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Role of Atf1 and Pap1 in the Induction of the Catalase Gene of Fission Yeast *Schizosaccharomyces pombe*

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We examined the induction of the catalase gene (*ctt1*$^+$) of fission yeast *Schizosaccharomyces pombe* in response to several stresses by using mutants of transcription factors (Atf1 and Pap1) and a series of deletion mutants of the *ctt1*$^+$ promoter region. A transcription factor, Atf1, and its binding site are necessary for the induction of *ctt1*$^+$ by osmotic stress, UV irradiation, and heat shock. Induction by menadione treatment, which produces superoxide anion, required element A, the region from –111 to –90 (numbered with the transcription start site as +1). The factor responsible for the induction of the gene by oxidative stress *via* element A was identified as the transcription factor Pap1. We also found that Atf1 is activated by menadione treatment in *pap1* mutant cells, although it is not activated by menadione treatment in *pap1*$^+$ cells. The activity of catalase is not increased in *pap1* cells by several stresses, despite mRNA induction, suggesting that Pap1 plays some role in the expression of catalase activity.

Key words: Atf1, catalase, Pap1, *Schizosaccharomyces pombe*, stress response.

Living organisms adapt to adverse environmental change by utilizing defensive systems. The key reaction of the adaptation is a transcriptional induction of the genes of defensive systems. Recently, the induction of defensive proteins mediated by stress activated protein kinases (SAPKs) has received much attention (*1–4*). This system is involved in resistance to reactive oxygen species, UV irradiation, and high osmolarity.

Fission yeast *Schizosaccharomyces pombe* are widely used to study fundamental cellular processes such as the cell cycle or gene expression. We studied the expression of the catalase gene (*ctt1*$^+$) as a model system of stress response of *S. pombe*. Catalase is an enzyme that degrades hydrogen peroxide and plays an important role in the adaptation of *S. pombe* cells to high concentrations of hydrogen peroxide (*5*). The expression of *ctt1*$^+$ in *S. pombe* is induced by several stresses including hydrogen peroxide treatment, UV irradiation, and osmotic stress (*6*). This induction is controlled at the transcriptional level and does not require *de novo* protein synthesis (*6*). Previously, we showed that at least two promoter elements are required for the induction of *ctt1*$^+$ by hydrogen peroxide. One is the binding site for the transcription factor Atf1 that resides at –57 to –50 (numbered with the transcription start site as +1), and the other is an element residing at –111 to –90 that we have called element A (*7*). The transcription factor that binds to element A has not been determined. Atf1, a bZIP transcription factor with homology to mammalian ATF/CREB, is activated by Spc1/Sty1 mitogen activated protein kinase (MAPK), the *S. pombe* homologue of mammalian SAPKs (*8–12*). A transcription factor, Pap1, a bZIP protein in *S. pombe* that shows homology to mammalian AP1 (*13*), plays an important role in the response to oxidative stress and a variety of cytotoxic agents (*14*, *15*). Pap1 activity is regulated by nuclear export of the protein (*14*) and the C-terminal cysteine rich domain of Pap1 is important for this nuclear export (*16*).

Here we report the deletion analysis of the promoter region of *ctt1*$^+$ and the role of Atf1 and Pap1 transcription factors in the induction of *ctt1*$^+$ by various stresses. We also present evidence that Pap1 plays some role in the expression of catalase activity.

MATERIALS AND METHODS

S. pombe Strains and Growth Conditions—*Schizosaccharomyces pombe* strain JY741 was a gift from Prof. Y. Hotta of the Nara Institute of Science and Technology. TP108-3C (*pap1* disruptant) (*13*) was a gift from Prof. M. Yanagida of Kyoto University. CN803 (*atf1* disruptant) and a series of deletion mutants of the *ctt1*$^+$ promoter region were constructed as described (*7*). CNd-116A (*atf1* and deletion of the catalase promoter region –672 to –117) and CNd-116P (*pap1* and deletion of the catalase promoter region –672 to –117) were constructed from CNd-116 by the one-step gene disruption method (*17*) using the DNA fragment containing *atf1::ura4*$^+$ and *pap1::ura4*$^+$, respectively. CNd-116DA, a deletion mutant of the *atf1* gene, was constructed from CNd-116A by recombination of chromosomal *atf1::ura4*$^+$ with a mutated *atf1* gene (a deletion between two *Hin*dIII sites in the Atf1 coding region), and was selected for in the presence of 5-fluoroorotic acid (*18*). CNd-116AP (*atf1*, *pap1*, and deletion of catalase promoter region –672 to –117) was constructed from CNd-116DA by the one step gene disruption method using the DNA fragment containing the *pap1::ura4*$^+$ gene. The mutations of these strains were con-

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Abbreviation: MAPK, mitogen activated protein kinase.

firmed by PCR as described (*7*). The genetic backgrounds of the strains used in this study are listed in Table I. *S. pombe* cells were grown in SD medium (0.67% yeast nitrogen base without amino acids, 2% glucose) supplemented with the required nutrients at a concentration of 50 μg/ml at 30°C with vigorous shaking. Stress treatments of the cells were performed as described (*6*). Cells were harvested 30 min after stress treatment for RNA analysis and 60 min after stress treatment for enzyme assay.

Hybridization Analysis of RNA—Total cellular RNA was extracted from logarithmically growing cells by the method described by Elder *et al.* (*19*). Forty micrograms of RNA was electrophoresed in an 0.8% agarose gel containing formaldehyde. The gel was stained with ethidium bromide to confirm that the same amount of RNA was electrophoresed in each lane by visualizing the ribosomal RNA. RNA was transferred to a nitrocellulose membrane and hybridized with a ^{32}P-labeled probe (*20*) synthesized by the random primed labeling method (*21*) using [α-^{32}P]dCTP (111 TBq/mmol) and a 3.5 kb *Hin*dIII fragment containing *ctt1*$^+$ (*6*), or a 1.1 kb *Nsi*I fragment containing the glutathione peroxidase gene (*gpx1*$^+$) (*22*).

Assay for Catalase Activity—Cell extracts were prepared by vortexing cells with glass beads as described (*6*). The catalase activity of the cell extract was assayed by the method of Beers and Sizer (*23*). The protein concentration of the cell extract was determined by the method of Lowry *et al.* (*24*) using bovine serum albumin as a standard.

RESULTS

Functional Elements and Role of Atf1 in the Induction of ctt1$^+$ *in Response to Several Stresses*—Several sequences that resemble the consensus sequences of transcription factor binding sites are found in the 5′-flanking region of *ctt1*$^+$ of *S. pombe* (Fig. 1). Previously, we reported that the induction of *ctt1*$^+$ mRNA by hydrogen peroxide treatment is

TABLE I. ***Schizosaccharomyces pombe* strains used.**

Strain	Genotype
JY741	*h*$^-$, *ade6-M216, leu1, ura4-D18*
CN-803	*h*$^-$, *ade6-M216, leu1, ura4-D18, atf1::ura4*$^+$
CNd-X	*h*$^-$, *ade6-M216, leu1, ura4-D18, deletion –672 to –(X +1) in catalase X;313, 285, 121, 116, 111, 89, 55*
CNd-116A	*h*$^-$, *ade6-M216, leu1, ura4-D18, atf1::ura4*$^+$, *deletion –672 to –117 in ctt1*$^+$
TP108-3C	*h*$^-$, *leu1, ura4, pap1::ura4*$^+$
CNd-116P	*h*$^-$, *ade6-M216, leu1, ura4, pap1::ura4*$^+$, *deletion –672 to –117 in ctt1*$^+$
CNd-116A	*h*$^-$, *ade6-M216, leu1, ura4, pap1::ura4*$^+$, *Δatf1, deletion –672 to –117 in ctt1*$^+$

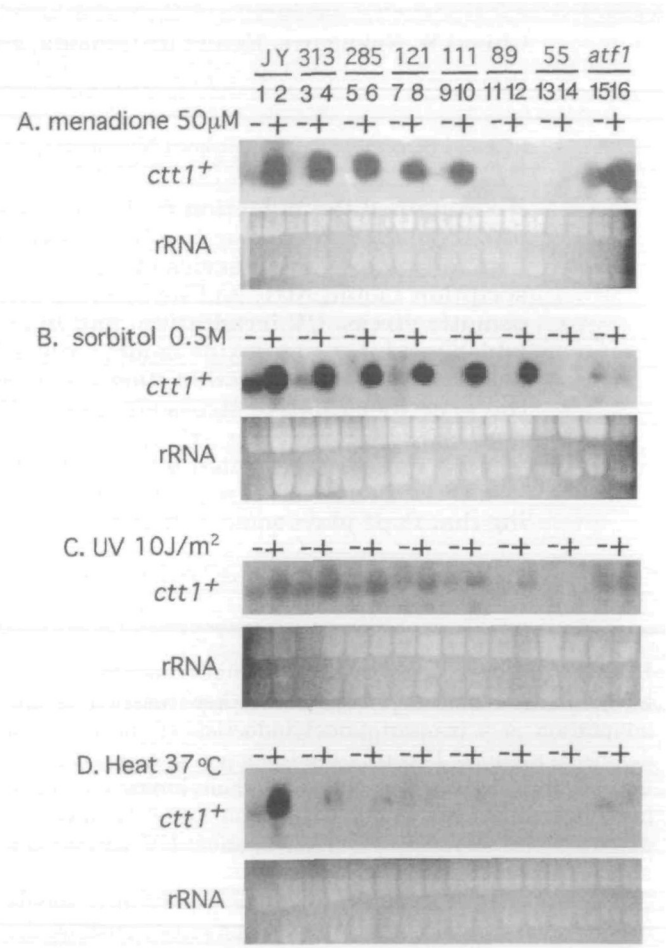

Fig. 2. **The *ctt1*$^+$ mRNA expression in response to menadione (50 μM) (A), osmotic stress (0.5 M sorbitol) (B), UV irradiation (255 nm 10 J/m^2) (C), and heat shock (37°C) (D).** Total cellular RNA was prepared from JY741 (wild type) (lanes 1 and 2); CNd-313 (lanes 3 and 4); CNd-285 (lanes 5 and 6); CNd-121 (lanes 7 and 8); CNd-111 (lanes 9 and 10); CNd-89 (lanes 11 and 12); CNd-55 (lanes 13 and 14); CN803 (*atf1*) (lanes 15 and 16) in the absence or presence of each stress. The amount of *ctt1*$^+$ mRNA was assessed by Northern hybridization analysis. The upper panel shows the amount of *ctt1*$^+$ mRNA, and the lower panel the ribosomal RNA stained with ethidium bromide, demonstrating that an equal amount (40 μg) of total cellular RNA was loaded in each lane.

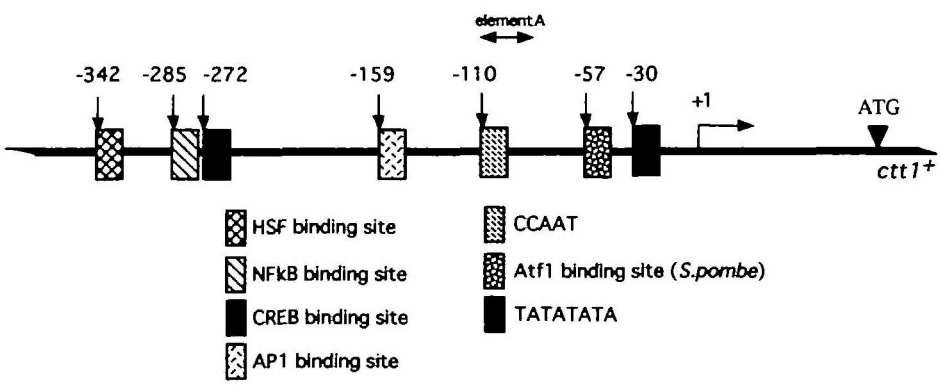

Fig. 1. **The 5′-flanking region of *ctt1*$^+$.** Putative transcription factor binding sites, Atf1 binding site, element A, and a TATA box in the promoter region are shown. Numbers indicate the nucleotide positions relative to the transcription start site (+1).

mediated through two elements, a region at –89 to –55 containing a binding site for the transcription factor Atf1, and a region at –111 to –90 that we named element A (7). We analyzed the promoter region that affects the induction of *ctt1*$^+$ under stress conditions involving menadione treatment (50 μM), osmotic stress (0.5 M sorbitol), UV (255 nm) irradiation (10 J/m^2), and heat shock (37°C). Northern blot analysis of wild type cells and a series of deletion mutants, named CNd-X, with deletions at –672 to –(X + 1) of the *ctt1*$^+$ promoter region where X represents the nucleotide number upstream from the transcription start site, was conducted and the results are shown in Fig. 2, A–D, lanes 1–14.

The induction of *ctt1*$^+$ mRNA by menadione treatment was not observed in CNd-89 (Fig. 2A, lanes 11 and 12). The induction of the *ctt1*$^+$ mRNA by menadione treatment in CNd-121 was less marked than in CNd-285 (Fig. 2A, lanes 5–8). This indicates that element A (–111 to –90) and the region –285 to –122 contain important transcription elements for the induction of *ctt1*$^+$ mRNA by menadione treatment. Catalase activity was low in CNd-89 (containing the Atf1 binding site but lacking element A), while in CNd-111 (containing element A) the catalase activity was increased upon menadione treatment (Fig. 3A). These results confirm that element A is important for the induction of *ctt1*$^+$ mRNA by menadione treatment.

In response to osmotic stress, *ctt1*$^+$ mRNA was induced in CNd-89, which contains the Atf1 binding site but lacks element A, while it was not observed in CNd-55, which lacks both the Atf1 binding site and element A (Fig. 2B, lanes 11–14). In response to UV irradiation, *ctt1*$^+$ mRNA was

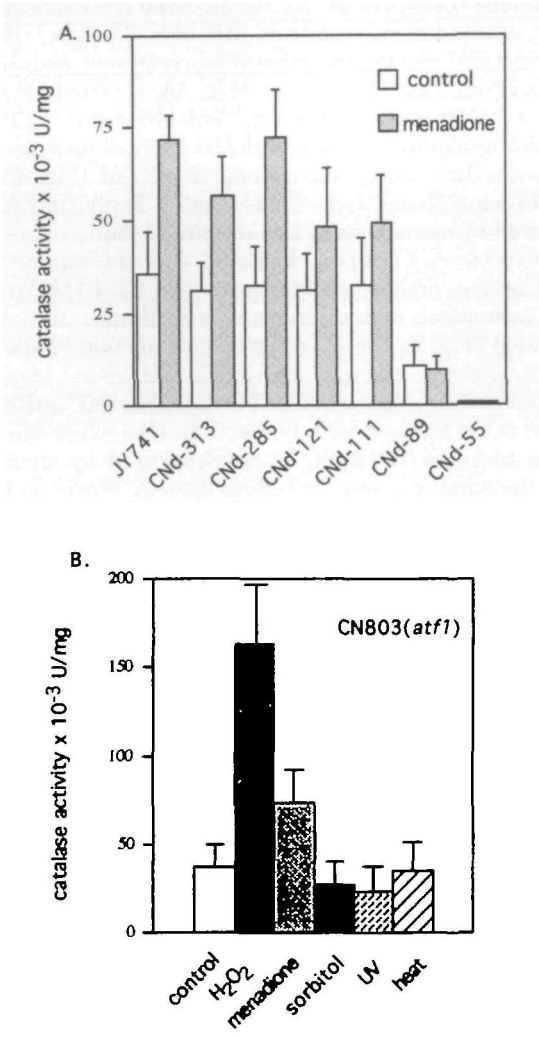

Fig. 3. **The catalase activity of the deletion mutants and JY741 in response to menadione (A). The catalase activity of CN803 (*atf1*) subjected to several stresses (B).** The cells were treated with hydrogen peroxide (0.2 mM), menadione (50 μM), osmotic stress (0.5 M sorbitol), UV irradiation (255 nm, 10 J/m^2), and heat shock (37°C). Catalase activity without stress is expressed by the open bars and stress-induced activity by a patterned bars. Results are means ± SE (vertical bar) of three independent experiments.

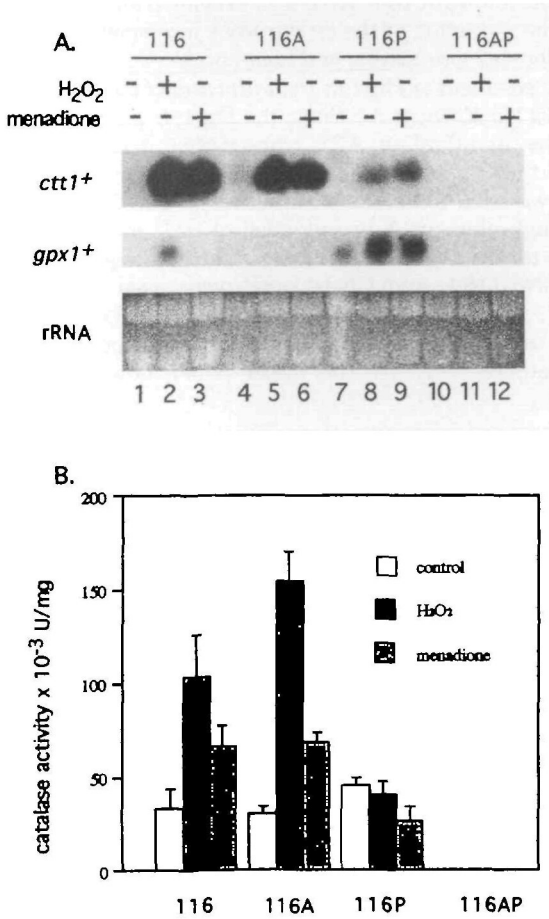

Fig. 4. **The effects of Atf1 and Pap1 on the induction of *ctt1*$^+$ *via* element A (A), and on catalase activity (B).** Total cellular RNA was prepared from CNd-116 (deletion of nucleotides –672 to –117) (lanes 1–3), CNd-116A (deletion of nucleotides –672 to –117 and *atf1*) (lanes 4–6), CNd-116P (deletion of nucleotides –672 to –117 and *pap1*) (lanes 7–9) and CNd-116AP (deletion of nucleotides –672 to –117, *pap1* and *atf1*) (lanes 10–12). The amount of *ctt1*$^+$ mRNA (the upper panel) and *gpx1*$^+$ mRNA (the middle panel) were assessed by Northern hybridization analysis. RNA was prepared from non-treated cells (lanes 1, 4, 7, and 10), hydrogen peroxide (0.2 mM)-treated cells (lanes 2, 5, 8, and 11), and menadione (50 μM)-treated cells (lanes 3, 6, 9, and 12). The lower panel shows ribosomal RNA stained with ethidium bromide, demonstrating that an equal amount (40 μg) of total cellular RNA was loaded in each lane. The catalase activity of each strain is mean ± SE (vertical bar) of three independent experiments.

weakly induced even in the wild type. The induction of *ctt1*$^+$ mRNA by UV irradiation decreased gradually as the deletion was extended, and was lost in CNd-55, which lacks the Atf1 binding site (Fig. 2C, lanes 13 and 14). In response to heat shock, there was almost no induction of *ctt1*$^+$ mRNA in CNd-313 (Fig. 2D, lanes 3 and 4). These results indicate that the region –89 to –55, which contains an Atf1 binding site, is involved in the induction of *ctt1*$^+$ mRNA by osmotic stress and UV irradiation. In order to clarify the role of the Atf1 transcription factor in the induction of *ctt1*$^+$ by these stresses, we assessed the amount of *ctt1*$^+$ mRNA in CN803 (*atf1*) (Fig. 2, A–D, lanes 15 and 16). The induction of *ctt1*$^+$ mRNA in CN803 by osmotic stress, heat shock, and UV irradiation was scarcely observed. Catalase activity was not increased by these stresses in CN803 (Fig. 3B), a finding consistent with the results of Northern blot analysis. These results indicate that Atf1 is a crucial transcription factor for the induction of the *ctt1*$^+$ mRNA in response to UV irradiation, osmotic stress, and heat shock.

Involvement of Pap1 in the Induction of ctt1$^+$ *by Oxidative Stress via Element A*—Since the Pap1, *S. pombe* homologue of the mammalian AP1 transcription factor, has been reported to be involved in the oxidative stress response (*14, 15*), we tried to determine whether or not the factor works through element A in induction of *ctt1*$^+$ is Pap1. To eliminate the effects of Atf1 and the upstream region of element A, CNd-116A, and CNd-116AP were used. In CNd-116A (*atf1*, *pap1*$^+$, and containing element A), the *ctt1*$^+$ mRNA was induced by treatment with hydrogen peroxide or menadione (Fig. 4A, the upper panel, lanes 4–6), but in CNd-116AP (*pap1*, *atf1*, and containing element A), it was not induced by treatment with hydrogen peroxide or menadione (Fig. 4A, the upper panel, lanes 10–12). The catalase activity in CNd-116A was increased under oxidative stress, while the activity in CNd-116AP was not detected (Fig. 4B). These results indicate that Pap1 is responsible for the induction of *ctt1*$^+$ by oxidative stress *via* element A.

From the results that Pap1 works *via* element A and that Atf1 is not affected by menadione treatment, we expected that *ctt1*$^+$ mRNA would not be induced by menadione in CNd-116P (*pap1*, *atf1*$^+$, and containing element A). The *ctt1*$^+$ mRNA in CNd-116P was induced by hydrogen peroxide treatment (Fig. 4A, the upper panel, lanes 7 and 8). This is reasonable given that hydrogen peroxide treatment induces *ctt1*$^+$ expression *via* the Atf1 binding site (*7*). Unexpectedly, the *ctt1*$^+$ mRNA was induced in CNd-116P by menadione treatment (Fig. 4A, the upper panel, lanes 7 and 9). To test whether Atf1 is activated in CNd-116P by menadione treatment or not, we assessed the expression of *gpx1*$^+$, which depends solely on Atf1 (*22*). The *gpx1*$^+$ mRNA in CNd-116P was highly induced by treatment with hydrogen peroxide and menadione (Fig. 4A, the middle panel, lanes 7–9). The *gpx1*$^+$ mRNA in CNd-116 (*pap1*$^+$, *atf1*$^+$, and containing element A), was induced by hydrogen peroxide treatment but not by menadione treatment (Fig. 4A, the middle panel, lanes 1–3). These results imply that Atf1 is activated by menadione in the absence of Pap1.

Involvement of Pap1 in the Expression of Catalase Activity—Catalase activity is not increased in CNd-116P treated with menadione or hydrogen peroxide despite the mRNA induction (Fig. 4). We examined the induction of the *ctt1*$^+$ mRNA and the enzyme activity in TP108-3C (*pap1*) in response to several stresses (Fig. 5). The *ctt1*$^+$ mRNA in TP108-3C is induced by stresses (Fig. 5A), while the catalase activity in TP108-3C is not increased by treatment with the same stresses, including osmotic stress and heat shock (Fig. 5B). These results suggest that Pap1 is involved in the induction of the enzyme activity in addition to mRNA induction.

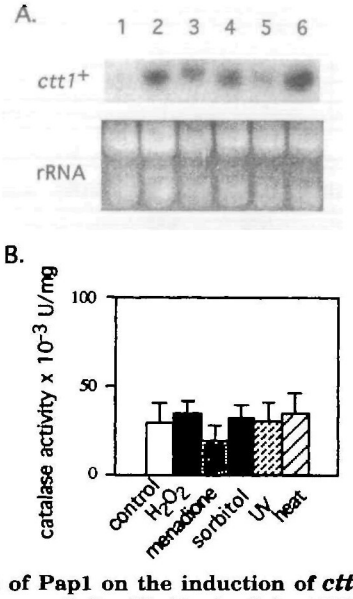

Fig. 5. **Effects of Pap1 on the induction of *ctt1*$^+$ mRNA (A) and on catalase activity (B).** Total cellular RNA was prepared from TP108-3C (*pap1*). The amount of *ctt1*$^+$ mRNA (the upper panel) was assessed by Northern hybridization analysis. RNA was prepared from non-treated cells (lane 1) and cells treated with hydrogen peroxide (0.2 mM) (lane 2), menadione (50 μM) (lane 3), sorbitol (0.5 M) (lane 4), UV irradiation (255 nm 10 J/m^2) (lane 5), and heat shock (37°C) (lane 6). The lower panel shows ribosomal RNA stained with ethidium bromide, demonstrating that an equal amount (40 μg) of total cellular RNA was loaded in each lane. The catalase activity of each strain is the mean ± SE (vertical bar) of three independent experiments.

DISCUSSION

We analyzed the induction of *ctt1*$^+$ in *S. pombe* in response to several stresses using mutants of transcription factors and a series of deletion mutants of the promoter region of *ctt1*$^+$. In response to menadione (Fig. 2A) and hydrogen peroxide (*7*), element A (–111 to –90) is important in the induction of the *ctt1*$^+$ mRNA by oxidative stress. To determine the factor functioning at element A, CNd-116A, which lacks Atf1, and *cis*-elements residing upstream of element A was constructed. The expression of the *ctt1*$^+$ mRNA and the enzyme activity in CNd-116A were induced as in CNd-116. Since Atf1 is working at –89 to –55 (*7*), the induction of *ctt1*$^+$ in CNd-116A is mediated by element A. In CNd-116AP, a *pap1* derivative of CNd-116A, neither the expression of *ctt1*$^+$ mRNA nor enzyme activity was detected. These findings indicate that the factor relevant to the induction of *ctt1*$^+$ mRNA *via* element A is Pap1. These results are consistent with the results reported by Toone *et al.* (*14*) that Pap1 is a transcription factor involved in the oxidative stress response in *S. pombe*. The consensus sequences of Pap1 binding site (*13, 25*) was not found in element A. More investigation is required to reveal the pre-

cise mechanism by which Pap1 induces *ctt1*$^+$ expression *via* element A.

We also showed that Atf1 is involved in the induction of *ctt1*$^+$ mRNA by hydrogen peroxide treatment, UV irradiation, osmotic stress, and heat shock in wild type cells. The failure to undergo Atf1 activation by menadione treatment (Fig. 2A) suggests that hydrogen peroxide and menadione, although both reactive oxygen species, use different signaling pathways to induce *ctt1*$^+$ mRNA. However, Atf1 is activated by menadione treatment in *pap1* cells (Fig. 4A). This suggests that Pap1 itself, or a product of the gene whose expression is under the control of Pap1, inhibits the cross talk between the intracellular signal transduction pathways which activate Atf1 and Pap1. Another possibility is that menadione treatment results in an accumulation of hydrogen peroxide or other stress agents, because of a lack of the enzyme whose expression is controlled under Pap1, to activate Atf1 in *pap1* cells.

Unexpectedly, catalase activity was not increased in *pap1* cells (Figs. 4B and 5B) even though the *ctt1*$^+$ mRNA was induced (Fig. 4A lanes 7–9 and Fig. 5A). This suggests that Pap1 or a gene product under Pap1 regulation plays some role in the post transcriptional regulation of *ctt1*$^+$ expression.

The results of *ctt1*$^+$ mRNA expression of promoter deletion mutants (Fig. 2) indicate that *cis*-elements besides element A and the Atf1 binding site are upstream of element A. Prr1 response regulator (*26*) which is an *S. pombe* homologue of *Saccharomyces cerevisiae* Skn7p transcription factor (*27, 28*) is involved in the induction of *ctt1*$^+$. The *cis*-elements that reside upstream of element A could include the sequence responsible for the induction of *ctt1*$^+$ by Prr1. Studies to identify the element responsible for the induction of *ctt1*$^+$ mediated by Prr1 are now in progress in our laboratory.

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