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Negative regulation of transcription of the *Saccharomyces cerevisiae* catalase T (*CTT1*) gene by cAMP is mediated by a positive control element

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Transcription of the *CTT1* (catalase T) gene of *Saccharomyces cerevisiae* is controlled by oxygen via heme, by nutrients via cAMP and by heat shock. Nitrogen limitation triggers a rapid, cycloheximide-insensitive derepression of the gene. Residual derepression in a cAMP-nonresponsive mutant with attenuated protein kinase activity (*bcy1 tpk1*^{w} *tpk2 tpk3*) demonstrates the existence of an alternative, cAMP-independent nutrient signaling mechanism. Deletion analysis using *CTT1-lacZ* fusion genes revealed the contribution of multiple control elements to derepression, not all of which respond to the cAMP signal. A positive promoter element responding to negative control by cAMP was inactivated by deletion of a DNA region between base pairs −340 and −364. Upstream fragments including this element confer negative cAMP control to a *LEU2-lacZ* fusion gene. Northern analysis of *CTT1* expression in the presence or absence of heme, in *RAS2*$^{+}$ (high cAMP) and *ras2* mutant (low cAMP) strains and in cells grown at low temperature (23°C) and in heat-shocked cells (37°C) shows that *CTT1* is only induced to an appreciable extent when at least two of the three factors contributing to its expression (oxidative stress signaled by heme, nutrient starvation (low cAMP) and heat stress) activate the *CTT1* promoter.

Key words: cAMP control element/cytosolic catalase/nutrient control/transcriptional regulation/yeast

Introduction

Saccharomyces cerevisiae cells are able to adjust their metabolic activity, their growth rate and the decision between the initiation of a new round of mitotic cell division and entry into a G_0-like state or into sporulation (in the case of **a**/α diploid cells) to the nutritional conditions (Pringle and Hartwell, 1981). Cyclic AMP plays a crucial role in the transduction of the nutrient signal to various intracellular sites (Matsumoto *et al.*, 1985). Whereas the nutrient sensing mechanisms of yeast cells are still unclear it has been demonstrated that *S.cerevisiae* RAS1 and RAS2 proteins are activated by a complete set of nutrients (Tatchell, 1986). These RAS proteins in turn activate adenylate cyclase and trigger an increase in cellular cAMP level when nutrients are available in sufficient amounts. As in other eukaryotes, cAMP activates protein kinase A (encoded by three genes, *TPK1*, *TPK2* and *TPK3*) by binding to its regulatory subunit, which is encoded by the *BCY1* (*SRA1*) gene. Protein kinase A-catalyzed phosphorylation of a number of proteins, most of them yet unknown, will then trigger a variety of responses on the transcriptional and metabolic level, stimulating cell growth and, at least indirectly, cell division. On the other hand, a low cAMP level signaling nutrient starvation causes an alternate pattern of transcription and metabolism and triggers entry of cells into the resting state.

As part of this pleiotropic response to nutrient conditions, a number of yeast genes encoding various stress proteins are under negative cAMP control. Their expression is low in the presence of a complete set of nutrients and is derepressed during nutrient starvation. Examples for such genes are *UBI4* encoding polyubiquitin (Tanaka *et al.*, 1988), *CTT1* (Bissinger *et al.*, 1989), which encodes the cytosolic catalase T (Spevak *et al.*, 1983, Hartig and Ruis, 1986), *SSA3*, one of the HSP70 genes of yeast (Werner-Washburne *et al.*, 1989) and *HSP12*, which encodes a 14.4 kd heat shock protein (Praekelt and Meacock, 1990).

The yeast *S.cerevisiae* produces two catalase proteins, the peroxisomal catalase A and the cytosolic catalase T (Seah and Kaplan, 1973, Seah *et al.*, 1973, Skoneczny *et al.*, 1988). In contrast to the *CTT1* gene, *CTA1*, the gene encoding catalase A (Cohen *et al.*, 1985), has been shown to be under glucose repression and to be induced by fatty acids (Hörtner *et al.*, 1982, Skoneczny *et al.*, 1988). Transcription of both catalase genes is controlled by heme (Hörtner *et al.*, 1982). Like other yeast genes demonstrated to be under negative cAMP control, *CTT1* was recently shown to be under heat shock control (Wieser,R., Adam,G., Wagner,A., Schüller,C., Marchler,G., Ruis,H., Krawiec,Z. and Bilinski,T., submitted for publication).

At present, the mechanisms involved in negative control of transcription by cAMP and in derepression by nutrient starvation are only poorly understood. Therefore, it is the aim of our studies to identify the factors involved in the transfer of a nutrient signal from cAMP-dependent protein kinases to the *CTT1* gene. These studies led us to the identification of a cAMP control element of this gene. The localization of this control element and its synergistic interaction with other elements of the promoter of the catalase T gene are the main topic of this paper. While these studies were in progress, a cAMP responsive transcriptional control element of the *SSA3* gene was described by Boorstein and Craig (1990).

Results

CTT1 expression during nitrogen starvation

Expression of the *CTT1* gene is repressed when yeast cells are grown on complete medium (YPD). Its transcription is derepressed when cells are transferred to a medium containing limiting amounts of nitrogen (STMD). This derepression is a consequence of low cAMP-dependent protein kinase activity (Bissinger *et al.*, 1989). In a characterization of control regions mediating this response

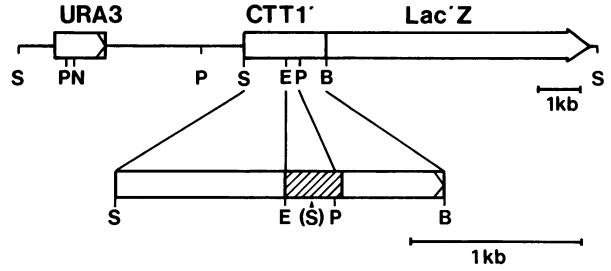

Fig. 1. Plasmid pTB3 used for single copy genomic integration of *CTT1-lacZ* genes. pTB3 was obtained by cloning a *CTT1-lacZ* fusion gene (Bissinger *et al.*, 1989) into the unique *SalI* site of a derivative of plasmid YIp5 (Struhl *et al.*, 1979) obtained by deletion of a 0.4 kb *Eco*RI−*Bam*HI fragment. The variable region modified in various deletion derivatives (see Figures 4 and 5) is indicated by hatching. The *SalI* site present in the deletion derivatives produced by linker insertion is indicated in parenthesis. Deletion derivatives were produced by replacing the small *Eco*RI−*Bam*HI fragment of pTB3 by corresponding deletion fragments. Genomic integration into the *URA3* locus was carried out after linearization of the plasmids with *NcoI*. B: *Bam*HI; E: *Eco*RI; N: *NcoI*; P: *PstI*; S: *SalI*.

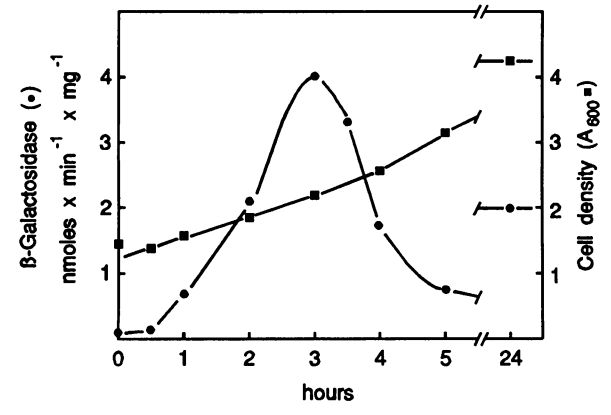

Fig. 2. Kinetics of derepression of *CTT1*-lacZ fusion gene after transfer from YPD to nitrogen starvation medium (STMD). Strain: TB3; circles: specific β-galactosidase activity; squares: absorbance of culture at 600 nm.

it was necessary to analyze whether the derepression observed is a fairly direct process or whether it is perhaps triggered by arrest of cells in a G_0-like state caused by nitrogen limitation. Furthermore, it was interesting to analyze whether derepression is entirely dependent on the RAS-cAMP pathway or whether there exists a cAMP-independent alternative signaling mechanism as in the case of other processes mediated by cAMP (Cameron *et al.*, 1988).

CTT1 expression was analyzed by single copy chromosomal integration of a *CTT1-lacZ* fusion gene available in plasmid pTB3 (Figure 1) or by Northern analysis of *CTT1* transcripts. As documented in Figure 2, derepression of *CTT1* expression occurs rapidly. Levels of β-galactosidase produced by expression of the fusion gene reach a maximum after 3 h, prior to arrest by nitrogen starvation. In agreement with this observation, high levels of *CTT1* transcript are observed after derepression for 30 min (Figure 3). Further experiments (data not shown) demonstrated that *CTT1* mRNA levels reach a saturation level after this time period. No inhibition of accumulation of *CTT1* transcripts by cycloheximide is observed under these conditions (Figure 3). These results indicate that derepression of the *CTT1* gene is not a consequence of G_0 arrest, but is

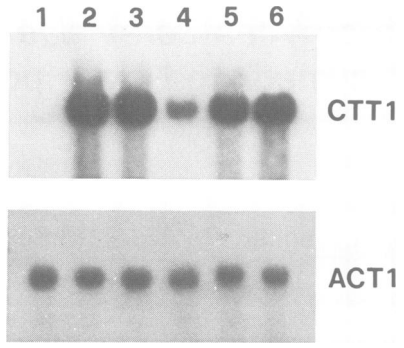

Fig. 3. Derepression of *CTT1* mRNA levels after transfer from YPD to STMD. Poly(A)$^+$ RNAs were probed with *CTT1* and actin (*ACT1*) fragments after electrophoretic separation and blotting. Lanes 1−3: RNA from strain S7-1A (*TPK1*$^+$ *tpk2 tpk3*); lanes 4−6: RNA from strain S13-58ArA (*bcy1 tpk1*^{w} *tpk2 tpk3*). Lanes 1, 4: RNA from cells grown on YPD; lanes 2, 3, 5, 6: RNA from cells transferred to STMD for 30 min in the absence (lanes 2, 5) or presence (lanes 3, 6) of cycloheximide (50 μg/ml culture).

one of the early and immediate events occurring under conditions where yeast cells enter a resting state.

It has previously been demonstrated that the characteristic pleiotropic response of yeast cells to changing nutrient levels (Pringle and Hartwell, 1981) is not exclusively mediated by the RAS-cAMP pathway. Cameron *et al.* (1988) have shown that cells lacking a functional regulatory protein kinase A subunit (*bcy1* mutants) can still respond to environmental changes. This became apparent when the protein kinase A activity of the mutants, which is constitutive because of the *bcy1* mutation, is reduced by mutations in protein kinase A structural genes (e.g. in a *bcy1 tpk1*^{w} *tpk2 tpk3* mutant with two protein kinase A genes inactivated by gene disruption and with a third *TPK* gene (*tpk1*^{w}) producing only low levels of protein kinase A activity). To test whether this cAMP-independent nutrient signaling pathway affects *CTT1* expression, *CTT1* mRNA levels of strains bearing the wild type allele of the *TPK1* gene as sole catalytic subunit of protein kinase A and of a *tpk1*^{w} *bcy1* mutant strain were compared (Figure 3). The results obtained show that *CTT1* expression on YPD medium is increased in the *tpk1*^{w} *bcy1* mutant, apparently because of the decrease in protein kinase A activity. Nevertheless, the gene still responds significantly to nutrient starvation in the absence of a functional regulatory subunit of protein kinase A. It can be concluded therefore that *CTT1* expression is partly controlled by nutrient levels via a cAMP-independent signaling pathway.

Localization of upstream sequences responding to nitrogen starvation

To localize control regions important for cAMP-mediated or cAMP-independent nutrient control of *CTT1* expression, a set of deletion derivatives of pTB3 (Figure 1) was constructed. Deletions tested for their effect on transcription cover the entire region previously demonstrated to be important for expression of the gene (Spevak *et al.*, 1986). Single copy chromosomal integrants were tested for expression of the *CTT1-lacZ* fusion gene on complete medium and after derepression on nitrogen starvation medium. The results summarized in Figure 4 demonstrate that a number of DNA elements contribute to derepression.

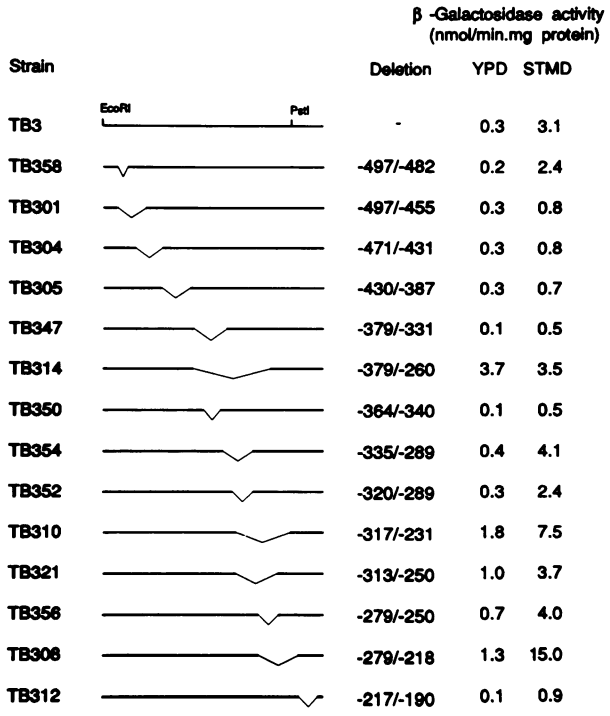

Fig. 4. Derepression of *CTT1-lacZ* fusion genes under nitrogen starvation conditions. After growth on YPD, cells were incubated on STMD (nitrogen starvation medium) for 16 h.

These fall into two categories: deletion of positive elements (e.g. in strains TB301, TB305 or TB350) reduces expression on nitrogen starvation medium (STMD), deletion of negative regions (e.g. in strains TB321 or TB308) mainly enhances expression on complete medium (YPD). Not all of these elements do necessarily directly respond to cAMP levels. Some might be targets of the cAMP-independent signaling pathway. Others, like the HAP1 (heme control) element (Winkler *et al.*, 1988) deleted in strains TB301 and TB304 act synergistically with those elements mediating nutrient control (see results presented in Figure 8).

Because of the apparent complexity of the *CTT1* promoter, further experiments described in this paper concentrated mainly on the localization of the DNA element(s) responding directly to the signal mediated by cAMP-dependent protein kinase. To assay for such elements, the *RAS2* gene of selected strains containing pTB3 or one of its derivatives was inactivated by gene disruption. The *ras2* disruption mutation reduces the cellular cAMP level on YPD medium. This leads to a (partial) derepression of *CTT1* transcription in *ras2* mutant strains compared to isogenic wild type cells on YPD medium (Bissinger *et al.*, 1989). It is therefore possible to use the extent of derepression of *CTT1* on YPD by a *ras2* gene disruption as a fairly direct indicator for negative cAMP control of transcription of the gene. Deletion mutants, which partly or completely loose the 'up'-phenotype of *ras2* strains characteristic for the wild type *CTT1* gene should contain a pTB3 derivative, in which a cAMP responsive DNA element of the *CTT1* promoter has been deleted. In these experiments, derepression by nitrogen starvation was also assayed. Initial experiments with *ras2* disruption strains (results not shown) demonstrated that these strains arrest growth on STMD medium much later than wild type cells. Derepression on STMD for 16 h as in earlier experiments

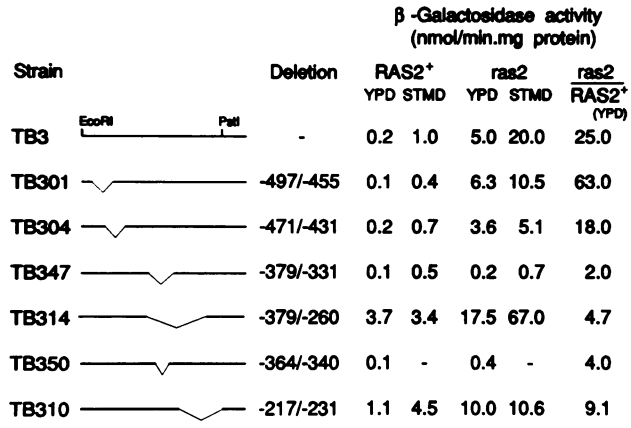

Fig. 5. Effect of *ras2* mutation on expression of *CTT1-lacZ* fusion genes. After growth on YPD, cells were incubated on STMD (nitrogen starvation medium) for 3 h.

(Figure 4) would therefore not allow a valid comparison of *CTT1* expression in *RAS2*$^+$ and *ras2* mutant strains. Thus, in further experiments derepression was carried out for a time period of 3 h. At this time point neither *RAS2*$^+$ nor *ras2* mutant strains had arrested growth and were still growing at a similar rate.

A comparison of β-galactosidase expression in wild type and *ras2* strains is presented in Figure 5. A group of deletion mutants exhibits a pronounced decrease in the *ras2*:*RAS2*$^+$ ratio of β-galactosidase activities on YPD. The largest of these deletions (TB314) is characterized by an increased expression apparently caused by elimination of a negative region also detected previously (Spevak *et al.*, 1986). The smaller two deletions (TB347, TB350) have very similar 'down'-phenotypes. This indicates the presence of one or more positive elements in the corresponding regions of the wild type gene. It can be concluded from these results that at least one positive element located within or overlapping with base pairs −340 to −364 (see deletion mutant TB350) is inactivated by cAMP.

In strain TB347, most of the *ras2* response of *CTT1* gene expression characteristic for the wild type gene (strain TB3) is lost. A *CTT1* promoter lacking base pairs −331 to −379 is therefore not significantly controlled by cAMP. Nevertheless, the derepression of *CTT1* expression by nitrogen starvation is still quite pronounced in this strain. This result demonstrates the existence of one or more DNA-elements which respond to cAMP-independent nutrient signaling and are separate from those responding to cAMP. Since derepression by nitrogen starvation in a *RAS2*$^+$ background is lost completely when base pairs −260 to −379 are deleted (TB314) it appears likely that cAMP-independent nutrient responsive elements are located between base pairs −260 and −330. However, this interpretation does not explain why strain TB314 does respond to nitrogen starvation after disruption of the *RAS2* gene. Further experiments are therefore necessary to locate cAMP-independent nutrient responsive elements.

Although the analysis of deletion mutants has led to the detection of a region of the *CTT1* promoter important for cAMP control it does not clarify whether this region is sufficient for a response to this signal. Therefore, *CTT1* upstream elements were tested in combination with a *S.cerevisiae LEU2* promoter lacking its own UAS element. The *LEU2-lacZ* integration vector pLS9 (Sarokin and

Carlson, 1985) was used for this purpose (see Figure 6). Single copy pLS9 derivatives were integrated into the *URA3* locus and β-galactosidase was assayed in extracts from the transformed cells. UAS activities of *CTT1* upstream regions and their response to nitrogen starvation and to the *ras2* genotype were tested (Table I). The *LEU2* promoter itself does not provide any cryptic sequences mediating negative cAMP control to UAS elements tested in this context, as was shown with a synthetic heat shock element (strain AW3). It exhibits temperature-dependent UAS activity, but is equally active in the isogenic *RAS2*$^+$ and *ras2* mutant strains. In contrast, those upstream fragments or synthetic oligonucleotides including the region deleted in strains TB347 and TB350 are sufficient for mediating the response to nitrogen starvation and to the *ras2* mutation to a heterologous promoter. Like other yeast UAS elements, the cAMP-responsive element is active in both orientations. Remarkably, strain MS226N, which contains the promoter region corresponding to base pairs −224 to −384 and should therefore activate transcription and respond to cAMP control, is almost entirely inactive. It is tempting to speculate that positive and negative elements in this construct cancel each other in their effects, whereas such constructs lacking the negative region of the *CTT1* promoter (e.g. AW1N) or those possessing additional positive elements (MS903) are active.

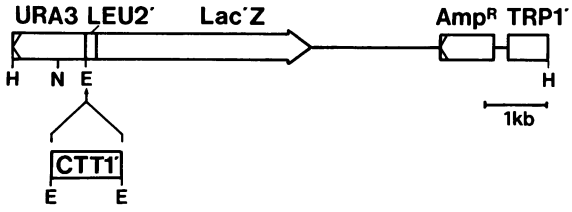

Fig. 6. Vector pLS9 (Sarokin and Carlson, 1985) and *CTT1* derivatives (see Table I). *CTT1* upstream fragments derived from *Sal*I linker insertion mutants and synthetic oligonucleotides were integrated into the unique *Eco*RI site of pLS9. In the case of *Sal*I linker-derived fragments, blunt ends were produced by treatment with Klenow polymerase and *Eco*RI linkers were added to fragments. Genomic integration into the *URA3* locus was carried out after linearization of the plasmids with *Nco*I. E: *Eco*RI; H: *Hin*dIII; N: *Nco*I.

In summary, deletion analysis and sufficiency experiments have demonstrated that the *CTT1* promoter contains a positive element, which is inactivated by cAMP control. They are consistent with the assumption that such an element is located within or at least overlapping with the region characterized by the smallest deletion tested (−364/−340).

Synergism between different types of UAS elements of the CTT1 gene

CTT1 expression is induced by heat shock (Wieser *et al.*, submitted for publication) and a pronounced interdependence of stimulation of transcription of this gene by low cAMP levels and by heat stress was noticed. This phenomenon was therefore analyzed in greater detail. The isogenic strains JC482 (wild type), JC302-26B (*ras2*) and JC303-79 (*ras2 sra1-13*) were used. The *sra1* mutation (Cannon *et al.*, 1990) present in strain JC303-79 is one of a number of suppressors of the growth defect of *ras2* mutants on non-fermentable carbon sources (Cannon *et al.*, 1986). It is allelic to *bcy1* mutations (protein kinase A regulatory subunit gene) (Matsumoto *et al.*, 1985). A defect in the regulatory subunit of protein kinase A has previously been shown to repress *CTT1* transcription almost completely compared to wild type or *ras2* mutants when cells are grown at 30°C (Bissinger *et al.*, 1989). When cells are grown at 23°C, however, catalase T levels are generally low and are virtually independent of the genotype of the three strains (Figure 7). Heat shock treatment of cells grown at 23°C restores their response to cAMP levels. Heat inducibility of catalase T is enhanced by the *ras2* mutation and is almost entirely abolished by the *sra1* mutation. Therefore, heat shock and cAMP control of *CTT1* expression are mutually interdependent: at 23°C, virtually no cAMP control is observed and heat shock induction is prevented by high protein kinase A activity. However, canonical heat shock elements (HSEs) are apparently not the targets of cAMP control: a number of genes controlled via HSEs are not under cAMP control (Werner-Washburne *et al.*, 1989), a synthetic HSE does not respond to cAMP (Table I) and HSEs and cAMP responsive elements have been shown to be separable in the case of the *SSA3* gene (Boorstein and Craig, 1990).

Table I. UAS activities of *CTT1* upstream elements

Strain		Insert	β-galactosidase activity (nmol/min/mg protein)				
			RAS2$^+$		*ras2*		$\frac{ras2}{RAS2^+}$ (YPD)
			YPD	STMD[a]	YPD	STMD[a]	
MS903N		−522/−142	1.4	15.7	4.7	25.7	3.4
AW1N		−382/−325	3.1	24.4	17.0	37.5	5.5
AW1I		−325/−382[b]	2.2	9.3	13.7	19.4	6.2
AW2X		−382/−325[c]	6.3	65.7	28.9	111.1	4.6
MS226N		−384/−224	0.4	1.2	0.4	1.6	1.0
	23°C[e]		2.8	–	2.6	–	0.9
AW3	30°C[e]	HSE[d]	4.3	–	4.7	–	1.1
	37°C[e]		55.6	–	56.4	–	1.0

[a]After growth on YPD, cells were incubated on STMD (nitrogen starvation medium) for 3 h.
[b]Reverted orientation.
[c]Two tandem copies.
[d]Synthetic heat shock element.
[e]Cells were grown at 23°C and 30°C, respectively; one half of the culture grown at 23°C was heat shocked at 37°C for 60 min.

The mechanistic basis of the synergistic response to the two regulatory signals will have to be investigated in more detail.

In a further experiment, we have extended our investigation to include the positive regulator heme. It has previously been shown to control *CTT1* expression at least partly via a HAP1 binding site located between base pairs −450 and −470 (Winkler *et al.*, 1988). A second HAP1 binding site has been localized between base pairs −200 and −220 (unpublished results). Deletions in both regions reduce expression of the gene under nitrogen starvation conditions (Figure 4). It was therefore attractive to test whether heme control, which is at least partly exerted via HAP1, also exhibits synergism with the two other mechanisms controlling *CTT1* expression. Levels of *CTT1* mRNA were analyzed in two isogenic strains (*hem1 RAS2*$^+$ and *hem1 ras2*). The *HEM1* gene encodes δ-aminolevulinate synthase, the enzyme catalyzing the first specific step in heme biosynthesis. The two strains were grown in the presence of concentrations of the heme precursor δ-aminolevulinate sufficient to compensate for the defect of the *hem1* mutant ('Heme$^+$') or, alternatively, under severe δ-aminolevulinate limitation ('Heme$^-$'). Cells were grown at 23°C and were tested for *CTT1* mRNA levels at this temperature and after a subsequent heat shock (37°C) (Figure 8). The Northern results obtained with heme-positive strains are consistent with the results obtained with another set of strains by assaying catalase T activities (Figure 7). Further, no positive heme control of the promoter is observed at 23°C and heat shock control is insignificant in the absence of heme in a *RAS2*$^+$ strain. However, the heme requirement of the promoter is lost in a *ras2* strain subjected to heat shock treatment. The results presented in Figures 7 and 8 demonstrate a pronounced synergism of heme control of *CTT1* expression with heat shock induction and derepression at low cAMP levels.

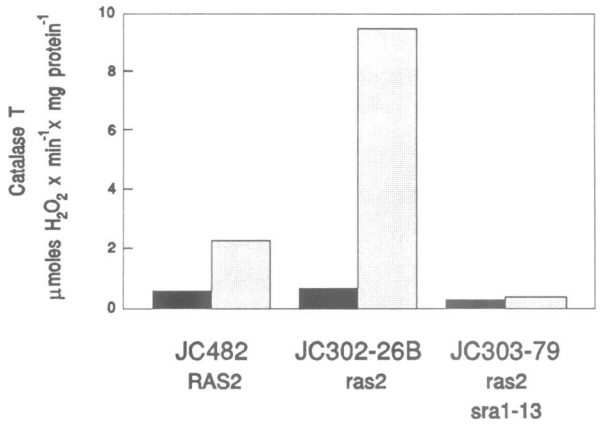

Fig. 7. Synergism between cAMP and heat shock control of catalase T levels. Strains were grown on YPD to an A_{600} of 1.5. The cultures were then divided and incubated for 20 min at 23°C (full bars) or 39°C (grey bars) followed by a recovery period of 1 h at 23°C. Catalase T activity was assayed in whole cell extracts.

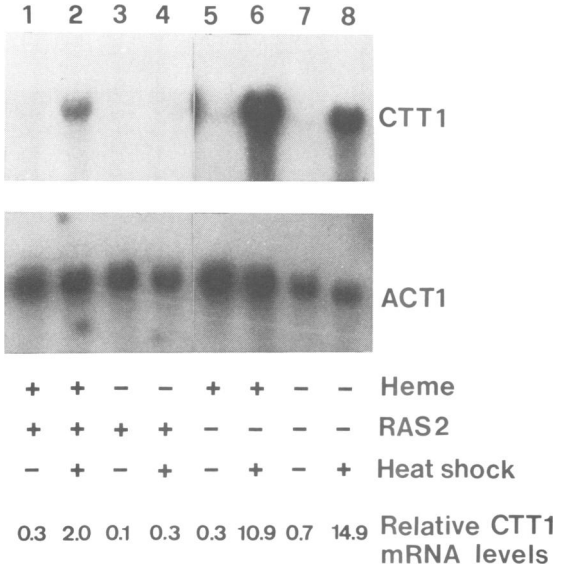

Fig. 8. Synergism between heme, heat shock and cAMP control of *CTT1* mRNA levels. Strains HP52 (lanes 1−4) and RW13-41 (lanes 5−8) were grown on YPD in the presence of 100 μg (lanes 1, 2, 5, 6) or 2 μg (lanes 3, 4, 7, 8) δ-aminolevulinate plus 2.6 mg Tween 80, 12 μg ergosterol per ml culture at 23°C to an A_{600} of 1.0. The cultures were then divided and incubated for 30 min at 23°C (lanes 1, 3, 5, 7) or 37°C (lanes 2, 4, 6, 8) before isolation of poly $(A)^+$ RNA. Poly $(A)^+$ RNAs were probed with *CTT1* and actin gene (*ACT1*) fragments after electrophoretic separation and blotting. *CTT1* band intensities were evaluated by densitometry and values were normalized against those obtained with the *ACT1* probe.

Discussion

Mechanisms involved in signaling of nutrient levels

Within cells of the yeast *S.cerevisiae*, nutrient levels are signaled by at least two different mechanisms. One of them, the RAS−cAMP pathway (Matsumoto *et al.*, 1985) has been studied extensively, an alternate, cAMP-independent signal transduction pathway (Cameron *et al.*, 1988) is presently only poorly understood. The transcription of several yeast genes has been demonstrated to be under negative control by cAMP (*UBI4* (Tanaka *et al.*, 1988); *CTT1* (Bissinger *et al.*, 1989); *ADH2* (Cherry *et al.*, 1989); *SSA3* (Werner-Washburne *et al.*, 1989); *HSP12* (Praekelt and Meacock, 1990); *IME1* (Matsuura *et al.*, 1990)). In contrast, no published information exists concerning yeast genes that are under positive transcriptional control by cAMP.

The experiments described in this publication demonstrate that derepression of *CTT1* transcription by nitrogen starvation is a rapid and fairly direct process. They do not necessarily imply, however, that protein kinase A directly inactivates a positive transcription factor by phosphorylation. The results obtained further demonstrate that the cAMP-independent alternative nutrient signaling pathway also contributes to transcription control of *CTT1* by nutrient levels and that this cAMP-independent control occurs at least partly via (a) DNA element(s) separate from the cAMP responsive element. No

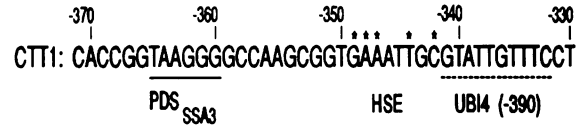

Fig. 9. Comparison of sequences (potentially) involved in cAMP control of transcription of *CTT1*, *SSA3* and *UBI4*. Bases in the *CTT1* cAMP control element conforming to the PDS element consensus derived from analysis of *SSA3* (Boorstein and Craig, 1990) are underlined, bases conforming to the canonical heat shock element (Bienz, 1985) are marked by asterisks. A DNA sequence identical to a *UBI4* upstream sequence flanking the heat shock element of this gene (Özkaynak *et al.*, 1987) is marked by a broken line.

published information is available concerning cAMP-independent nutrient control of transcription of any other yeast gene.

The cAMP responsive element of the CTT1 gene

The results of this investigation demonstrate that a positive element of the *CTT1* promoter responding to negative control by cAMP is located between or is at least overlapping with base pairs −340 and −364 (see Figure 9). This region, which also mediates heat shock control of *CTT1* (Wieser *et al.*, submitted for publication) contains three sequence motifs that could be involved in transcriptional control: (i) a sequence similar to the cAMP-responsive PDS element consensus of the *SSA3* gene (Boorstein and Craig, 1990) is located between base pairs −360 and −365 (see Figure 9). In accordance with the assumption that this type of DNA sequence is not only active in the *SSA3* promoter, but has more general importance as a cAMP responsive UAS element, regions with sequence similarity can also be detected in the *UBI4* (Özkaynak *et al.*, 1987) and *HSP12* (Praekelt and Meacock, 1990) upstream regions. (ii) A sequence element with limited similarity to heat shock elements (HSEs) binding heat shock transcription factor is located between base pairs −342 and −349. (iii) A further region that might be important for expression of *CTT1* and other genes, which are under similar control, is the 10 bp sequence immediately downstream of the HSE-like element (see Figure 9). An identical sequence flanks the heat shock element of the *UBI4* gene at its upstream side. The relevance of the similarities of these three elements with sequences detected in other promoters remains to be investigated. In any case, cAMP control of *CTT1* is not mediated by the *ADR1* protein, the activator of the *ADH2* gene shown to be controlled by cAMP-dependent protein phosphorylation (Cherry *et al.*, 1989). In contrast to the expression of *CTT1*, *SSA3* and *UBI4*, the expression of *ADH2*, which is mainly mediated by ADR1, strongly responds to carbon catabolite repression. *CTT1* is not controlled by *ADR1* (G.Adam and M.Simon, unpublished results) and the region sufficient for cAMP control does not contain sequence elements with any similarity to the ADR1 binding site of the *ADH2* gene (Eisen *et al.*, 1988).

Synergism between promoter elements of CTT1

Several yeast genes have been demonstrated to be under transcriptional control by heat shock and by cAMP. Whereas there is no evidence for a direct mechanistic link between the two modes of control, it is plausible to postulate a functional connection, since all genes affected encode proteins, which (could) have functions under stress conditions. If the effects of heat shock and nutrient starvation on transcription are independent of each other without any positive or negative interaction, dual control probably indicates that the gene product affected is needed by the cell under two different sets of physiological conditions. *UBI4* seems to be an example of a gene induced by heat shock and derepressed when cAMP levels are low without evidence for synergism between the two regulatory mechanisms (Tanaka *et al.*, 1988). However, a different regulatory pattern should arise if a gene product is important only when a combination of two physiologcal conditions (e.g. heat stress combined with nutrient starvation) occurs. Synergistic action of the regulatory factors or elements involved would then be appropriate. Such a pronounced synergism is evident in the case of *CTT1*. A similar effect has also been described for the *SSA3* gene (Boorstein and Craig, 1990), but the coupling between heat shock and cAMP control appears to be much less tight for the *SSA3* gene than for *CTT1*. Other heat shock genes appear to be also under this type of synergistic control (Shin *et al.*, 1987).

Among genes controlled by both heat shock and cAMP *CTT1* appears to be a special case not so much because of the extent of synergism observed, but because of the fact that a third regulatory factor, heme, which functions as an oxygen signal and acts via the transcription activator HAP1, also participates in this synergism. A negative region of the promoter located between base pairs −220 and −280 and probably consisting of several functional elements also contributes to the synergism exhibited by the promoter. It should be mentioned that a part of this negative region corresponding to base pairs −222 to −230 of *CTT1* has been demonstrated by Luche *et al.* (1990) to be similar in sequence to a negative element present in the promoter of the yeast *CAR1* gene and of a number of other yeast genes with a wide spectrum of functions. The element of the *CTT1* gene has been demonstrated by these authors to decrease expression of a heterologous yeast promoter and to form a complex with a factor also binding to the *CAR1* negative element. Results of our deletion experiments show that elimination of DNA elements of the negative region causes increased expression under repressed and derepressed conditions, but has a greater effect on the repressed promoter.

At the present stage it can only be speculated why different promoters apparently sharing the same types of regulatory elements differ in the extent of synergism between these elements. It has been demonstrated by Boorstein and Craig (1990) that the positions of these elements relative to each other are not important for synergism. Therefore, a direct interaction of the transcription factors binding to these elements seems an unlikely explanation. The quantity and the quality of the different types of positive elements and their interaction with negative elements attenuating their effects on transcription initiation could be more relevant. It seems worthwhile to point out that the HSE-like element of *CTT1* deviates considerably from the classical heat shock consensus (Bienz, 1985) as well as from the sequences defined more recently as functional HSEs of *Drosophila melanogaster* (Amin *et al.*, 1988, Xiao and Lis, 1988). It has to be tested by further experiments whether this element requires synergistic interaction with other positive elements to be functional.

Independent of the mechanisms determining the observed synergism of promoter elements this effect is probably relevant for the function of the cytosolic catalase encoded by the *CTT1* gene. In many eukaryotic organisms and tissues the existence of an extraperoxisomal catalase has been controversial. Therefore, there was hardly a basis for speculation about a specific function of such an enzyme. In *S.cerevisiae*, there is not only a separate gene encoding this extraperoxisomal catalase, but control of expression of this gene differs dramatically from that of the *CTA1* gene encoding the peroxisomal catalase A, which is induced by fatty acids, is not under heat shock control and is only moderately affected by cAMP levels (Simon,M., Adam,G., Rapatz,W., Spevak,W. and Ruis,H., in press). High level expression of the *CTT1* gene requires not only the presence of oxygen (heme), which causes oxidative stress, at least

partly by formation of hydrogen peroxide, but the combination of oxidative stress with other types of stress (heat shock, nutrient starvation). Our recent studies have demonstrated that catalase T provides some protection to yeast cells under 'lethal heat shock' conditions (50°C) and that pretreatment of cells containing a functional *CTT1* gene by a mild heat shock (37°C) protects these cells against exogenous hydrogen peroxide (Wieser *et al.*, submitted for publication). These data illustrate the functional importance of the cytosolic catalase T and of its mode of regulation.

Materials and methods

Yeast strains, media, growth conditions

S.cerevisiae strain WS17-5D (α *leu2 trp1 ura3 arg1*) was used as recipient for transformation with plasmids pTB3, pLS9 and their derivatives (see Figures 1 and 4) by integration into the *URA3* locus. Designations for the constructs integrated (e.g. TB3) are also used as designations for the corresponding yeast strains. Strains S7-1A (α *leu2 ura3 his3 trp1 ade8 TPK1*$^+$ *tpk2::HIS3 tpk3::TRP1*) and S13-58ArA (**a** *leu2 ura3 his3 trp1 ade8 bcy1::LEU2 tpk1*^{w} *tpk2::HIS3 tpk3::TRP1*) were obtained from C. Denis. The isogenic strains JC482 (α *ura3 leu2 his4*), JC302−26B (α *ura3 leu2 his4 ras2::LEU2*) and JC303-79 (α *ura3 leu2 his4 ras2::LEU2 sra1-13*) were obtained from K. Tatchell. Strain HP52 (**a** *leu2 trp1 ura3 his3 pep4 hem1*) was converted into the isogenic strain RW13-41 (**a** *leu2 trp1 ura3 his3 pep4 hem1 ras2::LEU2*) by gene disruption.

Strains were routinely grown on YPD medium (Fink, 1970) at 23°C or 30°C to an absorbance at 600 nm of 1.5 (for transfer to STMD medium) or 3 (for heat shock experiments). In nitrogen starvation experiments, cells were transferred to STMD medium (0.17% yeast nitrogen base without amino acids and ammonium sulfate, 2% glucose and limiting amounts of auxotrophic requirements (1 mg/l in the case of tryptophan, 5 mg/l in all other cases)) for the time periods indicated under 'Results'. In heat shock experiments, cells were incubated at 37°C or 39°C after growth at 23°C.

Plasmids, DNA constructions and oligonucleotides

The structure of plasmid pTB3 used for single copy genomic integration of *CTT1-lacZ* genes is illustrated in Figure 1. The mutated region of one of the plasmids constructed for deletion analysis of the *CTT1* upstream region (pTB310) was derived from a corresponding multicopy plasmid of the pF14/1 series used in a previous analysis (Spevak *et al.*, 1986). All other deletions are derived from products of *Sal*I linker (5′-GGTCGACC-3′; Amersham) insertion (Tatchell *et al.*, 1981) into a plasmid containing the *CTT1* upstream region in vector pUC13. Positions of the inserted linkers and size and position of deletions created during linker insertion were assayed by DNA sequencing. Linker insertion derivatives with appropriate deletions were used directly, other deletions were produced by recombining *Sal*I linker fragments with suitable end points. For this purpose, the small *Sal*I−*Bam*HI fragment of one pUC13 linker insertion derivative was replaced by another one with appropriate end point. In all cases, the small *Eco*RI−*Bam*HI fragment of plasmid pTB3 was then replaced by the corresponding deletion fragment isolated from the appropriate pUC13 derivative.

Vector pLS9 (Figure 4; Sarokin and Carlson, 1985) was donated to us by M.Carlson. Fragments inserted into its unique *Eco*RI site were synthetic oligonucleotides with *Eco*RI ends in the case of AW1 and AW2 plasmids. Oligonucleotides used were kindly supplied by R.Hauptmann, Ernst Boehringer Institut für Arzneimittelforschung, Vienna. For construction of the pMS plasmids, *CTT1* upstream fragments derived from linker insertion mutants were used. In the case of pMS903N, *Sal*I ends (position −142) were treated with Klenow polymerase, *Eco*RI linkers (5′-GGAATTCC-3′; Boehringer Mannheim) were added and the small fragment produced by cutting the resulting pUC13 derivative at the natural and at the artificial *Eco*RI site was cloned into pLS9. pMS226N was similarly constructed by converting a *Sal*I site (position−384) into an *Eco*RI site and by adding a *Pst*I-*Eco*RI adaptor (5′-CGAATTCGTGCA-3′; donated by H.Domdey) to the free end produced by cutting the insert with *Pst*I. A derivative of plasmid pUC19 containing a synthetic heat shock element, which was donated by P.Sorger, was cut with *Pst*I, a *Pst*I−*Eco*RI adaptor was again used to provide *Eco*RI ends and the fragment produced after cutting with *Eco*RI was cloned into pLS9 to obtain plasmid pAW3.

Plasmid pRa530 obtained from K.Tatchell (Tatchell *et al.*, 1984) was used for *LEU2* disruption of the *RAS2* gene.

Yeast transformation

Yeast transformation with linear fragments (plasmids linearized with *Nco*I in the case of pTB3 and pLS9 derivatives, a *Hin*dIII−*Xba*I fragment of plasmid pRa530 in the case of *RAS2* disruptions) was carried out by the method described by Beggs (1978) or by the procedure described by Ito *et al.* (1983). Transformants obtained by integration of derivatives of pTB3 or pLS9 into the *URA3* locus were tested by hybridization (Southern, 1975) and single copy integrants were used for further analysis.

RNA isolation and Northern analysis

Yeast poly (A)$^+$ RNA isolated as previously described (Richter *et al.*, 1980) was fractionated by electrophoresis, blotted and hybridized essentially as described by Thomas (1980). Band intensity on autoradiograms was evaluated using an Elscript-400 (Hirschmann) densitometer.

Enzyme activities

β-Galactosidase activity of extracts prepared by breakage of yeast transformants with glass beads (Rose and Botstein, 1983) was assayed using o-nitrophenyl-β-D-galactoside as substrate (Miller, 1972). Catalase activity of extracts was assayed spectrophotometrically at 240 nm (Beers and Sizer, 1952). Protein concentrations were assayed according to Bradford (1976).

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