Journal of Biochemistry and Molecular Biology, Vol. 33, No. 2, March 2000, pp. 166-171

JOURNAL OF
Biochemistry and Molecular Biology

Temperature-Dependent Expression of *Escherichia coli* Thioredoxin Gene

Jin-Joo Lee, Eun-Hee Park†, Ki-Sup Ahn‡ and Chang-Jin Lim*

Division of Life Sciences, Kangwon National University, Chuncheon 200-701, Korea,
†College of Pharmacy, Sookmyung Women's University, Seoul 140-742, Korea,
‡Department of Environment Managemental, Chonan College of Foreign Studies, Chonan 330-180, Korea

Received 10 December 1999, Accepted 20 January 2000

Thioredoxin is a multifunctional protein that is ubiquitous in microorganisms, animals and plants. Previously, the expression of the *Escherichia coli* thioredoxin gene (*trxA*) was found to be negatively regulated by cAMP. In the present study, the effect of temperature on the expression of the *E. coli trxA* gene was investigated. In order to examine the temperature effect, the fusion plasmid pCL70 that harbors the *E. coli trxA* P1P2 promoter was used. The other two fusion plasmids, pJH3 and pMG521 that were constructed in different vectors which harbor the *E. coli trxA* P2 promoter, were also used. When the *E. coli* strain MC1061/pCL70 was grown in a rich medium at 25°C, 34°C and 42°C, the cells grown at 42°C gave the highest ß-galactosidase activity. The *E. coli* MC1061/pJH3 cells also revealed similar results. The *E. coli* MC1061/pJH3 and MC1061/pMG521 cells showed increased β-galactosidase activity after the shift of the culture temperature to 42°C. The wild-type *trxA* gene of the *E. coli* MC1061 cells produced much higher thioredoxin activity at the higher temperature. These results support the conclusion that the *E. coli trxA* gene is regulated in a temperature-dependent manner. Especially the expression from its P2 promoter appeared to be sensitive to temperature.

Keywords: *Escherichia coli*, Regulation, Temperature, Thioredoxin gene.

Introduction

Thioredoxin(Trx) is a small, ubiquitous, heat-stable, and multifunctional protein that has a redox-active disulfide/dithiol within the conserved active site sequence -Trp-Cys-Gly-Pro-Cys-. Since thioredoxin was originally discovered in *Escherichia coli* as a reducing agent for ribonucleotide reductase (Laurent *et al.*, 1964), it has been shown to participate in the reduction of sulfate (Tsang and Schiff, 1976), methionine sulfoxide (Porgue *et al.*, 1970), and protein disulfide bonds (Holmgren, 1979a; Holmgren, 1979b; Holmgren, 1984). The oxidized form contains a disulfide bridge, which is reduced by thioredoxin reductase, or ferredoxin-thioredoxin reductase (Droux *et al.*, 1987). The NADPH-dependent reduction of the disulfide bond in 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (Moore *et al.*, 1964), or a protein such as insulin (Luthman and Holmgren, 1982), serves as a convenient assay of thioredoxin and thioredoxin reductase. In addition, it acts as an essential subunit of phage T7 DNA polymerase (Mark and Richardson, 1976) and is required for the assembly of the filamentous phages M13 and f1 (Lim *et al.*, 1985). In higher plants, it is involved in the modulations of enzymatic activities by thiol redox control (Buchanan *et al.*, 1979).

There are a few regulatory mechanisms identified in the expression of eukaryotic thioredoxin genes. Transcription of the *Rhodobacter sphaeroides* Y thioredoxin gene is regulated by high oxygen tension (Pasternak *et al.*, 1996). Oxidative agents increase the expression of a human thioredoxin gene. These include hydrogen peroxide, diamide and menadione, through a *cis*-acting regulatory element that is responsive to the oxidative stress (Taniguchi *et al.*, 1996). This type of regulation supports the fact that thioredoxin is involved in the regeneration of proteins inactivated by oxidative stress (Fernando *et al.*, 1992). Transcript levels of *Dictyostelium* thioredoxins are regulated during the development cycle, which are low in the growth phase and maximally high during development (Wetterrauer *et al.*, 1992). Thioredoxin gene expression was also reported to be transcriptionally up-regulated by retinol in monkey epithelial cells (An and Wu, 1992).

The gene encoding *E. coli* thioredoxin was designated as *trxA* and mapped at 84 minutes on the genetic map of the *E. coli* K-12 genome (Mark *et al.*, 1977). A *Hpa* II fragment, containing the *E. coli trxA* gene, was cloned and sequenced (Lim *et al.*, 1985). Here, the effect of temperature was

*To whom correspondence should be addressed.
Tel: 82-361-250-8514; Fax: 82-361-242-0459
E-mail: cjlim@cc.kangwon.ac.kr

Table 1. Bacterial strain and plasmids used in this study.

Strain or plasmid	Genotype or phenotype	Source
E. coli strain		
MC1016	*hsdR rglB araD139 Δ(araABC-leu) Δlac274 galU*	This Lab.
Plasmids		
pCL70	pMC1403 derivative containing *trxA P1P2*	This Lab.
pJH3	pMC1403 derivative containing *trxA P2*	This Lab.
pMG521	pJEL170 derivative containing *trxA P2*	This Lab.

investigated on the expression of the *E. coli* thioredoxin gene.

Materials and Methods

Materials Ampicillin, o-nitrophenyl-β-D-galactopyranoside (ONPG), bovine serum albumin (BSA), Bradford reagent, β-mercaptoethanol, NADPH, and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from the Sigma Chemical Co. (St. Louis, USA). Bacto tryrptone, Bacto yeast extract, and Bacto agar were obtained from Difco Laboratories (Detroit, USA). All other reagents were of analytical grade, or the highest quality commercially available. The *E. coli* thioredoxin reductase was a generous gift from Dr. J. A. Fuchs, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota.

Bacterial strain and plasmids The *E. coli* strain and plasmids, used in the present study, are listed in Table 1.

Bacterial growth The *E. coli* strains were routinely grown in Luria-Bertani broth (LB broth; 10 g tryptone, 5 g yeast extract and 10 g NaCl per liter), or LB solid agar (LB broth plus 1.5% agar). Ampicillin (50 μg/ml) was added to media if necessary. The *E. coli* strains used were grown at various temperatures, and the cells were harvested during the exponential and stationary phases. Bacterial growth was monitored by measuring optical density at 600 nm with a double-beam spectrophotometer.

Preparation of crude extract Crude extract was prepared from bacterial cultures grown in LB broth. Cells were harvested and resuspended in 50 mM Tris-HCl (pH7.4)-1 mM EDTA buffer (buffer A). The resuspended cells were disrupted by sonication, and then centrifuged to remove cell debris and membrane-bound enzymes. Protein concentrations in crude extracts were determined according to the methods of Lowry *et al.* (1951) and Bradford (1976) using bovine serum albumin as a standard.

β-Galactosidase assay The β-Galactosidase activity in crude extracts was determined according to a slight modification of the method of Miller (1972). The reaction mixture contained 0.1 M phosphate buffer (pH 7.5), 0.88 mg/ml ONPG, 1 mM $MgCl_2$, 45 mM β-mercaptoethanol and cell extract. Change in optical density at 420 nm was monitored using the microplate reader. The specific activities of β-galactosidase were represented as ΔA_{420}/min/mg protein.

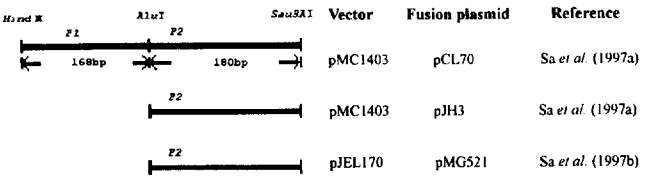
Thioredoxin assay Thioredoxin was assayed as a substrate of *E. coli* NADPH-thioredoxin reductase in the presence of NADPH and DTNB (Sa *et al.*, 1998; Park *et al.*, 1999). In the final volume of 0.2 ml, the reaction mixture contained 100 mM Tris-HCl buffer (pH 8.0), 2 mM EDTA, 0.5 mM DTNB (dissolved in 95% ethanol), 0.24 mM NADPH and cell extract. Adding *E. coli* thioredoxin reductase started the reaction, and the reduction of DTNB was determined by monitoring the absorbance change at 412 nm.

Results and Discussion

Thioredoxin is a conserved and compact protein that is found in every organism. In some organisms it exists in multiple forms. It is known to have various biological functions in the cells and its function is gradually broadening. Recently, it was discovered that thioredoxin levels were significantly increased in the inflamed joints of patients with rheumatoid arthritis (Maurice *et al.*, 1999). Possibly the elevated thioredoxin in rheumatoid arthritis patients is involved in the aggravation of rheumatoid inflammation by augmenting the NF-kB activation pathway (Yoshida *et al.*, 1999). The transgene expression of thioredoxin decreased the ischemic neuronal injury and thioredoxin and the redox state, modified by thioredoxin, played a crucial role in brain damage during stroke (Takagi *et al.*, 1999). Overexpression of thioredoxin negatively regulated p38 MAP kinase activation and the p38 MAP kinase-mediated IL-6 production by TNF-alpha-stimulated cells. This indicates that thioredoxin was critical for the p38 MAP kinase activation that regulated the cytokine expression (Hashimoto *et al.*, 1999).

Northern analysis and S1 mapping showed that the *E. coli trxA* gene contained two different promoters (Lim *et al.*, 1985). Their presence was confirmed by dissecting the two promoters into a promoter-cloning vector (Sa *et al.*, 1997). Previously, it was reported that the expression of the *E. coli trxA* gene was negatively regulated by cAMP (Sa *et al.*, 1997). This was reported in studies using the *trxA-lac* fusion plasmids. In this paper we describe the effect of temperature on the expression of the *E. coli trxA* gene.

Effect of growth temperature In these experiments, the effect of the growth temperature was examined on the β-galactosidase synthesis of the fusion plamid pCL70 and pJH3 (Fig. 1). The fusion plasmid pCL70 contains the P1P2 promoter of the *E. coli trxA* gene in the vector pMC1403, whereas the fusion plamid contains only the P2 promoter in



Vector	Fusion plasmid	Reference
pMC1403	pCL70	Sa *et al.* (1997a)
pMC1403	pJH3	Sa *et al.* (1997a)
pJEL170	pMG521	Sa *et al.* (1997b)

Fig. 1. Fusion plasmid constructs used in this study.

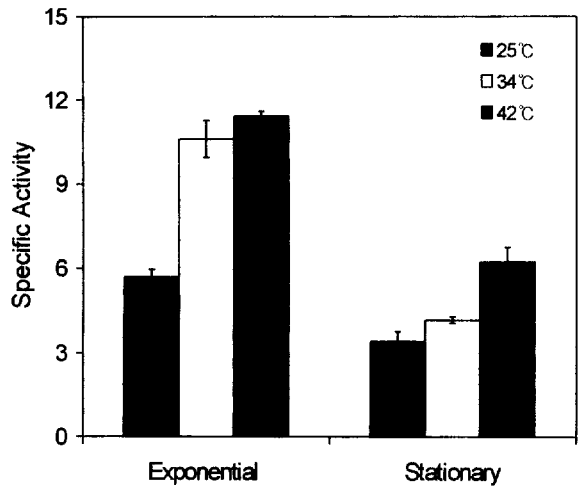


Fig. 2. Temperature-dependent synthesis of ß-galactosidase from the fusion plasmid pCL70, which carries the *E. coli trxA* P1P2 promoter. The *E. coli* MC1061/pCL70 cells were grown at 25°C, 34°C and 42°C, and harvested at the exponential and stationary phases. Specific activities of β-galactosidase were represented as ΔA_{420}/min/mg protein.

the same vector. The fusion plamids were introduced into the *E. coli lacZ* strain MC1061 that is suitable for detecting β-galactosidase synthesis from plasmids. The *E. coli* strain MC1061/pCL70 was grown in LB medium at 25°C, 34°C, and 42°C, and the cells were harvested at the exponential and stationary phases. As shown in Fig. 2, specific activities of the ß-galactosidase became higher as the temperature increased. The specific activity in the cells grown at 42°C was almost 2-fold compared with those in the cells grown at 25°C. This temperature effect is evident in both exponential and stationary phases. It was also found that the β-galactosidase specific activities were generally lower in the stationary phase than in the exponential phase. These results suggest that the expression of the *E. coli trxA* gene could be dependent on temperature. To verify the effect of the growth temperature on the P2 promoter of the *E. coli trxA* gene, the *E. coli* MC1061/pJH3 cells were grown at 25°C, 34°C, and 42°C. Then, the β-galactosidase activities were assayed using the cells harvested at the exponential and stationary phases. As shown in Fig. 3, the temperature effect became more evident. Specific activities of the β-galactosidase in the cells grown at 42°C appeared to be 2.5-fold and 3-fold in the exponential and stationary phases, respectively, compared with those in the cells grown at 25°C. This indicated that the expression from

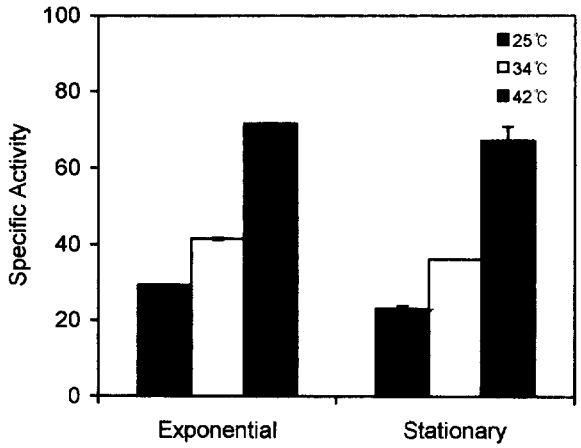


Fig. 3. Temperature-dependent synthesis of β-galactosidase from the fusion plasmid pJH3, which carries the *E. coli trxA* P2 promoter. The *E. coli* MC1061/pJH3 cells were grown at 25°C, 34°C and 42°C, and harvested at the exponential and stationary phases. Specific activities of β-galactosidase were represented as ΔA_{420}/min/mg protein.

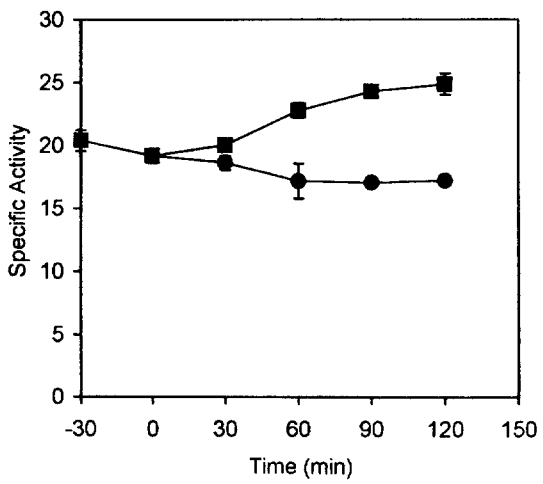


Fig. 4. Effect of temperature shift on the β-galactosidase from the fusion plasmid pJH3, which carries the *E. coli trxA* P2 promoter. The *E. coli* MC1061/pJH3 cells were grown at 25°C (-●-), and the culture was split at the exponential phase, and one culture flask was transferred to 42°C (-■-). Specific activities of β-galactosidase were represented as ΔA_{420}/min/mg protein.

the P2 promoter of the *E. coli trxA* gene was very sensitive to temperature. However, it did not make a large difference in the specific activities of the β-galactosidase in the cells harvested at the exponential and stationary phases.

Effect of temperature shift In these experiments, it is probable that temperature is one of the regulating factors in the expression of the *E. coli trxA* gene. The experiment detected the long-term effect of temperature in the expression of the *trxA* gene. In the temperature-shift experiment, the two-fusion plasmids, pJH3 and pMG521, were used. Both fusion

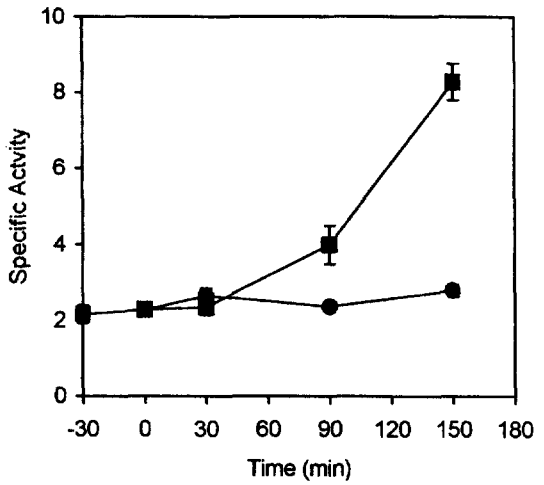


Fig. 5. Effect of temperature shift on the β-galactosidase synthesis from the *trxA-lacZ* fusion plasmid pMG521 harboring the *E. coli trxA* P2 promoter. The *E. coli* MC1061/pMG521 cells were grown at 34°C (-●-), and the culture was split at the exponential phase, and one culture flask was then shaken at 42°C (-■-). Specific activities of β-galactosidase were represented as ΔA_{420}/min/mg protein.

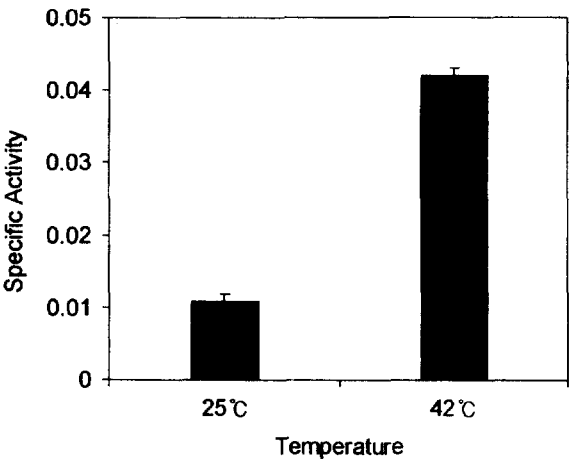


Fig. 6. Thioredoxin activities of the wild-type *E. coli* strain MC1061. The *E. coli* cells were grown at 25°C and 42°C, and then harvested at the stationary phase. Specific activities of thioredoxin were represented as ΔA_{412}/min/mg protein.

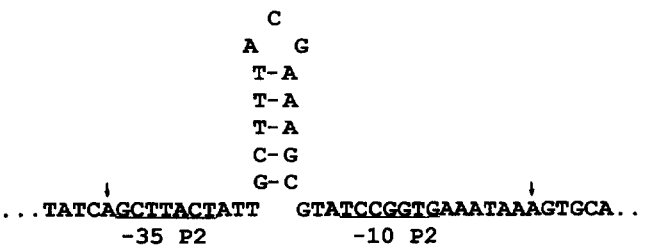


Fig. 7. Plausible stem-loop structure between -10 and -35 regions of the *E. coli trxA* P2 promoter. The arrows above the sequence indicate the two transcription initiation sites of the *E. coli trxA* gene.

plasmids contain only the P2 promoter of the *E. coli trxA* gene. The fusion plamid pJH3 was constructed in the vector pMC1403, whereas the fusion plamid pMG521 was constructed in the vector pJEL170 (Fig. 1). Their copy numbers seem to be different, since the different origins of replication were used in the construction of those vectors. In the exponential phase of the *E. coli* MC1061/pJH3 cells, specific activities of the ß-galactosidase elevated to 1.4-fold after the temperature shift of 25°C to 42°C (Fig. 4). This indicates that the temperature also has a short-term effect on the expression from the *E. coli trxA* gene. A similar effect was detected with the *E. coli* MC1061/pMG521 cells (Fig. 5). After shifting temperature to 42°C, it had up to 3-fold specific activity within 150 minutes. The shifting experiments strongly suggest that the P2 promoter is subject to temperature control. It could be at the transcriptional level, but it needs to be further examined on the RNA level.

Expression of the wild-type trxA gene Using the fusion plasmids, the possibility of temperature regulation was observed on the expression of the *E. coli trxA* gene. This was needed to detect the temperature effect in the expression of the wild-type *trxA* gene. The *E. coli* strain MC1061 contains the wild-type *trxA* gene. Therefore, the *E. coli* MC1061 cells were grown at 25°C and 42°C, and harvested at the stationary phase. The MC1061 cells produced a much higher thioredoxin activity in case of growth at 42°C (Fig. 6), thus confirming the temperature effect obtained from the experiments with the fusion plamids. The temperature-dependent expression of the wild-type *trxA* gene unambiguously supports the results obtained from the fusion plasmids.

Effect of hydrogen peroxide and cadmium Thioredoxin was reported to be involved in the response against oxidative stress. Therefore, the effect of hydrogen peroxide and cadmium chloride was examined on the synthesis of β-galactosidase from the fusion plasmid pCL70 (data not shown). The *E. coli* MC1061/pCL70 cells were grown in LB broth at 37°C, and the culture then split into three flasks. Hydrogen peroxide (1 mM) and cadmium chloride (0.005 mM) were added into the separate culture flasks. The cells were taken out at several time points and the β-galactosidase assay was performed. However, hydrogen peroxide and cadmium chloride made no change in the specific activities of the β-galactosidase, indicating that they do not play a role in the expression of *E. coli trxA* gene.

Our results indicate that the expression of the *E. coli trxA* gene is regulated by temperature presumably on a transcription level. The P2 promoter of the *E. coli trxA* gene particularly acts in a temperature-dependent manner. Previously the expression of the *Oenococcus oeni trxA* gene was induced by hydrogen peroxide and heat shock (Jobin *et*

al., 1999). The increased expression of thioredoxin genes may be related with responses against oxidative stress, since thioredoxin is involved in the regeneration mechanism for oxidative damages (Fernando *et al.*, 1992). The P2 promoter of the *E. coli trxA* gene contains a plausible stem-loop structure between its -10 and -35 regions (Fig. 7). Its stability does not appear to be strong, because five base pairs consist of the stem. The stem structure might be broken out at high temperature. The stem-loop structure may decrease the expression from the P2 promoter at the lower temperature. However, the detailed mechanism that is involved in the temperature regulation of the *E. coli trxA* gene remains to be elucidated and requires further study.

Acknowledgment The Basic Science Research Institute Program (BSRI-98-4439) supported this work.

References

An, G. and Wu, R. (1992) Thioredoxin gene expression is transcriptionally up-regulated by retinol in monkey conducting airway epithelial cells. *Biochem. Biophys. Res. Comm.* **183,** 170-175.

Bradford, M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein dye binding. *Anal. Biochem.* **72,** 245-254.

Buchanan, B. B., Wolosiuk, R. A. and Schürmann, P. (1979) Thioredoxin and enzyme regulation. *Trends Biochem. Sci.* **4,** 93-96.

Droux, M., Miginiac-Maslow, M., Jacquot, J. P., Gadal, P., Crawford, N. A., Kosower, N. S. and Buchanan, B. B. (1987) Ferredoxin-thioredoxin reductase: a catalytically active dithiol group links photoreduced ferredoxin to thioredoxin function in photosynthetic enzyme regulation. *Arch. Biochem. Biophys.* **256,** 372-380.

Fernando, M. R., Nanri, H., Yoshitage, S., Nagata-Kuno, K. and Minakami, S. (1992) Thioredoxin regenerates proteins inactivated by oxidative stress in endothelial cells. *Eur. J. Biochem.* **209,** 917-922.

Hashimoto, S., Matsumoto, K., Gon, Y., Furuichi, S., Maruoka, S., Takeshita, I., Hirota, K., Yodoi, J. and Horie, T. (1999) Thioredoxin negatively regulates p38 MAP kinase activation and IL-6 production by tumor necrosis factor-alpha. *Biochem. Biophys. Res. Comm.* **258,** 443-447.

Holmgren, A. (1979a) Reduction of disulfides by thioredoxin. Exceptional reactivity of insulin and suggested functions of thioredoxin in mechanism of hormone action. *J. Biol. Chem.* **254,** 9113-9119.

Holmgren, A. (1979b) Thioredoxin catalyzes the reduction of insulin disulfides by dithiothreitol. *J. Biol. Chem.* **254,** 9627-9632.

Holmgren, A. (1984) Enzymatic reduction-oxidation of protein disulfides by thioredoxin. *Methods Enzymol.* **107,** 295-300.

Jobin, M. P., Garmyn, D., Divies, C. and Guzzo, J. (1999) Expression of the *Oenococcus oeni trxA* gene is induced by hydrogen peroxide and heat shock. *Microbiol.* **145,** 1245-1251.

Larsen, J. E., Albrechtsen, B. and Valentin-Hansen, P. (1987) Analysis of the -terminator region after the *deoCABD* operon of *Escherichia coli* K-12 using a new class of single copy number operon-fusion vectors. *Nucleic Acids Res.* **15,** 5125-5140.

Laurent, T. C., Moore, E. C. and Reichard, P. (1964) Enzymatic synthesis of deoxyribonucleotides. IV. Isolation and characterization of thioredoxin, the hydrogen donor from *Escherichia coli* B. *J. Biol. Chem.* **239,** 3436-3443.

Lim, C.-J., Geraghty, D. and Fuchs, J. A. (1985) Cloning and nucleotide sequence of the *trxA* gene of *Escherichia coli* K-12. *J. Bacteriol.* **163,** 311-316.

Lim, C.-J., Haller, B. and Fuchs, J. A. (1985) Thioredoxin is the bacterial protein encoded by *fip* that is required for filamentous bacteriophage f1 assembly. *J. Bacteriol.* **161,** 799-802.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265-275.

Mark, D. F. and Richardson, C. C. (1976) *Escherichia coli* thioredoxin: a subunit of bacteriophage T7 DNA polymerase. *Proc. Natl. Acad. Sci. USA* **73,** 780-784.

Mark, D. F., Chase, J. W. and Richardson, C. C. (1977) Genetic mapping of *trxA*, a gene affecting thioredoxin in *Escherichia coli* K-12. *Mol. Gen. Genet.* **155,** 145-152.

Maurice, M. M., Nakamura, H., Gringhuis, S., Okamoto, T., Yoshida, S., Kullmann, F., Lechner, S., van der Voort, E. A., Leow, A., Versendaal, J., Muller-Ladner, U., Yodoi, J., Tak, P. P., Breedveld, F. C. and Verweij, C. L. (1999) Expression of the thioredoxin-thioredoxin reductase system in the inflamed joints of patients with rheumatoid arthritis. *Arthritis Rheum.* **42,** 2430-2439.

Miller, J. H. (1972) Experiments in molecular genetics, pp.352-355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Moore, E. E., Reichard, P. and Thelander, L. (1964) Enzymatic synthesis of deoxynucleotides. V. purification and properties of thioredoxin reductase from *Escherichia coli* B. *J. Biol. Chem.* **239,** 3445-3453.

Park, K.-N., Sa, J.-H. and Lim, C.-J. (1999) Sulfhydryl-related and phenylpropanoid-synthesizing enzymes in *Arabidopsis thaliana* leaves after treatments with hydrogen peroxide, heavy metals, and glyphosate. *J. Biochem. Mol. Biol.* **32,** 203-209.

Pasternak, C., Assemat, K., Breton, A. M., Clement-Metral, J. D. and Klug, G. (1996) Expression of the thioredoxin gene (*trxA*) in *Rhodobacter sphaeroides* Y is regulated by oxygen. *Mol. Gen. Genet.* **250,** 189-196.

Porque, P. G., Baldesten, A. and Reichard, P. (1970) The involvement of the thioredoxin system in the reduction of methionine sulfoxide and sulfate. *J. Biol. Chem.* **254,** 2371-2374.

Sa, J.-H., Fuchs, J. A. and Lim, C.-J. (1997) Effect of cyclic AMP on the two promoters of *Escherichia coli* thioredoxin gene. *J. Biochem. Mol. Biol.* **30,** 320-325.

Sa, J.-H., Namgung, M. A., Lim, C.-J. and Fuchs, J. A. (1997) Expression of the *Escherichia coli* thioredoxin gene is negatively regulated by cyclic AMP. *Biochem. Biophys. Res. Comm.* **234,** 564-567.

Sa, J.-H., Park, E.-H. and Lim, C.-J. (1998) Activities of sulfhydryl-related and phenylpropanoid-synthesizing enzymes

during leaf development of *Arabidopsis thaliana*. *J. Biochem. Mol. Biol.* **31**, 554-559.

Takagi, Y., Mitsui, A., Nishiyama, A., Nozaki, K., Sono, H., Gon, Y., Hashimoto, N. and Yodoi, J. (1999) Overexpression of thioredoxin in transgenic mice attenuated focal ischemic brain damage. *Proc. Natl. Acad. Sci. USA* **96**, 4131-4136.

Taniguchi, Y., Taniguchi-Ueda, Y., Mori, K. and Yodoi, J. (1996) A novel promoter sequence is involved in the oxidative stress-induced expression of the adult T-cell leukemia-derived factor (ADF)/human thioredoxin (Trx) gene. *Nucleic Acids Res.* **24**, 2746-2752.

Tsang, M. L.-S. and Schiff, J. A. (1976) Sulfate-reducing pathway in *Esherichia coli* involving bound intermediates. *J. Bacteriol.* **125**, 923-933.

Wetterrauer, B., Jacquot, J. P. and Veron, M. (1992) Thioredoxins from *Dictyostelium discoideum* are a developmentally regulated multigene family. *J. Biol. Chem.* **267**, 9895-9904.

Yoshida, S., Katoh, T., Tetsuka, T., Uno, K., Matsui, N. and Okamoto, T. (1999) Involvement of thioredoxin in rheumatoid arthritis: its costimulatory roles in the TNF-alpha-induced production of IL-6 and IL-8 from cultured synovial fibroblasts. *J. Immunol.* **163**, 351-358.