Regulation of Peroxiredoxins by Nitric Oxide in Immunostimulated Macrophages

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Reactive oxygen species and nitric oxide (NO) are capable of both mediating redox-sensitive signal transduction and eliciting cell injury. The interplay between these messengers is quite complex, and intersection of their signaling pathways as well as regulation of their fluxes requires tight control. In this regard, peroxiredoxins (Prxs), a recently identified family of six thiol peroxidases, are central because they reduce H₂O₂, organic peroxides, and peroxynitrite. Here we provide evidence that endogenously produced NO participates in protection of murine primary macrophages against oxidative and nitrosative stress by inducing Prx I and VI expression at mRNA and protein levels. We also show that NO prevented the sulfhydrylation-dependent inactivation of 2-Cys Prxs, a reversible overoxidation that controls H₂O₂ signaling. In addition, studies using macrophages from sulfiredoxin (Srx)-deficient mice indicated that regeneration of 2-Cys Prxs to the active form was dependent on Srx. Last, we show that NO increased Srx expression and hastened Srx-dependent recovery of 2-Cys Prxs. We therefore propose that modulation by NO of Prx expression and redox state, as well as up-regulation of Srx expression, constitutes a novel pathway that contributes to antioxidant response and control of H₂O₂-mediated signal transduction in mammals.

Macrophages participate in many important functions, including phagocytosis, iron recycling, and host defense, and produce the autacoid-like reactive oxygen species (ROS)3 and nitric oxide (NO) in response to inflammatory cytokines and bacterial products. It has long been reported that ROS and reactive nitrogen species are effectors of an innate immune response (1), but there is increasing evidence that both ROS, and particularly H₂O₂, and NO also operate as signaling molecules to mediate various responses, including cell growth, angiogenesis, and apoptosis (2, 3). Thus, H₂O₂ is now recognized as an important intracellular messenger that is physiologically produced by many cells in response to extracellular stimuli like cytokines and growth factors (4). Second messenger functions mediated by H₂O₂ signaling include activation of mitogen-activated protein kinase (5), modulation of the cell cycle (6, 7), inhibition of tyrosine and lipid phosphatases (8, 9), and protein sumoylation (10). Such signaling pathways imply a tight control of H₂O₂ production and elimination.

Peroxiredoxins (Prxs) constitute an important peroxidase family that uses the reactivity of the cysteine residues to reduce H₂O₂ and other peroxides. Reaction of H₂O₂ with Prxs is fast as indicated by recent reassessment of the kinetic values (11, 12). Further, in addition to their antioxidant function, Prxs have been shown to regulate cell signaling by H₂O₂ by modulating its fluxes and intracellular levels (13, 14). It is also worth noting that Prxs can reduce peroxynitrite (15, 16). Mammals carry six Prx enzymes that distribute in the three Prx subtypes with four typical 2-Cys Prxs (I-IV), one atypical 2-Cys Prx (Prx V), and one 1-Cys Prx (Prx VI) (17). Typical 2-Cys Prxs have the unique feature of undergoing substrate-mediated inactivation by overoxidation of their catalytic cysteine to a sulfonic acid (R-SO₂H). Overoxidation only occurs during enzymatic cycling and is proportional to the amount of substrate under both non-saturating and saturating conditions (15). The fact that inactivation by overoxidation is both unique to eukaryotic Prxs and reversible by ATP-dependent reduction of the Prx Cys-SO₂H by sulfiredoxin (Srx or npn3) and sestrins (18–22) had led to the suggestion that it is an acquired gain of function selected for regulating intracellular H₂O₂ fluxes and signaling (23). Hence, 2-Cys Prx activity is controlled both by the levels of its substrate H₂O₂ and by the activity of sulfhydryl reductases, and this dual control is likely important for regulating H₂O₂ signaling.

In this report, we have investigated the impact of NO on the expression of Prxs, Srx, and sestrins in murine macrophages. We provide a global view of the expression of the six mammalian Prxs in macrophages that produce NO upon stimulation with interferon-γ (IFN-γ) and lipopolysaccharide (LPS). We show that gene expression of Prx I, V, and VI and of Srx was increased in stimulated macrophages. Up-regulation of Prx I and VI, but not Prx V, was mediated by NO. We also report that NO decreases spontaneous and H₂O₂-induced Prx sulfhydrylation and hastens recovery upon H₂O₂-induced Prx sulfhydration, thus pointing to a role for NO in overoxidation prevention and reactivation of 2-Cys Prx.
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EXPERIMENTAL PROCEDURES

Reagents—Recombinant mouse IFN-γ (specific activity 2 × 10⁶ units/mg) was from R&D Systems, Abidgon, UK. Escherichia coli LPS, N-(3-aminomethyl-benzyl-acetamidine) (1400W), phorbol 12-myristate 13-acetate (PMA), tert-butyl hydroperoxide, and cycloheximide were from Sigma. S-ethylisothiourea and the nitric oxide donor diethylenetriamine NONOate (DETA-NO) were from Cayman Chemical (Ann Arbor, MI). Glucose oxidase was from Calbiochem.

Cell Culture and Treatments—Protocols involving animal experimentation were approved by a national animal care committee. Bone marrow cells were obtained by flushing femurs of WT C57BL/6 mice and of NOS2−/− or Srx−/− mice. Bone marrow-derived macrophages (BMM) were differentiated from bone marrow cells by culture in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and 10% L929 cell-conditioned medium. The phenotype of BMM was verified by fluorescence-activated cell sorter. It was shown that >95% of the cells expressed the monocyte/macrophage marker CD11b. BMM were stimulated or not with IFN-γ and/or E. coli LPS at the concentrations and for the times indicated in the figure legends. When indicated, BMM were treated with the nitric oxide donor DETA-NO. Its decomposition rate was determined by the loss of the chromophore at 252 nm.

Preparation of Cell Extracts—BMM cells were washed two times with cold phosphate-buffered saline and lysed in 0.5% Triton X-100 in 100 mM Tris, pH 7.4, containing protease inhibitor mixture Set III (Calbiochem). Cell lysate was then centrifuged at 10,000 × g at 4°C for 10 min, and the protein content of supernatant was determined spectrophotometrically at 595 nm by using the Bio-Rad protein assay.

Antibodies and Immunoblot Analysis—Anti-Prx I antibody was from Upstate/Chemicon, anti-Prx III antibody was from Abcam, and antibodies to Prx II, Prx VI, Prx (I-IV)-SO₂H, and Prx VI-SO₂H were from LabFrontier (Seoul, South Korea). The anti-Srx antibody was a purified rabbit polyclonal serum prepared by Neosystem (Strasbourg, France). Anti-vinculin antibody was a purified rabbit polyclonal serum prepared by Neosystem (Strasbourg, France). Anti-Prx I, Prx VI, and Prx (I-IV)-SO₂H, and Prx VI-SO₂H were from Abcam, and antibodies to Prx II, Prx VI, Prx (I-IV)-SO₂H, and Prx VI-SO₂H were from LabFrontier (Seoul, South Korea). The anti-Srx antibody was a purified rabbit polyclonal serum prepared by Neosystem (Strasbourg, France). Anti-vinculin antibody was a purified rabbit polyclonal serum prepared by Neosystem (Strasbourg, France).

RNA Extraction and Real-time Quantitative PCR—Total RNA was extracted from BMM cells using the SV Total RNA Isolation System (Promega) according to the manufacturer’s protocol. Transcription of total RNA was performed using the Moloney murine leukemia virus reverse transcriptase (Promega) and random primers. Quantitative real-time PCR was performed using a Light Cycler, and the detection of amplification products was carried out using the Light Cycler-DNA Master SYBR Green I kit (Roche Diagnostics). The generation of specific PCR products was confirmed by melting curve analysis. Data were analyzed with Light Cycler 3.5 software. Quantification was performed relative to the 18 S rRNA. All assays were performed in triplicate.

Nitrite Measurement—Nitrite, the stable end product of NO, was quantified in culture medium by using the Griess reagent. Briefly, 200 μl of medium were reacted with 800 μl of Griess reagent (0.5% sulfanilamide and 0.05% N-(1-naphthyl)ethylendiamine in 45% acetic acid), and the absorbance was measured at 543 nm. The nitrite concentration was determined from a sodium nitrite standard curve.

H₂O₂ Generation—Generation of H₂O₂ was assessed using the fluorescence indicator 2′,7′-di-chlorodihydrofluorescein diacetate (H₂DCFDA; Molecular Probes, Inc.). BMM were incubated in a 24-well microplate and loaded for 30 min with 20 μM H₂DCFDA in Hanks’-buffered solution. They were then stimulated for 30, 60, and 180 min with 500 nM PMA. Dichlorofluorescein fluorescence was measured with a Victor3 fluorescence microplate reader (PerkinElmer) by emission at 520 nm (excitation, 485 nm).

RESULTS

NO Increases Prx I and Prx VI Expression in Macrophages—BMM from WT or NOS2−/− mice were stimulated with IFN-γ and LPS. After 18 h, nitrite that had accumulated in the culture medium was quantified, and the expression of the six Prxs was analyzed by quantitative RT-PCR. In stimulated WT BMM, Prx I, Prx V, and Prx VI mRNA expression was noticeably increased compared with unstimulated cells, whereas Prx IV was significantly reduced (Fig. 1A). In contrast, in stimulated NOS2−/− BMM, mRNA level of Prx I, Prx IV, and Prx VI remained unchanged compared with stimulated WT cells, showing that regulation was dependent on NOS2. Still, Prx V mRNA levels remained increased in NOS2−/− BMM, indicating that the up-regulation of this gene by IFN-γ and LPS is independent of NOS2. Prx II and Prx III mRNA levels were not significantly altered by stimulation in either mouse strain. We also used DETA-NO, an NO donor with a long half-life that releases NO at nanomolar concentrations, in the range produced by IFN-γ and LPS-activated macrophages (24). DETA-NO treatment (500 μM) of resting macrophages during 18 h reproduced the effects of endogenous NO produced by BMM on Prx gene expression. Shorter exposures to DETA-NO revealed that Prx I and Prx VI mRNA levels increased 2- and 3-fold after 4 and 8 h, respectively (supplemental Fig. S1), suggesting that regulation is at least in part transcriptional. Moreover, the use of DETA-NO that had been left to decompose for 7 days at 37°C (>8 half-lives) had no effect on gene expression, indicating that neither DETA nor nitrite is responsible for the regulation of Prxs observed with DETA-NO (not shown). Altogether, these data point to a physiological role of NO in the selective regulation of Prx I, IV, and VI mRNA levels.

We further checked whether the NO-dependent increase in Prx I and VI transcript levels also occurred at protein level. Higher Prx I and VI protein levels were seen in BMM stimulated with IFN-γ and LPS, but not when cells were stimulated in the presence of NOS inhibitors or using cells explanted from NOS2−/−-deficient mice (Fig. 1B). Prx I and Prx VI protein levels were also increased in BMM incubated with DETA-NO,
in a dose-dependent manner. In contrast, Prx II and III protein levels were not sensitive to NO (Fig. 1C). On the whole, these results highlight the role of NO in up-regulation of Prx I and VI protein expression.

**Effect of NO on Prx Overoxidation**—The active site cysteine of eukaryotic Prxs undergoes substrate-mediated oxidation to the sulfenic acid form (SO$_2$H), which inactivates enzyme activity (25, 26). Based on the notion than NO induces cysteine sulfinylation in vitro (27, 28), we evaluated whether NO would also promote overoxidation of Prxs. Using an antibody that immunoreacts with the sulfinylated form of the four 2-Cys Prxs (I–IV), we thus monitored the level of overoxidized Prxs in macrophages that were stimulated to produce NO (Fig. 2A). In lysates of H$_2$O$_2$-challenged RAW 264.7 macrophage cell line was loaded (upper panel, right corner). Based on molecular mass and Refs. 19 and 29, the uppermost (and minor) band is Prx IV, the middle band is Prx III, and the lower (major) band is Prx I/II. Lower panel, WT BMM were exposed to 500 μM DETA-NO for increasing times ranging from 1 to 24 h, and cell lysates were assessed for expression of overoxidized Prx. Vinculin was used as a loading control. The experiment shown is representative of at least three performed.

![FIGURE 2. Endogenous and exogenous NO decrease basal overoxidation of Prxs.](image)

**FIGURE 2.** Endogenous and exogenous NO decrease basal overoxidation of Prxs. WT and NOS2$^{-/-}$ BMM were stimulated with 100 units/ml IFN-γ and 500 ng/ml LPS (I/L) in the presence or not of 25 μM 1400W and 100 μM S-ethylisothiourea, two NOS inhibitors (In), or exposed to 50 or 500 μM DETA-NO (B). As a positive control for the detection of Prx overoxidation, lysate of H$_2$O$_2$-challenged RAW 264.7 macrophage cell line was loaded (upper panel, right corner). Based on molecular mass and Refs. 19 and 29, the uppermost (and minor) band is Prx IV, the middle band is Prx III, and the lower (major) band is Prx I/II. Lower panel, WT BMM were exposed to 500 μM DETA-NO for increasing times ranging from 1 to 24 h, and cell lysates were assessed for expression of overoxidized Prx. Vinculin was used as a loading control. The experiment shown is representative of at least three performed.
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A Pre-treatment none DETA-NO IFN-γ/LPS

\[ \text{H}_2\text{O}_2 \ (\mu\text{M}) \]

\[ 0 \quad 100 \quad 0 \quad 100 \quad 0 \quad 100 \]

\( \alpha\text{-Prx(I-IV)} - \text{SO}_2 \text{H} \)

\( \alpha\text{-Vinculin} \)

| Glucose oxidase system (Fig. 3A) | Gene expression analysis (Fig. 3B) |
|---------------------------------|----------------------------------|
| Time (min) | IFN-γ/LPS | DETA-NO |
| BMM treated with IFN-γ/LPS | IFN-γ/LPS | DETA-NO |
| PMA treatment | 2 h | 4 h |
| PMA treatment | 2 h | 4 h |

as low as 50 \( \mu\text{M} \) and was markedly increased at 500 \( \mu\text{M} \). Time course experiments indicated that the effect of NO on Prx oxidation occurred early, with an \( \sim 50\% \) decrease at 2 h after addition of DETA-NO (Fig. 2B, lower panel).

We next evaluated whether NO would also alter the overoxidation of Prxs induced by exogenous \( \text{H}_2\text{O}_2 \). BMM were treated with IFN-γ and LPS or were exposed to DETA-NO for 16 h. After extensive washings, they were then challenged with a bolus of \( \text{H}_2\text{O}_2 \) (Fig. 3A) with the membrane-permeant pro-oxidant agent tert-butyl-hydroperoxide (Fig. 3B) or with \( \text{H}_2\text{O}_2 \) continuously produced at low concentrations by the glucose/glucose oxidase system (Fig. 3C). Again, \( \text{H}_2\text{O}_2 \)-induced Prx I/II, Prx III, and, to a lesser extent, Prx IV overoxidation was significantly decreased in DETA-NO-treated macrophages as compared with the untreated controls. At least with respect to Prx I/II, similar results were obtained with human epithelial carcinoma (HeLa) cells exposed to exogenous NO (supplemental Fig. S2), pointing to a more general effect on various cell populations and species. We also used PMA, a potent NOX2 activator that stimulates endogenous \( \text{H}_2\text{O}_2 \) production. Macrophage treatment with PMA indeed led to \( \text{H}_2\text{O}_2 \) production, as testified by oxidation of the fluorescent probe dichlorofluoroscein diacetate (not shown). PMA also increased the basal levels of sulfonlated 2-Cys Prxs, which was not seen when cells had been previously treated with DETA-NO (Fig. 3C).

We also checked whether 1-Cys Prx VI overoxidation was also affected by NO, using a Prx VI-SO\(_2\)H-specific antibody. Overoxidized Prx VI was detectable only after exposure of resting BMM to 100 \( \mu\text{M} \) \( \text{H}_2\text{O}_2 \), and prior stimulation of BMM by IFN-γ/LPS or exposure to DETA-NO did not alter Prx VI overoxidation (supplemental Fig. S3). Prx VI protein levels were increased by NO as shown above (Fig. 1B) and after reprotein membranes with an anti-Prx VI antibody (supplemental Fig. S3). Prx V was not evaluated, because this Prx isoform is not sensitive to overoxidation (20).

In summary, these data clearly indicate that NO decreases 2-Cys Prx sulfonlatedation rather than promoting this form of oxidation. These results could be explained by NO either preventing Prx sulfonlation or favoring its recycling. Based on the simultaneous effect of NO on up-regulation of Prx I expression, it is possible that the resulting increased \( \text{H}_2\text{O}_2 \)-scavenging capacity could act as a factor diminishing Prx I overoxidation. However, the fact that the translation inhibitor cycloheximide did not prevent DETA-NO from abating \( \text{H}_2\text{O}_2 \)-mediated Prx overoxidation (supplemental Fig. S4) indicates that Prx I up-regulation and overoxidation can be dissociated. This result also show that decrease in Prx overoxidation does not require de novo protein synthesis.

Effect of NO on Srx Expression—Sulfonlateded 2-Cys Prx (I–IV) can be reduced by two different types of enzymes with ATP-dependent sulfenic acid reductase activity, Srx (18, 21) and the sestrins (22). We therefore investigated the possible involvement of Srx and sestrins in the NO-dependent decline in Prx overoxidation. Sestrin mRNA levels were not significantly modified in NO-producing or DETA-NO-exposed BMM (not shown). In contrast, Srx mRNA levels were significantly up-regulated in IFN-γ/LPS-stimulated macrophages (Fig. 4A), and this increase was dependent on NOS2-derived NO because it was not observed in stimulated macrophages from NOS2\(^{-/-}\) mice. A time course experiment showed that the increase in Srx expression in DETA-NO-exposed BMM began as early as 1 h after DETA-NO exposure, peaked after \( \sim 5 \) h, and remained high after 24 h (Fig. 4B). Western blot analyses using an Srx-specific antibody showed that the increase in Srx mRNA levels was paralleled by an increase in Srx protein levels (Fig. 4C).

We sought to determine whether the NO-dependent up-regulation of Srx and the decline in Prx I–IV overoxidation were linked. To this aim, we analyzed the Prx oxidation status in BMM from WT or Srx-deficient mice that had been exposed to DETA-NO and then challenged with 100 \( \mu\text{M} \) \( \text{H}_2\text{O}_2 \). As already shown in Fig. 2, preincubation of WT BMM with DETA-NO decreased both constitutive and \( \text{H}_2\text{O}_2 \)-induced Prx overoxidation (Fig. 5A, compare lane 3 with lane 1 and lane 4 with lane 2). The same results were observed in Srx\(^{-/-}\) BMM (compare lane 7 with lane 5 and lane 8 with lane 6), indicating that the effect of NO on the decrease of Prx overoxidation does not involve Srx. To further investigate a possible role of Srx on Prx regeneration, we measured the rate of Prx sulfenic acid reversion after a brief exposure to \( \text{H}_2\text{O}_2 \). Prx sulfenication was measured at different time points in BMM preincubated with DETA-NO that were then exposed to \( \text{H}_2\text{O}_2 \) (100 \( \mu\text{M} \)) during 30 min and then washed.
and re-incubated in fresh medium (Fig. 5B). Densitometric analysis of immunoblots showed that the intensity of the H$_2$O$_2$-induced sulfinic acid signal had decreased by 50% after 1.5 h in WT control BMM and after only 45 min in DETA-NO-treated WT BMM. Furthermore, in lysates of Srx$^{-/-}$ BMM that had been incubated or not with NO, the signal of H$_2$O$_2$-induced sulfinylated Prx did not decrease, remaining high throughout the entire observation period. These results indicate that NO not only prevents Prx sulfinylation but also increases the efficiency of its in vivo reduction. They also confirm the crucial role of Srx in the reduction of sulfinylated 2-Cys Prxs in mammals.

**DISCUSSION**

Signaling by oxygen- and nitrogen-derived species is attracting growing attention as its involvement in diverse cellular responses is being disclosed (30, 31). As these mediators are reactive, they can also potentially damage various biomolecules, making their control of prime importance for cell physiology (1, 32, 33). Prxs are important cellular peroxide-scavenging enzymes and have been shown to also modulate signaling by H$_2$O$_2$. Classical 2-Cys Prxs, which comprise Prx I-Prx IV, undergo reversible hydroperoxide-mediated inactivation by overoxidation of their catalytic cysteine to the sulfinic acid form (16, 23) and reduction by ATP-dependent sulfiredoxin and sestrins (18, 21). This inactivation, being unique to eukaryotic Prxs, has led to suggestions that it is an acquired gain of function selected for regulatory purposes (23). In the present study, we have identified a novel cross-talk between the action of NO and H$_2$O$_2$ in primary macrophages. This cross-talk is based on the effect of NO of decreasing H$_2$O$_2$-induced 2-Cys Prx sulfinylation, of speeding up the regeneration of sulfinylated inactive enzymes, and of up-regulating Prx I, Prx VI, and Srx at mRNA and protein levels. These effects were observed whether NO was endogenously produced by NOS2 or delivered extracellularly by the slow NO donor DETA-NO and when Prxs were sulfinylated by H$_2$O$_2$ that was endogenously produced by stimulated macrophages or applied extracellularly. Our data also provide evidence that Prx V is induced in macrophages stimulated by IFN-γ and LPS, but in contrast to Prx I and Prx VI, this induction is independent of NO production. Also, in contrast to what was observed for 2-Cys Prxs, H$_2$O$_2$-mediated overoxidation of 1-Cys Prx VI was not significantly altered by endogenous or exogenous NO, which is consistent with the fact that Srx is not able to reduce sulfinyl-Prx VI (29). Taken together, these results indicate that NO production increases
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![Diagram](image)

**FIGURE 6. Two-level intersection of NO- and H₂O₂-signaling pathways.** In this proposed scheme, NO produced by immunologically stimulated macrophages first rapidly alleviates overoxidation of 2-Cys Prxs by post-translational modification and then mediates faster recovery to their reduced state via Srx up-regulation.

The question arises: How does NO affect 2-Cys Prx overoxidation? Our data indicate that NO both prevented catalytic cysteine overoxidation and accelerated the rate of its recovery by sulfiredoxin (18, 21). We found that NO-exposed WT BMM displayed much lower constitutive amounts of Prx-SO₂H (see Fig. 2A). As this effect was prominent as early as 2 h after exposing cells to NO (Fig. 2B), it could not be the consequence of faster reduction of the sulfylated form of Prxs by Srx because induction of the later gene by NO peaked much later, at 5 h (Fig. 4). We also observed that the overoxidation of Prxs that resulted from a 30-min exposure to H₂O₂ was significantly lower in NO-treated versus untreated macrophages, also implying that the effect could not be a consequence of a more efficient reduction by Srx. In addition, the effect of NO pretreatment on the overoxidation of Prxs by exogenous H₂O₂ was fully maintained in Srx⁻/⁻ BMM, further indicating that the effect of NO in preventing 2-Cys Prx overoxidation is unrelated to Srx (Fig. 4). Lipid hydroperoxide metabolites of arachidonic acid, which are commonly produced by stimulated macrophages, have recently been shown to reversibly overoxidize 2-Cys Prxs in cyclooxygenase- or lipoxygenase-overexpressing human cell lines (48). It would be worth considering that NO, by scavenging lipid peroxyl radicals (49, 50), decreases the level of 2-Cys Prx overoxidation. Alternatively, a plausible explanation is that Prxs from NO-producing macrophages are protected from overoxidation by H₂O₂ by a post-translational modification of its catalytic cysteine residues. Based on the role of S-glutathionylation in the adaptive response to oxidative stress (51, 52), protection of the Prx active site cysteine(s) by NO-induced disulfide formation with glutathione is an appealing hypothesis.

Nevertheless, NO also affected the rate of the Srx-dependent reversion of sulfynlated Prxs. This was deduced from the faster decline in the amount of PrxI/II-SO₂H generated by exogenous H₂O₂ in NO-treated versus untreated macrophages (Fig. 5B).

Our data also revealed that in Srx-deficient BMM, H₂O₂-induced Prx sulfenylation remained stable over time, indicating that Srx is the major macrophage sulfanyl reductase and that no other enzyme can compensate for its deficiency in these cells. This finding is in keeping with the work of Chang et al. (21) showing that small interfering RNA-mediated Srx silencing delays Prx regeneration in epithelial cells. It also supports the idea that the effect of NO in accelerating Prx regeneration is mediated by NO-induced Srx up-regulation, thus exemplifying the role of Srx in modulating the redox state of Prxs in a physiological setting.

To conclude, by pointing to Prxs as a cross-talk between NO and H₂O₂ signaling, we propose a novel control mechanism by which physiologically produced NO exerts an antioxidant effect. Increase in the amount of active Prxs is likely to represent a negative feedback loop to protect against excessive stress. It may also contribute to the redox control of the kinase/phosphatase balance. Further, the intrusion of the IFN-γ- and LPS-driven NOS2 as a new player in the Prx/Srx-regulating system of H₂O₂ flux opens a wide field for investigations of a connection between host defense and cell redox signaling.

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