Transcriptional regulation of the yeast vacuolar aminopeptidase yscI encoding gene (*APE1*) by carbon sources

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Abstract Transcription of the vacuolar aminopeptidase yscI-encoding gene (*APE1*) is regulated by the carbon source used for yeast growth, responding to carbon catabolite repression. By Northern blot analyses, we determined the kinetics of glucose repression in growth-shift experiments. When added to induced cells, glucose leads to the disappearance of hybridizable aminopeptidase yscI RNA sequences within 30 min. However, the amount of inmunoreactive protein, once induced, is not affected by the addition of glucose. By deletion analysis of the fusion gene *APE1–lacZ* we have identified a number of strong regulatory regions in the *APE1* promoter. Consensus sequences for the binding of yAP1 and the HAP2/HAP3/HAP4 complex are contained in those regions. Control of the *APE1* gene expression is not mediated by the *HXK2* regulatory gene, but a strain bearing a deletion in the *CAT1* gene can not derepress *APE1* transcription to wild-type levels.

Key words: Yeast; *Saccharomyces cerevisiae*; Vacuole; Aminopeptidase; Catabolite repression; Transcription regulation

1. Introduction

In the yeast *Saccharomyces cerevisiae*, growth on glucose results in the repression of transcription of a large number of genes encoding enzymes involved in the utilization of alternative carbon sources and in respiration (for a recent review see [1]). Carbon catabolite repression has also been shown to regulate the active enzyme levels of several vacuolar peptidases (reviewed in [2]) which are involved in protein turnover and cell survival under poor nutritional conditions [3].

In spite of the impresive amount of information gathered in the last few years, the molecular mechanisms underlaying catabolite repression are still poorly understood and seem to be different for different enzymes.

To better understand the mechanism of glucose repression we have selected the *APE1 (LAP4)* gene and its gene product, vacuolar aminopeptidase yscI. The *APE1* locus is on the left arm of chromosome XI [4,5] and encodes a 514 amino acid precursor containing an unsual amphiphilic α-helix in the amino terminal region [5,6]. Mature aminopeptidase yscI is located inside the vacuole as demonstrated by subcellular fractionation [7,8]. Unlike most soluble vacuolar hydrolases aminopeptidase yscI is not glycosylated and does not enter the secretory pathway. Instead, the aminopeptidase precursor remains in the cytoplasm and appears to translocate directly into the vacuole [9]. Aminopeptidase activity is repressed by glucose [8]. Glucose mediated repression is effected at least in part at the level of transcription [5]. In this paper we show that accumulation of the *APE1* transcript is tightly repressed by glucose and induced by ethanol. By deletion analysis of *APE1–LacZ* fusions we have identified promoter elements responsible for this regulation.

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2. Materials and methods

2.1. Strains, growth conditions and enzyme assays

Saccharomyces cerevisiae strains DBY746 (MATα *leu2–3,112 his3Δ1* ura3–52 trp1–289) (Yeast Genetic Stock Center), JS87.11–1A (MATα *trp1–289 cat1::HIS3 MAL2–8^c MAL3 SUC3*) and DBY2184 (MATα *hxk2–202 ura3–52 leu2–3,112 lys2–801 gal2*) were used in expression studies and transformed with different plasmids according to Ito et al. [10]. *Escherichia coli* XL1Blue was used for plasmid manipulations. Yeasts were grown on rich media based on 1% yeast extract and 2% peptone (YP), 2% glucose (D), 3% potasium acetate (A), 3% ethanol (E) or 3% glycerol (G), were added as carbon sources. β-galactosidase activity was assayed according to [11]. Protein was determined by the method of Lowry et al. [12].

2.2. Nucleic acid preparation and hybridization experiments

DNA manipulations were done by standard procedures [13]. Total RNA (10 μg) isolated from yeast cells according to [14] was separated on 1.5% agarose/MOPS/formaldehyde gels [15]. Northern blot analysis was performed by standard procedures [16].

2.3. Construction of gene fusions and deletions in the APE1 promoter

Plasmid pRAP1 was constructed as follows. A *Bam*HI–*Xho*I fragment containing 953 nucleotides from the 5′ noncoding region and 138 bp of the *APE1* coding sequence [5] was cloned in frame to *lacZ* into YIp357 [17]. Unidirectional 5′ deletions in the *APE1* promoter were performed in the pRAP1 plasmid using the Exo III nested-deletion kit of Pharmacia. The constructions were sequenced by the dideoxy chain-termination method [18] using Sequenase (USB, Cleveland, USA).

2.4. Inmunoblotting

Sodium dodecyl sulfate (SDS)-denatured samples (200 μg soluble yeast extract) were subjected to SDS-12% polyacrylamide gel electrophoresis (PAGE) and electroblotted onto nitrocellulose [19]. The transferred proteins were detected by probing the nitrocellulose filter with aminopeptidase yscI-specific antibodies [9] and visualization of bound antibody by colour reaction as described [20].

3. Results and discussion

3.1. Regulation of APE1 gene expression by carbon sources

To further investigate the previously reported regulation of vacuolar aminopeptidase yscI expression by glucose [5,8,21], we measured the stady-state levels of aminopeptidase yscI m-RNA isolated from cells grown on different carbon sources. Northern blot analysis (Fig. 1) revealed that *APE1* m-RNA

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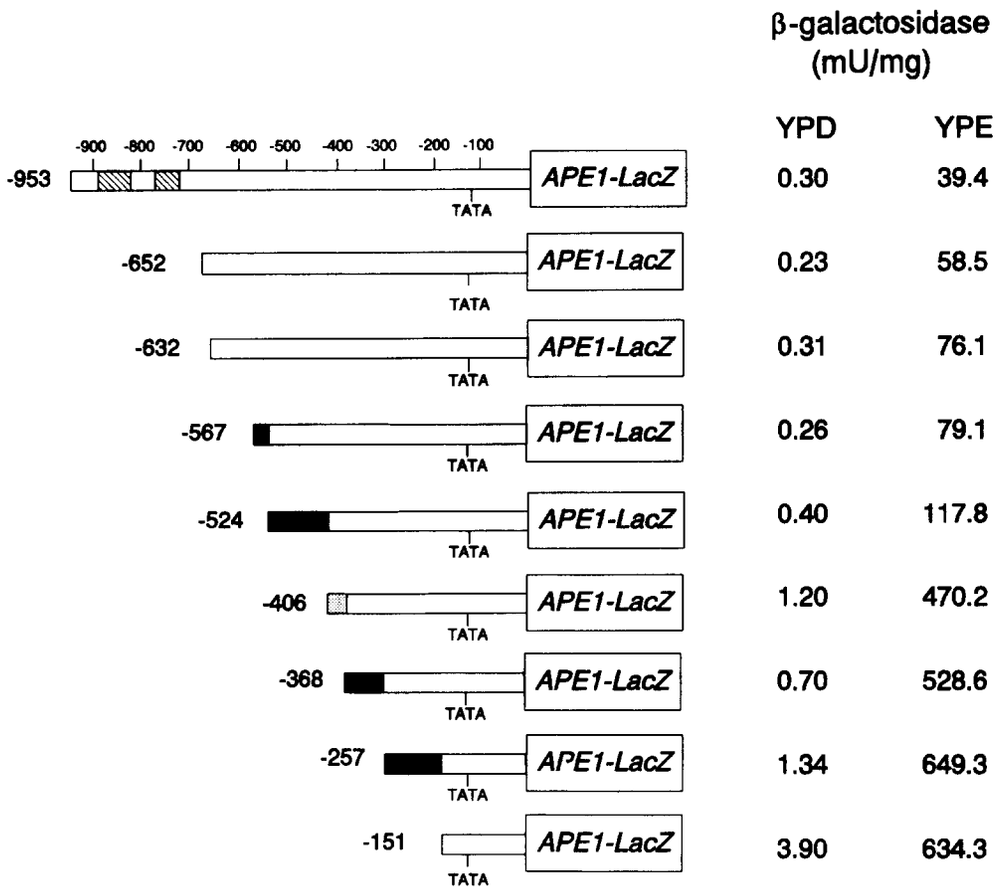

Fig. 1. Influence of carbon sources on *APE1* transcription. Total RNA (10 μg) was extracted from cells of strain DBY746 grown on rich media with glycerol (G), potasium acetate (A), ethanol (E) and glucose (D) as carbon sources, fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, hybridized with nick-translated *APE1* and *ACT1* probes, and subjected to autoradiography.

levels were hardly detectable in glucose grown cells, while high levels were seen in ethanol grown cells. Only a weak band was observed after growth of yeast cells on glycerol or acetate. These results are similar to those reported for other genes regulated by catabolite repression [22–25].

The kinetics of carbon catabolite repression by glucose on *APE1* m-RNA levels are shown in Fig. 2A. Ten minutes after shifting ethanol grown cells to glucose, the intensity of the band hybridizing to the *APE1* probe was reduced 2.6-fold as compared to the one from ethanol grown cells (0 min). After 30 min the signal was hardly detected. It seems likely that these results are the consequence of both a block of transcription and a decrease in m-RNA stability. The latter mechanism has been reported for the *MAL6S, CYC1* and *SDHip* genes [26–28].

In *Saccharomyces cerevisiae* glucose can affect enzyme levels not only through a decrease in the transcription rate but also through an irreversible inactivation of enzymes. This last process is known as catabolite inactivation [29] and has been reported for vacuolar aminopeptidase yscI [30]. To follow the fate of the polypeptide encoded by the *APE1* gene after shifting ethanol grown cells to glucose medium, we performed Western blot analysis of the corresponding cell extracts. Fig. 2B shows that the inmunoreactive band corresponding to the expression product of the *APE1* gene remains unaltered after 120 min, suggesting that proteolytic degradation is not the main event in the process of catabolite inactivation of aminopeptidase yscI activity. Rather, the disappearance of aminopeptidase activity might be due to a covalent modification of the protein induced by glucose addition.

3.2. *Regulatory regions in the* APE1 *promoter*

To identify cis-acting regulatory elements controling the transcription of the *APE1* gene, in-frame fusions of different 5′-truncated versions of the *APE1* promoter with the *E. coli lacZ* gene were constructed. Single copy transformants with the constructs were grown either on glucose or ethanol media to an $A_{600\ nm} = 1.0$ and β-galactosidase levels were measured (Fig. 3). A fusion containing 953 bp of the 5′-noncoding region showed a high level of expression when grown on ethanol that was repressed 130-fold on glucose media. This indicated that all essential promoter elements are located within this region. Deletions from −953 to −652 and from −652 to −632 increased progressively β-galactosidase activity in derepressing conditions, while β-galactosidase levels remained unaffected in glucose-grown cells, thus pointing to the existence of upstream repressing sequences in these regions. The next deletion up to position −567 had no effect, but a further regulatory site should be present between positions −567 and −524 since removal of this region increased expression in both repressing and de-

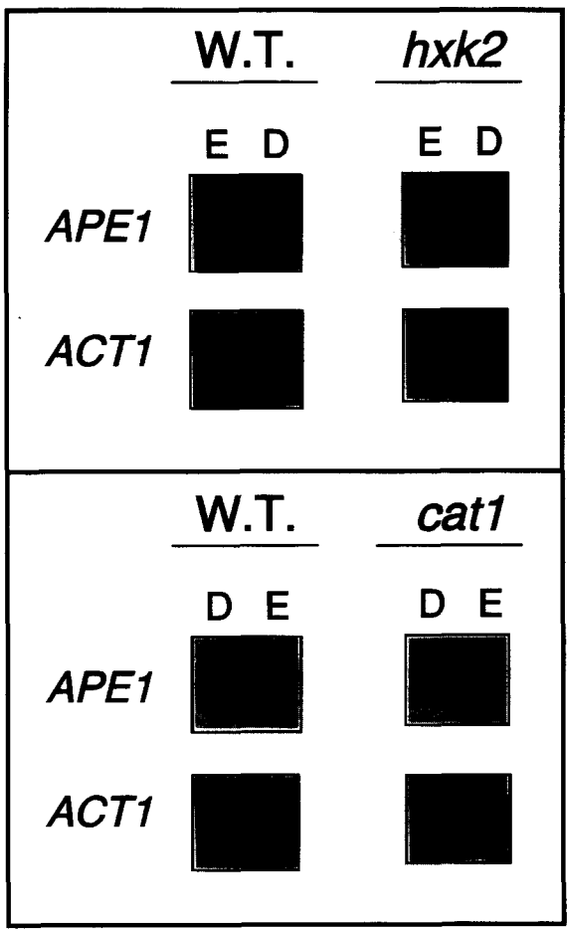

Fig. 2. Kinetics of carbon catabolite repression. Cells of strain DBY746 grown in YPE to an $A_{600\ nm}$ = 1.0 were harvested, washed and resuspended in YPD medium. Samples were taken at the times indicated. (A) Total RNA was isolated, blotted and hybridized. (B) Equal amounts of yeast cell extracts were subjected to SDS-PAGE, electroblotted and probed with aminopeptidase yscI-specific antiserum.

repressing conditions. There is a strong upstream repressing sequence between positions −524 and −406, since removal of this region increased β-galactosidase expression by 3-fold in glucose grown cells and by 4-fold in ethanol grown cells. Most interesting is the site located between −406 and −368. Removal of this site did not affect significantly β-galactosidase activity in derepressing conditions but decreased activity by 1.7-fold in glucose grown yeasts, leading to a repression ratio of more than 750-fold. Sucessive deletions between −368 and −151 had not a marked effect on β-galactosidase activity in derepressing conditions but increased progressively up to 5.5-fold its activity in conditions of repression, pointing to the existence of a site implicated in catabolite repression in this region.

It is worth noting that all the regulatory sequences identified, except the UAS region found between positions −406 and −368, are upstream repressing sequences (URS), a situation which is not very common in yeast genes. A possible explanation for these results might be the fact that aminopeptidase yspI expression is not only under carbon catabolite repression but also subjected to a strong repression by the nitrogen source of the growth medium (Bordallo, J. and Suárez-Rendueles, P., manuscript in preparation) and thus, some of the regulatory regions identified in this work, especially those leading to increased expression in both glucose and ethanol grown cells, might correspond to nitrogen regulatory elements. It is also remarkable the fact that the last deletion that leaves only about 25 bp in front of the TATA box still shows high levels of expression and that it is still sensitive to regulation by glucose. The high levels of expression may be due to transcriptional activation caused by flanking vector sequences, but the fact that it still responds to glucose regulation points to the existence of regulatory elements in that construct, that might be located in the promoter region left or even within the open reading frame.

A consensus binding site for the yeast activator protein yAP1 [31] is located in the putative UAS region of the *APE1* promoter at position −382. Similar sequences are found in UAS regions implicated in catabolite repression of the *FBP1* and *PCK1* promoters [32] . There is a consensus region for binding the regulatory protein complex HAP2/HAP3/HAP4 [33] at position −477 and a consensus binding site for the general transcription factor GRF1 [34] at position −445 within the strong URS region of the APE1 promoter. Such sequences have also been described in the *FBP1* and *PCK1* promoters [32]. It should be noted that the GRF1 binding site operates in many cases together with a CTTCC motif [35] which is also present at positions −707, −435 and −347 in the APE1 promoter. The motif situated far upstream in the *APE1* promoter might play at best a marginal role, since its removal causes only a slight decrease in β-galactosidase expression in glucose-grown cells (compare the first two constructions of Fig. 3). Removal of the region located between −524 and −406 which contains the *GRF1* binding site and the second CTTCC motif does not lead to a decrease in β-galactosidase expression but to a more than 3-fold increase in β-galactosidase expression (see Fig. 3). So, either the *GRF1* binding site is not functional in the *APE1* promoter or its effect is masked by a strong repressing binding site located within the removed region. Since the HAP2/HAP3/HAP4 complex has been described as an activating factor it was surprising to find consensus binding sites for it within the strong URS element of the *APE1* promoter. So, either the sequence is not relevant in connection with the large increase on the expression of the fusion gene observed upon removal of the fragment between −524 and −406, or there is some repress-

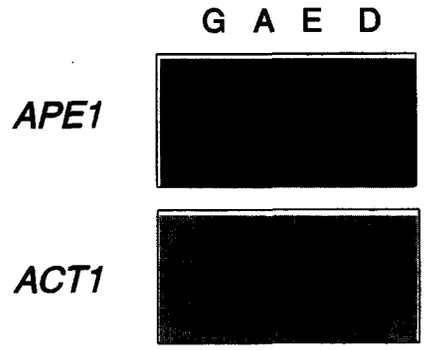

Fig. 3. Deletions in the *APE1* promoter and their effect on the expression of a fused *lacZ* gene. Deletions were performed in the pRAP1 plasmid as described in Section 2; their end points are indicated with relation to the first ATG in the coding sequence. Regulatory regions are indicated as: *hatched areas:* URS for ethanol induction; *black areas*: general URS; *dotted area*: UAS for catabolite repression; *grey areas*: URS for catabolite repression.

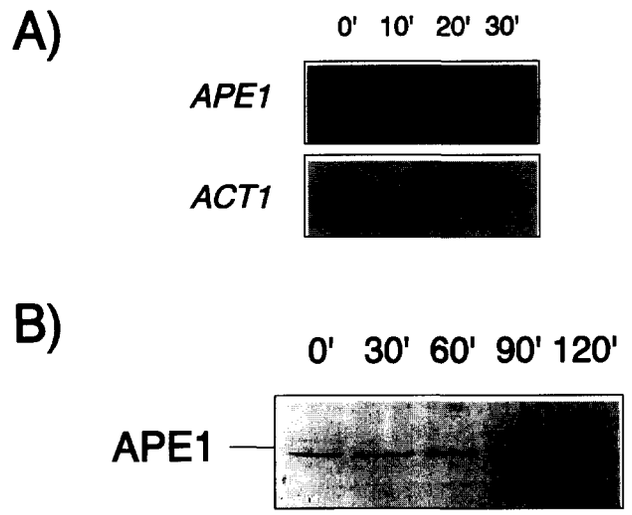

Fig. 4. Expression of the *APE1* gene in *hxk2* and *cat1* deletion mutants. *Upper panel*: total RNA was extracted from cells of the strains DBY746 (WT) and DBY2184 (*hxk2*) pregrown on YPE (E) and transferred to YPD (D) for 1 h. *lower panel*: total RNA was extracted from cells of the strains DBY746 (WT) and JS87.11–1A (*cat1*) pregrown on YPD (D) and transferred to YPE (E) for 6 h.

ing protein(s) with a Zn-finger sequence similar to that of the HAP complex.

There are several consensus binding sites for several transcription factors of different origin in the *APE1* promoter whose role in the control of *APE1* transcription remains to be established.

3.3. *Influence of* hxk2 *and* cat1 *mutants on* APE1 *gene expression*

An important part of glucose signal transduction in yeast occurs at the level of glucose uptake and the phosphorylation of hexoses (reviewed in [36]). Loss of the hexokinase PII isozyme causes the cell to become resistant to glucose-mediated repression of a number of genes (reviewed in [1]). On the other hand, *CAT1* encodes a protein kinase [37] necessary for derepression of several genes after a shift of cells to non-fermentable carbon sources [1,25]. Therefore we examined RNA prepared from *hxk2* as well as from *cat1* mutants for the presence of the *APE1* transcript by Northern blot analysis. Fig. 4 shows that a mutation in the *HXK2* gene does not abolish catabolite repression of the *APE1* transcription after a shift from ethanol to glucose. However, the protein encoded for by *CAT1* seems to play some role in the derepression of *APE1* transcription upon shifting glucose grown cells to ethanol medium.

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