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Research Paper

Mapping the phenotypic repertoire of the cytoplasmic 2-Cys peroxiredoxin – Thioredoxin system. 1. Understanding commonalities and differences among cell types

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ABSTRACT

The system (PTTRS) formed by typical 2-Cys peroxiredoxins (Prx), thioredoxin (Trx), Trx reductase (TrxR), and sulfiredoxin (Srx) is central in antioxidant protection and redox signaling in the cytoplasm of eukaryotic cells. Understanding how the PTTRS integrates these functions requires tracing phenotypes to molecular properties, which is non-trivial. Here we analyze this problem based on a model that captures the PTTRS' conserved features. We have mapped the conditions that generate each distinct response to H_2O_2 supply rates (v_{sup}), and estimated the parameters for thirteen human cell types and for *Saccharomyces cerevisiae*. The resulting composition-to-phenotype map yielded the following experimentally testable predictions. The PTTRS permits many distinct responses including ultra-sensitivity and hysteresis. However, nearly all tumor cell lines showed a similar response characterized by limited Trx-S^- depletion and a substantial but self-limited gradual accumulation of hyperoxidized Prx at high v_{sup}. This similarity ensues from strong correlations between the TrxR, Srx and Prx activities over cell lines, which contribute to maintain the Prx-SS reduction capacity in slight excess over the maximal steady state Prx-SS production. In turn, in erythrocytes, hepatocytes and HepG2 cells high v_{sup} depletes Trx-S^- and oxidizes Prx mainly to Prx-SS. In all nucleated human cells the Prx-SS reduction capacity defined a threshold separating two different regimes. At sub-threshold v_{sup} the cytoplasmic H_2O_2 concentration is determined by Prx, nM-range and spatially localized, whereas at supra-threshold v_{sup} it is determined by much less active alternative sinks and μM-range throughout the cytoplasm. The yeast shows a distinct response where the Prx Tsa1 accumulates in sulfenate form at high v_{sup}. This is mainly due to an exceptional stability of Tsa1's sulfenate. The implications of these findings for thiol redox regulation and cell physiology are discussed. All estimates were thoroughly documented and provided, together with analytical approximations for system properties, as a resource for quantitative redox biology.

1. Introduction

The PTTRS (Fig. 1) plays key roles in antioxidant protection and redox signaling in the cytoplasm of eukaryotic cells. This system controls cytoplasmic hydrogen peroxide (H_2O_2) concentrations at low oxidative loads [1–3], and plays prominent signaling roles in vascular adaptation [4], mitogenesis [5], inflammation [6], tumorigenesis [7] and apoptosis [8,9]. But despite the numerous studies associating the PTTRS to redox signaling, a consensus about the mechanisms conveying H_2O_2 signals to redox-regulated targets, and how this system integrates signaling and antioxidant protection is yet to emerge [10–13]. Clarifying how the dynamics of the PTTRS in cells relates to the properties and abundances of these proteins is a critical step towards understanding these problems.

This dynamics is largely determined by the redox behavior of the cytoplasmic Prx (Fig. 1). These are pentamers of dimers in antiparallel orientation. Each monomer carries a very H_2O_2-reactive thiolate (peroxidatic cysteine, C_P) and a less reactive thiol (resolving cysteine, C_R)·H_2O_2 oxidizes the peroxidatic cysteine to a sulfenate (Prx-SO^-), which then condenses with the resolving cysteine of the opposing

Abbreviations: ASK1, apoptosis signal-regulating kinase 1; Cat, catalase; GSH, glutathione; GPx1, glutathione peroxidase 1; Grx, glutaredoxin; KEAP1, Kelch-like ECH-associated protein 1; NRF2, nuclear factor erythroid 2-related factor 2; Prx, typical 2-Cys peroxiredoxin; PTTRS, peroxiredoxin / thioredoxin / thioredoxin reductase system; Srx, sulfiredoxin; Trx, thioredoxin; TrxR, thioredoxin reductase

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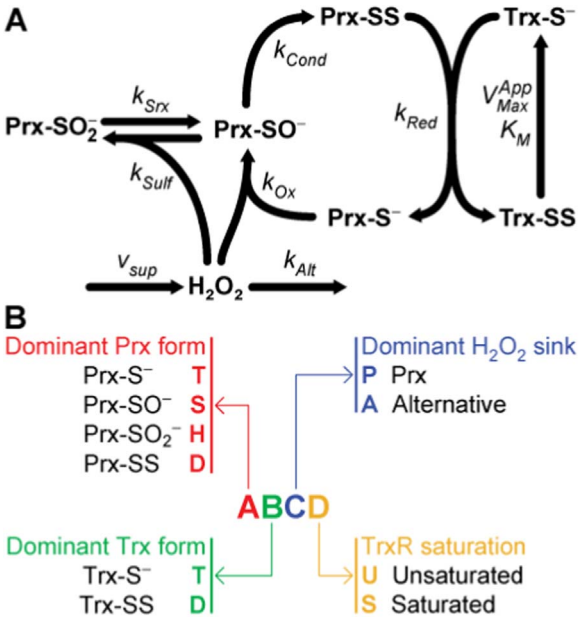

Fig. 1. A. A simple model of the peroxiredoxin / thioredoxin / thioredoxin reductase system. The kinetic parameters for each process are indicated near the respective arrow. B. Notation used to designate each phenotypic region.

monomer to form a disulfide (Prx-SS). In eukaryotic Prx the rate of this last step is limited by the local unfolding (LU) around the active site that is required to bring the sulfenate close enough to the resolving cysteine [12]. This delay prompts the accumulation of the sulfenate form, and thereby its further oxidation (called "hyperoxidation") by additional H_2O_2 molecules to sulfinate ($Prx\text{-}SO_2^-$) and sulfonate ($Prx\text{-}SO_3^-$). The conversion to sulfonate irreversibly inactivates the peroxidatic activity. However, $Prx\text{-}SO_2^-$ can be slowly reduced to $Prx\text{-}SO^-$ at the expense of ATP and reducing equivalents, under catalysis by sulfiredoxin (Srx) [14]. In turn, Prx-SS is reduced by thioredoxin ($Trx\text{-}S^-$) eventually returning Prx to its fully folded (FF) thiolate form. Thioredoxin becomes oxidized to a disulfide (Trx-SS) in the process, and this disulfide is reduced by NADPH under catalysis by thioredoxin reductase (TrxR).

The characterization of the responses of the PTTRS to H_2O_2 in a variety of conditions, cell types and organisms highlighted both commonalities and differences, as the following examples illustrate. Treatment of human erythrocytes (5×10^6 cells/mL) with H_2O_2 boluses up to 200 μM caused both PrxII and Trx1 to accumulate in disulfide form, with virtually no Prx hyperoxidation [1]. Hyperoxidation was detectable only in erythrocytes treated with ≥ 100 μM H_2O_2 boluses after Cat inhibition [1]. In contrast, treatment of Jurkat T cells (10^6 cells/mL) with ≥ 100 μM H_2O_2 caused extensive hyperoxidation even in absence of Cat inhibition [1]. Remarkably, this happens despite the predominant Prx in Jurkat T cells being PrxI [15], which is more resistant to hyperoxidation than the dominant Prx in erythrocytes, PrxII [5,16]. Extensive hyperoxidation of PrxI and PrxII was also observed when confluent cultures of human umbilical vein endothelial cells (HUVEC) were exposed to ≥ 30 μM H_2O_2 boluses [17]. In these cells hyperoxidation of PrxII is already extensive, and that of PrxI clearly observable, by 2 min after a 100 μM H_2O_2 bolus [17].

Sobotta et al. [18] exposed 10^6 HEK293 cells/mL to either 0.2–3.7 μM H_2O_2 steady states for 1 h or 2.5 μM – 5 mM H_2O_2 boluses for 5 min. In the former case the fraction of PrxII in disulfide form increased in a dose-dependent manner from ≈ 5% to 100%. In the latter case that fraction peaked at ≈80% for a 25 μM bolus and progressively decreased for increasing boluses, presumably due to increasing double hyperoxidation of the dimers preventing disulfide formation. Tomalin et al. [19] reported that treatment of 2×10^6 HEK293 cells/mL with 10–80 μM H_2O_2 boluses for 10 min caused a progressive increase in total hyperoxidation only after a ≈ 20 μM H_2O_2 bolus threshold.

These commonalities and differences prompt important questions with practical relevance for research in thiol redox regulation and for therapy. To what extent are results obtained in one organism or cell type generalizable? What among the many factors that may change form cell type to cell type explain the observed differences in the responses of the PTTRS? What are the determinants of the observed response thresholds? How can oxidative stress and apoptosis be most effectively induced in tumor cells? Will different types of tumor cells react in different ways? Again, understanding the answers to these questions requires clarifying how the dynamics of the PTTRS relates to the properties and cellular abundances of these proteins.

Mathematical modeling has consistently yielded useful insights about the operation of antioxidant and thiol redox systems [20–27] and is recognized as an important tool for the progress of redox biology [28]. Most previous computational studies have focused on accurately modeling specific cells. Instead, the present work seeks to identify generic principles connecting design and function in redox signaling and antioxidant protection by the PTTRS, and on understanding the underpinnings of differences among cell types. Further, we focus on overall dynamic properties, and not yet on details that hinge on further experimental characterization of the components. These distinct goals required a distinct modeling approach. Thus, we proceeded as follows. First, we extensively reviewed the literature and databases to identify (a) the features of the PTTRS in the cytoplasm of eukaryotic cells that are conserved and most relevant for its dynamics, and (b) the typical ranges of the kinetic and composition parameters. This preliminary analysis revealed that most current uncertainties — such as the contribution of GSH for Prx reduction, or a contribution of generic protein thiols for buffering H_2O_2 and oxidizing Trx1 — have a minor impact on the overall dynamics of the PTTRS and can be neglected in a first approach. The quantitative analyses supporting this conclusion are documented in the Supplementary Information Section 3 (SI3).

Second, we set up a simple coarse grained model of the PTTRS and analyzed it through a mathematical framework [29,30] that provides an approximated but intelligible comprehensive description of the relationship between system and molecular properties. The usefulness of this approach to clarify the functional significance of biological variability has been demonstrated [31]. This analysis permitted enumerating the qualitatively distinct states and responses available to the PTTRS and determine closed-form analytical relationships among H_2O_2 supply rates, protein concentrations and kinetic parameters that take the system to each state. Importantly, these results do not depend on numerical parameter values, but just on the order-of-magnitude considerations that informed model set up.

Third, based on selected data in the literature and quantitative proteomics datasets we estimated the kinetic parameters and cytoplasmic concentrations for human erythrocytes, hepatocytes, eleven human cell lines, and *S. cerevisiae*. (These estimates are thoroughly documented in the SI.) We validated the quantitative models by comparing computational predictions to the most comprehensive quantitative observations of the PTTRS' responses to H_2O_2 available. In light of the analysis mentioned in the previous paragraph, we then examined the functional implications of the variation in protein composition and properties among cell types, and we dissected the underpinnings of commonalities and differences among the predicted responses.

These analyses show that the PTTRS can *in principle* respond to H_2O_2 supply in many distinct ways. Nevertheless, once the actual parameter values and composition are considered distinct tumor cell lines are predicted to show a surprisingly similar response that (a) prevents strong $Trx\text{-}S^-$ depletion, (b) favors a gradual moderate accumulation of hyperoxidized Prx at high v_{sup}, and (c) avoids a run-away hyperoxidation of all the Prx. This response hinges on the Prx-SS reduction capacity just slightly exceeding the maximal steady state Prx-SS production. Its near-universality over cell lines with quite heterogeneous