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Review

Low molecular weight protein tyrosine phosphatases: small, but smart

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Abstract. Low molecular weight protein tyrosine phosphatases (LMW-PTPs) are a family of 18-kDa enzymes involved in cell growth regulation. Despite very limited sequence similarity to the PTP superfamily, they display a conserved signature motif in the catalytic site. LMW-PTP associates and dephosphorylate many growth factor receptors, such as platelet-derived growth factor receptor (PDGF-r), insulin receptor and ephrin receptor, thus downregulating many of the tyrosine kinase receptor functions that lead to cell division. In particular, LMW-PTP acts on both growth-factor-induced mitosis, through dephosphorylation of activated PDGF-r, and on cytoskeleton rearrangement, through dephosphorylation of p190RhoGAP and the consequent regulation of the small GTPase Rho. LMW-PTP activity is modulated by tyrosine phosphorylation on two specific residues, each of them with specific characteristics. LMW-PTP activity on specific substrates depends also on its localization. Moreover, LMW-PTP is reversibly oxidized during growth factor signaling, leading to inhibition of its enzymatic activity. Recovery of phosphatase activity depends on the availability of reduced glutathione and involves the formation of an S–S bridge between the two catalytic site cysteines. Furthermore, studies on the redox state of LMW-PTP in contact-inhibited cells and in mature myoblasts suggest that LMW-PTP is a general and versatile modulator of growth inhibition.

Key words. PTP; LMW-PTP; redox regulation; PDGF-r; cytoskeleton rearrangement; growth inhibition.

Introduction

Protein tyrosine phosphorylation plays a key role in the regulation of many cellular processes in eukaryotes, such as cellular metabolism, proliferation and differentiation [1]. Growing evidence indicates that the contribution of protein tyrosine phosphatases (PTPs) to control of the cell phosphorylation state is as relevant as that of phosphotyrosine protein kinases. The PTP superfamily is composed of almost 70 enzymes that, despite very limited sequence similarity, share a common CX_5R active-site motif and an identical catalytic mechanism. On the basis of their function, structure and sequence, PTPs can be classified into four main families: (i) tyrosine-specific phosphatases, (ii) VH1-like dual specificity PTPs, (iii) the cdc25 and (iv) low molecular weight phosphatases [2].

Low molecular weight PTPs (LMW-PTPs) are a group of 18-kDa enzymes with no particular tissue-specific expression [3]. Four different human LMW-PTP messenger RNA (mRNA) isoforms, derived by alternative splicing of a single transcript, have been characterized. Two of them correspond to the classical active isoforms 1 and 2, IF1 and IF2 according to Modesti et al. [4]. These two isoforms differ only in the sequence spanning residues 40–73 [5, 6]. Only for IF2 has strong in vitro activation by purine compounds been demonstrated such as guanosine and cGMP [3]. No differing physiological effects be-

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tween the two isoforms have so far been demonstrated. In addition, SV3 (splicing variant 3) [4] and LMW-PTP-C [7] inactive forms were characterized. For the latter, a possible role as a natural dominant negative has been proposed.

Previous studies on the molecular biology of LMW-PTP in NIH3T3 cells demonstrated a well-defined role of this enzyme in PDGF-induced mitogenesis, showing that activated platelet-derived growth factor receptor (PDGF-r) is a substrate for LMW-PTP [8, 9]. The most relevant phenotypic effects of LMW-PTP overexpression are strong reduction of cell growth rate in response to PDGF stimulation, together with upregulation of cell adhesion and chemotaxis [8]. The association and dephosphorylation of PDGF-r by LMW-PTP has been demonstrated in cells expressing wild-type LMW-PTP or a Cys^{12} to Ser mutant enzyme. This mutant is totally inactive and behaves as a dominant-negative enzyme for LMW-PTP, as it prevents dephosphorylation of PDGF-r from the native enzyme.

PDGF receptor is not the only substrate for LMW-PTP at the plasma membrane: insulin receptor [10] as well as ephrin receptors [11] and fibroblast growth factor receptor (FGF-r) [12] have been recognized as other specific substrates for LMW-PTP. Moreover, LMW-PTP is able to reduce FGF-r tyrosine phosphorylation and cell proliferation in response to basic FGF [12] and to macrophage colony-stimulating factor [13]. Finally, very recently Rigacci et al. [14] demonstrated that LMW-PTP associates with and dephosphorylates p125FAK, participating in focal adhesion remodeling and promoting cell motility in the absence of growth factors.

This review will mainly be focused on regulation of LMW-PTP, underlining how LMW-PTP activity may be positively modulated upon tyrosine phosphorylation and negatively regulated upon cysteine oxidation, and how LMW-PTP localization is important for its activity on different substrates.

Regulation of LMW-PTP by phosphorylation and subcellular localization

Figure 1 shows the three-dimensional structure of LMW-PTP, indicating the position of the most relevant amino acids. The catalytic loop structure of mammalian LMW-PTPs is totally conserved, presenting the characteristic CLGNICR sequence [3]. In this sequence, the first cysteine and the last arginine residues are both crucial for enzymatic activity, together with an aspartic acid, positioned far away in the primary structure (position 129), but located in the vicinity of the catalytic loop, as revealed by the analysis of the three-dimensional structure [15, 16]. Beyond these residues, two adjacent tyrosines, in position 131 and 132, are also conserved and have been recognized as the preferential sites of phosphorylation.

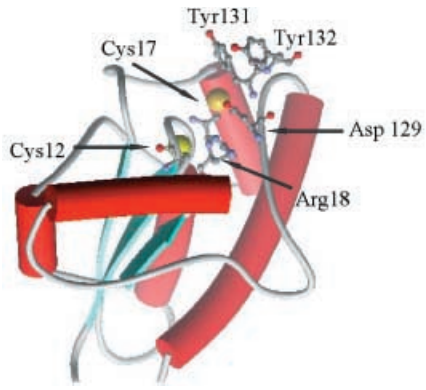

Figure 1. The structure of LMW-PTP with features of the catalytic site residues. The sulfur atoms of both Cys^{12} and Cys^{17} are shown in yellow.

LMW-PTP contains only five tyrosine residues. Tyr^{131} and Tyr^{132} have been recognized to be exposed on the surface of the molecule [15] and are preceded by two acidic residues at positions –3 and –2, a preferential site of phosphorylation for many PTKs. A first indication of LMW-PTP tyrosine phosphorylation was obtained in NIH-3T3 cells transformed with the *v-src* tyrosine kinase [17]. This kinase is able to phosphorylate LMW-PTP in vitro, leading to enzymatic activation. Later on, the phosphorylation of LMW-PTP by Lck and Fyn tyrosine kinases was shown in T lymphocytes: Tailor et al. [18] demonstrated phosphorylation at Tyr^{131} and, to a lesser extent, at Tyr^{132}. They also showed a twofold enzymatic activation upon phosphorylation, thus confirming the importance of residues lining the active-site pocket for regulation of enzyme activity.

Further studies on LMW-PTP tyrosine phosphorylation were performed by Bucciantini et al. [19]. Mutants were generated at positions 131 and/or 132, substituting tyrosine with alanine, in order to study their behavior upon phosphorylation. LMW-PTP phosphorylation was performed in vitro with the use of Src tyrosine kinase, which strongly and specifically phosphorylates Tyr^{131} and Tyr^{132}. In this way it was concluded that the phosphorylation at either residue has different effects on the enzyme behavior. Tyr^{131} phosphorylation is followed by a strong (about 25-fold) increase in enzyme-specific activity [19], much stronger that previously reported [17, 18]. This result highlights the importance of this mechanism in controlling LMW-PTP activity. Phosphorylation at Tyr^{132}, in contrast, does not affect enzyme activity. A different role for Tyr^{132} phosphorylation has been proposed. This residue is followed by the conserved sequence G-N-D, which conforms to the consensus motif for phosphotyrosine recognition by the Grb2 SH2 domain (*P*Y-X-N-X) [20]. Bucciantini et al. (19) showed that in vitro Grb2 binds synthetic phosphopeptides derived from the LMW-PTP sequence around Tyr^{131} and Tyr^{132}. Moreover, using

specific LMW-PTP mutants where either Tyr^{131} or Tyr^{132} were substituted with alanine, they demonstrated that Grb2 binds to LMW-PTP only when the phosphatase is phosphorylated in vitro on Tyr^{132} by Src. These results strongly suggest that Tyr^{132} is specifically recognized by Grb2 (most likely at the level of the SH2 domain).

It has long been reported that LMW-PTP is exclusively a cytosolic enzyme. On the contrary, LMW-PTP is not confined only to the cytoplasm but, rather, is localized also in the cytoskeleton- and plasma-membrane-associated structures, as demonstrated by Cirri et al. [21]. LMW-PTP phosphorylation depends on localization of the enzyme in the cell, independent of addition of growth factors such as PDGF [21], or FGF-r [12] or MCSF-r [13]. In fact, LMW-PTP tyrosine phosphorylation can be detected only in the cytoskeleton-associated fraction. Moreover, after PDGF stimulation c-Src is able to bind and to phosphorylate LMW-PTP only in this fraction (physical association of LMW-PTP with Src was demonstrated using coimmunoprecipitation analysis). While the LMW-PTP cytosolic pool, as already shown, binds and dephosphorylates activated (and autophosphorylated) PDGF-r, the cytoskeleton-associated pool, in contrast, specifically acts on substrates that become tyrosine phosphorylated upon PDGF treatment, surely distinct from PDGF-r. These results indicate that after PDGF stimulation, the two constitutive LMW-PTP pools possess very marked differences in enzyme-specific activity that could reflect distinct roles in signal transmission. Marked differences were also demonstrated in the duration of the two phenomena in which LMW-PTP is involved. LMW-PTP interaction with PDGF-r, restricted to the cytosolic LMW-PTP fraction, is transient and reaches a maximum 5 min after PDGF stimulation, while LMW-PTP phosphorylation in the cytoskeleton-associated fraction is a long-lasting phenomenon and decreases only after 40 min. These temporal and spatial differences between the two LMW-PTP pools already suggest the existence of a different specificity for each pool.

Further studies, using coimmunoprecipitation analysis indicate that p190Rho-GAP, a protein involved in the regulation of cytoskeleton rearrangement, is a possible substrate for the LMW-PTP associated to the cytoskeletal fraction [22]. Furthermore, a mutant form of LMW-PTP in which the c-Src phosphorylation sites (Tyr^{131} and Tyr^{132}) were mutated to alanine was transfected in NIH-3T3 cells. With this tool the authors established that LMW-PTP tyrosine phosphorylation, which is consequent to PDGF administration, is not essential for LMW-PTP translocation from the cytoplasm to the cytoskeleton. Moreover, LMW-PTP phosphorylation is a phenomenon that has a profound effect in integrin- and PDGF-mediated signaling, in particular on cytoskeleton rearrangements following mitotic stimulus. In fact, wtLMW-PTP overexpression in NIH-3T3 cells leads to a strong increase in cell adhesion, spreading and migration. On the other hand, cells expressing the Y131A/Y132A LMW-PTP double mutant have only a slight variation in these parameters with respect to control cells, indicating that LMW-PTP phosphorylation is required for regulating all these aspects of cytoskeleton rearrangement. Figure 2 presents a model that summarizes the different roles proposed for LMW-PTP in the cell.

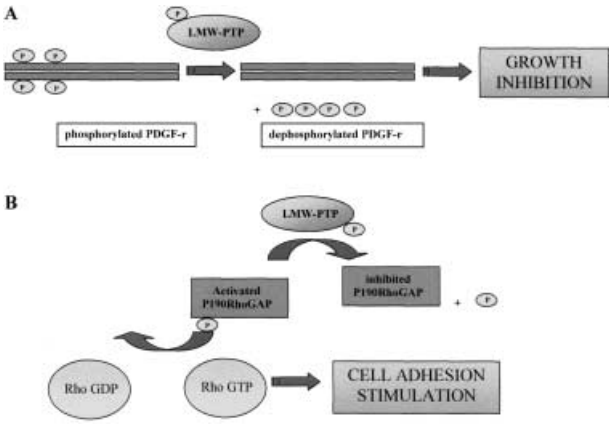

Figure 2. The effect of LMW-PTP on its substrates (*A*) PDGF-r and (*B*) p190 RhoGAP.

As emphasized above, it is very likely that p190Rho-GAP is a specific substrate of LMW-PTP. This observation correlates very well with evidence for the influence of LMW-PTP expression on phenomena such as cell adhesion, spreading and migration, all depending on integrin-dependent cytoskeletal rearrangements [8, 23]. In fact, Rho is upregulated in adherent cells, whereas its activity is decreased in suspended cells. The existence of a negative feedback loop that might prevent excessive contraction under physiological conditions has been hypothesized [24]. One of the candidates that could mediate this regulative effect on Rho activity is p190Rho-GAP: it has been shown that inhibition of p190Rho-GAP activity is sufficient for induction of Rho-mediated actin reorganization [25]. p190Rho-GAP itself becomes tyrosine phosphorylated by c-Src after epidermal growth factor stimulation, and this correlates with rapid disassembly of actin stress fibers [26, 27]. It has been proposed that p190Rho-GAP is a strong candidate effector of v-Src-induced cytoskeletal disruption, most likely mediated by inhibition of Rho [28]. It is interesting to notice that insulin treatment does not lead either to p190RhoGAP or to LMW-PTP phosphorylation: while LMW-PTP influences both PDGF, and insulin-induced mitogenesis (even if to different extents), it induces a decrease in cellular adhesion and chemotaxis only after PDGF treatment. For this reason it has been proposed that the differential effect of LMW-PTP on PDGF and insulin signaling may be due mainly to the absence of LMW-PTP phosphorylation [29].

The specific physiological role of Tyr^{131} and Tyr^{132} phosphorylation was further investigated, using of the specific tyrosine LMW-PTP mutants [30]. Both tyrosines are phosphorylated during PDGF signaling, but each tyrosine residue is involved in specific LMW-PTP functions. As far as Tyr^{131} phosphorylation is concerned, two distinct effects can be distinguished, one dealing with LMW-PTP-dependent regulation of cytoskeletal rearrangement mechanisms and a second involving the LMW-PTP-dependent regulation of cell growth on PDGF stimulation. The first effect of LMW-PTP Tyr^{131} phosphorylation is increased fibronectin-mediated cell adhesion and PDGF-induced cell migration. The increased LMW-PTP activity due to phosphorylation leads to a decrease in p190RhoGAP phosphorylation and hence to induction of Rho-mediated actin reorganization. The second effect of LMW-PTP Tyr^{131} phosphorylation is due to PDGF-r dephosphorylation and mediates the LMW-PTP-dependent inhibition of mitosis [21].

As far as Tyr^{132} is concerned, it has already been hypothesized that this phosphorylated residue may interact with Grb2 [19]. Physical association of Grb2 with LMW-PTP was also observed [30]. Phosphorylation on Tyr^{132} leads to an increase in the strength of cell substrate adhesion. This effect is most likely due to downregulation of matrix metalloprotease (MMP) expression, through inhibition of the Grb2/MAPK pathway. It is well known that many cellular processes such as cell migration, adhesion and proliferation require the collaboration of growth factor and extracellular matrix (ECM) stimuli [31, 32]. Cell adhesion to ECM, on the other hand, is a balance between focal adhesion formation and proteolysis of ECM. The addition of a growth factor that induces cell migration has a double role: (i) it promotes cell substratum adhesion through the regulation of Rho-mediated cytoskeleton rearrangement and (ii) induces MMP expression, which in turns promotes the degradation of basement membranes and stromal ECM. These concomitant events allow cells to assume an adhesive condition that permits migration [32]. Chiarugi et al. [30] propose that LMW-PTP is a bifunctional phosphatase that regulates both the strength and rate of formation of cell adhesion through alternative phosphorylation of LMW-PTP in Tyr^{131} or Tyr^{132}.

We have already stressed that LMW-PTP is not restricted only to the cytosolic compartment, but is present also in cytoskeleton-associated fractions. Very recently it has also been found associated to caveolin, suggesting a possible involvement in regulating caveolin action. Caselli et al. [33] identified tyrosine phosphorylated caveolin as a new potential substrate for LMW-PTP. Caveolin is tyrosine phosphorylated in vivo by kinases of the Src family, recruits into caveolae and hence regulates the activity of several proteins involved in cellular signaling cascades. Caselli et al. showed that a fraction of LMW-PTP localizes in caveolae and that the two molecules physically associate. Moreover, overexpression of the C12S dominant-negative mutant of LMW-PTP causes enhancement of tyrosine-phosphorylated caveolin. This result, together with the fact that LMW-PTP very rapidly dephosphorylates phosphocaveolin in vitro, suggests a direct action of LMW-PTP in dephosphorylating caveolin. In the same work it is also suggested that the interaction with caveolin is very likely mediated by a LMW-PTP sequence motif similar to those found in other caveolin-binding proteins. Carman et al. [34] have reported that all known G-protein-coupled receptor kinases contain the conserved caveolin-binding motif (I/L)*XXXX*F*XX*F. This caveolin-binding motif is also present in LMW-PTP (residues 77–85) [35], suggesting that caveolin interacts with this LMW-PTP region. The localization of LMW-PTP in caveolae, the in vivo interaction between this enzyme and caveolin, and the capacity of this enzyme to rapidly dephosphorylate phosphocaveolin indicate that tyrosine-phosphorylated caveolin is a relevant substrate for this phosphatase.

Redox downregulation of LMW-PTP during PDGF signaling

Both human LMW-PTP isoenzymes are inactivated by H_2O_2 and NO. The two isoenzymes are protected from inactivation by Pi, a competitive inhibitor, suggesting that the H_2O_2 action is directed towards the active site [36, 37]. Only two out of the eight cysteine residues present in LMW-PTP are modified by H_2O_2 treatment. High-performance liquid chromatography-electrospray spectroscopy, together with specific radiolabeling and tryptic fingerprint analyses, demonstrated that H_2O_2 causes the oxidation of LMW-PTP to form a disulphide bond. The activity is restored after treatment with thiols, such as dithiothreitol or reduced glutathione [36, 37].

It has been shown that reactive oxygen species (ROS), such as O_2^- or H_2O_2, are transiently generated intracellularly when cells are stimulated with cytokines or growth factors [38]. In addition, an exogenous oxidative stress could be produced in various physiological conditions, such as the lymphocytic and macrophagic oxidative burst or in pathological conditions, such as reperfusion and so on [38]. Recent findings indicate that treatment with growth factors or H_2O_2 induces elevation of tyrosine-phosphorylated proteins. This elevation can be achieved by the activation of protein tyrosine kinases (PTKs) and/or inactivation of PTPs. While there is no convincing evidence that PTKs are activated by ROS, PTPs have been shown to be regulated by a redox mechanism [39–41]. Oxidation of PTPs always takes place at the catalytic site cysteine, where the sulphydrylic residue is transformed in sulphenic acid. Oxidized PTPs are catalytically inactive due to their inability to form the cysteinyl-phosphate intermediate during the first step of the

catalysis. Lee et al. recently reported that PTP1B is reversibly inactivated in A431 cells stimulated with epidermal growth factor (EGF) [40]. These observations suggest that PTPs might undergo H_2O_2-dependent inactivation in cells, resulting in a shift, in the balance with PTKs, towards phosphorylation [42, 43].

It has been assessed that LMW-PTP is oxidized in vivo by an exogenous oxidative stress, such as H_2O_2 produced by glucose oxidase or sodium pervanadate [44]. Quantitation of the LMW-PTP in vivo activity directly on its natural phosphorylated substrates (PDGF-r and p190RhoGAP) demonstrated that oxidation of LMW-PTP leads to inactivation of the enzyme, preventing dephosphorylation of both PDGF-r and p190Rho-GAP. However, in vivo LMW-PTP is able to rescue its catalytic activity on PDGF-r after removal of the oxidative stress. The reversibility of oxidation and the consequent recovery of enzymatic activity are generally recognized as key points in the redox regulation of a protein. In fact, it has been widely discussed that protein oxidation should have two different meanings in cell behavior. Oxidation of the protein backbone or the simple oxidation of methionine and/or cysteine residues could lead to direct protein fragmentation or to irreversibly oxidized dead products [38]. On the other hand, regulated oxidation of amino acid side chains, and in particular cysteine residues, could be considered a reversible functional regulation of proteins, as the oxidation might be reversed by the redox cellular systems (thioredoxin and GSH/glutaredoxin). The reduction process, which is responsible for the recovery of LMW-PTP activity, depends on the cellular reduced glutathione content, as indicated by data obtained using glutathione depletants [44]. The two major redox cellular systems are the thioredoxin/thioredoxin reductase/NADPH and the glutaredoxin/glutathione/glutathione reductase/NADPH complexes [38]. In vivo LMW-PTP reactivation upon exogenous oxidative stress removal appears to be under the control of the glutaredoxin/glutathione/glutathione reductase/NADPH system, as demonstrated by our group [44]. Moreover, it was proved that the redox regulation of LMW-PTP in vivo activity (i) is active upon PDGF stimulation, (ii) is performed by the endogenously produced H_2O_2 and (iii) is required for LMW-PTP dephosphorylation of PDGF-r itself. Upon PDGF treatment almost 80% of LMW-PTP is oxidized and inhibited after 10 min, but after 45 min almost 70% of the phosphatase has been reduced and consequently recovers its catalytic activity. Also, in these conditions the reduction process is dependent on the availability of reduced glutathione.

It has been proposed that the transient increase of ROS observed in response to growth factor administration is due to the induced activation of a membrane NADPH oxidase complex [45]. This event depends on administration of PDGF and is probably under the control of the signal transduction pathway of phosphatidyl-inositol-3 kinase (PI3K) [46]. LMW-PTP oxidation is performed by an endogenously produced oxidative stress, as indicated by the use of dyphenyl iodide, an inhibitor of the NADPH oxidase that prevents the oxidation of LMW-PTP in response to PDGF. Interestingly, both the oxidation and the subsequent reduction of LMW-PTP during PDGF stimulation are required for PDGF-r dephosphorylation. In fact, treatment with glutathione depletants severely impairs the action of LMW-PTP on phosphorylated receptor. These data, together with the time-dependent oxidation/inactivation and reduction/reactivation of LMW-PTP upon PDGF stimulation, indicate a specific role of the LMW-PTP inhibition during PDGF-r signal transduction (fig. 3). In fact, LMW-PTP is oxidized or inactivated at the very beginning of agonist stimulation, and reduced or reactivated later on, when receptor signal switching off is required. Lee et al. [40] proposed a different explanation for a similar behavior of PTP1B during EGF stimulation: they propose that growth factor stimulation alone may not be sufficient to increase the steady-state level of protein tyrosine phosphorylation in the cell, and that concurrent inhibition of PTPs by H_2O_2 could also be required. This second hypothesis is supported by previous data about PDGF-r activation by ultraviolet rays through PTP inactivation/oxidation [47]. In any event, PTP inhibition permits transduction of the signal through maintenance of a high receptor phosphorylation level. Hence, PTPs could be seen either as regulators for turning off the growth factor signal or as concurrent activators of receptor phosphorylation. Recent evidence suggests that the main action of LMW-PTP follows the redox reactions resulting from PDGF stimulation and hence in the late phase of the receptor signaling, suggesting that LMW-PTP only terminates of growth factor signaling [44].

Furthermore, inhibition of growth-factor-induced LMW-PTP oxidation during PDGF signaling, by treatment with catalase or DPI, causes a decrease in the phosphorylation level of PDGF-r [44]. We have recently obtained evidence strongly suggesting that LMW-PTP acts on PDGF-r by dephosphorylating Tyr^{857} (kinase domain), Tyr^{716} (Grb2

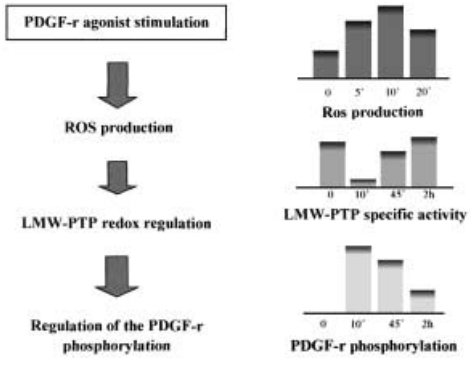

Figure 3. Role of LMW-PTP redox regulation in the kinetics of dephosphorylation of PDGF-r.

binding site) and Tyr[751] (PI3K binding site), and affecting the recruitment of SH2-containing proteins such as phospholipase-C-γ (PLCγ), PTP1D and PI3K [unpublished results from this group].We propose LMW-PTP for a key role in PDGF-r downregulation: while in the first minutes of stimulation it regulates the kinase activity of the receptor, in the descending phase of stimulation LMW-PTP leads to a general attenuation of the signals elicited from the receptor.

LMW-PTP redox regulation shows a peculiarity above all other PTPs. The function of glutathione in the redox regulation of PTP1B is to protect the cysteine sulphenic derivative from further oxidation, as it can easily be oxidized to form the irreversible sulphinic and sulphonic products. In fact, cysteine sulphenic acids are highly unstable and readily undergo condensation with a thiol. In the presence of reduced glutathione, the sulphenic derivative can be converted in a more stable S-thiolated product. This reaction prevents a further oxidation to sulphinic or sulphonic acid. This is a general mechanism that should bona fide operate on every PTP which possesses the catalytic cysteine (fig. 4). The different behavior of glutathione in the protection of PTP1B or LMW-PTP from further oxidation could be due to differences in the structure of the catalytic sites of the two phosphatases. In fact, the PTP1B active-site cleft is surrounded by positively charged lysine residues that could interact with the negatively charged glutathione. On the other hand, LMW-PTP active site crevice is rich in aromatic residues, a structure that should not facilitate the entry of reduced glutathione. We propose that LMW-PTP is able to undergo a different mechanism, due to the presence of an additional cysteine in the catalytic site, which can form an intramolecular S–S bridge. This intramolecular S–S bond between Cys[12] and Cys[17] has been confirmed in vitro, by mass spectroscopy analysis of NO-oxidized LMW-PTP, and retains a key role in the protection of LMW-PTP from excessive oxidation. We suggest that the presence of this additional cysteine in the catalytic site, which allows intramolecular S–S bond formation, confers a particular rapidity and efficiency in the redox regulation of the phosphatase. The formation of an intramolecular disulfide bond in Yab1 transcription factor has been already proposed to be responsible for the rapid

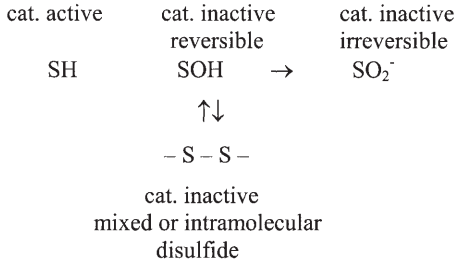

Figure 4. Scheme of the protection mechanism of catalytic site cysteines from excessive oxidations.

response to stress condition in H_2O_2 sensing [48]. Although the role of an intramolecular bridge has been postulated but never demonstrated for the regulation of CDC25 phosphotyrosine protein phosphatase [49], this particular safety mechanisms appears to be a unique feature of LMW-PTP.

Redox upregulation of LMW-PTP during cell growth inhibition

Growing evidence suggests the involvement of LMW-PTP in the delivery of antiproliferative signals causing growth inhibition. The biochemical mechanisms at the basis of the intracellular delivery of antiproliferative signals are poorly understood in comparison with the well-established signaling cascades that lead to mitosis. Contact and differentiation-induced growth inhibition are typical examples of conditions to which normal cells grown in a monolayer are exposed. Although the molecular events responsible for these processes remain largely unknown, the involvement of PTPs in both contact inhibition and cell differentiation was supposed. Previous findings showed that total PTP activity is increased in total lysates or membrane fractions derived from high-density cultures [50, 51]. The level of receptor-like PTP density-enhanced phosphatase-1 (DEP-1) increases gradually with cell density in WI-38 and AG1518 cells [51]. Furthermore, decreased tyrosine phosphorylation of PDGF-β, and EGF receptors are shown in cells of dense culture in comparison with sparse ones. It was recently postulated that during growth inhibition conditions, in addition to increase in the expression level of several PTPs, the specific activity of these enzymes could be increased via redox upregulation. In fact, recent results demonstrate reduced production of endogenous ROS and impaired redox signaling by growth factor receptors upon cell-cell contact. In particular, it has been demonstrated that cell growth arrest induced by cell density is associated, at least in part, to a decrease in the steady-state levels of intracellular ROS [52]. Fiaschi et al. [53] demonstrated that muscle differentiation induces a great decrease in reactive oxygen species, as well as cell confluence. Similarly, myogenesis leads to an increase in the reduced form of LMW-PTP in myotubes with respect to myoblasts [53]. In addition, Taddei et al. have demonstrated that LMW-PTP is involved in cell contact formation. In particular, the authors propose that LMW-PTP increases the cadherin-mediated cell-cell adhesion and report a redox upregulation of LMW-PTP when adherens junctions are formed [54]. The redox state of LMW-PTP in contact-inhibited cells and in differentiated cultures is associated with enzymatic activity, since LMW-PTP increases its specific activity in myotubes or in dense cultures three- to five-fold with respect to undifferentiated or

sparse cells, respectively [53]. These data support the hypothesis that the endogenous ROS concentration is an important element which participates in the arrest of cell growth, either in cell-cell contact or during cell differentiation.

Phosphorylation level evaluation of PDGF-r in dense and sparse cultures showed that it appears more dephosphorylated in contact-inhibited C2C12 cells in comparison with sparse cultures. Like cell-cell contact, cell differentiation increases LMW-PTP/PDGF-r association in comparison with control cells, resulting in a strong decrease in the tyrosine phosphorylation level of the receptor. We postulated that reduced PDGF-r activation in contact-inhibited cells and in terminally differentiated cells is due to the increase in LMW-PTP content and association with the receptor [53]. Previous results indicate that PDGF can regulate myoblast proliferation and differentiation in vitro, suggesting that PDGF has a role in increasing the number of myoblasts during skeletal muscle regeneration by stimulating proliferation and/or inhibiting differentiation. In particular, a role for PDGF has been suggested in the growth of satellite muscle cells, supporting the regeneration of adult muscle under stress, and wound or trauma [55]. Recently, a large decrease in the expression of PDGF-r mRNA and cell surface protein levels following hormonal induction of differentiation in 3T3-L1 fibroblasts was demonstrated. This suggests that downregulation of growth factor receptors might be one mechanism whereby differentiating cells become desensitized to the biological action of growth factors. The role of PTPs in the downregulation of the mitogenic signals has long been accepted. Many PTPs have been found to be associated with cell differentiation, for example rVH6 or MKP1 [56, 57], PRL-1 [58], PTP-β2 [59], DEP-1 [60] and PTP20 [61]. Little is known about the role of these phosphatases with the exception of rVH6 and MKP1, which specifically dephosphorylate the activated MAPKs. Among those mentioned above, the only PTP which acts on activated PDGF-r, is DEP1. DEP1 increases in dense cultures but not in differentiated cells and displays site-selective dephosphorylation of PDGF-r [51, 60]. Thus, DEP1 is likely to have a modulatory function on the activated receptor rather than a general dowregulating role for the mitogenic signal. It is very likely that LMW-PTP acts directly on the Tyr^{857} in phosphorylated PDGF-r, in the tyrosine kinase activation loop [unpublished results of this lab], thus causing general attenuation of PDGF signaling. In this light, the role of LMW-PTP in the downregulation of PDGF-r signaling, both in cell contact inhibition and cell differentiation, could be different and wider with respect to DEP1. On the basis of all these observations, we suggest that LMW-PTP behaves as a growth arrest protein since (i) its cellular content increases in contact and differentiation-inhibited cells, (ii) its specific activity is upregulated by reduction of oxidative stress in the enviroment and (iii) its association with and dephosphorylation of PDGF-r is therefore enhanced. In conclusion, because the mitogenic delivery of growth factor signals involves LMW-PTP redox downregulation, growth inhibition conditions such as cell density and terminal differentiation are connected with redox upregulation of LMW-PTP activity (fig. 5). In this light, LMW-PTP could be considered to be a general modulator of cell growth, which is differentially regulated during mitosis commitment or growth inhibition.

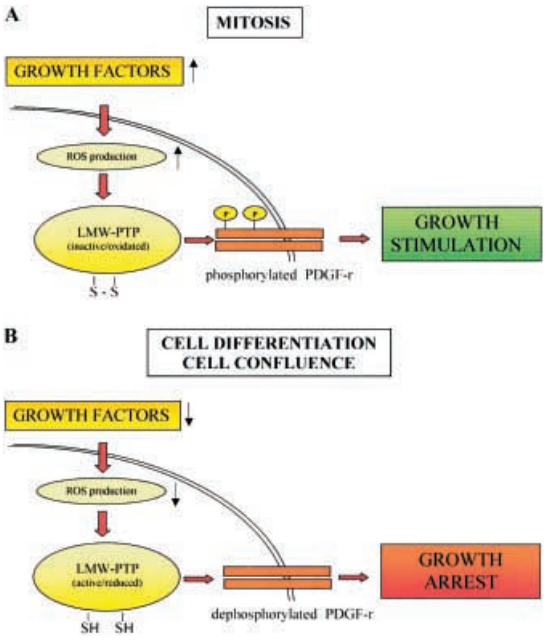

Figure 5. Redox regulation of LMW-PTP during proliferative (*A*) or antiproliferative (*B*) conditions.

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