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NOTE

Carbon Source-Dependent Regulation of the *Schizosaccharomyces pombe pbh1* Gene

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Pbh1, from the fission yeast *Schizosaccharomyces pombe*, is a baculoviral inhibitor of apoptosis (IAP) repeat (BIR) domain-containing protein. Its unique encoding gene was previously found to be regulated by nitric oxide and nitrogen starvation. In the current work, the Pbh1-*lacZ* fusion gene was used to elucidate the transcriptional regulation of the *pbh1* gene under various carbon sources. When fermentable carbon sources, such as glucose (at a low concentration of 0.2%), sucrose (2.0%) and lactose (2.0%), were the sole carbon source, the synthesis of β-galactosidase from the Pbh1-*lacZ* fusion gene was reasonably enhanced. However, the induction by these fermentable carbon sources was abolished in the Pap1-negative *S. pombe* cells, implying that this type of induction of the *pbh1* gene is mediated by Pap1. Ethanol (2.0%), a nonfermentable carbon source, was also able to enhance the synthesis of β-galactosidase from the fusion gene in wild-type cells but not in Pap1-negative cells. The results indicate that the *S. pombe pbh1* gene is up-regulated under metabolic oxidative stress in a Pap1-dependent manner.

Keywords: carbon source, fission yeast, Pap1, Pbh1, *Schizosaccharomyces pombe*, transcriptional regulation

The inhibitor of apoptosis (IAP), originally identified in baculoviruses and present in diverse organisms from viruses to humans, regulates apoptosis by binding and inhibiting caspases (Uren *et al.*, 1998). IAPs are characterized by one to three copies of baculovirus IAP repeat (BIR) domains, which are zinc-binding domains of approximately 65 residues (Salvesen and Duckett, 2002). Although some BIR domain-containing proteins do not have anti-apoptotic properties, they still play an important role in fundamental cellular processes (Adams *et al.* 2000; Reed and Bischoff, 2000). For example, IAPs are known to have roles in the control of cell division and a variety of signaling cascades, such as transforming growth factor β activation, c-Jun N-terminal kinase regulation and nuclear factor κB activation (Salvesen and Duckett, 2002).

The unique gene encoding a BIR domain-containing protein, Pbh1 (*p*ombe *b*ir *h*omolog), was previously identified by the *Schizosaccharomyces pombe* genome sequencing project. Since the fission yeast does not contain caspase-like proteins, Pbh1p is thought to play a fundamental role in cellular regulation. In fact, Pbh1p is essential for yeast viability. Cells devoid of Pbh1p are defective in chromosome condensation and chromosome segregation (Rajagopalan and Balasubramanian, 1999). Functional Pbh1p was shown to be essential for localization of the *S. pombe* aurora kinase homolog Aim1p, a protein essential for mitosis, to the kinetochores and the spindle midzone, which indicates the importance of Pbh1p for multiple processes during mitosis in *S. pombe* (Rajagopalan and Balasubramanian, 2002).

Yeast cells ferment glucose producing ethanol and CO_2 even under aerobic conditions (Johnston and Carlson, 1992). They are able to sense the presence of glucose and regulate the expression of genes required for glucose uptake and metabolism (Johnston, 1999). In the budding yeast *S. cerevisiae*, the Snf1 kinase is known to play a role in adaptation to glu-

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cose depletion and in growth on carbon sources other than glucose, and has also been implicated in various stress responses (Schüller, 2003; Wiatrowski and Carlson, 2003). It was suggested that Yap1, a transcription factor of the AP-1 family required for the adaptive response to oxidative stress in *S. cerevisiae*, has a functional connection to Snf1 kinase. This supported the association of adaptation to carbon source with the increased response to oxidative stress (Wiatrowski and Carlson, 2003). In other words, glucose depletion or respiratory growth on nonfermentable carbon sources leads to increased oxidative stress response via Yap1 (Grant *et al.*, 1996).

We have previously shown that the *S. pombe pbh1* gene participates in the response to oxidative stress, and its transcription is up-regulated by nitric oxide (NO)-generating sodium nitroprusside (SNP) and nitrogen starvation (Cho *et al.*, 2006). In this continuing work, we pursued the regulatory mechanism of the *pbh1* gene under metabolic stress conditions induced by low glucose concentration or by carbon sources other than glucose. In order to conveniently monitor the expression of the *pbh1* gene, it was previously fused into the promoterless β-galactosidase gene of the *E. coli*-yeast shuttle vector YEp367R (Myers *et al.*, 1986). The fusion plasmid pPbh04 carries the Pbh1-*lacZ* fusion gene, containing the 1,223 bp upstream from the translational initiation point of the *pbh1* gene (Cho *et al.*, 2006). The *S. pombe* KP1 (*h*$^+$ *leu1-32 ura4-294*) and TP108-3C (*h*$^-$ *leu1-32 ura4D18 pap1::ura4*$^+$) strains were used as hosts for regulation studies. The fusion plasmid pPbh04 was introduced into the wild-type strain KP1 and Pap1-negative TP108-3C cells. The yeast cells harboring pPbh04 were grown in minimal medium, and then split at the early exponential phase and treated with suitable carbon sources instead of 2% glucose as a sole carbon source. β-Galactosidase activity in extracts was measured at 25°C by spectrophotometry using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate (Guarente, 1983). Protein concentration in extracts was determined according to the procedure of Bradford using bovine serum albumin (BSA) as a standard (Bradford, 1976).

Glucose is a powerful signaling molecule which causes major metabolic changes (Rolland *et al.*, 2001). Yeast cells growing in the presence of glucose or related rapidly-fermented sugars differ greatly in a variety of physiological properties when compared to cells grown in the absence of glucose (Thevelein *et al.*, 2000). Some of these differences are due to protein kinase A (PKA) and related signal transduction pathways. Deprivation of glucose causes a strong metabolic oxidative stress which results in increased steady-state levels of intracellular hydroperoxide and oxidized glutathione (Lee *et al.*, 1998; Song *et al.*, 2002). To see whether carbon sources other than glucose are able to influence the expression of the *pbh1* gene, yeast cells harboring the fusion plasmid pPbh04 were forced to switch their sole carbon source to one of various carbon compounds after removing the normal concentration of glucose. β-Galactosidase activity was measured 3 and 6 h after the switch. Low glucose concentration significantly enhanced the synthesis of β-galactosidase from the fusion plasmid pPbh04. Its specific activity had increased 2.4-fold 6 h after reducing the glucose concentration to 0.2% (Fig. 1A). A switch to this low glucose concentration (0.2%) did not delay the normal growth of the yeast (data not shown). Other fermentable carbon sources, such as lactose and sucrose, were also able to enhance the synthesis of β-galactosidase from the fusion plasmid

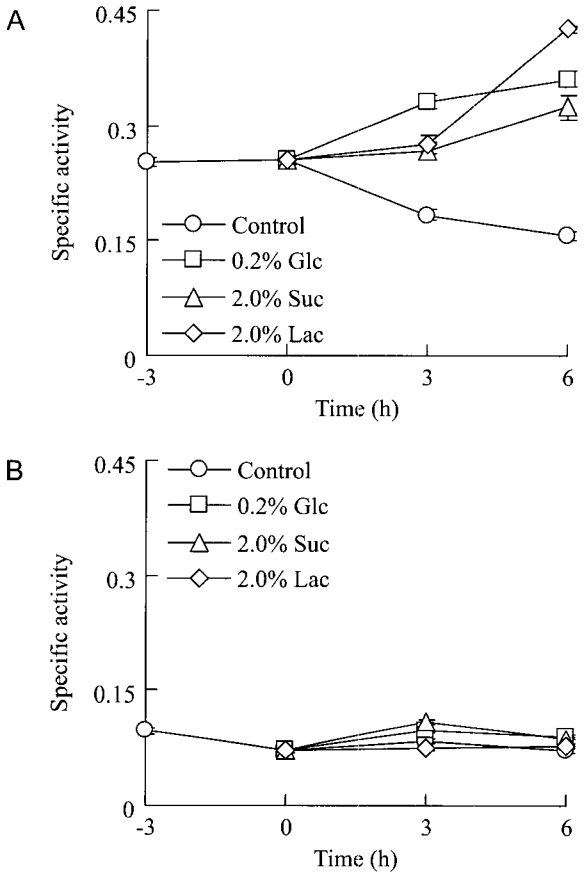

Fig. 1. The effects of fermentable carbon sources such as D-glucose (0.2% Glc), sucrose (2.0% Suc) and lactose (2.0% Lac) as sole carbon sources on the synthesis of β-galactosidase from the Pbh1-*lacZ* gene in the wild-type KP1 (A) and the Pap1-negative TP108-3C (B) cells. The yeast cells harboring the fusion plasmid pPbh04 were grown in minimal medium, and split at the early exponential phase. β-Galactosidase activity in the extracts was measured at 25°C by the spectrophotometric method, using ONPG as a substrate (Guarente, 1983). Its specific activity was represented in A_{420}/min/mg protein.

(Fig. 1A). β-Galactosidase activity increased 2.1- and 2.7-fold 6 h after treatment with 2.0% sucrose and 2.0% lactose, respectively (Fig. 1A). However, 2.0% sucrose and 2.0% lactose slightly delayed the growth of the yeast cultures (data not shown). The results suggest that the *pbh1* gene is subject to regulation by fermentable carbon sources.

Growth of the budding yeast *S. cerevisiae* on nonfermentable carbon sources, such as lactate, ethanol or acetate, requires the coordinate expression of several genes which are dispensable in the presence of substrates easily utilized by glycolysis (e.g. glucose or fructose) (Hiesinger *et al.*, 2001). In particular, growth with a nonfermentable carbon source requires coordinate transcriptional activation of gluconeogenic structural genes by an upstream activation site (UAS) element, designated CSRE (carbon source-responsive element). The four plausible CSRE motifs, CATACATTCCG, TCTCAAGTCCG, CCATCGCATCG and CCAGTG AACCT, can be detected within 1,223 bp upstream from the translational initiation point of the *pbh1* gene. They are identical to 10 out of 11 nucleotides with the consensus CCN_6CCG typical of a subfamily among zinc cluster proteins (Walther and Schüller, 2001). For this reason expression of the *pbh1* gene was examined after being switched to ethanol, a nonfermentable carbon source. Incubation with 2.0% ethanol completely arrested yeast growth (data not shown). However, 2.0% ethanol gave rise to an enhancement in the synthesis of β-galactosidase from the fusion gene (Fig. 2A). This result implies that the *pbh1* gene is regulated also by nonfermentable carbon sources.

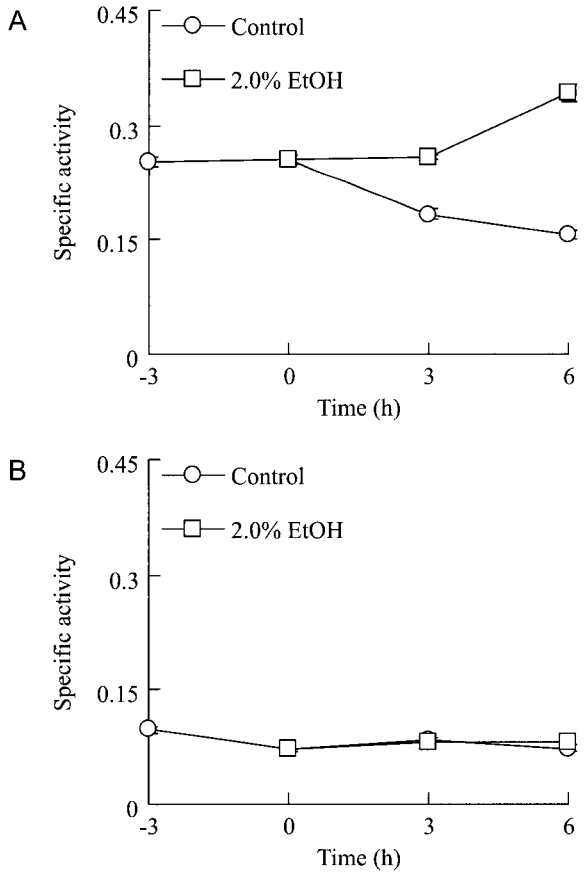

Fig. 2. The effect of ethanol (2.0% EtOH), a nonfermentable carbon source, on the synthesis of β-galactosidase from the Pbh1 -*lacZ* gene in wild-type KP1 (A) and Pap1-negative TP108-3C (B) cells. Yeast cells harboring the fusion plasmid pPbh04 were grown in minimal medium, and split at the early exponential phase. The β-galactosidase activity in extracts was determined as described in the legend of Fig. 1.

In response to various stresses, yeast cells induce the transcription of the genes required for detoxification of stressful agents. The transcription factor Pap1 is known to be responsible for the induction of some stress-related genes in the fission yeast *S. pombe*, which is widely used to study fundamental processes such as cell cycle or gene expression (Nguyen *et al.*, 2000). Pap1, an *S. pombe* bZIP protein homologous to mammalian AP-1, has been shown to play an important role in the response to oxidative stress and to a variety of cytotoxic agents, and it binds to the DNA sequence containing the consensus sequence TTACGTAA (Toone *et al.*, 1998; Fujii *et al.*, 2000). Synthesis of β-galactosidase from the Pbh1-*lacZ* fusion gene was previously compared in Pap1-negative TP108-3C and Pap1-positive KP1 cells in order to determine whether the basal expression of the *S. pombe pbh1* gene is regulated by Pap1 (Cho *et al.*, 2006). The synthesis of β-galactosidase from the Pbh1-*lacZ* fusion gene was significantly lower in the TP108-3C cells than in the KP1 cells (Cho *et al.*, 2006). These findings indicated that Pap1 is involved in the basal expression of the *pbh1* gene under uninduced conditions. With these results in mind, the Pap1-negative *S. pombe* strain TP108-3C was used to examine whether Pap1 is involved in the induction of the *pbh1* gene by various carbon sources. TP108-3C cells harboring the fusion plasmid pPbh04 were exposed to fermentable and nonfermentable carbon sources and the β-galactosidase activities in the treated cells were measured. TP108-3C cells harboring the fusion plasmid did not show any changes in the synthesis of β-galactosidase from the Pbh1-*lacZ* fusion gene (Fig. 1B, Fig. 2B). These results indicate that the *pbh1* gene is regulated by carbon sources in a Pap1-dependent manner.

In the upstream region of the *pbh1* gene, two plausible Pap1 binding sites, TTACCTAG and TTACG CAA, can be identified. The present study has shown that the *S. pombe pbh1* gene is up-regulated by both fermentable and nonfermentable carbon sources only in

the presence of the stress response-related transcription factor Pap1. These results imply that Pbh1p is one of the stress response-related proteins, although its mechanism of action in the stress response remains unclear. The presence of ethanol however, concomitantly arrests the normal growth of the yeast cultures. This might indicate that nonfermentable carbon sources induce up-regulation of the *pbh1* gene in a different way. Consequently, the *pbh1* gene is up-regulated under a switch to poor nutrient conditions. Also, the function of the plausible CSRE motifs in the up-regulation of the *S. pombe pbh1* gene by nonfermentable carbon sources remains to be explained. Recently, Bir1p deletion mutants have been found to show typical apoptotic hallmarks and decreased survival rates after the induction of apoptosis by hydrogen peroxide (Walter *et al.*, 2006). This finding might correlate with the up-regulation of Pbh1 under the stressful conditions that has been elucidated in this work. Additionally, it suggests that Pbh1 may play a protective role against oxidative stress.

In conclusion, the expression of the *S. pombe pbh1* gene is up-regulated under metabolic oxidative stress, which might help the fission yeast cells survive under poor nutrient conditions.

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