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# Transcription of *Schizosaccharomyces pombe* Thioltransferase-1 in Response to Stress Conditions

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**Thioltransferase, also known as glutaredoxin, is an enzyme that catalyzes the reduction of a variety of disulfide compounds. In *Schizosaccharomyces pombe*, two thioltransferases were reported and the cDNA of one of the thioltransferases (thioltransferase-1) was cloned. Using a Northern blot assay, we investigated the thioltransferase transcription in response to various stress conditions. When the culture was shifted to a high temperature, the thioltransferase transcription was not significantly changed compared to the unshifted 30°C culture. Treatment of zinc chloride to exponentially-growing cells remarkably increased the thioltransferase transcription, whereas the treatment of mercury chloride greatly reduced the transcription. Treatment of hydrogen peroxide and cadmium chloride caused no significant effects on the transcription of the thioltransferase. These results suggest that the transcription of thioltransferase-1 in *S. pombe* is induced in response to metal stress that is caused by zinc chloride, but not in response to heat stress or oxidative stress that is caused by hydrogen peroxide.**

**Keywords:** Thioltransferase; Transcription; Stress condition, *Schizosaccharomyces pombe*

Thioltransferase, also known as glutaredoxin, is a small heat-stable oxidoreductase, which has been highly conserved throughout evolution (Holmgren, 1989; Holmgren and Aslund, 1995). It forms part of the glutaredoxin system that consists of NADPH, glutathione (GSH), and glutathione reductase. The electrons are transferred from NADPH to thioltransferase via GSH (Holmgren, 1990). Thioltransferase was originally identified as a hydrogen donor for ribonucleotide reductase, but it is also required for the reduction of sulfate (Tsang, 1981), methionine sulfoxide (Fuchs, 1977), and arsenate (Shi *et al*., 1999). Some thioltransferases are able to reduce non-disulfide substrates, such as dehydroascobic acid (Wells *et al*., 1990) and alloxan (Washburn and Wells, 1997).

Thioltransferase can catalyze the cleavage of mixed disulfides that may be formed as a result of oxidative stress (Chrestensen *et al*., 1995; Raghavachari *et al*., 1996). In addition, it can reactivate a number of oxidized proteins by reducing the mixed disulfides that are formed as a result of thiol oxidation (Terada *et al*., 1992; Terada, 1994; Yoshitake *et al*., 1994). The OxyR transcription factor, which is activated through the formation of disulfide bond, is deactivated by thioltransferase (Zheng *et al*., 1998). The thioltransferase gene expression is induced in response to various stress conditions, including oxidative, osmotic, and heat stress (Grant *et al*., 2000). Thioltransferase may be involved in the regulation and maintenance of protease activity in HIV-1 infected cells (Davis *et al*., 1997).

Thioltransferases contain the conserved sequence of Cys-Pro-Phe(Tyr)-Cys in their active site (Hoog *et al*., 1983). Recently, thioltransferases were isolated and characterized from various organisms. In *S. cerevisiae*, five thioltransferases (Grx1, Grx2, Grx3, Grx4, and Grx5) were characterized as protecting yeast cells against various damages that are caused by oxidative, osmotic, and heat stress (Luikenhuis *et al*., 1988; Rodriguez-Manzaneque *et al*., 1999; Grant *et al*., 2000). It has been reported that the fission yeast *S. pombe* contains two thioltransferases (Kim *et al*., 1998; Kim *et al*., 1999a); one of these thioltransferases (thioltransferase-1) was recently cloned (Kim *et al*., 1999b; Cho *et al*., 2000a). In attempt to identify the respective biological roles of these thioltransferases in *S. pombe*, we analyzed the transcription of the thioltransferase-1 gene in response to several stress conditions. We demonstrated that the transcription of the gene is increased by zinc chloride, but not in response to the oxidative stress that is caused by hydrogen peroxide.

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## Materials and Methods

**Yeast strain and growth conditions** The *S. pombe* strain that was used in this study was wild-type 972. Yeast cells were grown at 30°C in a YEALU or PM medium (Alfa *et al*., 1993). The YEALU medium is a standard YE medium that is supplemented with 75 mg per liter of adenine, leucine and uracil.

**Treatment of chemicals** The cells were grown to the mid-log phase and treated with the respective compound, which was directly added to the growth medium at the concentrations that was indicated for each experiment. Untreated cultures were incubated in parallel over the same periods.

**RNA preparation** Total RNA was prepared by the modified method of Nischt *et al*. (1986). The cells were harvested by centrifugation, resuspended in a TLSE solution (10 mM Tris-HCl, 10 mM LiCl, 1% lithium lauryl sulfate, pH 7.5), and disrupted by glass beads. After the phenol/chloroform extraction of the supernatant, the RNA was precipitated by ethanol with 5 M LiCl. The total RNA concentration in these preparations was estimated by measuring the absorbance at 260 nm.

**Northern hybridization** Total RNA (20 μg) was separated by electrophoresis on a 1.5% formaldehyde gel. The RNA on the gel was transferred to a nylon membrane by electroblotting. For detection of thioltransferase-1 mRNA, the blotted membranes were hybridized with a DIG-labeled thioltransferase-1 DNA probe that was synthesized by a polymerase chain reaction. Hybridization in 50% formamide and luminescent detection using a DIG luminescent detection kit (Boehringer, Mannheim, Germany) were performed according to the manufacturers manual.

**PCR** The probe was synthesized by PCR using a DIG Probe Synthesis Kit (Boehringer, Mannheim, Germany). The *S. pombe* thioltransferase-1 clone, pKU10 (Kim *et al*., 1999b), was used as a template for the PCR reaction. Two primers (MK2F: 5'-ATGTCTA GTGRRGAATCATTTGT-3'; MK2R: 5'-TTTATTGCTCATAGAA TTTCATT-3') were used to amplify the fragment (327 bp) that covered the entire thioltransferase-1 cDNA sequence. PCR was performed for 30 cycles that consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min.

## Results and Discussion

Thioltransferase was purified in several organisms and was found to have multiple biological functions. However, the precise regulation in cells remains to be elucidated. Recently, the expression of the *S. cerevisiae* thioltransferase gene was identified as being induced by various stress conditions (including oxidative, osmotic and heat stress) in response to the stationary phase and growth on non-fermentable carbon sources (Grant *et al*., 2000). In this study, we examined the transcription of the *S. pombe* thioltransferase-1 gene in response to various stress conditions.

**Heat stress** Heat is probably one of the factors in thioltransferase regulation. To address the question of whether the *S. pombe* thioltransferase-1 is induced by heat or not, the extent of the thioltransferase-1 transcription was observed in a temperature-shift experiment. The wild-type *S. pombe* cells that were grown in a rich medium at 30°C were split at the mid-log phase and the incubation temperature was shifted to 35°C. The thioltransferase gene transcription did not increase significantly by the temperature shift to 35°C when compared to the untreated cells (Fig. 1). This indicates that high temperatures may not induce the thioltransferase gene transcription. A similar experiment was done with the *S. pombe* cells that were grown in a minimal medium. We performed this experiment because some types of medium could affect the temperature dependence of thioltransferase transcription. Again, the extent of the transcription was not increased by the temperature shift, even to 40°C (Fig. 2). Therefore, these results strongly suggest that the thioltransferase transcription is not induced by high heat. Cho *et al*., (2000b) reported that the thioltransferase activity was significantly increased by high heat in either rich or minimal mediums. Therefore, their increased thioltransferase activity could be at the enzyme level rather than the transcriptional level.

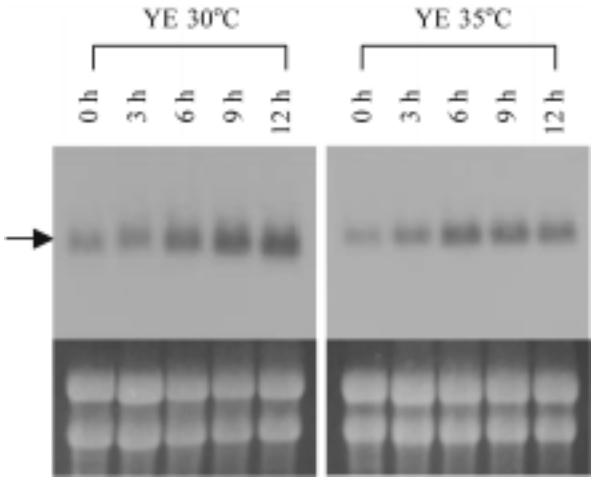


**Fig. 1.** Effect of temperature shift on thioltransferase transcription in a rich medium. The yeast cells, grown in a rich medium, were split in the mid-log phase and transferred to 35°C. The thioltransferase transcription was assayed in a Northern blot (described in Materials and Methods). An arrow indicates thioltransferase-1 mRNA. The 28S and 18S rRNA bands in ethidium bromide-stained gel are shown as loading controls.

**Metal stress** We next examined the effect of various metals on the thioltransferase-1 transcription (Fig. 3). The *S. pombe* culture was grown in a rich medium at 30°C and the culture was split at the early stationary phase. Zinc chloride was added into a separate culture flask at a final concentration of 550 mM. The *S. pombe* culture was harvested at 3, 6, 9, and 12 h after the addition of zinc chloride. The zinc chloride

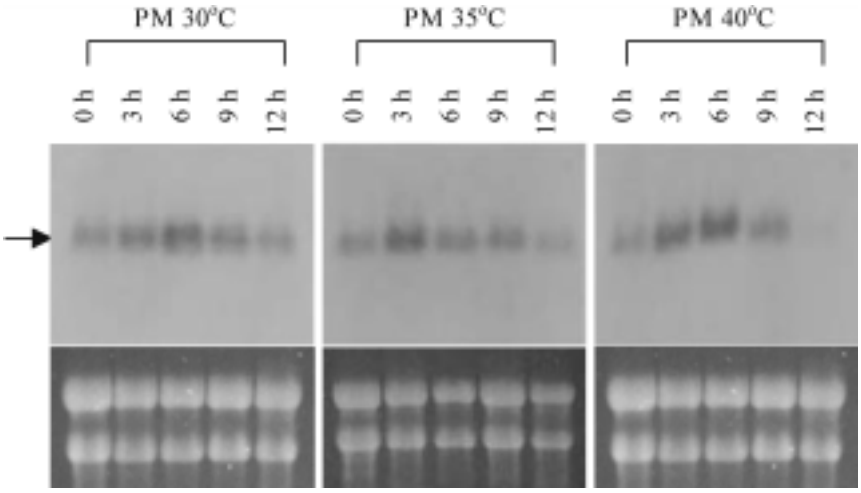


**Fig. 2.** Effect of temperature shift on thioltransferase transcription in a minimal medium. The yeast cells, grown in minimal medium, were split in the mid-log phase and transferred to 35ºC and 40ºC. The thioltransferase transcription was assayed in a Northern blot (described in Materials and Methods). An arrow indicates thioltransferase-1 mRNA. The 28S and 18S rRNA bands in ethidium bromide-stained gel are shown as loading controls.

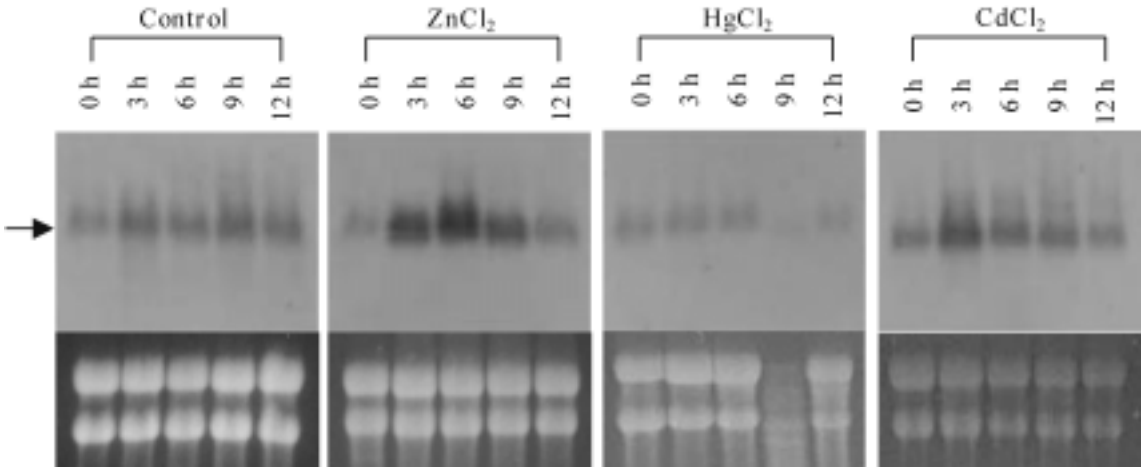


**Fig. 3.** Effects of metals on thioltransferase transcription. The yeast cells, grown in a rich medium, were split in the mid-log phase, then zinc chloride, mercuric chloride, and cadmium chloride were added to 0.55 mM, 0.3 mM, and 0.3 mM, respectively. The thioltransferase transcription was assayed in a Northern blot (described in Materials and Methods). An arrow indicates thioltransferase-1 mRNA. The 28S and 18S rRNA bands in ethidium bromide-stained gel are shown as loading controls.

significantly enhanced the transcription of the thioltransferase gene. This suggests that the expression of *S. pombe* thioltransferase gene may be directly related to the stress response that is caused by zinc chloride.

Treatment of 0.3 mM mercury chloride almost completely inactivates the thioltransferase gene expression in *S. pombe* cells. However, treatments with 0.3 mM cadmium chloride had no significant effect on the thioltransferase gene transcription. These results correlate well with the report that the thioltransferase activity of *S. pombe* cells was completely abolished by mercuric chloride, but was unaffected by the treatment of cadmium chloride (Cho *et al*., 2000c).

These results, therefore, suggest that a zinc-containing protein might mediate the transcriptional activation of thioltransferase. It has been reported that a zinc-finger protein regulates the *ste11* gene transcription, which encodes a transcription factor for sexual development (Kunitomo *et al*., 2000). It is conceivable that the treatment of zinc chloride could induce the activation of such a zinc-finger transcriptional activator. On the other hand, the treatment of mercury chloride, a potent inhibitor of many enzymes, could inhibit the activity of a protein that is involved in the thioltransferase gene transcription.

**Oxidative stress** To test whether or not the thioltransferase expression is induced in response to oxidative stress conditions, *S. pombe* cells were grown in a rich medium to the mid-log phase and challenged with 1.5 mM hydrogen

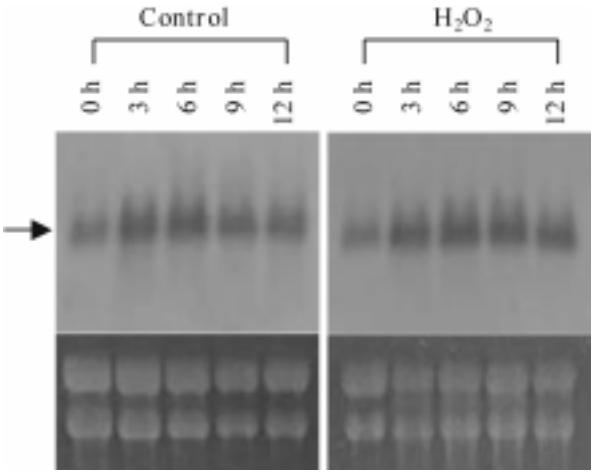


**Fig. 4.** Effect of hydrogen peroxide on thioltransferase transcription. The yeast cells, grown in a rich medium, were split in the mid-log phase and hydrogen peroxide was added to 1.5 mM. The thioltransferase transcription was assayed in a Northern blot (described in Materials and Methods). An arrow indicates thioltransferase-1 mRNA. The 28S and 18S rRNA bands in ethidium bromide-stained gel are shown as loading controls.

peroxide. The thioltransferase gene transcription was not significantly changed following exposure to hydrogen peroxide (Fig. 4). This suggests that the thioltransferase-1 expression in *S. pombe* is not induced in response to the oxidative stress that is caused by hydrogen peroxide. It has been reported that treatment with hydrogen peroxide has no effect on the thioltransferase activity in *S. pombe* (Cho *et al*., 2000c). However, in *S. cerevisiae*, the expression of both the GRX1 and GRX2 genes was elevated approximately three- to four-fold in response to the 0.3 mM $H_2O_2$ treatment (Grant *et al*., 2000). This apparent paradox might be due to the different organisms that were used in the experiments.

In this communication, it was found that the thioltransferase-1 gene transcription is induced by the treatment of zinc chloride, but not by heat or hydrogen peroxide. This suggests that the thioltransferase-1 in *S. pombe* might not be involved in the oxidative stress response that is caused by hydrogen peroxide. It has been reported that in *S. cerevisiae*, the *grx1* disruption mutant cells are particularly sensitive to oxidative stress that is caused by menadione (a generator of superoxide anions), while the *grx2* mutant is hypersensitive to hydrogen peroxide (Luikenhuis *et al*., 1998). This suggests separate roles for the Grx1 and Grx2 proteins against several types of oxidative stress. In *S. cerevisiae*, another subfamily of glutaredoxins (Grx3, Grx4, and Grx5) is described that differs from Grx1 and Grx2 in that it contains a single cystein residue at the putative active site (Rodriguez-Manzaneque *et al*., 1999). Therefore, there could be more genes that encode proteins with thioltransferase activity in *S. pombe*. Recently, a second thioltransferase (thioltransferase-2) was purified in *S. pombe* (Kim *et al*., 1999a) and its gene was cloned (personal communication). Further studies on these new thioltransferase genes could determine the relationship between the expression of thioltransferase genes and various stress conditions in *S. pombe*.

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