfide bond in response to oxidative stress and the recovery to its thiols are proposed to be involved in regulating protein stability, it is necessary to detect the in vivo oxidized species and reveal whether their oxidized species contain a disulfide bond or not.

It is a generally accepted mechanism of disulfide bond formation by H_2O_2 that the reactive cysteine thiol is directly oxidized to Cys-SOH by H_2O_2, then Cys-SOH is attacked by a neighboring cysteine thiol, forming a disulfide bond between them. However, in Yap1, the reactive Cys-SH is not directly oxidized to Cys-SOH by H_2O_2. It is indirectly oxidized by Cys-SOH of oxidized Gpx3 to form an intermolecular disulfide bond between two molecules, followed by the disulfide bond rearrangement to form an intramolecular disulfide bond in Yap1 [57]. Therefore, the aforementioned PTPs might also be oxidized by mediation of a certain thiol peroxidase like Gpx3 (Fig. 3). Gpx3 is a phospholipid hydroperoxidase and has a cysteine residue at the conserved active site instead of the selenocysteine most commonly found in other glutathione peroxidases [58]. It is suggested that it works as a sensor and transducer of the hydroperoxide signal [57]. Thiol peroxidases like Gpx3 have merits as mediators of H_2O_2 signaling since they have high hydroperoxide reactivity as selenoproteins [58] and might have the specificity in cellular function.

According to the models of Jhoti and coworkers, sulfenylamide intermediate is formed by dehydration of water from

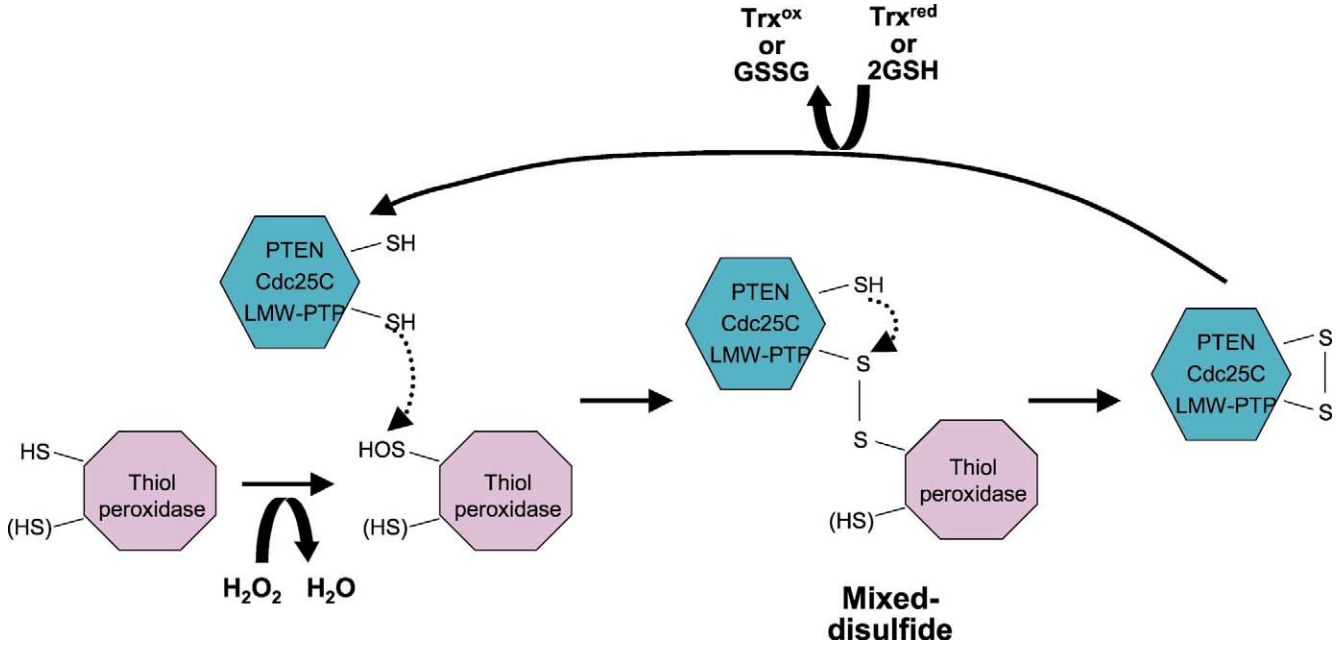

Fig. 3. Putative models for the formation of intramolecular disulfide bonds in PTEN, Cdc25C, and LMW-PTP. Thiol peroxidase senses H_2O_2 to yield Cys-SOH. Cys-SOH reacts with PTPs to form thiol peroxidase-PTP mixed disulfide. Another cysteine pair in PTP then resolves the bond to result in the production of a disulfide bond in PTP and the recovery of thiol peroxidase to the reduced form. Trx^{ox} and Trx^{red} represent oxidized and reduced thioredoxins, respectively. GSSG and GSH represent oxidized glutathione and glutathione, respectively.

Cys-SOH species, but it could be alternatively formed by the oxidation of Cys-SOH by H_2O_2 or oxidized glutathione (oxidative mechanism) [49]. Therefore, it is also possible that an oxidized thiol peroxidase can play a role in the oxidation of Cys-SOH in PTP1B, replacing glutathione. This idea might also be applicable to the formation of disulfide bonds in aforementioned three PTPs. The proposed mechanism adopts the nucleophilic attack of the S_γ atom in vicinal cysteine instead of backbone nitrogen on the S_γ atom of oxidized cysteine. The involvement of an oxidized thiol peroxidase might offer specificity to cellular functions in addition to catalytic efficiency, compared to small molecular thiols such as glutathione.

The oxidation products of reactive cysteines in PTPs are Cys-SOH, glutathionylated cysteine, disulfide bond with neighboring cysteine, and the recently reported sulfenyl-amide intermediate. While there are very diverse oxidized species of cysteines and the oxidation mode to produce them, it seems that the chance of stable existence of Cys-SOH is excluded. There is a possibility that other kinds of oxidation product exist in redox-regulated proteins. We have mentioned the irreversibility of Cys-SO_2H of PTPs. But according to Rhee and coworkers, the Cys-SO_2H form of peroxiredoxin I, produced during the exposure of cells to H_2O_2, was rapidly reduced to form a catalytically active thiol species [59]. Also, a sulfiredoxin that could reduce the Cys-SO_2H form of peroxiredoxin using ATP and magnesium was found in yeast by Toledano and coworkers [60]. Therefore, in PTPs, the reversibility of Cys-SO_2H as another mechanism should not be completely excluded and the irreversibility in the cell should be verified.

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