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Minireview

The Roles of Peroxiredoxin and Thioredoxin in Hydrogen Peroxide Sensing and in Signal Transduction

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A challenge in the redox field is the elucidation of the molecular mechanisms, by which H_2O_2 mediates signal transduction in cells. This is relevant since redox pathways are disturbed in some pathologies. The transcription factor OxyR is the H_2O_2 sensor in bacteria, whereas Cys-based peroxidases are involved in the perception of this oxidant in eukaryotic cells. Three possible mechanisms may be involved in H_2O_2 signaling that are not mutually exclusive. In the simplest pathway, H_2O_2 signals through direct oxidation of the signaling protein, such as a phosphatase or a transcription factor. Although signaling proteins are frequently observed in the oxidized state in biological systems, in most cases their direct oxidation by H_2O_2 is too slow (10^1 $M^{-1}s^{-1}$ range) to outcompete Cys-based peroxidases and glutathione. In some particular cellular compartments (such as vicinity of NADPH oxidases), it is possible that a signaling protein faces extremely high H_2O_2 concentrations, making the direct oxidation feasible. Alternatively, high H_2O_2 levels can hyperoxidize peroxiredoxins leading to local building up of H_2O_2 that then could oxidize a signaling protein (floodgate hypothesis). In a second model, H_2O_2 oxidizes Cys-based peroxidases that then through thiol-disulfide reshuffling would transmit the oxidized equivalents to the signaling protein. The third model of signaling is centered on the reducing substrate of Cys-based peroxidases that in most cases is thioredoxin. Is this model, peroxiredoxins would signal by modulating the thioredoxin redox status. More kinetic data is required to allow the identification of the complex network of thiol switches.

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INTRODUCTION

H_2O_2, an endogenous oxidant present in all aerobic cells, was traditionally viewed as a toxic substance (Winterbourn, 2008). However, research during the last two decades built a compelling case supporting the role of H_2O_2 as a diffusible signaling messenger that controls many cellular processes by modulating the redox state of cysteine residues in proteins (Ying et al., 2007). Accordingly, disruption of signaling pathways mediated by H_2O_2 and other oxidants would be a mechanism underlying diseases (Jones, 2006).

H_2O_2 occurs in aerobic cells typically at steady state concentration in the 10 nM range; and NADPH oxidases and/or complex III of the mitochondrial respiratory chain are frequently considered as the major sources, whereas catalase and multiple Cys-based peroxidases exert a fine control of its levels (reviewed by Marinho et al., 2014; Sies, 2014). H_2O_2 are neutral molecules that can cross biological membranes, although its diffusivity can be modulated by the lipid composition of biological membranes (Branco et al., 2004; Pedroso et al., 2009) and aquaporins provide facilitated transport channels across bilayers (Miller et al., 2010).

A challenge in the field is the elucidation of the molecular mechanisms by which H_2O_2 is sensed and how this signal is transduced in cells. Furthermore, several chemical and biological aspects appear to be contradictory (Winterbourn, 2008). Elucidation of these aspects is very relevant since redox regulated proteins are implicated in diverse pathologies. For instance, redox medicine is emerging as an innovative field supported by molecular evidence showing deregulated redox signaling pathways dependent on H_2O_2 to be at the basis of several pathologies, including obesity (Kaszubska et al., 2002) and cardiovascular diseases (Schröder et al., 2012). Furthermore, peroxiredoxins (Prxs) and its partner proteins, thioredoxins (Trxs), have been associated to cancer (Irwin et al., 2013), neurodegenerative (Krapfenbauer et al., 2003), cardiovascular (Ahsan et al., 2009), and metabolic diseases (Abbasi et al., 2014). In principle, understanding the mechanisms of H_2O_2 sensing and signal transduction may provide the basis for therapeutic interventions in redox-related diseases (Harald et al., 2015; Jones, 2006).

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MODELS OF H_2O_2 SENSING AND SIGNALING

Prxs are highly abundant Cys-based peroxidases with crucial roles in maintaining genome stability, protecting cells against cancer and promoting longevity (Nyström et al., 2012; Rhee and Woo, 2011). Prxs are subdivided into subfamilies that present very divergent amino acid sequences but uniformly possess at least one conserved cysteine residue at the active site, the so-called peroxidatic cysteine, C_P (Nelson et al., 2011). Reaction of C_P with H_2O_2 is very fast, with second-order rate constants ranging from 10^6 to 10^8 $M^{-1}s^{-1}$ (Ogusucu et al., 2007; Parsonage et al., 2005; Peskin et al., 2007; Toledo et al., 2011; Trujillo et al., 2007). This represents an extraordinary catalytic power, since free Cys reacts with hydroperoxides with a rate constant equivalent to 10^1 $M^{-1}s^{-1}$ (Winterbourn and Metodiewa, 1999).

Remarkably for a protein involved in signal transduction, Prx rapidly reacts with hydroperoxides, but not with other electrophiles such as iodoacetamides or chloroamines (Peskin et al., 2007). Specificity is a desirable feature for a sensor protein. High reactivity and specificity are related to the fact that Prxs stabilize the transition state of a S_N2 type of nucleophilic substitution reaction through the activation of not only C_P, but also H_2O_2 (Hall et al., 2011). Besides reactivity and specificity, Prxs are abundant proteins so they present several features appropriate for a sensor protein.

Aspects related to how the oxidation signal is transmitted to other proteins are less understood. It is well known that most Prxs transmit oxidizing equivalents to Trxs, especially in the case of typical 2-Cys Prx, which are Prxs with intermolecular disulfide bonds (in the context of this proposal, we refer to typical 2-Cys Prx as the proteins that belong to the AhpC/Prx1 subgroup, according to Nelson et al., 2011). For some typical 2-Cys Prx, dual function (moonlight property) was reported as thiol peroxidase and chaperone (holdase) (Jang et al., 2004; Rhee and Woo, 2011).

In principle, Prxs may mediate H_2O_2 signaling through three mechanisms that are not mutually exclusive (Fig. 1A) : (1, blue) H_2O_2 reacts directly with the target signaling protein (SP), and Prxs control this process by regulating H_2O_2 tone in cells; (2, green) H_2O_2 reacts with a Prx which is oxidized and then relays this oxidation to the target signaling protein (SP, for example a transcription factor or a phosphatase); and (3, orange) H_2O_2 reacts with a Prx which is oxidized, then this Prx is reduced back by its partner thioredoxin (Trx), and it is the oxidized Trx that relays the oxidation to the target SP. Some variations occur when Prx enzymes are hyperoxidized, as will be further described below. For instance, mechanism (1) also encompasses the so-called floodgate hypothesis (Rhee and Woo, 2011; Wood et al., 2003). In this case, when H_2O_2 accumulates at high levels, C_P could be hyperoxidized to sulfinic or sulfonic states (Fig. 1B), allowing accumulation of H_2O_2, which could then attack other targets. Noteworthy, in this case levels of reduced Trx also increase. Adding more complexity to these mechanisms, it was found recently that glutaredoxin 1 (Grx 1) can also sustain the peroxidase activity of Prxs by reducing the glutathionylated form of Prx formed in the presence of H_2O_2 and glutathione (GSH) (Peskin et al. 2015). Such pathway may, in addition, prevent the hyperoxidation of Prxs.

Concerning the mechanisms described in Fig. 1, there are evidences supporting them, and most probably all three mechanism operate for different signal transduction proteins and probably depend on several conditions such as second-order rate constants, pH, as well as concentration of oxidants, reductants, SP and Prx, among other factors. In principle, these three mechanistic models are not mutually exclusive, but obviously they should agree with chemical and biological data.

H_2O_2 SIGNALING THOURGH DIRECT OXIDATION OF SIGNALING PROTEINS (SP)

Reactivities of H_2O_2 towards SP, like the phosphatases Cdc25B, SHP-2 and protein tyrosine phosphatase 1B (PTB1B), are five to seven orders of magnitude lower than the reactivity towards Prxs or glutathione peroxidases (GPxs) (Winterbourn and Hampton, 2008). In addition, Prxs are much more abundant than SP like phosphatases or transcription factors. The consequence is that SP cannot outcompete with Prxs or GPxs for H_2O_2.

Noteworthy, other thiol proteins than Prxs and Gpxs can also react faster with H_2O_2 than ordinary Cys residues present in most SP. Two cases deserve special attention here: glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and OxyR. OxyR is a transcription factor with highly reactive Cys residues capable to react with H_2O_2 with a second order rate constant of 10^5 M^{-1} s^{-1} (Aslund et al., 1999; Lee et al., 2004). Upon oxidation of reactive Cys, OxyR conformation changes and activates transcription of target genes. Due to these properties, OxyR is considered the H_2O_2 sensor in bacteria (reviewed by Marinho et al., 2014). GAPDH can be reversible inhibited by H_2O_2 at more moderate rates (500 M^{-1} s^{-1}) (Little and O'Brien, 1969; Winterbourn and Hampton, 2008; Peralta et al., 2015), still more reactive than free Cys; and GAPDH is highly abundant (~240 μM) (Sneider, 2013). Furthermore, most cells contain high amounts of GSH that can also outcompete for H_2O_2 (Winterbourne and Hampton, 2008).

Therefore, there are multiple competitive routes that can detour H_2O_2 from the direct reaction toward ordinary thiols present in most SP. However, SP like PTP1B and PTEN, which display low reactivity towards H_2O_2 (Denu and Tanner, 1998), are found oxidized in biological tissues (Kwon et al., 2004; Lee et al., 1998; Mahadev et al., 2001; Meng et al., 2002). Therefore, attempts were made to reconcile both biological data (showing that Cys-based proteins are oxidized in cellular systems) and chemical information (low reactivity and low abundance of these Cys-based proteins). The so- called “floodgate hypothesis” is one of the most popular models (Rhee and Woo, 2011; Wood et al., 2003) that could be considered a variation to pathway 1, and it is schematically represented in (Fig. 1B, blue pathway). First observation that gave support to this hypothesis, was that typical 2-Cys Prx can be divided into sensitive and robust enzymes and that this property is related with a “gain of function” (Wood et al., 2003). Sensitive Prx are enzymes that are more easily hyperoxidized to sulfinic acids (C_P-SO_2H^-), inactivating these peroxidases and allowing the accumulation of H_2O_2 in cells. At higher levels, H_2O_2 would be able to attack other targets that in basal conditions would be outcompeted by sensitive Prx. The build-up of H_2O_2 is possible because sulfinic acid is not reducible by standard reductants such as Trx and Grx.

The identification of an enzyme capable to specifically reduce typical 2-Cys Prx sulfinic acid at the C_P also supported the signaling role of their oxidative inactivation (Biteau et al., 2003). Besides hyperoxidation of human Prx2, phosphorylation of Prx1 also leads to its inactivation (Rawat et al., 2013; Woo et al., 2010), proving an alternative pathway for H_2O_2 accumulation inside cells. Further experimental evidences for the pathway described in Fig. 1B are presented by Rhee et al. (2012). A problem with this hypothesis is that even in the absence of highly reactive peroxidases, cells still have high amounts of GSH (mM levels) and GAPDH that on chemical grounds can

outcompete PTP-1B and PTEN for H_2O_2 (Winterbourne and Hampton, 2008).

Another attempt to reconcile the low reactivity of H_2O_2 with SP and redox signaling is based on an extended kinetic analysis that focused not only on the routes of H_2O_2 elimination but also on the transmission of the oxidation signal. The key notion is whether the fraction of H_2O_2 that escapes the action of Prxs, glutathione peroxidases and also GSH is sufficient to alter the oxidation state of low-reactive SP, thus transmitting the oxidation signal to these target proteins (Marinho et al., 2014). The H_2O_2 concentration needed to oxidized 50% of these targets within 5 minutes and in competition with all cellular H_2O_2-removing systems was estimated in the range 14 to 120 μM for Cdc25B and PTP1B, respectively (Marinho et al. 2014). These H_2O_2 concentrations are much higher than the sub-micromolar concentrations usually assumed to occur intracellularly, but they may be reached in the vicinity of SP during signaling dependent on the local activation of NADPH oxidases (Chen, 2008; Mishina, 2011; Paulsen, 2012). This analysis was extended by fitting PTP1B and SHP-2 oxidation profiles obtained experimentally to a mathematical model that included not only the oxidation component of redox signaling, but also the reductive part, in which target SPs are reduced back, switching-off the oxidation signal, thus taken into account all possible cellular biochemical reactions. The results obtained suggest that while for PTP1B the redox signaling is compatible with a direct reaction between H_2O_2 and PTP1B, for SHP-2 an alternative mechanism is more likely (Brito and Antunes, 2014). Thus, pathway 1 (Fig. 1, blue pathway) may operate *in vivo* under some conditions, namely under relative high local H_2O_2 concentrations, even if SP display low reactivity towards H_2O_2.

H_2O_2 SIGNALING THROUGH MESSENGER PROTEIN OXIDIZED BY PRX

Most of the knowledge related to pathway 2 (Fig. 1A, green pathway) was initially gained through studies on the activation mechanism of Yap1 transcription factors from *Saccharomyces cerevisiae* (Boronat et al., 2014). Like OxyR, the transcriptional activity of Yap1 is redox regulated. However, apparently Cys residues of Yap1 are not highly reactive and therefore Yap1 does not react directly with H_2O_2, but instead undergo oxidative activation through thiol-disulfide reshuffling mediated by a Cys-based peroxidase: glutathione peroxidase 3 (Gpx3), also called "Oxidant Receptor Protein" (Orp1) (Delaunay et al., 2002). In this signaling pathway, H_2O_2 oxidizes Gpx3/Orp1 into a sulfenic acid (Cys-SOH) that then condenses with a Cys residue of Yap1, giving rise to a transient, mixed disulfide between the two proteins. Through thiol – disulfide exchange reactions, an intramolecular disulfide is formed in Yap1. This oxidized form of Yap1 has an altered structure that cannot leave the nucleus. The nuclear accumulation of Yap1 facilitates its ability to induce the transcription of target genes, such as Prx and catalase genes (Delaunay et al., 2002). Ybp1 is a third protein involved in this pathway that physically interacts with Yap1 and thereby stimulates nuclear accumulation of this transcription factor in response to H_2O_2 (Veal et al., 2003). Interestingly, in strains where Ybp1 gene is mutated, Tsa1 (by far, the most abundant Cys-based peroxidase in yeast) and not Gpx3/Orp1 is the protein that senses H_2O_2 and activates Yap1 through thiol-disulfide exchange reactions (Tachibana et al., 2009). These studies represented a breakthrough in the field, since they provide a pathway to redox regulate a SP presenting low-reactive thiols through a protein oxidation relay by a Cys-based peroxidase.

Therefore, in contrast to bacteria, Cys-based peroxidases and not transcription factors are the major sensors of H_2O_2 in yeast. Indeed, a yeast strain deleted for the five Prx genes and three Gpx genes is still viable, but lost its capacity to transcriptionally respond to H_2O_2 stimuli (Fomenko et al., 2011), suggesting that green pathway (Fig. 1A) is preponderant in the adaptive response of *Saccharomyces cerevisiae* to H_2O_2.

According to this mechanism, H_2O_2 signaling is mediated through sequential transfers of oxidizing equivalents. Upon oxidation, all Prx enzymes are oxidized to sulfenic acids (Cys-SOH) that in most cases can be converted to intra- or intermolecular disulfides. Oxidized Prxs (Cys-SOH or Cys-SS-Cys) probably selectively transfers oxidizing equivalents to downstream regulatory proteins through specific protein-protein interactions and thiol-disulfide exchange reactions (Gutscher et al., 2009; Netto et al., 2015).

So far, the number of cases in which a high-reactive peroxidase was found to selectively transfer oxidizing equivalents to a SP is relatively low, but the descriptions of new cases are increasing. Remarkably, Prx1 from mammals was shown to physically interact in a redox dependent manner with SP such as: (1) PTEN, a protein with phosphatase activity (Cao et al.,

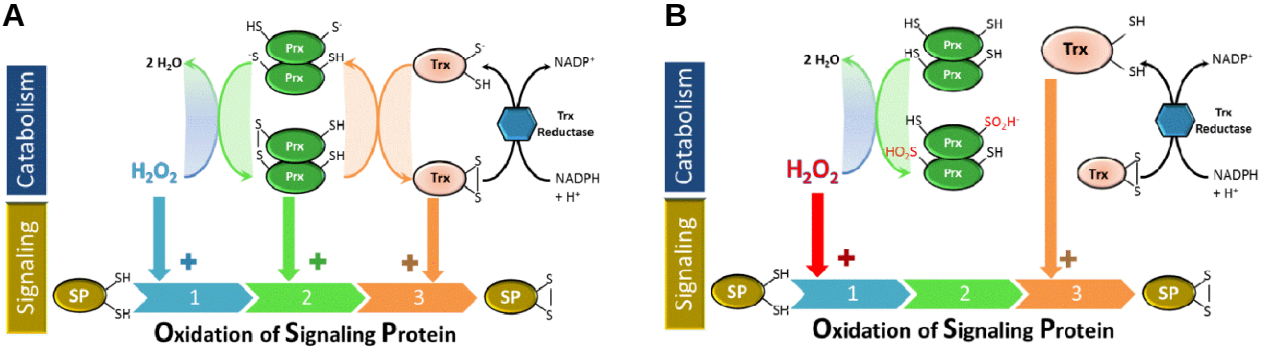

Fig. 1. Mechanisms of H_2O_2 sensing. In (A) the three mechanisms investigated in these proposal are shown: (1) direct oxidation of the signaling protein (SP) by H_2O_2; oxidation of SP is mediated by (2) a peroxiredoxin (Prx) or by (3) a thioredoxin (Trx). In (B) the overoxidation of a Prx is shown, which leads to H_2O_2 accumulation.

2009); (2) apoptosis signaling kinase1 (ASK1) (Jarvis et al., 2012); and (3) MAPK phosphatases (Turner-Ivey et al., 2013).

Recently, the activation of a transcription factor (STAT3) by mammalian peroxiredoxin2 (Prx2) was also reported and present several features similar to the Gpx3/Orp1 –Yap1 pathway (Sobotta et al., 2015). In all cases, the transfer of oxidizing equivalents involves physical interaction and thiol-disulfide exchange between the two partners.

TRX-PRX MODEL

A lot of attention has been centered on H_2O_2 in the signaling pathways that Prx take place. In contrast, the roles play by Prx enzymes in regulating the redox state of its other substrate (Trx) has been somehow overlooked. This is surprising, since it is well established for a long time that several signal transduction pathways are activated by the oxidized but not by the reduced form of Trx (reviewed by Berndt et al., 2007). For instance, only reduced Trx1 and Trx2 bind Ask-1, thereby inhibiting its kinase activity. The oxidation of Trx1 leads to the physical dissociation of the Trx1-Ask1 complex and, consequently, to the activation of Ask-1 (Saitoh et al., 1998). Another example of redox regulation is the activation of NF-κB by Trx1. The binding of NF-κB subunit p50 to its target DNA sequence requires the reduction of a single cysteinyl residue by Trx1 (Hayashi et al., 1993; Matthews et al., 1992). Indeed, Trx enzymes recognize the oxidized form of their target proteins with higher selectivity, than their corresponding reduced forms (Palde and Carroll, 2015). It should be referred that Trx itself reacts slowly with H_2O_2 (Chae et al., 1994; Winterbourne and Hampton, 2008). Therefore, typical 2-Cys Prxs that catalyze the oxidation of Trx by hydroperoxides (Netto et al., 1996; Tairum et al., 2012) can impact redox regulation by modulating the redox status of Trx.

Therefore, another model can be envisaged, in which the redox pathway between H_2O_2 and the SP would be mediated not only by Prx, but also by Trx (Fig. 1A, pathway 3, red). According to this Prx-Trx signaling pathway, the oxidizing equivalent from H_2O_2 would be transferred to Prx and then to Trx and finally to the SP. Alternatively, Prx could oxidize Trx, and thereby avoid the interaction of this thiol-disulfide oxidoreductase with a SP.

More examples of redox-regulation by Prx-Trx pathway came from studies using fission yeast. In these organisms, two transcription factors have been found to be redox regulated by H_2O_2: Pap1 (Brown et al., 2013; Calvo et al., 2013) and Msn2 (Boisnard et al., 2009). Relevant for the adaptive response of fission yeast to H_2O_2, the formation of 2-Cys Prx disulfide triggers a signaling cascade that activates the otherwise unresponsive Pap1 protein, but only when Trx enzymes become transiently depleted (Brown et al., 2013; Calvo et al., 2013).

As described for the model presented in the section 2.2 (green pathway, Fig. 1), a feature underlying these pathways is the specificity provided by protein-protein interactions and thiol-disulfide exchange reactions. Proteins and their corresponding partners require to share complementary physico-chemical properties (i.e. hydrophobicity, polarity) in order to interact. As a matter of fact, yeast Trx reductase can only reduce yeast but not bacterial or mammalian Trx, due to complementary properties (Oliveira et al., 2010). Noteworthy, a protuberance (containing residues Glu50, Arg146 and Cys170 of yeast Tsa1) present only in the oxidized form of 2-Cys Prx is involved in its recognition by yeast Trx1 (Tairum et al., 2012).

Therefore, physical proximity between proteins is a requirement to allow the transfer of oxidizing equivalents through pathways 2 and 3. The construction of artificial quimeras between Cys-based proteins and redox sensitive GFP, Gutscher et al. (2009) elegantly demonstrated the "proximity-based Protein Thiol Oxidation by H_2O_2-scavenging peroxidases" concept that also form the basis of several genetically encoded fluorescent probes.

This Prx-Trx model also applies in conditions where cells are exposed to high levels of H_2O_2, provoking Prx hyperoxidation (Fig. 1B, pathway 3; red). Because TrxR (thioredoxin reductase) levels are limiting, several proteins (including disulfide form of 2-Cys Prx; transcription factors and methionine sulfoxide reductase) compete for reduced Trx1. For instance, the hyperoxidation of Tpx1 (2-Cys Prx from *Schizosaccharomyces pombe*) allowed accumulation of reduced Trx (since this oxidoreductase is not consumed by Prx in the disulfide form) that could be then used to repair other damaged proteins (Day et al., 2012). In these conditions, the activity of enzymes such as methionine sulfoxide reductase and ribonucleotide reductase would not be limited by shortage of reduced Trx. Remarkably, when Tpx1 is hyperoxidized, Pap1 (a transcription factor) is no longer activated possibly because there is enough Trx to keep this transcription factor in the reduced state and consequently out of the nucleus (reviewed by Veal et al., 2014).

Other observations that supported the notion that Prx participates in redox signaling by regulating the Trx status were gained by studies with *Saccharomyces cerevisiae*. For instance, mutations that destroy cytosolic TrxR activity rescue the growth of *tsa1*Δ *rad51*Δ double mutant and also suppress the genomic instability phenotype of *tsa1*Δ mutants (Ragu et al., 2014). Furthermore, a mutant of *S. cerevisiae* for cytosolic TrxR suppressed the growth defect of *tsa1*Δ mutants in zinc-deficient conditions (MacDiarmid et al., 2013). These studies are consistent with the idea that deletion of Prx genes decreases the rates of Trx oxidation, whereas deletion of TrxR genes decreases rates of Trx reduction and thereby compensates the absence of Cys-based peroxidase by keeping the Trx redox state similar to the levels observed in the wild type strain. These studies suggest that the main role of Prx enzymes at least in some cases may be regulating Trx redox levels, rather than reducing hydroperoxides (Veal et al., 2014).

Probably the redox status of Trx is probably not affected by 2-Cys Prx peroxidase activity in bacteria, because in these microorganism there is a reducing system (AhpF) that is specifically dedicated to reduce this Cys-based peroxidase (Veal et al., 2014). This phenomenon is probably related to the fact that most 2-Cys Prx in bacteria are robust, meaning that they resist to hyperoxidation, even when exposed to high H_2O_2 levels. In contrast, in eukaryotes, a selection pressure towards the evolution of 2-Cys Prxs that are sensitive to inactivation by H_2O_2 arose and according to this model could be involved to maintain Trx in the reduced form, available to mediate repair damaged proteins under acute stress conditions (Veal et al., 2014).

Not many examples of the Prx-Trx model are available in mammals. But noteworthy, HsTrx1 (Thioredoxin 1 from humans) contains five Cys residues: Cys32 and Cys35 are the two residues that belong to the conserved CGPC motif, whereas Cys62, Cys69 and Cys73 are three non-conserved residues. HsPrx1 (2-Cys Prx from *Homo sapiens*) specifically oxidizes Cys32 and Cys35 into an intramolecular disulfide. In contrast, Cys62 and Cys69 is an additional dithiol-disulfide redox center. When a disulfide is formed between Cys62 and Cys69, the ability of HsTrx1 to bind a target is impaired (Watson et al., 2003). Indeed, Cys62-Cys69 non-active site disulfide is not a substrate for HsTrxR1, but to the GSH/HsGrx1 system, whereas the Cys62-Cys69 dithiol can be oxidized by HsPrx1 (Du et al., 2013). These multiple oxidative states of HsTrx1 involving its five Cys residues play a role in the fine tune of the redox

regulation of SPs such as Ask1 (kinases), PTEN (phosphatases) and Ref-1 (transcription factor) in redox pathways that 2-Cys Prx enzymes also take place (reviewed by Lee et al., 2013; Meyer et al., 2009).

CONCLUSION

A long way has come since H_2O_2 was seen as a toxicant, and today thiol switches are established cellular partners in signaling networks. Although the identification of the thiol redox proteome is advancing quickly, there is a massive lack of quantification, namely kinetic parameters. These parameters entail dynamic information that helps to identify in the complex network of thiol switches, those that operate during physiology and those whose disruption is responsible for a pathological state. The reactivity with H_2O_2 is known for only a few thiol SP (Ferrer-Sueta et al., 2011; Tanner et al., 2011); rate constants for the thiol-disulfide exchange reactions in which a peroxidase transfers oxidizing equivalents to a SP are virtually unknown; the reduction of the oxidized form of thiol SPs, switching-off the oxidation signal (Dagnell et al., 2013), is known only for a couple of interactions (Parsons and Gates, 2013). Determining true rate constants for these processes entails experiments with purified systems that are complex to preform and cannot cope with the rapid identification of new thiol switches provided by large-scale thiol proteomic experiments. As an alternative, the determination of apparent kinetic parameters under the typical redox experiments with intact cells or tissues (Brito and Antunes, 2014) may provide a more practical approach so that quantitative knowledge can follow suit the rapidly expanding redox proteome landscape, establishing the basis for redox kinectomics.

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