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NOTE

Transcriptional Regulation of the *Schizosaccharomyces pombe* Gene Encoding Glutathione S-Transferase I by a Transcription Factor Pap1

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In a previous study, a *gst* gene was isolated from the fission yeast *Schizosaccharomyces pombe*. This gene was dubbed *gst*I, and was characterized using the *gstI-lacZ* fusion plasmid pYSH2000. In this work, four additional fusion plasmids, pYSHSD1, pYSHSD2, pYSHSD3 and pYSHSD4, were constructed, in order to carry (respectively) 770, 551, 358 and 151 bp upstream regions from the translational initiation point. The sequence responsible for induction by aluminum, mercury and hydrogen peroxide was located in the range between -1,088 and -770 bp upstream of the *S. pombe gst*I gene. The same region was identified to contain the nucleotide sequence responsible for regulation by Pap1, and has one putative Pap1 binding site, TTACGTAT, located in the range between -954 ~ -947 bp upstream of the *gst*I gene. Negatively acting sequences are located between -1,088 and -151 bp. These findings imply that the Pap1 protein is involved in basal and inducible transcription of the *gst*I gene in the fission yeast *S. pombe*.

Key words: fission yeast, glutathione S-transferase, Pap1, *Schizosaccharomyces pombe*, transcriptional regulation

Glutathione S-transferases (GSTs; EC 2.5.1.18), a multigene family of cytosolic multi-functional proteins, are detoxifying enzymes which are present in all aerobic organisms. All cytosolic GSTs are, structurally, composed of homo- or hetero-dimers of subunits (MW, 23-27 kDa), and show a generally similar conformation, two domains joined by a short linker of several amino acid residues (Rossjohn *et al*., 1998; Angelucci *et al*., 2000). GSTs are involved in the intracellular binding and transport of a wide number of hydrophobic compounds, such as heme, drugs, and carcinogens (Bhargava *et al*., 1978; Homma *et al*., 1985).

The two types of *GST* gene (*GST*I and *GST*II) in the budding yeast *Saccharomyces cerevisiae* have previously been isolated and characterized (Choi *et al*., 1998). Notable findings in this research included the discovery that the expression of the *S. cerevisiae GST*I gene is induced after diauxic shift, and that the deletion of the *GST*I gene increased sensitivity to heat shock in stationary phase cells (Choi *et al*., 1998). In the yeast *Issatchenkia orientalis*, the level of expression of two *GST* genes was induced by *o*-dinitrobenzene, an electrophilic xenobiotic (Tamaki *et al*., 1999).

The fission yeast *Schizosaccharomyces pombe* resembles higher eukaryotes with respect to its genetics and physiology. Three *gst* genes encoding GSTI, GSTII and GSTIII were recently cloned from *S. pombe*, and their regulational characteristics have been studied under various stress conditions (Kim *et al*., 2001; Cho *et al*., 2002; Shin *et al*., 2002). The expression of the *S. pombe gst*I gene is induced by cadmium, mercury, hydrogen peroxide, menadione, and high temperature (Kim *et al*., 2001). The three *S. pombe gst* genes have also been reported to be induced in response to hydrogen peroxide, via Sty1 stress-activated protein kinase (Veal *et al*., 2002).

The yeast cells induce the transcription of genes required for detoxification of stressful agents, in response to various stresses (Lee *et al*., 2003). The two transcription factors, Pap1 and Atf1, have been established to be responsible for the induction of stress-related genes in the

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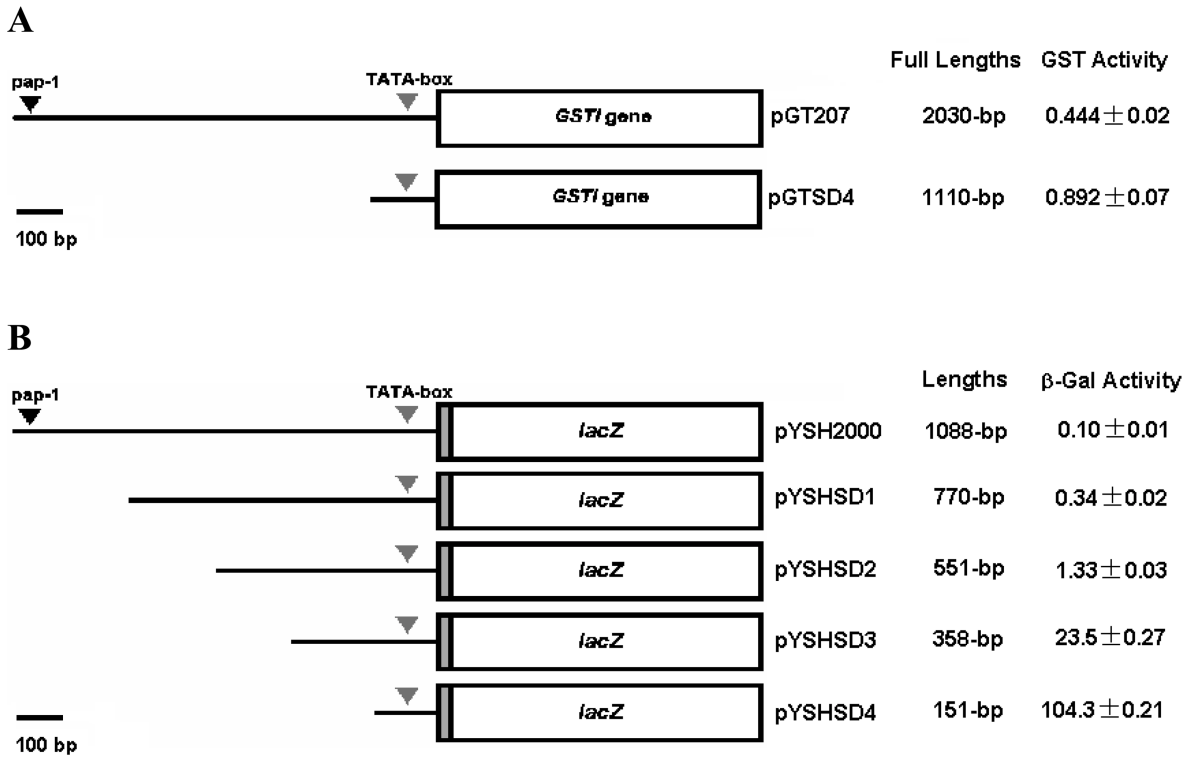

Fig. 1. A. Construction of the recombinant plasmids harboring two different sizes of the upstream region with the intact GSTI coding region. The *E. coli*-yeast shuttle vector pRS316 was used. The GST activity of the fission yeast cells harboring the two recombinant plasmids carrying different sizes of the upstream region. The yeast cells harboring recombinant plasmid were grown in minimal medium, and harvested at mid-exponential phase. The GST activity was determined at 25°C by spectrophotometric assay, using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate (Habig *et al.*, 1974), and its specific activity was represented as ΔA_{340}/min/mg protein. **B**. Constructions of serially deleted plasmids from the original fusion plasmid pYSH2000 using the promoterless yeast-*E. coli* shuttle vector YEp357R. β-Galactosidase activity was determined at 25°C by spectrophotometric assay, using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate (Guarente, 1983), and its specific activity was represented as ΔA_{420}/min/mg protein.

fission yeast *S. pombe* (Nguyen *et al.*, 2000). The transcription factor Pap1, an *S. pombe* bZIP protein homologous to mammalian AP1, was shown to play an important role in responses to both oxidative stress and a variety of cytotoxic agents, and was also shown to bind DNA containing the consensus sequence TTACGTAA (Toone *et al.*, 1998; Fujii *et al.*, 2000). One plausible Pap1 binding site TTACGTAT, 7 out of 8 nucleotides identical with the consensus sequence, is found at the -954 ~ -947 bp upstream region of the *S. pombe gst*I gene (Fig. 1A). It could be responsible for Pap1 regulation of the *S. pombe gst*I gene. To find out whether transcription factor Pap1 is involved in the transcription of the *S. pombe gst*I gene, we used two constructs, containing different lengths of the upstream regions, together with the intact *gstI* coding region (Fig. 1A), and the five *gst*I-*lacZ* fusion plasmids, containing serially deleted upstream regions of the *gst*I gene (Fig. 1B). As shown in Fig. 1A, the *S. pombe* KP1 cells (h^+ *leu*1-32 *ura*4-294) harboring plasmid pGTSD4 showed 2.01-fold higher GST activity than did cells harboring pGT207, and their growth was more rapid than that of the fission yeast cells harboring plasmid pGT207 (data not shown). These results imply that negatively acting sequence(s) might exist between -1,088 and -151 base pairs. However, the mechanisms underlying the activities of these sequences remain unclear.

To identify the upstream region responsible for the regulation of the *gst*I gene, its upstream regions were serially deleted from the original fusion plasmid pYSH2000 by PCR (Fig. 1B). The appropriately synthesized five primers (primer 1, 5'-acagttgaaaatcccattgtggatccctagcatg-3'; primer 2, 5'-tctgaaaactacatgtcttaggatcctgaaacag-3'; primer 3, 5'-tagcgtgtaaccggctaacaggatccaaatgta-3'; primer 4, 5'-ccaaaattttttcataaatattggatccgcaatgga-3'; primer 5, 5'-ttgaaccaccttccatgaattcggaccatgagc-3') were used for PCR amplification by *Pyrobest*® DNA polymerase (Takara shuzo co., Japan). Primer 5 was used in common to generate four additional fusion plasmids. The amplified DNA fragments contained *Bam*HI and *Eco*RI restriction sites, which originated from the five synthetic primers. The fusion plasmids pYSHSD1, pYSHSD2, pYSHSD3, and pYSHSD4 were confirmed to contain (respectively) 770, 551, 358, and 151 base pairs of the upstream region of the *gst*I gene (Fig. 1B). The five fusion plasmids, one original and four newly-constructed

Table 1. Effects of aluminum, mercury and hydrogen peroxide on the β-galactosidase activity of the serially deleted *gst*I-*lacZ* fusion genes in *S. pombe*

Agents	Concentration (mM)	Induction Fold[a]				
		pYSH2000	pYSHSD1	pYSHSD2	pYSHSD3	pYSHSD4
Aluminum	5.0	1.92 ± 0.15	1.09 ± 0.08	1.14 ± 0.06	1.31 ± 0.01	0.77 ± 0.01
Mercury	0.001	1.81 ± 0.16	1.15 ± 0.09	1.25 ± 0.06	1.07 ± 0.03	0.91 ± 0.01
Hydrogen peroxide	2.0	2.03 ± 0.13	1.09 ± 0.03	1.02 ± 0.09	1.01 ± 0.07	0.96 ± 0.05

The yeast cells harboring individual *gst*I-*lacZ* fusion gene were grown in minimal medium, and split at the early exponential phase. The yeast cells were harvested 6 h after the treatment. β-Galactosidase activity was determined at 25°C by spectrophotometric assay, using ONPG as a substrate, and its specific activity was expressed in ΔA_{420}/min/mg protein.

[a] Induction fold was calculated by considering the specific activity of the corresponding untreated cultures as 1.

fusion plasmids were individually introduced into the KP1 strain of *S. pombe*. The *S. pombe* cells containing each fusion plasmid were grown in minimal media, and harvested at the mid-exponential phase. β-Galactosidase synthesis from four newly-constructed fusion plasmids, pYSHSD1, pYSHSD2, pYSHSD3 and pYSHSD4, was found to occur at rates (respectively) 3.34-, 13.3-, 235- and 1043-fold higher than from the β-galactosidase synthesis rate of pYSH2000 (Fig. 1B). β-Galactosidase synthesis from the fusion plasmid pYSHSD4 appeared to be much higher than that from pYSH2000, which is consistent with the finding of significant elevation of GST activity in the fission yeast cells harboring plasmid pGTSD4 under normal growth conditions. Since the basal expression of the *S. pombe gst*I gene was markedly lower than that of the *gst*II and *gst*III gene (data not shown), the increase in GST activity in the yeast cells harboring plasmid pGTSD4 was observed to be relatively lower than the increase in β-galactosidase activity in the yeast cells harboring the fusion plasmid pYSHSD4. These results support the existence of negatively acting sequence(s) located in the upstream region of the *S. pombe gst*I gene.

Since the expression of the *gst*I gene was induced by heavy metals and ROS (Kim *et al*., 2001), the responsible region for the induction was identified. The yeast cells harboring each fusion plasmid were treated with 5.0 mM aluminum chloride, 1 μM mercuric chloride, or 2.0 mM hydrogen peroxide, and the cells were harvested 6 h after the treatments. As shown in Table 1, the synthesis of β-galactosidase from the fusion pYSH2000 in the cells treated with aluminum (5.0 mM) and mercury (1 μM) were about 1.92- and 1.81-fold higher than that in the corresponding untreated cells, respectively. However, in the yeast cells harboring other fusion plasmids, β-galactosidase synthesis was shown to be uninduced by the same agents (Table 1). The same pattern was manifested with respect to hydrogen peroxide (2 mM, Table 1), cadmium and superoxide-generating menadione (data not shown). Taken together, our results suggest that the upstream region responsible for induction by metal ions and ROS resides in the range between -1,088 and -770 bp from the translational initiation point.

To verify whether transcription of the *S. pombe gst*I gene is regulated by the transcription factor Pap1, the yeast cells harboring the five fusion plasmids with the Pap1-overexpressed plasmid pST1 (Toda *et al*., 1991) were individually grown in minimal medium, and harvested at mid-exponential phase. β-Galactosidase synthesis in the yeast cells harboring pYSH2000 and pST1, was found to occur at a level about twice as high as in cells with pYSH2000 only, while those in yeast cells containing each of the other fusion plasmids and pST1 were not enhanced (Fig. 2). Basal β-galactosidase synthesis in the four fusion plasmids, pYSHSD4, pYSHSD3, pYSHSD2 and pYSHSD1, was higher than in pYSH2000 (Fig. 1B); however, these β-galactosidase activities were clearly not induced by Pap1, due to the lack of the putative Pap1 binding site in their upstream regions. These results suggest that the basal expression of the *S. pombe gst*I gene is dependent on the presence of Pap1, and that the responsible upstream sequence is located between -1,088 and -770 bp from the translational initiation point, in which a plausible Pap1 binding site can be identified.

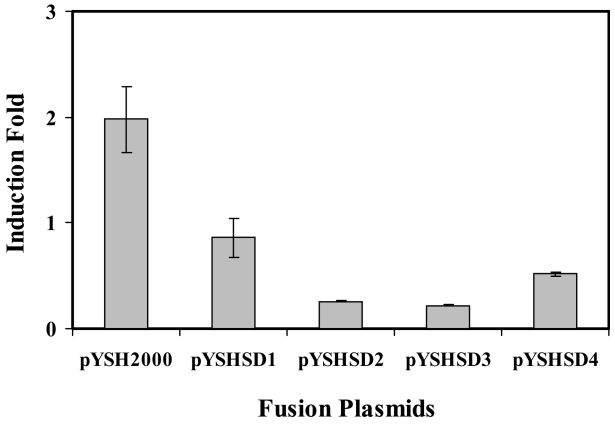

Fig. 2. β-Galactosidase activity of the serially deleted fusion plasmids in the presence of pST1, a Pap1-overexpressed plasmid in *S. pombe*. The yeast cells were grown in minimal medium, and harvested at mid-exponential phase. β-Galactosidase activity was determined at 25°C by spectrophotometric assay, using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate (Guarente, 1983), and its specific activity was represented as ΔA_{420}/min/mg protein. Induction fold was calculated by considering the specific activity of the corresponding yeast cultures without a Pap1-overexpressed plasmid as 1.

Analysis on the upstream sequence of the *S. pombe gst*I gene shows that it contains a plausible binding site for Pap1, which is known to be involved in stress responses (Nakagawa *et al*., 2000). The Pap1 binding site of *S. pombe gst*I gene is functional, and involved in the responses of *S. pombe gst*I gene to various stresses. Also, the basal and induced levels of *S. pombe* GSTI mRNA are totally dependent on the Pap1 transcription factor, under low levels of hydrogen peroxide (Veal *et al*., 2002). In previous studies, the expression of the *S. pombe gst*II gene was also shown to be regulated by the Pap1-dependent signal pathway (Lim *et al*., 2002), and the induction of the *gst*III gene by metal ions was regulated in a Pap1-independent manner (Sa *et al*., 2002). The findings obtained in this work, strongly suggest that the induction of the *S. pombe gst*I gene could be linked with Pap1. However, other upstream sequences may also be responsible for the mechanisms underlying regulation of the *S. pombe gst*I gene. Therefore, further study is indicated, to elucidate the precise regulatory mechanisms involving the transcription of the *S. pombe gst*I gene.

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