Selenoprotein H Suppresses Cellular Senescence through Genome Maintenance and Redox Regulation

Received for publication, September 14, 2014, and in revised form, October 16, 2014. Published, JBC Papers in Press, October 21, 2014, DOI 10.1074/jbc.M114.611970

Ryan T. Y. Wu, Lei Cao, Benjamin P. C. Chen, and Wen-Hsing Cheng

From the Department of Nutrition and Food Science, University of Maryland, College Park, Maryland 20742, Department of Food Science, Nutrition, and Health Promotion, Mississippi State University, Mississippi State, Mississippi 39762, and Division of Molecular Radiation Biology, Department of Radiation Oncology, Southwestern Medical Center at Dallas, University of Texas, Dallas, Texas 75390

Background: Selenoprotein H (SelH), a proposed redox-responsive DNA-binding protein, is little-studied.

Results: SelH shRNA cells display severe proliferation defects and accelerated senescence with abnormal responses to DNA damage and oxidative stress.

Conclusion: SelH suppresses senescence and may have important roles in gatekeeping genomic integrity.

Significance: Learning how SelH keeps senescence in check is crucial for advancing our understanding linking selenium and aging intervention.

Oxidative stress and persistent DNA damage response contribute to cellular senescence, a degeneration process critically involving ataxia telangiectasia-mutated (ATM) and p53. Selenoprotein H (SelH), a nuclear selenoprotein, is proposed to carry redox and transactivation domains. To determine the role of SelH in genome maintenance, shRNA knockdown was employed in human normal and immortalized cell lines. SelH shRNA MRC-5 diploid fibroblasts under ambient O2 displayed a distinct profile of senescence including β-galactosidase expression, autofluorescence, growth inhibition, and ATM pathway activation. Such senescence phenotypes were alleviated in the presence of ATM kinase inhibitors, by p53 shRNA knockdown, or by maintaining the cells under 3% O2. During the course of 5-day recovery, the induction of phospho-ATM on Ser-1981 and H2AX by H2O2 treatment (20 μM) subsided in scrambled shRNA but exacerbated in SelH shRNA MRC-5 cells. Results from clonogenic assays demonstrated hypersensitivity of SelH shRNA HeLa cells to paraquat and H2O2, but not to hydroxyurea, neocarzinostatin, or camptothecin. While SelH mRNA expression was induced by H2O2 treatment, SelH-GFP did not mobilize to sites of oxidative DNA damage. The glutathione level was lower in SelH shRNA than scrambled shRNA HeLa cells, and the H2O2-induced cell death was rescued in the presence of N-acetylcyesteine, a glutathione precursor. Altogether, SelH protects against cellular senescence to oxidative stress through a genome maintenance pathway involving ATM and p53.

Cellular senescence restricts cell proliferation through permanent withdrawal from the cell cycle and plays dual physiological roles (1–4). While cellular senescence can curb tumorigenesis at the precancerous stage and control fibrosis during cutaneous healing early in life (5, 6), it contributes to cellular and tissue aging and age-related disorders later in life (7–10). Although replicative senescence is induced by gradual telomere dysfunction in proliferating cells, stress-induced senescence occurs in essentially any cell type. Reactive oxygen species (ROS) can induce the formation of oxidative and broken DNA, resulting in persistent activation of the DNA damage response and senescence if left unrepaired. Ataxia telangiectasia-mutated (ATM) is a key DNA damage response kinase coordinating checkpoint and senescence responses. ATM is activated by either DNA breaks or oxidative stress (11, 12), and plays an essential role in the senescence response by phosphorylating and stabilizing p53 (13–15).

In mammals, most selenoproteins carry antioxidative functions (16, 17). Selenoprotein expression requires the essential trace element, selenium. Selenoprotein H (SelH), glutathione peroxidase-1, selenoprotein W, and selenoprotein M are more sensitive than other selenoproteins to body selenium fluctuations (18–20). Tissue-specific knockout of selenocysteine tRNA for global suppression of selenoproteins in epidermal cells or osteo-chondroprogenitor cells renders the mice susceptible to age-related disorders including alopecia and bone abnormality (21, 22). These observations are consistent with an estimation linking eleven selenoproteins to aging or age-related disorders (23).

SelH is a thioredoxin-like nuclear protein exhibiting glutathione peroxidase activity (24). Furthermore, the homologue of human SelH in Drosophila is critical for embryogenesis through its antioxidative activity (25). Studies of human SelH in HT22 mouse neuronal cells have implicated this selenoprotein in the protection against UVB-induced apoptosis and as a...
transactivator for GSH biosynthesis (26–29). Nonetheless, a role of SelH in the senescence response to DNA damage and oxidative stress has not been explored. Because SelH expression is enriched in nucleoli, and this organelle has been proposed as a stress-sensing center in the nucleus (24, 30, 31), we hypothesized that SelH protects against oxidative stress through genome maintenance and the limitation of cellular senescence. Thus, we stably knocked down SelH expression in human normal diploid fibroblasts and cancerous cells to evaluate their cellular and biochemical responses to various DNA-damaging agents. Our results suggested a new role of SelH specifically in the cellular response to oxidative stress that suppresses senescence and gatekeeps genomic integrity in a manner depending on ATM and p53.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Reagents**—The MRC-5 diploid lung fibroblasts (Coriell Institute, Camden, NJ), HeLa cervical cancer cells (ATCC, Manassas, VA), and HCT116 colorectal adenocarcinoma cells complemented with hMLH1 (HCT116 + hMLH1) (32, 33) were cultured as described previously in 20% or 3% O2 incubators (34, 35). However, no additional selenium was supplemented in the current study. Because selenium inevitably appears in FBS, a typical cell culture medium containing 10–15% FBS can support selenoprotein expression at nutritional level. By analysis, the batch of FBS used in this study contains selenium at 355 nm. N-Acetylcysteine (NAC), a GSH precursor, was dissolved in water. KU 60019 and KU 55933 (Tocris, Ellisville, MO) were dissolved in DMSO. All chemicals were from Sigma-Aldrich unless otherwise indicated.

**shRNA Knockdown**—SelH and SelH2 shRNA sequences targeting the 3′ SelH mRNA at nucleotides 333–353 and 503–523 were designed based on Invitrogen Block-it™ RNAi designer, and the human non-target scrambled sequence (5′-GGGAATGTCGCCCTGC-3′) was adapted from Addgene Organization. The lentiviral particles containing shRNA cassette were produced by BLOCK-it™ Lentiviral RNAi Expression System (Life Technologies) and used to infect cells in culture. After viral infection (1 day), recovery (1 day), and blasticidin selection (14 days), 12 viable clones were picked from each viral infection and sub-cultured to confirm the knockdown efficiency by using quantitative RT-PCR. Human TaqMan probes (FAM-tagged) and primers were purchased from Applied Biosystems using inventoried TaqMan gene expression assays: SelH (Hs0015057_m1) and GAPDH (Hs99999905_m1). Cells containing SelH or SelH2 shRNA sequences expressed ~80% less SelH mRNA as compared with scrambled shRNA cells. A viable colony is defined as one containing more than 50 cells. Passage 2 SelH shRNA MRC-5 cells were employed for experiments unless otherwise indicated. p53 shRNA knockdown was performed as described previously (36).

**Detection of ROS, Immunofluorescence, and Immunoblotting**—Levels of intracellular ROS were assessed by using 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H2DCFDA, Invitrogen). CM-H2DCFDA stock (1 M) was prepared in DMSO. MRC-5 cells on coverslips were washed twice with PBS, incubated in phenol red-free RPMI medium containing 5 mM CM-H2DCFDA for 15 min, washed twice with PBS, and then incubated in phenol red-free RPMI medium for an additional 15 min in an incubator. Cells were then imaged under a Zeiss AxioObserver 100 fluorescence microscope for image acquisition using the FITC 488-nm excitation spectra setting. Immunofluorescence and immunoblotting analyses were performed as described previously (34, 35). Focus positive cells were defined as those containing at least five foci within the nucleus (34, 35, 37). Briefly, permeabilized cells were incubated overnight at 4 °C with antibodies against phospho-H2AX on Ser-139 (γH2AX, 1:200; Abcam, Cambridge, MA), phospho-ATM on Ser-1981 (pATM Ser-1981, 1:500; Rockland, Gilbertsville, PA), H2AX (1:500; Abcam), and ATM (1:500; Epitomics, Burlingame, CA). γH2AX and pATM Ser-1981 are well-defined markers for DNA breaks and ATM pathway activation, respectively (38, 39). Six pictures were randomly taken from each slide. Nuclear fraction was prepared by using a Nuclear and Cytoplasmic Extraction Kit (G-Bioscience, MO), separated by SDS-PAGE, and transferred onto PVDF membranes. Blots were incubated with antibodies against phospho-Nrf2 on Ser-40 (pNrf2, 1:1000; Epitomics), Nrf2 (1:1000; Santa Cruz Biotechnology), and lamin B (1:1000; Santa Cruz Biotechnology), followed by incubation with HRP-conjugated secondary antibodies and chemiluminescent reagents (Super Signal, Pierce) for signal acquisition. All experiments were performed in duplicate and a minimum of three times.

**Senescence and Autofluorescence Assays**—The activity of senescence-associated β-galactosidase (SA-β-gal), a senescence marker undetectable in actively proliferating cells (10), was determined using a Senescence Detection Kit (BioVision, San Francisco, CA) according to manufacturer’s instruction. MRC-5 cells were seeded onto 12-well plates (104 cells/well) in the presence or absence of H2O2 (20 μM) in a 3% or a 20% O2 incubator. Images were captured under a light microscope for quantification. Autofluorescence is another index of aging in cultured human diploid fibroblasts (40). Passage 4 SelH and scrambled shRNA MRC-5 cells were subjected to Zeiss AxioObserver 100 fluorescence microscope at 200× magnification. The fluorescence signal was acquired by using filter cube set 49 (excitation, 365 nm; filter, 395 nm; emission, 445 nm), and the intensity of autofluorescence was analyzed by Axiosvision software.

**Clonogenic Assay**—After being seeded (750 cells/6-cm dish) for 24 h, SelH and scrambled shRNA HeLa and HCT116 + hMLH1 cells were incubated with a gradient concentration of DNA-damaging agents, including hydroxyurea, neocarzinostatin, camptothecin, paclitaxel, and H2O2 (Fisher Scientific) for 24 h. Then, the drug-containing medium was replaced by a complete medium and cultured for additional 7 days. Cells were washed with 1× PBS, fixed in 90% methanol, and stained by 0.5% crystal violet (Alfa Aesar, MA) in 25% methanol. A viable colony is defined as one consisting of more than 50 cells.

**Laser Microirradiation and Live Cell Imaging**—A mixture of oxidative and broken DNA damage was generated in live cell nuclei by laser-induced microirradiation using a pulsed nitrogen laser as previously described (41). SelH-GFP (24) and proliferating cell nuclear antigen (PCNA)-DsRed plasmids were transiently transfected into MRC-5 cells. The laser system was coupled to a Zeiss Axiovert microscope for live cell, time lapse image capture.
Detection of GSH and Apoptotic Cells—Total intracellular GSH was estimated using monochlorobimane (mBCL), which formed fluorescent adducts with GSH (42, 43). Adherent cells were collected and preloaded with mBCL (40 μM) in PBS for 10 min, followed by incubation with propyl iodide permeable only to dead cells. The stained cells were applied to FACSCanto II flow cytometer (BD Bioscience, CA) immediately to determine the content of GSH per 10,000 live cells. Apoptotic cells were determined by a Mitocapture kit (Biovision) on the basis of the disruption of mitochondrial membrane potential. The Mitocapture reagent accumulated in the mitochondria and showed red fluorescence only in live cells, but could not aggregate and showed green fluorescence in apoptotic cells. Cells on coverslips were incubated with 1:1000 Mitocapture reagent (diluted in pre-warmed incubation buffer) in an incubator for 15 min, and washed three times with incubation buffer. The stained cells were subjected to fluorescent microscopic analyses of GFP and DsRed signals. Eight pictures were randomly taken from each slide.

RESULTS

Essential Role of SelH in the Inhibition of Replicative Senescence and Oxidative Stress—Strikingly, SelH shRNA MRC-5 cells barely proliferated, accumulated non-dividing large and flat cells, and showed completely stalled growth by passage 4.
or 36 days after clonal selection, whereas scrambled shRNA MRC-5 cells proliferated exponentially (Fig. 1, A and B). These results indicate that SelH deficiency restricts replicative lifespan in human diploid fibroblasts. Old or senescent cells in culture have previously been shown to exhibit elevated autofluorescence (40, 44). Consistent with these observations, microscopic analyses of passage 4, non-proliferating SelH shRNA MRC-5 cells showed 7-fold greater autofluorescence signal as compared with scrambled shRNA cells (Fig. 1B). Further microscopic analyses of CM-H2DCFDA fluorescence demonstrated that level of intracellular ROS was 2-fold greater in SelH than in scrambled shRNA MRC-5 cells at passage 4 when cultured under 20% O2 (Fig. 1C). Overall, these results implicate ROS in the slow proliferation and the formation of senescence-like phenotypes in SelH shRNA MRC-5 cells.

Because fibroblasts cultured in a typical 20% O2 incubator is believed to be under chronic oxygen tension and can accelerate replicative senescence (45, 46), features of senescence in MRC-5 cells were also assessed under a 3% O2 (physiological level) culture condition. While SA-β-gal expression was significantly greater (p < 0.05) in SelH than in scrambled shRNA MRC-5 cells being cultured either in a 3% or a 20% O2 incubator for 7 days (Fig. 1D), the extent of which was significantly reduced when cultured under 3% O2 in both SelH and scrambled shRNA MRC-5 cells. Moreover, the complete growth inhibition of SelH shRNA MRC-5 cells maintained under 20% O2 for 5 weeks could be partially rescued when grown in a 3% O2 incubator, although SelH shRNA MRC-5 cells remained to proliferate poorly (~300-fold slower) as compared with scrambled shRNA MRC-5 cells (Fig. 1, A and E). Similarly, scrambled shRNA MRC-5 cells proliferated 55-fold faster in a 3% than in a 20% O2 incubator. Altogether, these results suggest that SelH is required for cellular proliferation and the suppression of replicative senescence in a manner depending on ROS in human diploid fibroblasts.

**SelH shRNA MRC-5 Cells Display Sustained DNA Damage Response and Exacerbated Senescence Induction after H2O2 Treatment**—Senescence in human diploid fibroblasts is associated with persistent broken and oxidative DNA damage (47, 48), both of which can result in ATM pathway activation (11, 12, 49). Thus, SelH and scrambled shRNA MRC-5 cells were treated with H2O2 (20 μM) for 1 day, followed by 0–5 days recovery. The percent γH2AX and pATM Ser-1981 positive cells were greater (p < 0.05) in SelH than in scrambled shRNA cells before and 1 day after H2O2 treatment (Fig. 2, A and B). During the course of 5-day recovery, the abundance of γH2AX and pATM Ser-1981 positive cells subsided in the scrambled shRNA cells whereas these DNA damage markers accumulated further in SelH shRNA cells. Interestingly, the recovery of pATM Ser-1981 expression was complete and happened faster than that of γH2AX in scrambled shRNA MRC-5 cells, suggesting that 20 μM was a physiological dose of H2O2 in normal MRC-5 cells and that the ATM pathway activation preceded γH2AX formation in this scenario. The expression and distribution of H2AX and ATM did not differ before and after H2O2 treatment, as shown in the representative pictures (Fig. 2, A and B). Furthermore, the percent SA-β-gal-positive cells 5 days after recovery were 17 and 70% in scrambled shRNA and SelH shRNA MRC-5 cells, respectively, in a 20% O2 incubator (Fig. 2C). When maintained under 3% O2, they dropped (p < 0.05) to 2 and 34% in scrambled and SelH shRNA MRC-5 cells, respectively. Taken together, these results suggest that SelH plays an
essential role in gatekeeping genomic integrity and suppressing senescence in the response of MRC-5 normal diploid fibroblasts to oxidative stress. 

SelH Deficiency Specifically Sensitizes Cells to DNA-damaging Agents That Directly Contribute to Oxidative Stress—Next, we asked whether SelH protected against genotoxic agents other than H$_2$O$_2$. Although clonogenic assay is considered a gold standard for assessing cell proliferation after DNA damage, not all cells, including MRC-5 cells, can effectively form colonies when seeded at very low density. To circumvent this limitation and to evaluate the protective role of SelH in other cell types, SelH shRNA and scrambled shRNA HeLa and HCT116 colorectal cancer cells were generated. Results from clonogenic assays showed that SelH shRNA HeLa cells displayed increased sensitivity to oxidative stress inducers paraquat and H$_2$O$_2$ (Fig. 3, A and B), but not to replication stress inducers hydroxyurea and camptothecin or a potent γ-irradiation mimicry, neocarzinostatin (Fig. 3, C–E). In addition, SelH shRNA HCT116+hMLH1 cells (35) displayed increased sensitivity to H$_2$O$_2$ exposure dose-dependently (data not shown). Interestingly, SelH mRNA levels were induced by H$_2$O$_2$ exposure in a dose-dependent manner (Fig. 3F). To further investigate the role of SelH in cellular response to DNA damage, live cell imaging was performed to track SelH translocation to an area containing a mixture of locally and freshly induced oxidative and broken DNA. Although DsRed-tagged PCNA, a sensitive marker of DNA synthesis and repair (50, 51), was rapidly recruited to and enriched at the site of DNA damage, SelH-GFP did not mobilize in the MRC-5 cells (Fig. 4). Therefore, SelH specifically protects against genotoxic agents that induce oxidative stress, but does not seem to be directly involved in the repair of or early response to the DNA damage.

The Slow Proliferation Phenotype in SelH shRNA MRC-5 Cells Depends on ATM Kinase Activity and p53 Expression—The ATM kinase and p53 protein are known to modulate the senescence response to oxidative stress in fibroblasts and endothelial cells (34, 52). Treatment with Ku 60019 (5 μM), a specific ATM kinase inhibitor (53), for 28 days alleviated the slow proliferation phenotype by 3-fold in SelH shRNA MRC-5 cells, but
slightly suppressed the proliferation in scrambled shRNA MRC-5 cells (Fig. 5A). SelH shRNA MRC-5 cells proliferated 14-fold slower than scrambled shRNA cells, but such a difference was reduced to 3.6-fold in the presence of Ku 60019. Similar proliferation and senescence results were observed when the cells were treated with Ku 55933 (Fig. 5B), a popular but less specific ATM kinase inhibitor; however, the extent of slow proliferation alleviation in SelH shRNA MRC-5 cells was much greater compared with Ku 60019. Analyses of SA-β-gal staining confirmed that the ATM kinase is required to senesce SelH shRNA cells (40% versus 5%) after being cultured for 28 days (Fig. 5C). SelH shRNA MRC-5 cells significantly accumulated (*, p < 0.05) additional γH2AX and pATM Ser-1981 after 28 days in a 20% O2 incubator, but such induction was completely reversed or inhibited (p < 0.05) in the presence of Ku 60019 (Fig. 5D and E). Next, SelH and p53 double shRNA MRC-5 cells were generated to test a role of p53 in the slow proliferation phenotype. After puromycin selection, the amount of colonies was 5-fold greater in SelH shRNA and p53 double shRNA than in SelH shRNA MRC-5 cells (Fig. 5F). In particular, the cell size was much smaller and the shape was rounded in the double shRNA cells, as opposed to those in SelH shRNA MRC-5 cells displaying enlarged and flattened senescence phenotypes, under a 40-fold light microscope. Altogether, the ATM kinase activity and p53 protein are necessary for the induction of replicative senescence in SelH shRNA MRC-5 diploid fibroblasts under chronic oxidative stress.

**FIGURE 5.** The ATM kinase and p53 protein are involved in the protection of SelH against genome instability and replicative senescence in MRC-5 cells. SelH shRNA and scrambled shRNA MRC-5 cells in 12-well plates (2 × 10^4 cells/well) were cultured in the presence or absence of Ku 60019 (A) and Ku 55933 (B) at 5 μM for 28 days, followed by cell counting. Immunofluorescent analyses of γH2AX and pATM Ser-1981 expression and microscopic capturing of SA-β-gal appearance were performed in SelH shRNA MRC-5 cells (C–E, *, p < 0.05, compared with Day 0 or with Ku 60019 or Ku 55933 treatment). F. SelH and p53 double shRNA and the control MRC-5 cells were generated 14 days after puromycin selection, followed by counting viable colonies stained with crystal violet. A colony is defined as one containing at least 50 cells. Values are means ± S.E. (n = 3) (*, p < 0.05). Representative pictures taken under a 40-fold bright field were shown.

GSH Deficiency and Nuclear pNrf2 Accumulation in the Apoptotic Response of SelH shRNA HeLa Cells to H₂O₂ Treatment—The expression of glutamylcysteine synthetase, a key enzyme for de novo GSH biosynthesis, is increased in murine hippocampal HT22 cells overexpressing human SelH (27). Here we showed that the level of intracellular GSH was significantly lower (p < 0.05) in SelH shRNA than in scrambled shRNA HeLa cells before and after exposure with H₂O₂ for 24 h (Fig. 6A). H₂O₂ treatment linearly increased GSH level up to a concentration of 80 and 160 μM in the SelH and scrambled shRNA cells, respectively. While H₂O₂ treatment (160 μM) resulted in a time-dependent induction of mitochondrial membrane potential disruption, an indicator of apoptosis, in both SelH shRNA and scrambled shRNA cells, the extent of which was significantly greater (p < 0.05) in the former than the latter cells (Fig. 6B). Further statistical analyses indicated that the intracellular GSH level was inversely correlated (p < 0.05) with apoptotic death after H₂O₂ treatment. To further understand a role of GSH in the response of SelH shRNA cells to H₂O₂ treatment, clonogenic assays were performed in the presence or absence of NAC, a GSH precursor. Although SelH shRNA cells were more sensitive than scrambled shRNA cells to H₂O₂ treatment, the supplement of NAC (10 mM) rescued the retarded proliferation of SelH shRNA cells to a level similar to that of the scrambled shRNA cells (Fig. 6C). The Nrf2-Keap1 pathway also regulates GSH biosynthesis (54). Upon oxidative stress, Nrf2 is phosphorylated and dissociates from Keap1, followed by nuclear
translocation and transactivation of genes assisting GSH synthesis. Results from Western and immunofluorescent analyses showed that levels of pNrf2 and Nrf2 in the nucleus were increased after treatment of the cells with a gradient concentration of H$_2$O$_2$ (0–80 μM, Fig. 7, A and B) and during a 5-day recovery (Fig. 7C), the extent of which was significantly greater ($p < 0.05$) in SelH shRNA than in scrambled shRNA cells. Nuclear lamin B level did not differ by H$_2$O$_2$ treatment or between cell types. Because nuclear Nrf2 level also increased as a result of the pNrf2 translocation (55), lamin B was used as a...
also participate in the senescence response, as treatment of SelH shRNA cells with the less-specific ATM kinase inhibitor Ku 55933 alleviates the growth retardation phenotype to a greater extent compared with Ku 60019 (Fig. 5, A and B). Because unrepaired DNA damage and persistent DNA damage response are hallmarks of cellular senescence (47, 57, 58), a fair question to ask is whether SelH plays a direct role in the repair of DNA damage. Results from analysis of live cell imaging of SelH mobilization to localized DNA damage do not support such a hypothesis. As the expression of recombinant wild-type selenoprotein (i.e. without substituting Sec with Cys) is becoming technically feasible (59), it is of future interest to purify SelH for enzymatic and kinetic analyses of oligonucleotide substrates representing various types of DNA damage.

SelH shRNA cells are hypersensitive to H₂O₂ and paraquat, but not hydroxyurea, camptothecin, and necarzinostatin. As a pro-oxidant, paraquat initiates the generation of superoxide, which in turn is reduced to H₂O₂ in vivo. Interestingly, SelH siRNA cells display increased sensitivity to H₂O₂, but not other peroxides (24). Furthermore, although hydroxyurea is best known as an inhibitor of ribonucleotide reductase, it can also increase superoxide level and iron availability for the formation of hydroxyl radicals (60). Similarly, both camptothecin, an inhibitor of topoisomerase I (61), and necarzinostatin, an ionizing radiation mimicry, have been reported to be capable of inducing ROS formation (62). Thus, it is surprising why SelH shRNA cells do not show increased sensitivity to these three DNA-damaging agents. They may indirectly induce the formation of ROS in forms not recognized by SelH, thus resulting in a “secondary burst” of DNA damage independent of SelH status. It is also possible that SelH does not play direct roles clearing H₂O₂ at later time points. Whatever the reason, data support a direct and upstream role of SelH in the dealing of H₂O₂ (24, 27), resulting in the protection against ROS-induced genome instability and senescence.

How does SelH protect against replicative senescence under chronic oxidative stress? Accumulation of ROS (63, 64) and reduction of antioxidant capacity, DNA repair efficacy and selenoprotein expression (65–72) have been linked to cellular and organismal aging. However, damage accumulation early in life can also contribute to or accelerate cellular senescence, as DNA breaks and ATM pathway activation are prominent and there are 3-fold more viable clones when SelH shRNA MRC-5 cells are maintained in 3% O₂ than in 20% O₂ incubators (data not shown) as early as two passages after clonal selection. Our data are in line with previous findings demonstrating that the ATM-p53 pathway contributes to persistent amplification of DNA damage signal and replicative senescence (15). Moreover, increased intracellular ROS may activate replicative senescence by guanine oxidation on telomeres, subsequently resulting in the disruption of telomeric T-loops and telomere dysfunction (73, 74). Although the expression of SelH, among other eight selenoproteins, has been shown to be decreased by senescence or by maintenance under a low Se medium in WI-38 cells (71), our data provide the first evidence of SelH in the direct protection against replicative senescence under chronic oxidative stress through the maintenance of genomic stability.

SelH may play a dual role in the response to oxidative stress. Burk et al. have demonstrated that the activation of Nrf2-ARE...
Protection of Selenoprotein H against Cellular Senescence

pathway in mouse liver is specifically induced by dietary depriva-
tion of selenium, but not other dietary antioxidants such as vita-
min E (75). SelH may confer the intersection between selenium and
Nrf2 as this selenoprotein is among the few most sensitive ones to
dietary selenium deficiency (19, 76). Consistent with this observa-
tion, SelH-deficient cells display increased nuclear pNrf2 expres-
sion endogenously or dose-dependently after H$_2$O$_2$ treatment.
Furthermore, pNrf2 expression is known to be correlated with
GSH content (77). We propose that, with increased concentra-
tions of H$_2$O$_2$, GSH is dispensed faster than those synthesized de
novo through Nrf2 translocation in SelH shRNA cells when ROS
scavenger by SelH is hampered. Increased pNrf2 expression in SelH-
deficient cells may further activate the compensatory expres-
sion of other antioxidant selenoproteins including thiore-
doxin reductase-1 and glutathione peroxidase-2 (78, 79). On the
other hand, SelH may directly induce the de novo synthesis of GSH
(27). Indeed, SelH may play an important role in sensing oxidative
stress as the majority of SelH-GFP is localized in the nucleolus
(Ref. 24 and Fig. 4), a nuclear region being proposed as a sensor of
multiple forms of stress including H$_2$O$_2$ (30, 31).

How does SelH, assumed to be localized in the nucleolus (24),
protect genomic integrity? As the nuclear domain accommodat-
ing the tandem repeat rDNA gene, nucleolus is a proposed sen-
or of nuclear oxidative stress (30, 31). First, JNK2 activation by
oxidative stress promptly phosphorylates and inactivates a
RNA polymerase I transcription factor TIF-IA in the nucleoli to
down-regulate rRNA synthesis (31). Second, decreased rDNA
copy numbers can hamper genomic integrity (81). Thus, part of
the gatekeeper functions of SelH may be attributed to its prox-
imity to rDNA and preventing TIF-IA phosphorylation by
JNK2 such that rRNA down-regulation is limited. Through its
antioxidative motif, SelH may also decompose local H$_2$O$_2$
and prevent rDNA oxidation in the nucleoli. Furthermore, there
is no membrane boundary separating nucleolus from the sur-
rounding nucleoplasm. Considering the topological and
dynamic properties of a genome (82), long-range contacts of
chromosomes in nucleoloplasm with SelH in nucleolus may
enable this selenoprotein to interact with and protect the
genome distantly.

As depicted in Fig. 5, SelH is proposed to play dual roles in
gatekeeping genomic integrity against a senescence response to
oxidative stress. SelH may enzymatically decompose H$_2$O$_2$, as
well as directly increase the expression of GSH as a transactiva-
tor and indirectly up-regulate other antioxidant enzymes
through Nrf2. Considering the specialized nucleolar localization
and function (24), SelH in principle can keep ROS levels in
check, thus suppressing the accumulation of oxidative and inci-
sion damage on DNA. Such impact of SelH on the maintenance
of genomic stability is consistent with the recent, refined dam-
age theory of aging (80). In this regard, SelH could serve as a
potential intervention target for the protection against genomic
damage during the aging process.

Acknowledgments—We thank Dr. Vadim Gladyshev for SelH-GFP
construct, Dr. Thomas Wang for sharing some of the reagents and
equipment for performing quantitative RT-PCR, and Elliot Mattson
and Erica Lee for helping with some cellular assays.

REFERENCES

1. Rodier, F., and Campisi, J. (2011) Four faces of cellular senescence. J. Cell
Biol. 192, 547–556
2. Fumagalli, M., Rossiello, F., Clerici, M., Barozzi, S., Cittaro, D., Kaplunov,
J. M., Bucci, G., Dobrea, M., Matti, V., Bausejour, C. M., Herbig, U.,
Longhese, M. P., and d’Adda di Fagagna, F. (2012) Telomeric DNA dam-
age is irreparable and causes persistent DNA-damage-response activa-
tion. Nat. Cell Biol. 14, 355–365
3. Mallette, F. A., and Ferbeyre, G. (2007) The DNA damage signaling path-
way connects oncogenic stress to cellular senescence. Cell Cycle 6,
1831–1836
4. Takahashi, A., Ohtani, N., Yamakoshi, K., Iida, S., Tahara, H., Nakayama,
K., Nakayama, K. I., Ide, T., Saya, H., and Hara, E. (2006) Mitogenic sig-
nalling and the p16INK4a-Rb pathway cooperate to enforce irreversible
cellular senescence. Nat. Cell Biol. 8, 1291–1297
5. Halazonetis, T. D., Gorgoulis, V. G., and Bartek, J. (2008) An oncogene-
induced DNA damage model for cancer development. Science 319,
1352–1355
6. Jun, J. L., and Lau, L. F. (2010) The matricellular protein CCN1 induces
fibroblast senescence and restricts fibrosis in cutaneous wound healing.
Nat. Cell Biol. 12, 676–685
7. Ressler, S., Bartkova, J., Niederegerg, H., Bartek, J., Scharffetter-Kochanek,
K., Jansen-Durr, P., and Wlaschek, M. (2006) p16INK4a is a robust in vivo
biomarker of cellular aging in human skin. Aging Cell 5, 379–389
8. Voghel, G., Thorin-Trescases, N., Farhat, N., Nguyen, A., Villeneuve, L.
Mammarbachi, A. M., Fortier, A., Perrault, L. P., Carrier, M., and, Thorin,
E. (2007) Cellular senescence in endothelial cells from atherosclerotic pa-
tients is accelerated by oxidative stress associated with cardiovascular risk
factors. Mech Ageing Dev. 128, 662–671
9. Wang, C., Jurk, D., Maddick, M., Nelson, G., Martin-Ruiz, C., and von
Zglinicki, T. (2009) DNA damage response and cellular senescence in
tissues of aging mice. Aging Cell 8, 311–323
10. Dimri, G. P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Me-
drano, E. E., Linksrens, M., Rubelj, I., and Pereira-Smith, O. (1995) A bio-
marker that identifies senescent human cells in culture and in aging skin in
vivo. Proc. Natl. Acad. Sci. U.S.A. 92, 9363–9367
11. Lee, J. H., and Paull, T. T. (2005) ATM activation by DNA double-strand
breaks through the Mre11-Rad50-Nbs1 complex. Science 308, 551–554
12. Guo, Z., Kozlov, S., Lavin, M. F., Person, M. D., and Paull, T. T. (2010)
ATM activation by oxidative stress. Science 330, 517–521
13. Canman, C. E., Lim, S. D., Cimprich, K. A., Taya, Y., Tamai, K., Sakaguchi,
K., Appella, E., Kastan, M. B., and Siliciano, J. D. (1998) Activation of the
ATM kinase by ionizing radiation and phosphorylation of p53. Science
281, 1677–1679
14. Bartkova, J., Rezaei, N., Lintons, M., Karakaidos, P., Kletas, D., Issaeva,
N., Vassilou, L. V., Kolettas, E., Niforou, K., Zoumpoulis, V. C., Takaoka,
M., Nakagawa, H., Tort, F., Fugger, K., Johansson, F., Sehsted, M., Anderssen,
C. L., Dryskot, L., Ørntoft, T., Lukas, J., Kittas, C., Helleday, T., Halzone-
tis, T. D., Bartek, J., and Gorgoulis, V. G. (2006) Oncogene-induced senes-
cence is part of the tumorigenesis barrier imposed by DNA damage check-
points. Nature 444, 633–637
15. Suzuki, M., Suzuki, K., Kodama, S., Yamashita, S., and Watanabe, M.
(2012) Persistent amplification of DNA damage signal involved in replica-
tive senescence of normal human diploid fibroblasts. Oxid. Med. Cell Long-
gev. 2012:310534
16. Kryukov, G. V., Castellano, S., Novoselov, S. V., Lobanov, A. V., Zehtab,
O., Guigó, R., and Gladyshev, V. N. (2003) Characterization of mammalian
selenoproteomes. Science 300, 1439–1443
17. Lu, J., and Holmgren, A. (2009) Selenoproteins. J. Biol. Chem. 284,
723–727
18. Sunde, R. A. (2010) Molecular biomarker panels for assessment of sele-
nium status in rats. Exp. Biol. Med. 235, 1046–1052
19. Raines, A. M., and Sunde, R. A. (2011) Selenium toxicity but not deficient
or super-nutritional selenium status vastly alters the transcriptome in
rodents. BMC Genomics 12, 26
20. Kipp, A. P., Banning, A., van Schothorst, E. M., Mépl an, C., Coort, S. L.,
Evelo, C. T., Keijer, J., Hesketh, J., and Brigelius-Flohe, R. (2012) Marginal
selenium deficiency down-regulates inflammation-related genes in spleen leukocytes of the mouse. J. Nutr. Biochem. 23, 1170–1177
21. Sengupta, A., Lichti, U. F., Carlson, B. A., Ryscavage, A. O., Gladyshev, V. N., Yuspa, S. H., and Hatfield, D. L. (2010) Selenoproteins are essential for proper keratinocyte function and skin development. PLoS ONE 5, e12249
22. Downey, C. M., Horton, C. R., Carlson, B. A., Parsons, T. E., Hatfield, D. L., Hallgrimsson, B., and Jirik, F. R. (2009) Osteo-chondroprogenitor-specific deletion of the selenocysteine tRNA gene, Trsp, leads to chondrocnecrosis and abnormal skeletal development: a putative model for Kashin-Beck disease. PLoS Genet 5, e1000616
23. McCann, J. C., and Ames, B. N. (2011) Adaptive dysfunction of selenoproteins from the perspective of the triage theory: why modest selenium deficiency may increase risk of diseases of aging. FASEB J. 25, 1793–1814
24. Novoselov, S. V., Kryukov, G. V., Xu, X. M., Carlson, B. A., Hatfield, D. L., and Gladyshev, V. N. (2007) Selenoprotein H is a nuclear thioredoxin-like protein with a unique expression pattern. J. Biol. Chem. 282, 11960–11968
25. Morozova, N., Forry, E. P., Shahid, E., Zavacki, A. M., Harney, I. W., Kraytsberg, Y., and Berry, M. J. (2003) Antioxidant function of a novel selenoprotein in Dro sophila melanogaster. Genes Cells 8, 963–971
26. Ben Lilani, K. E., Panee, J., He, Q., Berry, M. J., and Li, P. A. (2007) Overexpression of selenoprotein H reduces H2O2 neuronal cell death after UVB irradiation by preventing superoxide formation. Int. J. Biol. Sci. 3, 198–204
27. Panee, J., Stoytcheva, Z. R., Liu, W., and Berry, M. J. (2007) Selenoprotein H is a redox-sensing high mobility group family DNA-binding protein that up-regulates genes involved in glutathione synthesis and phase II detoxification. J. Biol. Chem. 282, 23759–23765
28. Mendelev, N., Witherspoon, S., and Li, P. A. (2009) Overexpression of human selenoprotein H in neuronal cells ameliorates ultraviolet irradiation-induced damage by modulating cell signaling pathways. Exp. Neurol. 220, 328–334
29. Mendelev, N., Mehta, S. L., Witherspoon, S., He, Q., Sexton, J. Z., and Li, P. A. (2011) Upregulation of human selenoprotein H in murine hippocampal neuronal cells promotes mitochondrial biogenesis and functional performance. Mitochondrion 11, 76–82
30. Lewinska, A., Wnuk, M., Grzelak, A., and Bartosz, G. (2010) Nucleolus as a stress sensor in the yeast Saccharomyces cerevisiae. Redox Rep. 15, 87–96
31. Mayer, C., Bierhoff, H., and Grummt, I. (2005) The nucleolus as a stress sensor: JNK2 inactivates the transcription factor TIF-IA and down-regulates rRNA synthesis. Genes Dev. 19, 933–941
32. Koi, M., Umar, A., Chauhan, D. P., Cherian, S. P., Carethers, J. M., Kunkel, T. A., and Boland, C. R. (1994) Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces N-methyl-N’-nitro-N-nitrosoguanidine tolerance in colon tumor cells with homozygous hMLH1 mutation. Cancer Res. 54, 4308–4312
33. Yanamadala, S., and Ljungman, M. (2003) Potential role of MLH1 in the induction of p53 and apoptosis by blocking transcription on damaged DNA templates. Mol. Cancer Res. 1, 747–754
34. Wu, M., Kang, M. M., Schoene, N. W., and Cheng, W. H. (2010) Selenium compounds activate early barri er of tumorigenesis. J. Biol. Chem. 285, 12055–12062
35. Qi, Y., Schoene, N. W., Larter, F. M., and Cheng, W. H. (2010) Selenium compounds activate ATM-dependent DNA damage response via the mismatch repair protein hMLH1 in colorectal cancer cells. J. Biol. Chem. 285, 33010–33017
36. Wu, M., Wu, R. T., Wang, T. T., and Cheng, W. H. (2011) Role for p53 in Selenium-Induced Senescence. J. Agric. Food Chem. 59, 11882–11887
37. Maude, S. L., and Enders, G. H. (2005) Cdk inhibition in human cells. J. Biol. Chem. 280, 1170–1177
38. Wu, M., Wu, R. T., Wang, T. T., and Cheng, W. H. (2011) Role for p53 in Selenium-Induced Senescence. J. Agric. Food Chem. 59, 11882–11887
39. Bakkenist, C. J., and Kastan, M. B. (2003) DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature 421, 499–506
40. Wilhelm, J., Vytrásek, R., Ostádalová, I., and Vajner, L. (2009) Evaluation of different methods detecting intracellular generation of free radicals. Mol. Cell Biochem. 328, 167–176
41. Uematsu, N., Weterings, E., Yano, K., Morotomi-Yano, K., Jakob, B., Taucher-Scholz, G., Mari, P. O., van Gent, D. C., Chen, B. P., and Chen, D. J. (2007) Autophosphorylation of DNA-PKCS regulates its dynamics at DNA double-strand breaks. J. Cell Biol. 177, 219–229
42. Sebastiá, J., Cristófol, R., Martín, R., Rodríguez-Farré, E., and Sanfelici, C. (2003) Evaluation of fluorescent dyes for measuring intracellular glutathione content in primary cultures of human neurons and neuroblastoma SH-SYSY. Cytometry A 51, 16–25
43. Franco, R., and Cidlowski, J. A. (2006) SLCO/OATP-like transport of glutathione in Fasl-induced apoptosis: glutathione efflux is coupled to an organic anion exchange and is necessary for the progression of the execution phase of apoptosis. J. Biol. Chem. 281, 29542–29557
44. Kuijlan, M., Michaloglou, C., Mooi, W. J., and Peep, D. S. (2010) The essence of senescence. Genes Dev. 24, 2463–2479
45. Balajee, A. S., and Geard, C. R. (2001) Chromatin-bound PCNA complex formation triggered by DNA damage occurs independent of the ATM gene product in human cells. Nucleic Acids Res. 29, 1341–1351
46. Mortuszewicz, O., Fouquerel, E., Amé, J. C., Leonhardt, H., and Schreiber, V. (2011) PARG is recruited to DNA damage sites through poly(ADP-ribose)- and PCNA-dependent mechanisms. Nucleic Acids Res. 39, 5045–5056
47. Zhan, H., Suzuki, T., Aizawa, K., Miyagawa, K., and Nagai, R. (2010) Ataxia telangiectasia mutated (ATM)-mediated DNA damage response in oxidative stress-induced vascular endothelial cell senescence. J. Biol. Chem. 285, 29662–29670
48. Moinova, H. R., and Mulcahy, R. T. (1999) Up-regulation of the human gamma-glutamylcysteine synthetase regulatory subunit gene involves binding of NF-κ2 to an electrophile responsive element. Biochem. Biophys. Res. Commun. 261, 661–668
49. Huang, H. C., Nguyen, T., and Pickett, C. B. (2000) Regulation of the antioxidant response element by protein kinase C-mediated phosphorylation of NF-E2-related factor 2. Proc. Natl. Acad. Sci. U.S.A. 97, 12475–12480
50. Moussavi-Harami, F., Duwayri, Y., Martin, J. A., and Buckwalter, J. A. (2011) Adaptive dysfunction of selenoproteins from the perspective of the triage theory: why modest selenium deficiency may increase risk of diseases of aging. J. Biol. Chem. 286, 1793–1814
51. Mortuszewicz, O., Fouquerel, E., Amé, J. C., Leonhardt, H., and Schreiber, V. (2011) PARG is recruited to DNA damage sites through poly(ADP-ribose)- and PCNA-dependent mechanisms. Nucleic Acids Res. 39, 5045–5056
52. Balajee, A. S., and Geard, C. R. (2001) Chromatin-bound PCNA complex formation triggered by DNA damage occurs independent of the ATM gene product in human cells. Nucleic Acids Res. 29, 1341–1351
53. Mortuszewicz, O., Fouquerel, E., Amé, J. C., Leonhardt, H., and Schreiber, V. (2011) PARG is recruited to DNA damage sites through poly(ADP-ribose)- and PCNA-dependent mechanisms. Nucleic Acids Res. 39, 5045–5056
54. Rico, C., Cichelese, A., Fumagalli, M., Dobreva, M., Verrecchia, A., Pelicci, P. G., and di Fagagna, F. (2008) DNA damage response activation in mouse embryonic fibroblasts undergoing replicative senescence and following spontaneous immortalization. Cell Cycle 7, 3601–3606
55. Rodier, F., Coppé, J. P., Patil, C. K., Hoeijmakers, W. A., Muñoz, D. P., Raza, S. R., Freund, A., Campeau, E., Davalos, A. R., and Campisi, J. (2009) Persistent DNA damage signalling triggers senescence-associated inflam-
Protection of Selenoprotein H against Cellular Senescence

59. Han, X., Fan, Z., Yu, Y., Liu, S., Hao, Y., Huo, R., and Wei, J. (2013) Expression and characterization of recombinant human phospholipid hydroperoxide glutathione peroxidase. *JBIOMB Life* **65**, 951–956

60. Davies, B. W., Kohanski, M. A., Simmons, L. A., Winkler, J. A., Collins, J. I., and Walker, G. C. (2009) Hydroxyurea induces hydroxyl radical-mediated cell death in *Escherichia coli*. *Mol. Cell* **36**, 845–860

61. Wall, M. E., Wani, M. C., Cook, C. E., Palmer, K. H., McPhail, A. I., and Sim, G. A. (1966) Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from camptotheca acuminate. *J. Am. Chem. Soc.* **88**, 3888–3890

62. Kang, M. A., So, E. Y., Simons, A. L., Spitz, D. R., and Ouchi, T. (2012) DNA damage induces reactive oxygen species generation through the H2AX- Nox1/Rac1 pathway. *Cell Death Dis* **3**, e249

63. Harman, D. (1960) The free radical theory of aging: the effect of age on serum mercaptan levels. *J. Gerontol* **15**, 38–40

64. Lawless, C., Jurk, D., Gillespie, C. S., Shanley, D., Saretzki, G., von Zglinicki, T., and Passos, J. F. (2012) A stochastic step model of replicative senescence explains ROS production rate in ageing cell populations. *PLoS ONE* **7**, e32117

65. Williams, J. B., Roberts, S. P., and Elekonich, M. M. (2008) Age and natural metabolically-intensive behavior affect oxidative stress and antioxidant mechanisms. *Exp Gerontol* **43**, 538–549

66. Sivonová, M., Tatarková, Z., Duracková, Z., Dobrota, D., Lehotský, J., Martáková, T., and Kaplán, P. (2007) Relationship between antioxidant potential and oxidative damage to lipids, proteins and DNA in aged rats. *Physiol. Res.* **56**, 757–764

67. Kim, J. W., No, J. K., Ikeno, Y., Yu, B. P., Choi, J. S., Yokozawa, T., and Chung, H. Y. (2002) Age-related changes in redox status of rat serum. *Arch Gerontol Geriatr* **34**, 9–17

68. Pieri, C., Testa, R., Marra, M., Bonfigli, A. R., Manfrini, S., and Testa, I. (2001) Age-dependent changes of serum oxygen radical scavenger capacity and haemoglobin glycosylation in non-insulin-dependent diabetic patients. *Gerontology* **47**, 88–92

69. Kimoto-Kinoshita, S., Nishida, S., and Tomura, T. T. (1999) Age-related change of antioxidant capacities in the cerebral cortex and hippocampus of stroke-prone spontaneously hypertensive rats. *Neurosci. Lett.* **273**, 41–44

70. Pérez, R., López, M., and Barja de Quiroga, G. (1991) Aging and lung antioxidant enzymes, glutathione, and lipid peroxidation in the rat. *Free Radic Biol. Med.* **10**, 35–39

71. Legravity, Y., Touat-Hamici, Z., and Chavatte, L. (2014) Interplay between selenium levels, selenoprotein expression, and replicative senescence in WI-38 human fibroblasts. *J. Biol. Chem.* **289**, 6299–6310

72. Garm, C., Moreno-Villanueva, M., Bürkle, A., Petersen, I., Bohr, V. A., Christensen, K., and Stevnsner, T. (2013) Age and gender effects on DNA strand break repair in peripheral blood mononuclear cells. *Aging Cell* **12**, 58–66

73. Opresko, P. L., Fan, J., Danzy, S., Wilson, D. M., 3rd, and Bohr, V. A. (2005) Oxidative damage in telomeric DNA is disrupted by TRF1 and TRF2. *Nucleic Acids Res.* **33**, 1230–1239

74. Richter, T., and Proctor, C. (2007) The role of intracellular peroxide levels on the development and maintenance of telomere-dependent senescence. *Exp. Gerontol* **42**, 1043–1052

75. Burk, R. F., Hill, K. E., Nakayama, A., Mostert, V., Levander, X. A., Motley, A. K., Johnson, D. A., Johnson, J. A., Freeman, M. L., and Austin, L. M. (2008) Selenium deficiency activates mouse liver Nrf2-ARE but vitamin E deficiency does not. *Free Radic Biol. Med.* **44**, 1617–1623

76. Kipp, A., Banning, A., van Schothorst, E. M., Méplan, C., Schomburg, L., Evelo, C., Coort, S., Gaj, S., Keijer, J., Hesketh, J., and Brigelius-Flohé, R. (2009) Four selenoproteins, protein biosynthesis, and Wnt signalling are particularly sensitive to limited selenium intake in mouse colon. *Mol. Nutr. Food Res.* **53**, 1561–1572

77. Suh, J. H., Shenoi, S. V., Dixon, B. M., Liu, H., Jaiswal, A. K., Liu, R. M., and Hagen, T. M. (2004) Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipic acid. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 3381–3386

78. Sakurai, A., Nishimoto, M., Himeno, S., Imura, N., Tsujimoto, M., Kunimoto, M., and Hara, S. (2005) Transcriptional regulation of thioredoxin reductase 1 expression by cadmium in vascular endothelial cells: role of NF-E2-related factor-2. *J. Cell. Physiol.* **203**, 529–537

79. Singh, A., Rangasamy, T., Thimmulappa, R. K., Lee, H., Osburn, W. O., Brigelius-Flohé, R., Kessler, T. W., Yamamoto, M., and Biswal, S. (2006) Glutathione peroxidase 2, the major cigarette smoke-inducible isoform of GPX in lungs, is regulated by Nrf2. *Am. J. Respir. Cell Mol. Biol.* **35**, 639–650

80. Gladyshev, V. N. (2014) The free radical theory of aging is dead. Long live the damage theory! *Antioxid. Redox Signal.* **20**, 727–731

81. Ide, S., Miyazaki, T., Maki, H., and Kobayashi, T. (2010) Abundance of ribosomal RNA gene copies maintains genome integrity. *Science* **326**, 693–696

82. Dixon, J.R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J.S., and Ren, B. (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* **485**, 376–380