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보 문

A unique thioredoxin reductase plays defensive roles against oxidative, nitrosative and nutritional stresses in *Schizosaccharomyces pombe*

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*Schizosaccharomyces pombe*의 유일한 치오레독신 환원효소의 산화적, 일산화질소 및 영양 스트레스에 대한 방어적 역할

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ABSTRACT: A unique *Schizosaccharomyces pombe TrxR*$^+$ gene encoding thioredoxin reductase (TrxR) was found to be positively regulated by stress-inducing agents through the stress-responsive transcription factor Pap1. In the present study, the protective roles of *S. pombe* TrxR were evaluated using the TrxR-overexpressing recombinant plasmid pHSM10. In the presence of hydrogen peroxide (H_2O_2) and superoxide anion-generating menadione (MD), *S. pombe* TrxR increased cellular growth and the total glutathione (GSH) level, while it reduced levels of intracellular reactive oxygen species (ROS). The nitric oxide (NO) levels of the TrxR-overexpressing cells, in the presence of H_2O_2 and MD, were maintained to be similar to those of the corresponding non-treated cells. Although *S. pombe* TrxR was able to scavenge NO generated by sodium nitroprusside (SNP), it had no significant modulating effects on cellular growth, ROS levels, or the total GSH level of SNP-exposed yeast cells, compared with the differences in those of the two non-treated cell cultures. TrxR increased the cellular growth and total GSH level, which were diminished by nitrogen starvation. It also scavenged ROS and NO produced during nitrogen starvation. Taken together, the *S. pombe* TrxR protects against oxidative, nitrosative, and nutritional stresses.

Key words: *Schizosaccharomyces pombe*, glutathione, hydrogen peroxide, nitric oxide, nitrogen starvation, reactive oxygen species, superoxide anion

Microbial cells must maintain specific internal conditions for appropriate cellular growth and function. Variations in the external environment directly disturb the internal conditions of living cells, thereby perturbing physiological processes, such as optimal enzyme activities, cellular structures, metabolic fluxes, and chemical gradients (Gasch *et al.*, 2000). Yeast cells must be able to survive abrupt and sometimes severe variations in their external environments. They are often exposed to variations in temperature and osmolarity, nutrient starvation, acidity, radiation, and toxic chemicals (Gasch *et al.*, 2000).

Aerobically proliferating cells are continuously challenged by reactive oxygen species (ROS), which derive from incomplete reductions of molecular oxygen during respiration. Excess amounts of ROS are deleterious to living cells because they damage various cellular components (Halliwell and Gutteridge, 1999). When anaerobic cells, such as anaerobic bacteria, are exposed to aerobic conditions, they are also challenged by ROS. Different families of anaerobes adapt to aerobic conditions differently. Although some bacteria remain sensitive to aerobic

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conditions, others develop diverse mechanisms to solve the problem of thiol oxidation and to challenge the presence of oxygen (Fahey, 2001). Some are equipped with a complex oxidative stress response mechanism, which is required to preserve their extended aerotolerance (Rocha *et al.*, 2007). Many cells are able to adapt to oxidative stress by increasing their levels of antioxidant enzymes (Carmel-Harel *et al.*, 2001).

Although some concentrations of ROS are involved in normal physiological functions, such as intracellular signaling pathways and redox regulation, the excessive ROS generated during abnormal metabolic reactions damages macromolecules and lead to genetic mutation, physiological dysfunction, and ultimately, cell death (Nordberg and Arnér, 2001). The level of intracellular ROS is significantly elevated in presence of oxidative stress-inducing agents. This elevation subsequently threatens the integrity of various biomolecules. When cellular defense systems cannot cope with exogenously-added stress agents, the cells experience oxidative stress. Because the intracellular ROS level is elevated under various stresses, including oxidative stress, it is considered to be a crucial cellular indicator of intracellular stress.

The internal environment of a living cell is generally maintained in a reduced state. The thiol/disulfide redox system is an intracellular system that is sensitive to oxygen. In the presence of oxidative stress, thiols are oxidized, and thiol groups in proteins cannot be reduced. Various antioxidant proteins, such as superoxide dismutase (SOD), catalase, thioredoxin (Trx), glutaredoxin (Grx), and peroxiredoxin (Prx), participate in maintaining a normal thiol/disulfide balance in living cells (Holmgren, 1985). Thioredoxin reductase (TrxR), an additional antioxidant protein, was originally discovered for its NADPH-dependent ability to reduce an active site disulfide in oxidized Trx to a dithiol in reduced Trx. Since then, it has been found to be involved in protection against oxidative stress, redox regulation of cell signaling, regulation of apoptosis, and control of cell growth and proliferation (Yoshitake *et al.*, 1994; Ejima *et al.*, 1999).

Staphylococcus aureus TrxR is required for growth. It is upregulated following exposure to oxidative and disulfide stresses, which lead to increased disulfide bond formation (Uziel *et al.*, 2004). *Candida neoformans* TrxR1, which has little homology with that of its mammalian host, is required for viability of this pathogenic fungus (Missall and Lodge, 2005). The crucial thiol/disufide redox system (which includes TrxR and Trx) in the anaerobe *Bacteroides fragilis* is necessary for survival and abscess formation in a peritoneal infection model. It also plays an important role in aerobic proliferation of the facultative anaerobe *Lactobacillus casei* (Rocha *et al.*, 2007; Serata *et al.*, 2012). Overexpression of TrxR1 in *Lactobacillus plantarum* increases oxidative stress tolerance to hydrogen peroxide (H_2O_2), which suggests that it functions as a redox sensor in the cell (Serrano *et al.*, 2007). Yeast cells lacking TrxR have reduced abilities to detoxify oxidants and to repair oxidative damage (Carmel-Harel *et al.*, 2001). Disruption of TrxR elevates oxidative stress, mitochondrial dysfunction, and cell death in dopaminergic cells (Lopert *et al.*, 2012). TrxR inhibitors cause growth inhibition and apoptosis in various cancer cells, indicating a relationship between TrxR inactivation and apoptosis or inhibition of cell growth (Zhao *et al.*, 2005).

Schizosaccharomyces pombe cells containing extra copies of $TrxR^+$ display enhanced survival on solid media supplemented with mercuric chloride or aluminum chloride (Hong *et al.*, 2004). *S. pombe* $TrxR^+$ is upregulated by stressors, such as superoxide anion, H_2O_2, mercuric chloride, sodium selenite, and aluminum chloride, through Pap1 (Hong *et al.*, 2004). Induction of Pap1-dependent transcriptional regulation by nitrogen starvation and nitrosative stress was confirmed using a $TrxR^+$-*lacZ* fusion and semiquantitative RT-PCR (Park *et al.*, 2012). Although regulation of *S. pombe* $TrxR^+$ has been studied in detail, studies on its roles against stresses have been limited. In this work, the roles of *S. pombe* TrxR against diverse stresses, including oxidative and nitrosative stresses and nitrogen starvation, were evaluated.

Materials and Methods

Chemicals

Bradford reagent, bovine serum albumin (BSA), NADPH, Griess reagent, 5, 5′-dithio-(2-nitrobenzoic acid) (DTNB), 2′, 7′-dichlorodihydrofluorescein-diacetate (DCFH-DA), D-glucose, sodium nitrite, glutathione (GSH), and glutathione reductase (GR) were obtained from Sigma Chemical Co. Agar, yeast extract, and peptone were obtained from Amersham Life Science.

All other chemicals used in this work were of the highest grade commercially available.

Strain and growth conditions

S. pombe KP1 (*h*$^+$ *leu1-32 ura4-294*), a derivative of the *S. pombe* heterothallic haploid strain 975*h*$^+$, was used in this work. Yeast cells were cultured in yeast extract peptone dextrose medium (pH 6.5; YEPD), which contained 1% yeast extract, 2% peptone, and 1% glucose. Cells were also grown in minimal medium (pH 5.8) containing the following (per L): KH phthalate (3 g), Na_2HPO_4 (1.8 g), NH_4Cl (5 g), D-glucose (20 g), 1,000× vitamin stock (1 ml), 10,000× mineral stock (0.1 ml), 50× salt stock (20 ml), and L-leucine (250 mg). The 50× salt stock contained 5.2 mM $MgCl_2·6H_2O$, 0.1 mM $CaCl_2·2H_2O$, 13.4 mM KCl, and 0.28 mM Na_2SO_4. The 10,000× mineral stock contained 8.1 μM H_3BO_3, 2.37 μM $MnSO_4$, 1.39 μM $ZnSO_4·7H_2O$, 0.74 μM $FeCl_3·6H_2O$, 0.25 μM $MoO_4·2H_2O$, 0.6 μM KI, 0.16 μM $CuSO_4·5H_2O$, and 4.76 μM citric acid. The 1,000× vitamin stock contained 81.2 μM nicotinic acid, 55.5 μM inositol, 40.8 μM biotin, and 4.2 μM pantothenic acid. Yeast cells were grown under shaking at 30°C. Growth was monitored by measuring absorbance at 600 nm. Yeast cells were treated with stress-inducing agents when they were in the early exponential growth phase.

Plasmids

In our previous work, we cloned a unique *S. pombe* gene encoding TrxR into the shuttle vector pRS315 using PCR amplification, which resulted in the recombinant plasmid pHSM10 (Hong *et al.*, 2004). *S. pombe* cells containing pHSM10 exhibit ~2-fold higher TrxR activity compared to vector-only control cells (Hong *et al.*, 2004). In this work, the plasmids pHSM10 and pRS315 were transformed into *S. pombe* KP1 cells.

Preparation of the cellular extracts

Yeast cells were centrifuged, resuspended in 20 mM Tris buffer (pH 8.0) with 2 mM EDTA, and lysed using a glass bead beater. Lysed cells were centrifuged, and supernatants were used as the crude cell extracts for total GSH and protein quantitation, as detailed below.

Determination of intracellular ROS

For analysis of the intracellular ROS, the redox-sensitive fluorescent probe DCFH-DA was used, as previously described (Royall and Ischiropoulos, 1993). When DCFH-DA enters cells, the diacetate group is cleaved by cellular nonspecific esterases to produce nonfluorescent DCFH, which is then oxidized to fluorescent dichlorofluorescein (DCF) in the presence of ROS, such as H_2O_2 (Kiani-Esfahani *et al.*, 2012). Cells were incubated with 5 μM DCFH-DA for 30 min at 30°C and were analyzed immediately using a Multi-Mode Microplate Reader (Synergy^{TM} Mx, BioTek Instruments).

Determination of nitrite in culture supernatants

Accumulated nitrite (NO_2^-), a nitric oxide (NO) indicator, in culture supernatants was quantified using a colorimetric assay based on the Griess reaction (Sherman *et al.*, 1993). Culture supernatants (100 μl) were incubated with 100 μl of Griess reagent (6 mg/ml) at room temperature for 10 min. Then, the NO_2^- concentrations were determined by measuring the absorbance at 540 nm. The standard curve was generated using known concentrations (0−160 μl) of sodium nitrite.

Determination of total GSH

As previously described (Nakagawa *et al.*, 1990), the total GSH in crude extracts was quantified using an enzyme recycling assay based on GR. Reaction mixtures (200 μl) containing 175 mM KH_2PO_4, 6.3 mM EDTA, 0.21 mM NADPH, 0.6 mM DTNB, 0.5 units/ml GR, and crude extract were incubated at 25°C. The absorbance values at 412 nm were measured using a microplate reader. The total GSH content was expressed as μg/mg protein. Protein concentrations of crude extracts were determined by Bradford assay (Bradford, 1976), with BSA as the standard.

Statistical analysis

Results were presented as the mean ± standard deviation (SD). Comparisons between experimental groups were statistically analyzed using unpaired Student's *t*-tests. A *P* value less than 0.05 was considered statistically significant.

Results

Oxidative stress

To test the growth of yeast in the presence of oxidative stress-inducing agents, *S. pombe* cells containing pHSM10 and vector-only control cells were grown to the early exponential phase in rich media, and then the cell cultures were shifted to fresh rich media containing 100 μM H_2O_2 or 50 μM menadione (MD). Growth was measured at 3 and 6 h after exposure to oxidative stress agents. In the absence of stress-inducing agents, *S. pombe* cells containing pHSM10 grew at higher growth rates than vector-only control cells (Fig. 1A). In the presence of 100 μM H_2O_2 or 50 μM MD, the growth of vector-only control cells was nearly arrested, whereas the growth of *S. pombe* cells containing pHSM10 was attenuated (Fig. 1B and C). This result demonstrates that *S. pombe* TrxR participates in cellular proliferation under oxidative stress.

Next, the effect of TrxR on the intracellular ROS levels under oxidative stress was tested. Cells in the exponential growth phase were shifted to fresh liquid media with or without oxidative stress-inducing agents. *S. pombe* cells containing pHSM10 and vector-only control cells were subjected to 100 μM H_2O_2 or 50 μM MD for 6 h. *S. pombe* cells containing pHSM10 had lower intracellular ROS levels than vector-only control cells in the absence of stress-inducing agents (Fig. 2A). This finding can be explained by the ability of *S. pombe* TrxR to reduce ROS production or to scavenge ROS that are produced in the absence of exogenous stress-inducing agents. In the presence of 100 μM H_2O_2 or 50 μM MD, the vector-only control cells had markedly higher ROS levels than nontreated cells (Fig. 2A). *S. pombe* cells containing pHSM10 had lower ROS levels in the presence of 100 μM H_2O_2 or 50 μM MD than vector-only control cells (Fig. 2A). Although the ratio of ROS levels between vector only and TrxR-overexpressing cells was 0.71, it decreased to 0.67 and 0.62, respectively, in the presence of H_2O_2 and MD (Fig. 2A). This finding can be explained by the ability of TrxR to reduce intracellular ROS levels that are enhanced by the exogenous addition of 100 μM H_2O_2 or 50 μM MD.

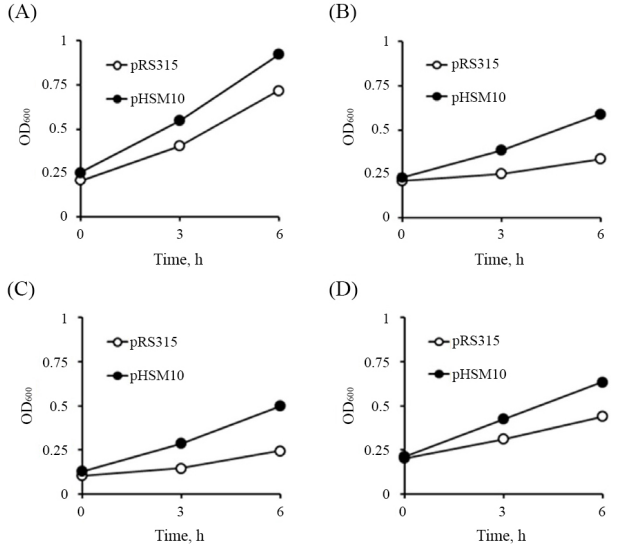

Fig. 1. Effects of TrxR on *S. pombe* proliferation in the presence of stress-inducing agents. *S. pombe* cells containing pRS315 or pHSM10 were grown to exponential phase in rich media, and then were shifted to fresh rich media without stress-inducing agent (Control, A), with 100 μM hydrogen peroxide (H_2O_2, B), with 50 μM menadione (MD, C), or with 100 μM sodium nitroprusside (SNP, D). Growth was measured at 3 and 6 h after the shift.

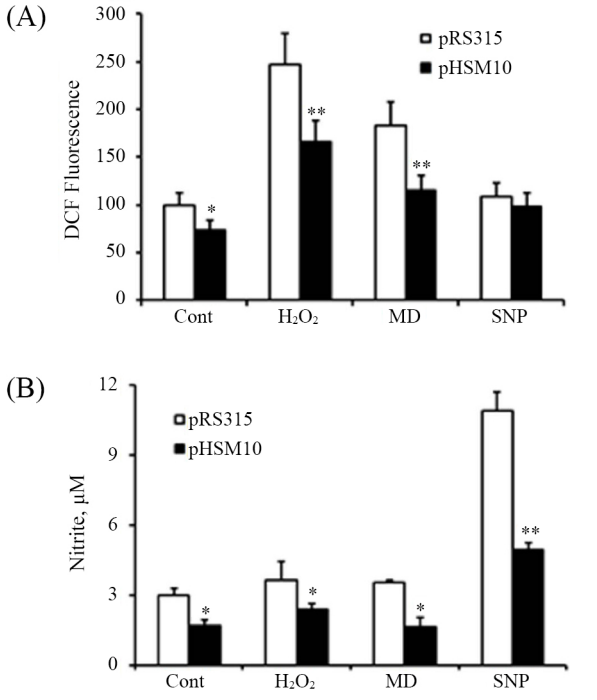

Fig. 2. Effects of TrxR on reactive oxygen species (ROS, A) and nitric oxide (NO, B) levels in *S. pombe* in the presence of stress-inducing agents. *S. pombe* cells containing pRS315 or pHSM10 were grown to exponential phase in rich media, and then were subjected to no stress-inducing agent (Cont), 100 μM hydrogen peroxide (H_2O_2), 50 μM menadione (MD), or 100 μM sodium nitroprusside (SNP) for 6 h. Intracellular ROS levels were detected by fluorometry, and are presented as relative DCF fluorescence (A). Levels of nitrite, which is an indicator of NO, were measured in culture supernatants from yeast treated with no stress agent (Cont), with H_2O_2, MD, or SNP (B). **P*<0.05; ***P*<0.01 versus the corresponding pRS316-containing cells.

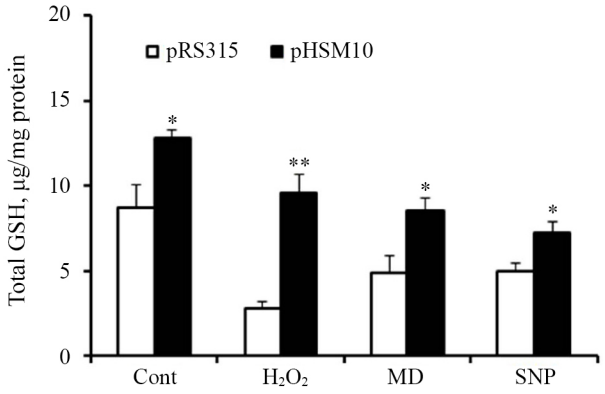

Fig. 3. Effects of TrxR on total glutathione (GSH) in *S. pombe* in the presence of stress-inducing agents. *S. pombe* cells containing pRS315 or pHSM10 were grown to exponential phase in rich media, and then were subjected to no stress-inducing agent (Cont), 100 μM hydrogen peroxide (H_2O_2), 50 μM menadione (MD), or 100 μM sodium nitroprusside (SNP) for 6 h. Total GSH levels, determined using a spectrophotometric recycling assay, are presented as μg/mg protein. **P*<0.05; ***P*<0.01 versus the corresponding pRS316-containing cells.

The NO levels were compared in culture supernatants from yeasts exposed to 100 μM H_2O_2 or 50 μM MD. Although the *S. pombe* cells containing pHSM10 exhibited lower NO levels than vector-only cells, 100 μM H_2O_2 and 50 μM MD couldn't display significant modulating effects, compared to those of the non-treated cells (Fig. 2B).

Living cells are equipped with powerful defense mechanisms, including free-radical scavengers and antioxidants, to protect against oxidative damage. GSH, a principal nonenzyme antioxidant, plays a major role in defending against many types of oxidative stresses. We determined whether *S. pombe* TrxR affects the total GSH level in the absence or presence of stress. The total GSH level was higher in *S. pombe* cells containing pHSM10 than in vector-only control cells in the absence of stress-inducing agents (Fig. 3). In the presence of 100 μM H_2O_2 or 50 μM MD, the total GSH levels were still higher in *S. pombe* cells containing pHSM10 than in vector-only control cells (Fig. 3). In summary, *S. pombe* TrxR increases cellular proliferation and the total GSH level and reduces intracellular ROS under oxidative stress, demonstrating its defensive role against oxidative stress.

Nitrosative stress

Nitric oxide (NO•, NO) has normal physiological effects when it is generated in minute quantities by constitutive nitric oxide synthases (NOSs), whereas it has pathological effects when it is generated in excess by inducible NOSs. NO can directly or indirectly interact with biological targets. Its indirect effects are mediated by reactive nitrogen species (RNS) that undergo further reactions with biological targets, including proteins, lipids, and DNA. RNS play crucial roles in cellular signaling, but at high RNS concentrations, cells are subjected to nitrosative stress, which may lead to cell death.

A protective role for TrxR in yeast cells under nitrosative stress was examined. When *S. pombe* cells containing pHSM10 and vector-only control cells were subjected to 50 μM NO-generating sodium nitroprusside (SNP) for up to 6 h, both groups exhibited similar reduced growth patterns 3 and 6 h after treatment (Fig. 1D). No significant differences in ROS levels were detected between *S. pombe* cells containing pHSM10 and vector-only control cells after treatment with SNP (Fig. 2A). As expected, SNP caused the NO level to increase markedly in vector-only control cells (Fig. 2B). In the presence of SNP, the NO level in culture supernatants of *S. pombe* cells containing pHSM10 was lower than the level in vector-only control cells (Fig. 2B). In the presence of SNP, the total GSH levels in *S. pombe* cells containing pHSM10 and in vector-only control cells were reduced compared to the total GSH levels in nontreated cells (Fig. 3). In summary, *S. pombe* TrxR is able to scavenge artificially generated NO, suggesting that it has a defensive role against nitrosative stress.

Nitrogen starvation

Nitrogen is required for the reproduction of all organisms, and living cells are stressed when deprived of nitrogen. To assess the role of *S. pombe* TrxR under nitrogen starvation, the nitrogen source NH_4Cl was depleted from fresh minimal medium. The growth of *S. pombe* cells containing pHSM10 and vector-only control cells was measured at 3 and 6 h after nitrogen depletion. In both cultures, growth was lower in nitrogen-depleted media compared to growth in minimal media containing a nitrogen source (Fig. 4A and B). *S. pombe* cells containing pHSM10 grew better than vector-only control cells under nitrogen-starved conditions (Fig. 4B).

Because TrxR was shown to participate in the cellular proliferation of yeast cells under nitrogen starvation, we compared stress-related factors, such as ROS, NO, and GSH, in

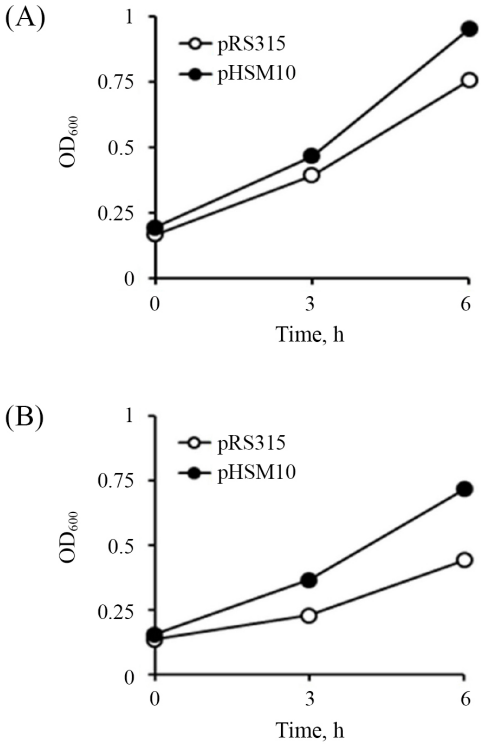

Fig. 4. Effects of TrxR on *S. pombe* proliferation under nitrogen starvation. *S. pombe* cells containing pRS315 or pHSM10 were grown to exponential phase in minimal medium, and then were shifted to fresh minimal medium with NH_4Cl (A) or without NH_4Cl (B) as a nitrogen source. Growth was measured at 3 and 6 h after the shift.

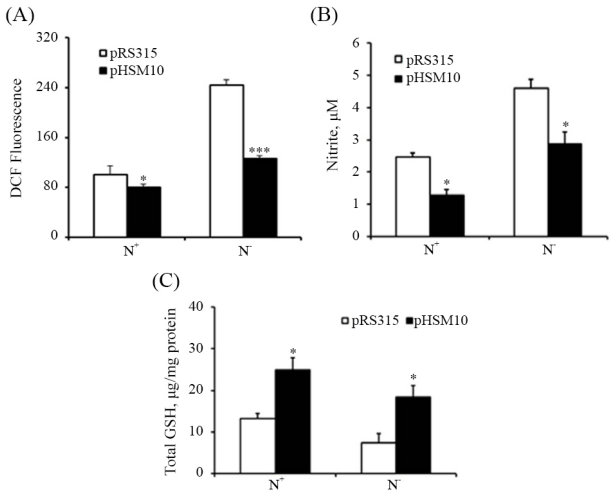

Fig. 5. Effects of TrxR on levels of reactive oxygen species (ROS, A), nitric oxide (NO, B), and total glutathione (GSH, C) in *S. pombe* under nitrogen starvation. *S. pombe* cells containing pRS315 or pHSM10 were grown to exponential phase in minimal media, and then were shifted to fresh minimal media lacking NH_4Cl as a nitrogen source for 6 h. Intracellular ROS levels were detected by fluorometry and are presented as relative DCF fluorescence (A). Levels of nitrite, an indicator of NO, were measured in culture supernatants from yeast under nitrogen starvation (B). Total GSH levels, determined using a spectrophotometric recycling assay, are presented as μg/mg protein (C). **P*<0.05; ****P*<0.001 versus the corresponding pRS315-containing cells.

nitrogen-starved and nonstarved yeast cells. The ROS level in vector-only control cells subjected to nitrogen starvation for 6 h was 2.4-fold higher than the level in nonstarved vector-only control cells (Fig. 5A). Under nitrogen starvation, the ROS level in *S. pombe* cells containing pHSM10 was 50% lower than the level in vector-only control cells (Fig. 5A). The NO level in vector-only control cells subjected to nitrogen starvation for 6 h was 1.9-fold higher than the level in nonstarved vector-only control cells (Fig. 5B). Under nitrogen starvation, the NO level in *S. pombe* cells containing pHSM10 was 58% lower than the level in vector-only control cells (Fig. 5B). In contrast, the total GSH level in nitrogen-starved vector-only control cells was 57% lower than the level in nonstarved vector-only control cells. Under nitrogen starvation, the total GSH level in *S. pombe* cells containing pHSM10 was 2.4-fold higher than the level in vector-only control cells (Fig. 5C).

In summary, nitrogen starvation increases the ROS and NO levels and reduces the total GSH level in the fission yeast *S. pombe*. TrxR reduces the ROS and NO levels that were enhanced by nitrogen starvation and increases the total GSH level that was diminished by nitrogen starvation.

Discussion

Since TrxR was first discovered in *E. coli*, it has been found to have novel physiological functions, with roles against stresses in various organisms. In *S. cerevisiae*, tolerance to freezing, heating, or MD-induced oxidative stress is significantly improved by expression of the *Chlorella vulgaris* NADPH-dependent TrxR (Machida *et al.*, 2012). This improvement is further enhanced when 2-Cys peroxiredoxin is coexpressed, suggesting that the *C. vulgaris* NADPH-dependent TrxR acts as an antioxidant system complementary to 2-Cys peroxiredoxin (Machida *et al.*, 2012). In *S. cerevisiae*, TrxR has less of an effect during exponential growth when there is a strong requirement for the active Yap1 transcription factor, but it is important during stationary phase growth (Drakulic *et al.*, 2005).

Larva of the aquatic midge *Chironomus riparius* is widely

considered to be a test organism for aquatic ecotoxicological studies. It represents an important link in the aquatic food web and is associated with benthic sediments (Bouché *et al.*, 2000). *C. riparius* TrxR1 was found to be upregulated upon paraquat exposure (Nair and Choi, 2011) and can potentially be used as a biomarker for identifying oxidative stress-inducing environmental contaminations. TrxR has been implicated in the proliferation, survival, and pathogenicity of certain microbes and has been suggested as a potential therapeutic target for *Pneumocystis jiroveci* opportunistic infections (Kutty *et al.*, 2003). Because TrxR overactivation and dysfunction are closely related to tumor development, TrxR inhibitors have been considered as promising cancer chemotherapy agents (Cai *et al.*, 2012).

This work demonstrates that the unique *S. pombe* TrxR functions during responses to oxidative, nitrosative and nutritional stresses. This finding may be indirectly supported by previous results demonstrating that *S. pombe* $TrxR^+$ is positively regulated by oxidative, nitrosative, and nutritional stresses in a Pap1-dependent manner (Hong *et al.*, 2004; Park *et al.*, 2012). However, *S. pombe* TrxR seems to protect against these stresses in different ways. In response to oxidative stress, although *S. pombe* TrxR increases cellular proliferation, elevates the total GSH level, and reduces the ROS level, it has no significant modulating effect on the NO levels. In contrast, under nitrosative stress, *S. pombe* TrxR affects NO levels, but does not affect cellular proliferation or the ROS or total GSH level. In response to nitrogen starvation, *S. pombe* TrxR increases cellular proliferation and the total GSH level, and it reduces the ROS and NO levels. These results suggest that *S. pombe* TrxR protects against diverse stresses in different ways. Determining the precise defense mechanisms of *S. pombe* TrxR to these stresses will require further detailed studies.

Some stress response-related signaling pathways are dependent on the type and severity of the stress. *S. pombe* triggers different signaling pathways depending on the severity of the oxidative stress; for example, Pap1 is more sensitive to H_2O_2 than the Sty pathway. These pathways with distinct sensitivities are designed for adaptive, rather than survival, responses. The Sty1 pathway remains fully functional in the presence of high H_2O_2 concentrations. The antioxidant pathways of *S. pombe* are triggered by different signaling pathways and do not overlap (Vivancos *et al.*, 2006). In response to H_2O_2, the *S. pombe* transcription factor Pap1 regulates the transcription of genes required for adaptation to oxidative stress and tolerance to toxic drugs (Calvo *et al.*, 2012). Pap1 is oxidized and accumulates in the nucleus when TrxR is inhibited. A subset of Pap1-dependent genes, such as those coding for the efflux pump Caf5 and the ubiquitin-like protein Obr1, only require nuclear Pap1 for activation, whereas another subset of genes, such as those coding for the antioxidants catalase, sulfiredoxin, and TrxR, require oxidized Pap1 to form a heterodimer with the constitutive nuclear transcription factor Prr1 (Calvo *et al.*, 2012).

Adaptations of living cells to nutritional stresses include alterations in gene expression, which may be associated with the selective degradation of superfluous proteins. Yeasts can utilize GSH as an endogenous sulfur source, and GSH stored in yeast vacuoles can be used as an alternative nitrogen source during nitrogen starvation (Hiesinger *et al.*, 2001). As *S. pombe* cells are quiescent when cultured under nitrogen-starved conditions, *S. pombe* is a good model organism for investigating the mechanisms responsible for the transition from proliferation to quiescence (Sajiki *et al.*, 2009). When *S. pombe* cells are not supplied with exogenous nitrogen, they undergo autophagy to obtain nitrogen (Kohda *et al.*, 2007). Several proteins, including some antioxidant proteins, have been reported to participate in the response to nitrogen starvation. The *S. pombe* bacterioferritin comigratory protein, a member of the thiol-specific antioxidant/alkyl hydroperoxide peroxidase C family, plays a defensive role in the response to nitrogen starvation by upregulating total and reduced GSH levels (Kang *et al.*, 2009). *S. pombe* Spy1, a histidine-containing phosphotransfer protein, plays defensive roles against nitrosative and nutritional stresses, such as nitrogen starvation. Spy1 is transcriptionally upregulated by those stresses in a Pap1-dependent manner (Kang *et al.*, 2011). Similarly, overexpression of a second protein disulfide isomerase, PDI2, increases *S. pombe* survival on nitrogen-depleted minimal medium plates supplemented with NO-generating SNP. In addition, *S. pombe Pdi2* is positively regulated by NO and nitrogen starvation in a Pap1-dependent fashion (Lee *et al.*, 2010). Our work also demonstrates that TrxR is involved in the response to nitrogen starvation in *S. pombe*.

Nitrogen starvation increases ROS and NO levels in *S. pombe*, and the elevated ROS and NO levels upregulate expression of $TrxR^+$. During nitrogen starvation, GSH serves as

the nitrogen source. In *S. pombe*, nitrogen starvation appears to be a critical nutritional stress, which induces NO and subsequently causes nitrosative stress. Many genes in *S. pombe* respond similarly to stress agents that produce NO, such as SNP, as to nitrogen starvation. For example, *S. pombe* $TrxR^+$, *Spy1*, and *Pdi2* are transcriptionally upregulated by both nitrosative stress and nitrogen starvation. Interestingly, two well-known stress-responsive *S. pombe* transcription factors, Pap1 and Atf1, are also upregulated by nitrosative stress and nitrogen starvation (Kim *et al.*, 2008; Song *et al.*, 2009). Pap1-dependent upregulation of *S. pombe* $TrxR^+$ by nitrosative stress might result from transcriptional activation of Pap1 (Kim *et al.*, 2008). Currently, the transcriptional regulation of ROS-dependent and NO-dependent inducible genes responsible for stress defense is unknown.

적 요

치오레독신 환원효소(TrxR)를 encoding하는 *Schizosaccharomyces pombe*의 유일한 $TrxR^+$ 유전자는 스트레스 반응 전사인자인 Pap1의 매개에 의하여 스트레스 유발 인자들에 의하여 양성적으로 조절됨이 발견되었다. 본 연구에서는, TrxR 과잉 발현 재조합 플라스미드 pHSM10을 사용하여 *S. pombe* TrxR의 방어적 역할들이 평가되었다. 과산화수소(H_2O_2)와 superoxide anion을 생성하는 menadione (MD)의 존재 하에서, *S. pombe* TrxR은 세포성장과 총 글루타치온 (GSH) 수준을 증강시키나, 세포 내 활성산소종(ROS) 수준은 감소시켰다. H_2O_2와 MD에 의하여 크게 영향 받지 않는 일산화질소(NO) 수준에는 유의성 없는 효과를 보였다. *S. pombe* TrxR은 sodium nitroprusside (SNP)에 의하여 생성되는 NO를 소거할 수 있었으나, SNP에 노출된 세포들의 성장, ROS 수준이나 총 글루타치온 수준에는 영향을 보이지 않았다. *S. pombe* TrxR은 질소 결핍 (nitrogen starvation)에 의하여 감소되는 세포 성장 및 총 글루타치온 수준을 증가시키며, 질소 결핍에 의하여 생성되는 ROS와 NO를 소거하였다. 요약하건대, *S. pombe* TrxR은 산화적, 일산화질소 및 영양 스트레스로부터 효모 세포를 보호하지만, 공통적인 기전에 의하지는 않는다.

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