A redox-sensitive peroxiredoxin that is important for longevity has tissue- and stress-specific roles in stress resistance

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Oxidative damage caused by reactive oxygen species (ROS) is implicated in many diseases and in aging. Removal of ROS by antioxidant enzymes plays an important part in limiting this damage. For instance, peroxiredoxins (Prx) are conserved, abundant, thioredoxin peroxidase enzymes that function as tumor suppressors. In addition to detoxifying peroxides, studies in single-cell systems have revealed that Prx act as chaperones and redox sensors. However, it is unknown in what manner the different activities of Prx influence stress resistance or longevity in the context of whole animals. Here, we reveal three distinct roles for the 2-Cys Prx, PRDX-2, in the stress resistance of the nematode worm *Caenorhabditis elegans*. (*i*) The thioredoxin peroxidase activity of PRDX-2 protects against hydrogen peroxide. (*ii*) Consistent with a chaperone activity for hyperoxidized PRDX-2, peroxide-induced oxidation of PRDX-2 increases resistance to heat stress. (*iii*) Unexpectedly, loss of PRDX-2 increases the resistance of *C. elegans* to some oxidative stress-causing agents, such as arsenite, apparently through a signaling mechanism that increases the levels of other antioxidants and phase II detoxification enzymes. Despite their increased resistance to some forms of oxidative stress, *prdx-2* mutants are short-lived. Moreover, intestinal expression of PRDX-2 accounts for its role in detoxification of exogenous peroxide, but not its influence on either arsenite resistance or longevity, suggesting that PRDX-2 may promote longevity and protect against environmental stress through different mechanisms. Together the data reveal that in metazoans Prx act through multiple biochemical activities, and have tissue-specific functions in stress resistance and longevity.

aging | *Caenorhabditis elegans* | oxidative stress | signaling | chaperone

Oxidative stress-associated cellular damage has a fundamental role in the initiation and development of many diseases, including age-associated diseases such as diabetes, cardiovascular disease, and cancer. Antioxidant enzymes provide an important cellular defense against oxidative stress by removing reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, before they cause oxidative damage. Alterations in the activity of signaling pathways that result in increased levels of antioxidant enzymes are also frequently associated with increased oxidative stress resistance and longevity (1, 2). However, it has not been conclusively established that any single antioxidant enzyme is essential for mediating this increased oxidative stress resistance and lifespan (for a review, see ref. 3).

Peroxiredoxins (Prx) are a group of ubiquitous peroxidase enzymes in which redox-active cysteine residues participate in the reduction of hydrogen peroxide (4). Based on their catalytic mechanism, Prx have been separated into three classes: typical 2-Cys, atypical 2-Cys, and 1-Cys Prx (for a review, see ref. 5). Typical 2-Cys Prx contain two catalytic cysteine residues. The "peroxidatic" cysteine is oxidized directly by hydrogen peroxide, generating a "sulfenic" derivative that is stabilized by the formation of a disulfide bond with the other "resolving" cysteine in a neighboring Prx molecule [supporting information (SI) Fig. S1*A*]. Typical 2-Cys Prx are thioredoxin peroxidases, in that the oxidoreductase thioredoxin is required to restore their activity by reducing the disulfide between the catalytic cysteines. The thioredoxin peroxidase activity of typical 2-Cys Prx is important for protection against the toxic effects of hydrogen peroxide. However, thioredoxin peroxidase-independent activities have also been identified for typical 2-Cys Prx in signal transduction (6–8) and as chaperones (9, 10).

Genetic studies have revealed that typical 2-Cys Prx have important roles in protecting against DNA damage, oxidative stress, and cancer (11–13). However, it is not clear in what way the multiple activities of typical 2-Cys Prx as thioredoxin peroxidases, chaperones, or signaling molecules contribute to the oxidative-stress defenses of multicellular organisms. To begin to address these questions, we have used the nematode worm *Caenorhabditis elegans* as a genetically amenable metazoan in which to examine the role of typical 2-Cys Prx in stress responses and aging.

Here we report that the 2-Cys Prx, PRDX-2, has important roles in stress resistance, stress-signaling, and aging. Our data suggest that PRDX-2 affects stress resistance by three distinct molecular mechanisms: (*i*) The thioredoxin peroxidase activity of PRDX-2 protects against hydrogen peroxide, and (*ii*) a hyperoxidized form of PRDX-2 that lacks peroxidase activity protects against heat stress. Conversely, (*iii*) loss of PRDX-2 increases resistance to the oxidative stress-causing arsenite, apparently through a signaling mechanism that allows phase II detoxification gene expression to be increased independently of the canonical SKN-1 (Nrf2) pathway (14). Together, these data imply that the thioredoxin peroxidase, chaperone, and signaling activities of PRDX-2 all make important contributions to the stress resistance of *C. elegans*.

We have also identified tissue-specific roles for PRDX-2. For instance, whereas intestinal PRDX-2 protects against oxidative stress caused by hydrogen peroxide or arsenite, PRDX-2 in other tissues acts to inhibit expression of phase II detoxification genes and limit arsenite resistance. Furthermore, although intestinal expression of PRDX-2 protects against oxidative stress, it does not restore normal growth and fecundity or increase the lifespan of short-lived *prdx-2* mutants. We conclude that, while PRDX-2 acts in the intestine to protect against environmental stress, PRDX-2 in other tissues influences endogenous mechanisms that promote longevity.

Results

PRDX-2 Influences Oxidative and Heat-Stress Resistance Through Multiple Mechanisms. Two typical 2-Cys Prx are present in *C. elegans*, PRDX-2 and PRDX-3 (Fig. S1*B*), which both share extensive

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homology with human typical 2-Cys Prx. However, phylogenetic analysis identifies PRDX-2 as the most likely ortholog of the tumor suppressor Prdx1 (Fig. S1*C*). The *prdx-2* (*gk169*) and *prdx-3* (*gk529*) mutant alleles are each predicted nulls that result in complete loss of the PRDX-2 and PRDX-3 proteins, respectively (Fig. S2 *A–D*). We observed that exposure to hydrogen peroxide levels that were sublethal for either wild-type or *prdx-3* animals was highly toxic to *prdx-2* mutants (Fig. S2*E*), suggesting that PRDX-2 is important for protecting against hydrogen peroxide. Thus, we chose to investigate further the biological functions of *C. elegans* PRDX-2.

The thioredoxin peroxidase activity of typical eukaryotic 2-Cys Prx is sensitive to inactivation by hyperoxidation of the peroxidatic cysteine to sulfinic acid (15, 16) (Fig. S1*A*). We examined the sensitivity of PRDX-2 to oxidation. Under nonstress conditions, PRDX-2 was present in the reduced monomeric form, along with PRDX-2 disulfide catalytic cycle intermediates (Fig. 1*A* and Fig. S3*A*). In contrast, following 5-min exposure to 5.0 mM hydrogen peroxide, reduced PRDX-2 was no longer detectable and PRDX-2 was present instead as hyperoxidized-monomer and oxidized-disulfide forms, including a new PRDX-2 disulfide form (⋆ in Fig. 1*A* and Fig. S3A). During a 2-h recovery in the absence of hydrogen peroxide, the peroxide-induced PRDX-2 disulfide form (⋆) disappeared, and some reduced PRDX-2 reappeared, consistent with this PRDX-2 disulfide being reduced (Fig. 1*A*). However, importantly, the amount of hyperoxidized, monomeric PRDX-2 did not decrease significantly, even after a 4-h recovery period, suggesting that the hyperoxidation of PRDX-2 is not readily reversed (Fig. 1*A*). Pretreatment with hydrogen peroxide levels that cause complete oxidation of PRDX-2, including apparently irreversible hyperoxidation of some PRDX-2, rendered wild-type animals approximately as sensitive to hydrogen peroxide as *prdx-2* mutants (Fig. 1*B*). Moreover, there is no difference between the sensitivity of wild type and *prdx-2* mutants to higher concentrations of hydrogen peroxide, at which PRDX-2 is hyperoxidized (Fig. S2*F*) (17). Taken together, these data suggest that the thioredoxin peroxidase activity of reduced PRDX-2 plays an important role in protecting *C. elegans* against hydrogen peroxide.

Studies in vitro and in single cells have suggested that, in addition to their thioredoxin peroxidase activity, 2-Cys Prx have a chaperone activity that protects against heat-induced protein aggregation (9, 10). Moreover, it has been suggested that hydrogen peroxide-induced hyperoxidation promotes structural changes that enhance this chaperone activity (9, 10). The stability of hyperoxidized PRDX-2 (Fig. 1*A*) allowed us to examine whether hyperoxidized PRDX-2 could protect intact *C. elegans* against heat stress. Remarkably, although pretreatment with 5.0 mM hydrogen peroxide decreased resistance to a subsequent peroxide challenge (Fig. 1*B*), this regimen actually increased the survival of wild-type, but not *prdx-2* mutant, worms at 37 °C (Fig. 1*C*). Importantly, only pretreatment with sufficient hydrogen peroxide to generate substantial levels of hyperoxidized PRDX-2 produced significant levels of thermal protection (Fig. S3). This finding, together with the comparable heat-stress sensitivities of untreated wild-type and *prdx-2* mutant animals (Fig. 1*C*), suggests that hyperoxidation of PRDX-2 is required for its heat-protective activity. Importantly, resistance to arsenite (AsO_2^-) was not increased by pretreatment with 5.0 mM hydrogen peroxide, suggesting that hyperoxidized PRDX-2 shows some specificity for protecting against heat stress (Fig. 1*D*). We conclude that hyperoxidized PRDX-2 may protect against heat stress through its predicted chaperone activity (Fig. 1*E*).

Unexpectedly, this analysis also revealed that *prdx-2* mutants have increased resistance to arsenite (Fig. 1*D*). This was surprising because the cytotoxicity of metalloids and heavy metals, such as arsenic and cadmium, is believed to be in part through the generation of ROS (18). However, in marked contrast to their sensitivity to hydrogen peroxide, *prdx-2* mutant animals were significantly more resistant to arsenite and cadmium toxicity than wild type (Fig. 1*D* and data not shown). PRDX-2 therefore protects *C. elegans*

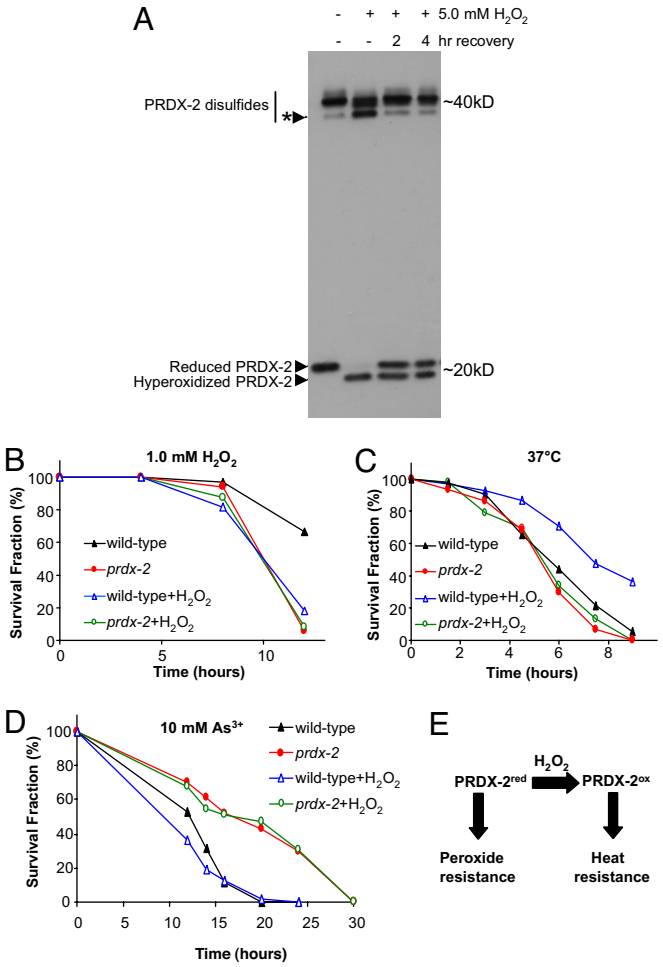

Fig. 1. PRDX-2 is sensitive to hydrogen peroxide-induced oxidation (*A*) and has distinct stress-specific roles in resistance to peroxide, heat stress, and arsenite (*B–E*). (*A*) The redox state of PRDX-2 was examined in wild-type (N2) worms treated as indicated for 5 min with 5.0 mM H_2O_2, then allowed to recover for 0, 2, or 4 h in the absence of H_2O_2. Proteins extracted from these animals were treated with the thiol-reactive agent AMS, then examined by nonreducing Western blot analysis using anti-PRDX-2 antibodies. A nonreducing Western blot analysis allows electrophoretic separation of reduced and hyperoxidized PRDX-2 from PRDX-2 disulfide dimers (PRDX-2 disulfide) (Fig. S1*A*). In addition, AMS modification of the peroxidatic cysteine thiol present in reduced PRDX-2, but absent from hyperoxidized PRDX-2 (Fig. S1*A*), allows separation of reduced and hyperoxidized PRDX-2 on the basis of the reduced electrophoretic mobility of PRDX-2 after AMS modification. In untreated animals PRDX-2 is present as reduced monomer (≈20 kDa) and a PRDX-2 disulfide form (≈40 kDa), likely to be a catalytic cycle intermediate (Fig. S1*A*). After treatment with 5.0 mM H_2O_2, reduced PRDX-2 is replaced with a hyperoxidized monomeric form and an additional peroxide-induced PRDX-2 disulfide (⋆). The reappearance of some reduced PRDX-2 within 2 h of recovery is coincident with loss of H_2O_2-induced PRDX-2 disulfide*, consistent with reduction of PRDX-2 disulfide* to yield reduced PRDX-2. However, levels of hyperoxidized monomeric PRDX-2 do not decrease even after 4 h of recovery, suggesting that hyperoxidized PRDX-2 is stable. (*B* and *C*) After pretreatment for 5 min with 5.0 mM H_2O_2 ($+H_2O_2$): (*B*) the resistance of wild-type (N2) animals to 1.0 mM H_2O_2 is reduced to levels similar to those in a *prdx-2* (*gk169*) mutant, and (*C*) the thermotolerance of wild-type (N2) animals (at 37 °C) is significantly increased ($P < 0.0001$), but the thermotolerance of *prdx-2* (*gk169*) mutant worms is unaffected. (*D*) *prdx-2* (*gk169*) mutant animals are more resistant to 10 mM sodium arsenite (As^{3+}) than wild-type (N2) animals ($P < 0.0001$), but the arsenite resistance of either wild-type (N2) or *prdx-2* (*gk169*) animals is not increased by 5-min pretreatment with 5.0 mM H_2O_2 ($+H_2O_2$). All of the viability experiments were repeated ≥3 times, giving similar results. Representative experiments are shown. (*E*) The thioredoxin peroxidase activity of PRDX-2 ($PRDX\text{-}2^{red}$) protects against H_2O_2, but is inactivated by oxidation. Oxidation of PRDX-2 is associated with an increase in thermal tolerance that implies a chaperone activity for hyperoxidized PRDX-2 ($PRDX\text{-}2^{ox}$).

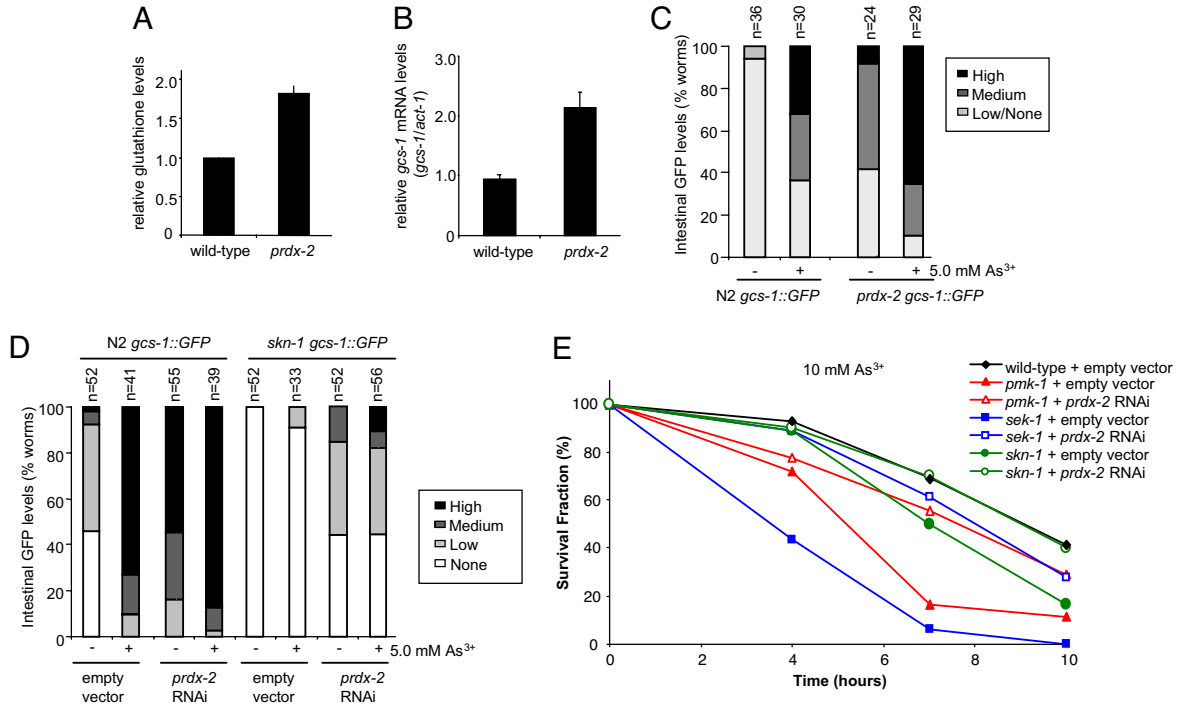

Fig. 2. Loss of PRDX-2 increases glutathione levels, phase II gene expression, and arsenite resistance through SEK-1/PMK-1/SKN-1-dependent and independent mechanisms. (*A*) The level of total glutathione is increased in *prdx-2* (*gk169*) mutant animals. Levels of glutathione in 3 different extracts from wild-type (N2) and *prdx-2* (*gk169*) animals were determined. Mean values relative to the wild type (N2) are shown. Error bars indicate the range in values among extracts. (*B*) Loss of PRDX-2 leads to increased expression of *gcs-1* mRNA. qRT-PCR quantification of *gcs-1* mRNA relative to *act-1* mRNA (control) revealed elevated *gcs-1* mRNA levels in *prdx-2* (*gk169*) animals compared with wild type (N2). Error bars indicate the SEM between samples. (*C*) Loss of PRDX-2 leads to increased intestinal expression of a stress-induced *gcs-1*::*GFP* reporter gene. The intestinal expression of *gcs-1*::*GFP* is high/medium in a larger proportion of *prdx-2* (*gk169*) mutant animals (*prdx-2 gcs-1*::*GFP*) than wild type (N2 *gcs-1*::*GFP*), both before and after treatment with 5.0 mM sodium arsenite. (*D*) *prdx-2* RNAi, by feeding with bacteria containing pL4440+*prdx-2*, leads to increased expression of *gcs-1*::*GFP* in wild-type (N2 *gcs-1*::*GFP*) and *skn-1* (*zu67*) (*skn-1 gcs-1*::*GFP*) animals compared with empty vector (pL4440). Animals were exposed to 5.0 mM sodium arsenite as indicated As^{3+} in the figure. (*E*) *prdx-2* RNAi, by feeding with bacteria containing pL4440+*prdx-2*, increases the arsenite resistance of *pmk-1* (*km25*), *sek-1* (*km4*), and *skn-1* (*zu67*) animals, compared with empty vector (pL4440). [Cox's regression analysis; *pmk-1* empty vector vs. *pmk-1 prdx-2* RNAi ($P < 0.0001$), *sek-1* empty vector vs. *sek-1 prdx-2* RNAi ($P < 0.0001$), *skn-1* empty vector vs. *skn-1 prdx-2* RNAi ($P = 0.022$)]. Experiments were repeated several times with similar results and representative experiments are shown. In *C* and *D* the number of worms in each group is indicated (*n*).

against hydrogen peroxide, but conversely *reduces* resistance to heavy metals. Importantly, resistance to arsenite was increased by loss of PRDX-2 but not by hyperoxidation-induced inactivation of the PRDX-2 peroxidase activity (Fig. 1*D*). Taken together, the data suggest that the PRDX-2 protein might alter other stress-resistance activities, perhaps through a signaling mechanism.

PRDX-2 Inhibits the Expression of Phase II Detoxification Genes. To investigate further why *prdx-2* mutants are resistant to heavy metals, we examined levels of the antioxidant glutathione, which is important in the detoxification of heavy metals. Indeed, *prdx-2* mutants contained approximately twice as much total glutathione as wild type, and they were also more resistant to the glutathione-oxidizing agent diethyl maleate (DEM) (Fig. 2*A* and data not shown). The committing step in glutathione synthesis is catalyzed by the γ-glutamine cysteine-synthetase heavy chain (GCS-1). *gcs-1* is one of the phase II detoxification genes, which encode a battery of enzymes that defend against oxidative damage and are rapidly induced in response to arsenite and various other stress conditions (14, 19). Consistent with the increased glutathione levels we observed, loss of *prdx-2* also increased *gcs-1* mRNA levels and expression of other phase II detoxification genes (Fig. 2*B*, Table S1, and data not shown). Moreover, in the absence of PRDX-2, the intestinal expression of a transgene-encoding GFP under the control of the *gcs-1* promoter (*gcs-1*::*GFP*) (14, 19) was increased, both under unstressed conditions and after treatment with sodium arsenite or hydrogen peroxide (Fig. 2 *C* and *D*, and data not shown).

Basal and stress-induced intestinal expression of *gcs-1* is normally dependent on the Nrf2-related transcription factor, SKN-1, which is activated through phosphorylation by the p38-related mitogen-activated protein kinase (MAPK) PMK-1 (14, 19) (Fig. S4*A*). Thus, it was possible that the absence of the antioxidant activity of PRDX-2 increased *gcs-1*::*GFP* expression by increasing the activation of the p38 pathway. However, we did not detect any increase in either basal or stress-induced phosphorylation of PMK-1 in *prdx-2* mutant worms (Fig. S4 *B* and *C*). In fact, the activation of PMK-1 by arsenite was reduced in *prdx-2* mutants, suggesting that PRDX-2 is *required* for normal arsenite-induced activation of PMK-1. This suggests that loss of PRDX-2 must increase phase II gene expression by a different mechanism. SKN-1 activity is also subject to negative regulation by GSK-3 and insulin-like growth factor signaling pathways (20, 21). Hence it was possible that PRDX-2 regulates *gcs-1* expression by promoting inhibition of SKN-1 by these pathways. Therefore we examined whether SKN-1 is required for the increased intestinal *gcs-1*::*GFP* expression associated with loss of PRDX-2. As expected, intestinal *gcs-1*::*GFP* expression was barely detected in the *skn-1* (*zu67*) mutant background either before or after stress (Fig. 2*D*). Indeed, the increase in *gcs-1*::*GFP* expression produced by loss of PRDX-2 was substantially lower in the *skn-1* (*zu67*) mutant background than in wild-type animals, underlining the important role of this transcription factor in the activity of this promoter. However, loss of PRDX-2 by RNAi significantly increased the number of *skn-1* (*zu67*) animals in which intestinal *gcs-1*::*GFP* was detected (*skn-1 gcs-1*::*GFP* empty vector vs. *skn-1 gcs-1*::*GFP prdx-2* RNAi χ^2 test,

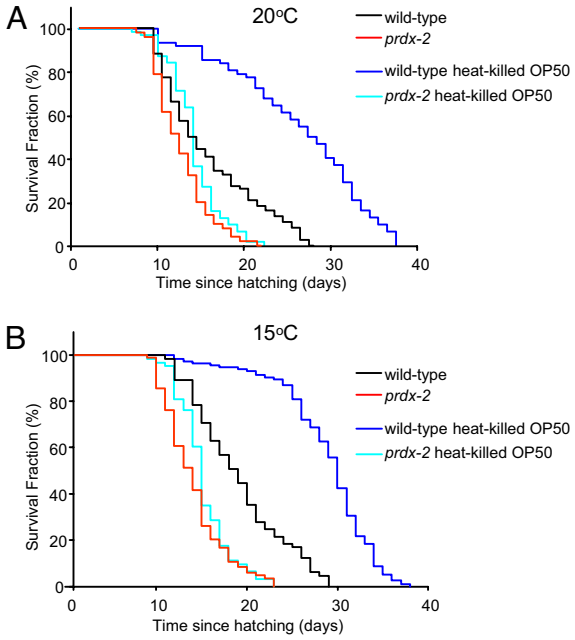

Fig. 3. PRDX-2 is required for normal longevity. *prdx-2* (*gk169*) mutant animals maintained at 20 °C (*A*) and 15 °C (*B*) on either proliferative or nonproliferative (heat-killed) *E. coli* OP50 have a much shorter lifespan than wild-type (N2) animals. Lifespan assays were performed at least twice giving similar results, and a representative experiment is shown above and in Table S2.

$P < 0.001$), suggesting that in the absence of PRDX-2, *gcs-1* is also activated by a *skn-1*-independent mechanism (Fig. 2*D*).

The increased *gcs-1*::*GFP* expression following loss of PRDX-2 in a *skn-1* mutant lacking intestinal forms of SKN-1 suggests the existence of an alternative pathway for regulating the expression of *gcs-1* and other phase II detoxification genes that is normally inhibited by PRDX-2. We tested whether this PRDX-2-inhibited pathway could bypass SEK-1/PMK-1/SKN-1 to increase arsenite resistance. Consistent with reduced-stress signaling, *sek-1*, *pmk-1*, and *skn-1* mutants are all more sensitive to sodium arsenite than are wild-type animals (19). However, loss of PRDX-2 substantially increased the arsenite resistance of these mutants (Fig. 2*E* and Fig. S5). This strongly suggests that, although some of the increased *gcs-1*::*GFP* expression and arsenite resistance of PRDX-2-deficient animals depends on SKN-1 (Fig. 2*D* and S5*A*), loss of PRDX-2 can also lead to increased arsenite resistance through mechanisms that are independent of the canonical p38/SKN-1 pathway.

PRDX-2 Is Required for Normal Longevity. A large body of evidence links oxidative stress-induced damage with aging and suggests that increased oxidative stress resistance is associated with greater longevity (for a review, see ref. 22). However, although *prdx-2* mutants were resistant to some forms of oxidative stress, their lifespans were decreased substantially (Fig. 3 and Table S2). Accordingly, *prdx-2* mutants accumulated the age-associated autofluorescent lipofuscin more quickly and exhibited a faster decline in mobility than wild-type animals (Fig. S6), indicating that their reduced lifespans derive from an increased rate of aging rather than a general reduction in health (23, 24). In sharp contrast to wild type, *prdx-2* mutants maintained on nonproliferative (heat-killed) bacteria did not live any longer, and hence their average lifespan was far shorter than wild type (Fig. 3 and Table S2). This observation indicates that the reduced longevity of *prdx-2* mutants does not result from increased susceptibility to bacterial pathogenesis, a potentially important factor in *C. elegans* longevity (25–27), and, moreover, that *prdx-2* is required for processes that allow wild-type animals to live longer under nonpathogenic conditions. Consistent with other studies, we found that wild-type *C. elegans* maintained on proliferative bacteria lived longer at 15 °C than at 20 °C (Fig. 3 and Table S2) (24). Interestingly, this effect was minimal when animals were maintained on heat-killed bacteria, suggesting that overlapping mechanisms may be involved in producing the lifespan increases seen under each of these conditions. In contrast to wild-type, *prdx-2* mutant animals did not live longer at 15 °C, indicating that PRDX-2 is required for the increased longevity at low temperature (Fig. 3 and Table S2). Together, our data suggest that PRDX-2 promotes longevity by influencing fundamental mechanisms that affect aging.

Tissue-Specific Functions of PRDX-2 in Stress Resistance and Longevity. PRDX-2 is expressed in several tissues, including the intestine, a primary site of exposure to environmental toxins (Fig. S7) (28). To determine whether intestinal PRDX-2 was sufficient to rescue the phenotypes of a *prdx-2* mutant, we examined whether expression of PRDX-2 from an intestinal-specific promoter (*Pges-1*) restored peroxide resistance and normal longevity to *prdx-2* mutant worms. Introduction of this transgene into wild-type worms substantially increased their resistance to hydrogen peroxide (Fig. 4*A*). In contrast, hydrogen peroxide resistance was not increased by intestinal expression of a PRDX-2 mutant protein (PRDX-2^{C170S}) predicted to lack thioredoxin peroxidase activity (Fig. 4*A*). Together, these data suggest that the presence of the thioredoxin peroxidase activity of PRDX-2 in the intestine confers increased resistance to hydrogen peroxide. Moreover, expression of PRDX-2 from an intestinal-specific promoter restored wild-type levels of hydrogen peroxide resistance to a *prdx-2* mutant (Fig. 4*C*), suggesting that intestinal PRDX-2 is both necessary and sufficient for the survival of *C. elegans* exposed to hydrogen peroxide.

Since loss of PRDX-2 increases arsenite resistance, it might be expected that overexpression of PRDX-2 in wild-type animals would have the opposite effect. However, instead we found that increased intestinal expression of PRDX-2 produced a small but significant increase in arsenite resistance (Fig. 4*B*). These data suggest that intestinal PRDX-2 promotes resistance to both hydrogen peroxide and heavy-metal stress (Fig. 4 *A* and *B*). However, despite promoting stress resistance, transgenic expression of PRDX-2 in the intestine of wild-type animals did not increase lifespan (Fig. 4*E* and Table S2).

Next, we examined the extent to which intestinal PRDX-2 expression was able to rescue the phenotypes of the *prdx-2* mutant. In addition to reduced peroxide resistance, reduced lifespan, increased expression of phase II detoxification genes, and increased arsenite resistance (Figs. 1–3 and Table S1), *prdx-2* mutant animals have a reduced size and fecundity (17). Intestinal PRDX-2 did not restore normal growth or fecundity or indeed rescue any of these phenotypes, aside from peroxide sensitivity (Fig. 4 *C*–*E*, Table S1, and data not shown). In particular, intestinal expression of PRDX-2 failed to restore normal longevity to *prdx-2* mutant worms (Fig. 4*E* and Table S2). It is possible that the level of intestinal PRDX-2 expression achieved was simply insufficient to rescue the other phenotypes of a *prdx-2* mutant. However, this transgene was able to greatly increase the hydrogen peroxide resistance of wild-type animals (Fig. 4*A*), suggesting a substantial level of expression. Moreover, intestinal expression of PRDX-2 did not simply fail to restore sensitivity to arsenite, but actually *increased* the hyperresistance of *prdx-2* mutant worms to arsenite still further (Fig. 4*D*). We conclude that PRDX-2 has functions in influencing heavy-metal resistance and longevity that are distinct from its role in protecting against exogenous hydrogen peroxide, and furthermore that these involve its action in tissues outside of the intestine (Fig. 4*F*).

Discussion

In summary, our data reveal that in *C. elegans* the typical 2-Cys Prx, PRDX-2, has a multiplicity of roles in influencing oxidative stress

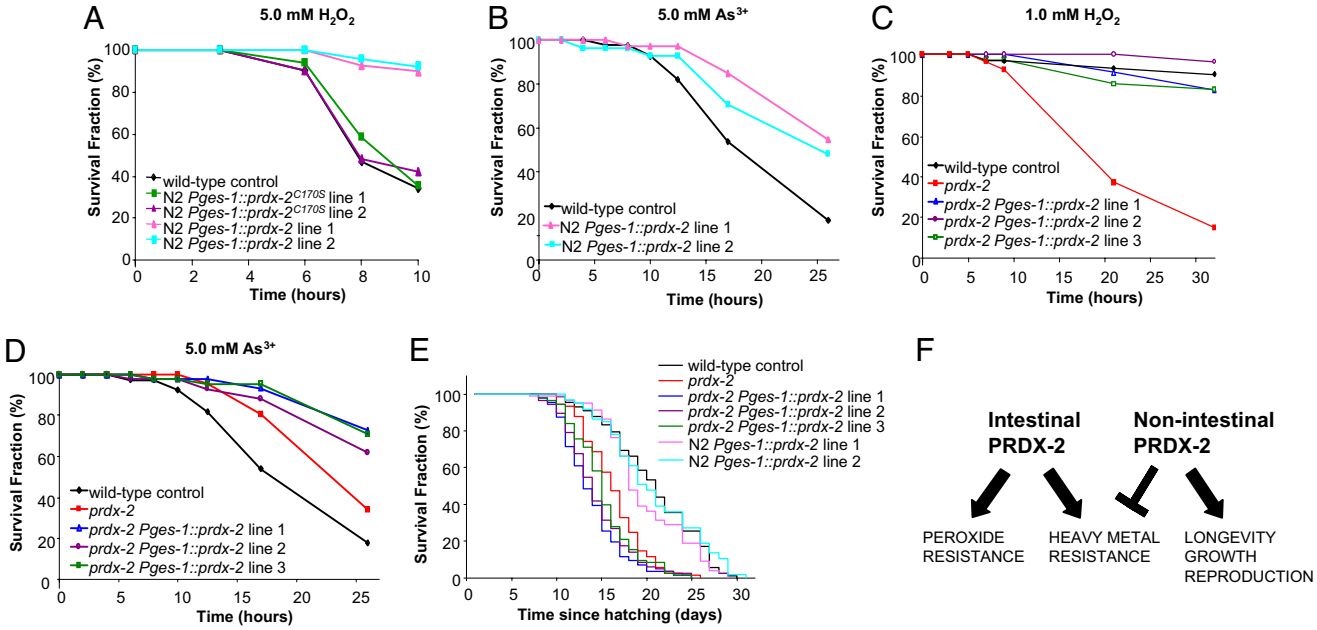

Fig. 4. Intestinal PRDX-2 increases oxidative stress resistance but does not increase the lifespan of short-lived *prdx-2* mutants. (*A*) Animals from independently generated lines containing the *Pges-1*::*prdx-2* transgene (N2 *Pges-1*::*prdx-2*) showed improved survival on plates containing 5.0 mM H_2O_2 compared with wild type (N2) or lines containing the *Pges-1*::*prdx-2*^{C170S} transgene (N2 *Pges-1*::*prdx-2*^{C170S}) [Cox's regression analysis; N2 vs. N2 *Pges-1*::*prdx-2* lines ($P \leq 0.003$)]. (*B*) The survival of animals containing the *Pges-1*::*prdx-2* transgene (N2 *Pges-1*::*prdx-2*) on plates containing 5.0 mM sodium arsenite was improved compared with wild type (N2) [Cox's regression analysis; N2 vs. N2 *Pges-1*::*prdx-2* lines ($P \leq 0.002$)]. (*C–E*) Intestinal PRDX-2 rescues the H_2O_2 sensitivity of *prdx-2* (*gk169*) (*C*) but does not restore normal arsenite sensitivity (*D*) or increase the longevity of either wild-type (N2) or short-lived *prdx-2* (*gk169*) mutant animals (*E*). The survival of worms from 3 transgenic *prdx-2* (*gk169*) *Pges-1*::*prdx-2* lines on plates containing 1.0 mM H_2O_2 (*C*) or 5 mM sodium arsenite (*D*) was compared with wild type (N2) and *prdx-2* (*gk169*). In *D*, *prdx-2* (*gk169*) *Pges-1*::*prdx-2* lines were even more resistant to sodium arsenite than *prdx-2* mutant animals [Cox's regression analysis; *prdx-2* vs. *prdx-2 Pges-1*::*prdx-2* lines ($P < 0.0001$)]. In *E*, the lifespan of N2 *Pges-1*::*prdx-2* and *prdx-2* (*gk169*) *Pges-1*::*prdx-2* lines was compared with wild-type (N2) and *prdx-2* (*gk169*) animals maintained at 15 °C. All transgenic animals also contained pRF4. Survival assays were performed ≥3 times with 25–35 animals in each group. Representative experiments are shown. Lifespan assays were performed at least twice with similar results, and a representative experiment is shown above and in Table S2. (*F*) PRDX-2 has tissue-specific roles. Whereas intestinal PRDX-2 protects against exogenous hydrogen peroxide and heavy-metal stress, nonintestinal PRDX-2 reduces resistance to heavy-metal stress. In addition, nonintestinal PRDX-2 is required for normal growth, reproduction, and longevity.

resistance. For example, we have defined different activities for PRDX-2 in response to different types of stress. Whereas the thioredoxin peroxidase activity of PRDX-2 protects against hydrogen peroxide, our data suggest that inactivation of this activity by oxidation promotes a heat-protective chaperone activity for PRDX-2 (Fig. 1*E*). We also find that PRDX-2 has tissue-specific roles in the growth, reproduction, stress responses, and longevity of *C. elegans* (Fig. 4*F*). For example, whereas intestinal PRDX-2 increases resistance to arsenite, PRDX-2 in other tissues acts to reduce arsenite resistance.

Our data suggest that nonintestinal PRDX-2 may reduce arsenite resistance by inhibiting expression of phase II detoxification genes (Fig. 2 and Table S1). Phase II detoxification genes are induced by a conserved pathway involving activation of the SKN-1 (Nrf2) transcription factor by the PMK-1 (p38) MAPK (19). PRDX-2 is required for normal activation of PMK-1 by arsenite (Fig. S4), but our data imply that in the *prdx-2* mutant phase II gene expression is nevertheless induced in response to stress (Fig. 2). Moreover, our genetic analysis suggests that in the absence of PRDX-2, an alternative pathway that does not require SKN-1 activity can activate expression of phase II detoxification genes (Fig. 2*D*). Strikingly, loss of PRDX-2 increases the arsenite resistance of mutants lacking vital components of the PMK-1 stress-signaling pathway (Fig. 2*E* and Fig. S5), suggesting that activation of this alternative pathway may partly bypass the requirement for PMK-1/SKN-1 in heavy-metal tolerance.

Although it is widely accepted that oxidative stress-induced damage is an important cause of aging, there is little direct evidence that particular antioxidant enzymes are required to prevent aging (3). Here we show that loss of a single cellular antioxidant enzyme can significantly reduce the lifespan of a metazoan. In mice the PRDX-2 ortholog, Prdx1, is required for a normal lifespan, suggesting that the essential role of Prx in longevity may be conserved (13). It may be that the thioredoxin peroxidase activity of Prx plays a critical role in preventing aging. However, Prx have other biochemical activities, including as chaperones, that are promoted by oxidative inactivation of their thioredoxin peroxidase activity (9, 29) (Fig. 1). Indeed, because aging is associated with increased protein aggregation (30, 31), it is possible that the chaperone activity of PRDX-2 may contribute to its role in longevity.

Some genes that are important for stress resistance, such as *pmk-1*, contribute to the longevity of *C. elegans* through their essential role in innate immunity against bacterial infection in the gut (27). However, although *prdx-2* is important for peroxide resistance (Fig. 1) and stress-induced activation of PMK-1 (Fig. S4), the lifespan of *prdx-2* mutant was similar on proliferating and heat-killed bacteria, arguing against a critical requirement for PRDX-2 in immunity. Moreover, it is intriguing that transgenic expression of PRDX-2 in the intestine restored hydrogen peroxide resistance and increased arsenite resistance of *prdx-2* mutants, but did not rescue their reduced lifespan. We conclude that PRDX-2 acts outside of the intestine to promote longevity through mechanisms that are distinct from its intestinal function in protection against environmental stress. PRDX-2 is expressed in neurons and in the reproductive system (Fig. S7 *B* and *C*), both of which are important for determining longevity (32–34). Therefore, it is possible that PRDX-2 promotes longevity by protecting these tissues against endogenously produced ROS or other stresses. Indeed, these data raise the intriguing possibility that PRDX-2 in neurons and/or the gonad participates in regulatory mechanisms that limit

aging and promote longevity. It will be interesting to determine the mechanism(s) by which PRDX-2 acts in tissues outside of the intestine to increase lifespan, while also paradoxically inhibiting expression of phase II enzymes that protect against exogenous-arsenite stress.

Studies in single-cell systems have revealed a variety of biochemical activities for 2-Cys Prx; as thioredoxin peroxidases, chaperones, and both activators and repressors of signaling (for a review, see ref. 35). Here, we have shown that in *C. elegans* PRDX-2 similarly acts through multiple biochemical mechanisms and also has tissue-specific functions (Fig. 4*F*). Although sufficient to rescue the hydrogen peroxide sensitivity of a *prdx-2* mutant, the failure of intestinal expression of PRDX-2 to complement the other phenotypes of a *prdx-2* mutant suggests that PRDX-2 acts in other tissues to profoundly influence growth, reproduction, heavy-metal sensitivity, and longevity. Indeed, our data suggest that intestinal and nonintestinal PRDX-2 have opposite effects on the resistance to heavy-metal stress (Fig. 4*F*). Previous studies have identified mechanisms for differentially regulating the thioredoxin peroxidase, signaling, and chaperone activities of 2-Cys Prx (for a review, see ref. 35). Our data suggest that 2-Cys Prx may also have different activities in different tissues, and thus have important implications for our understanding of these abundant, multifunctional proteins in mammals.

Materials and Methods

***C. elegans* Strains.** The following *C. elegans* alleles were used in this study: N2 Bristol as wild type, *sek-1* (*km4*), *pmk-1* (*km25*), *skn-1* (*zu67*), and *prdx-2* (*gk169*). Strains containing these alleles and strains containing *gcs-1*::*GFP*, *eavEx1Pges-1*::*prdx-2*, or *eavEx2Pges-1*::*prdx-2* ^{C170S} transgenes were generated and maintained as described in *SI Materials and Methods*.

In Vivo Oxidation of PRDX-2. Approximately 3,000 synchronized L4 worms were washed from plates by using M9 buffer (36) containing the indicated concentrations of H_2O_2, then harvested or washed 3 times with M9, and allowed to recover on plates at 20 °C, or used in viability assays (see *SI Materials and Methods*). Worms were harvested in 20% trichloroacetic acid (TCA), then snap-frozen. Thawed pellets were washed with acetone to remove TCA, dried, and resuspended in 30 μl of 1.0% SDS; 100 mM Tris·HCl, pH 8.0; and 1.0 mM EDTA containing 25 mM 4-acetamido-4′-((iodoacetyl)amino)stilbene-2,2′-disulfonic acid (AMS, Invitrogen). Proteins were separated on SDS/15% PAGE gels and analyzed by Western blotting using anti-PRDX-2 antibodies (*SI Materials and Methods*).

Glutathione Assay. Lysate was prepared from young adult worms (*SI Materials and Methods*) and total glutathione levels relative to protein concentration were determined by using a glutathione assay kit (Sigma).

Analysis of *gcs-1* Expression. RNA was extracted from wild-type and *prdx-2* mutant animals, and *gcs-1* and *act-1* mRNA levels were determined as described previously (21) by using Supercript III Platinum SYBR Green One-Step qRT-PCR kit (Invitrogen) and Applied Biosystems StepOnePlus Real-Time PCR system. *gcs-1*::*GFP* expression was assessed as described previously (14) in L2–L3-stage animals after incubation for 30 min, in M9 buffer or on plates, containing the indicated concentrations of sodium arsenite ($NaAsO_2$), followed by 1-h recovery on plates.

***prdx-2* RNA Interference.** L4-stage worms were placed on *prdx-2* RNAi (pL4440+*prdx-2*) or empty vector control (pL4440) RNAi plates (*SI Materials and Methods*). F_1 progeny were scored for intestinal GFP expression or heavy-metal resistance (*SI Materials and Methods*).

Lifespan Assays. Synchronized animals were transferred to plates seeded with live or heat-killed (65 °C for 30 min) *E. coli* (OP50). The worms were transferred away from their progeny to a fresh plate every second day until they stopped laying eggs. Worms that lacked pharyngeal pumping and did not move after repeated prodding with a pick were scored as dead and removed from the plate.

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