MOLECULAR AND CELLULAR BIOLOGY, Mar. 1989, p. 1309–1315
0270-7306/89/031309-07$02.00/0
Copyright © 1989, American Society for Microbiology
Vol. 9, No. 3

Control of *Saccharomyces cerevisiae* Catalase T Gene (*CTT1*) Expression by Nutrient Supply via the *RAS*-Cyclic AMP Pathway

PETER H. BISSINGER, ROTRAUD WIESER, BARBARA HAMILTON, AND HELMUT RUIS*

Institut für Allgemeine Biochemie der Universität Wien and Ludwig Boltzmann-Forschungsstelle für Biochemie, A-1090 Vienna, Austria

Received 3 October 1988/Accepted 6 December 1988

In *Saccharomyces cerevisiae*, lack of nutrients triggers a pleiotropic response characterized by accumulation of storage carbohydrates, early G1 arrest, and sporulation of a/α diploids. This response is thought to be mediated by RAS proteins, adenylate cyclase, and cyclic AMP (cAMP)-dependent protein kinases. This study shows that expression of the *S. cerevisiae* gene coding for a cytoplasmic catalase T (*CTT1*) is controlled by this pathway: it is regulated by the availability of nutrients. Lack of a nitrogen, sulfur, or phosphorus source causes a high-level expression of the gene. Studies with strains with mutations in the *RAS*-cAMP pathway and supplementation of a *rca1* mutant with cAMP show that *CTT1* expression is under negative control by a cAMP-dependent protein kinase and that nutrient control of *CTT1* gene expression is mediated by this pathway. Strains containing a *CTT1-Escherichia coli lacZ* fusion gene have been used to isolate mutants with mutations in the pathway. Mutants characterized in this investigation fall into five complementation groups. Both *cdc25* and *ras2* alleles were identified among these mutants.

The yeast *Saccharomyces cerevisiae* contains two different hemoprotein catalases, the peroxisomal catalase A (32), which is encoded by the *CTA1* gene (11), and the cytoplasmic catalase T (33), encoded by *CTT1* (35). Like the *CYC1* (iso-1-cytochrome *c*) gene, the two catalase genes have been reported to be controlled positively by oxygen and heme and negatively by glucose repression (18). Whereas heme control of *CTT1* expression has been studied in some detail (35) and has recently been shown (44) to be coordinated with that of the *CYC1* and *CYC7* (iso-2-cytochrome *c*) genes (27) by the HAP1 protein, virtually nothing is known about the mechanism of glucose repression of catalase genes. Chvojka et al. demonstrated some years ago (10) that *CTT1* gene expression is controlled by the *GLC1* gene, which also affects metabolism of storage carbohydrates (30). This gene has been reported to code for a phosphoprotein phosphatase and to be a component of the *RAS* gene-cyclic AMP (cAMP) pathway of *S. cerevisiae* (24). However, involvement of the gene in glucose repression of *CTT1* expression appeared unlikely, since cAMP has been reported not to be involved in glucose (carbon catabolite) repression of *S. cerevisiae* (23).

The results of this study show that expression of the *CTT1* gene is controlled not only by the quality of the carbon and energy source but also by other nutrients such as nitrogen, phosphorus, and sulfur sources. The availability of these nutrients is signalled to control regions of the gene by a pathway involving the RAS2 protein and a cAMP-dependent protein kinase.

MATERIALS AND METHODS

Yeast strains and media. The *S. cerevisiae* strains used in this study are listed in Table 1. The strains were grown on the following media: yeast extract-peptone medium (YP) (15) with 2 or 10% glucose (YPD) or 2% glycerol plus 2% ethanol (YPGE); yeast nitrogen base (Difco Laboratories) complete medium (YNBC) (15); nitrogen starvation medium with 0.17% yeast nitrogen base without amino acids and ammonium sulfate, 2% glucose, and limiting amounts (1 mg/liter in the case of tryptophan, 5 mg/liter in all other cases) of auxotrophic requirements (STMD2%); and a synthetic complete medium (SM) (19). Cells were grown on YP and YNB media at 30°C to an optical density at 600 nm of 3.5 to 4.0. For growth on STMD2%, cells grown on YPD2% plates or in liquid YPD2% were transferred to STMD2% (optical density at 600 nm, 1.0) and were incubated at 30°C for 36 h (after this period, all cells were arrested unbudded in early G1). Synthetic α-factor (kindly provided by K. Nasmyth) was added in some experiments to a final concentration of 0.1 μg per ml of culture. Cells were grown and incubated on SM in the same way as on STMD2%. Strains bearing *cdc* mutations were pregrown at 23°C on YPD2% to an optical density at 600 nm of 2.5, and the cultures were divided, transferred to STMD2% in some experiments, and incubated for 5 h at 23 and 35°C on YPD2% or STMD2%, respectively, before being harvested.

Enzyme activities. The specific activity of β-galactosidase of crude extracts from yeast strains bearing a single-copy *CTT1-lacZ* fusion gene (36) integrated into the chromosomal *URA3* locus (see Fig. 1) was assayed as described by Miller (26).

β-Galactosidase plate assays were carried out with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) as the chromogenic substrate (29). Specific activities of catalase A and catalase T in crude extracts from yeast strains were assayed after removing the other enzyme by immunoprecipitation as described previously (37). Protein concentrations of extracts were assayed by the method of Bradford (3).

Northern (RNA) hybridization. Poly(A)$^+$ RNA for hybridization experiments was prepared from total RNA by oligo(dT)-cellulose chromatography (1). Denaturation of RNA, agarose gel electrophoresis, transfer to nitrocellulose, and hybridization were carried out as described by Thomas (41). Blots were probed with gene fragments labelled by random-primed labeling (14).

Mutagenesis and mutant characterization. Strains A2-201 and PY1 were mutagenized by UV irradiation (35). After plating of mutagenized cells and incubation at 30°C, mutants

* Corresponding author.

TABLE 1. *S. cerevisiae* strains

Strain	Genotype	Source or reference
A2-200	α *leu2-3,112 his3-11,115 ura3::CTT1-lacZ can1*	This study
A2-201	α *leu2-3,112 his3-11,115 ura3::CTT1*(Δ−517/−431)*-lacZ can1*	This study
PM57	α *leu2-3,112 his3-11,115 ctn1-1*[a] *ura3::CTT1*(Δ−517/−431)*-lacZ can1*	This study
PM116	α *leu2-3,112 his3-11,115 ctn2-1 ura3::CTT1*(Δ−517/−431)*-lacZ can1*	This study
PM416	α *leu2-3,112 his3-11,115 ctn3-1 ura3::CTT1*(Δ−517/−431)*-lacZ can1*	This study
PY1	**a** *trp1 ura3::CTT1*(Δ−517/−431)*-lacZ can1 cyh*	This study
PYM801	**a** *trp1 ura3::CTT1*(Δ−517/−431)*-lacZ ctn4-1 can1 cyh*	This study
PYM864	**a** *trp1 ura3::CTT1*(Δ−517/−431)*-lacZ ctn5-1*[b] *can1 cyh*	This study
JC482	α *ura3-52 leu2 his4 RAS2*$^+$	9
JC302-26B	α *ura3-52 leu2 his4 ras2-530::LEU2*	8
JC303-79	α *ura3-52 leu2 his4 ras2-530::LEU2 sra1-13*	8
JC303-31	α *ura3-52 leu2 his4 ras2-530::LEU2 SRA4-5*	8
JC303-18	α *ura3-52 leu2 his4 ras2-530::LEU2 sra5-5*	8
JC303-46	α *ura3-52 leu2 his4 ras2-530::LEU2 sra6-15*	8
EG81-40A	α *ura3 his4 lys2 RAS2*$^+$	40
EG81-40B	α *leu2 ura3 his4 lys2 lys1 ras2-530::LEU2*	40
EG81-40C	**a** *leu2 ura3 his4 his3 lys2 ras2-530::LEU2*	40
EG81-40D	**a** *ura3 his4 lys2 lys1 his3 RAS2*$^+$	40
KT308	α *leu2 ura3 trp1 his3 lys1 lys2*	K. Tatchell
KT131	α *leu2 ura3 trp1 his3 lys1 lys2 ras2-530::LEU2*	K. Tatchell
KT301	α *leu2 ura3 trp1 his3 lys1 lys2 RAS2*$^{Val-19}$	K. Tatchell
OL86	α *ade2 leu2 trp1 cdc25-5*	7
321	**a** *ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc25-1*	Yeast Genetic Stock Center, Berkeley
185-3-4	**a** *ade1 ade2 ura1 his7 lys2 tyr1 leu2 gal1 cdc28-1*	Yeast Genetic Stock Center
OL170-4B	α *his3 leu2 ura1 rca1*	M. Jacquet
BP30-6A	α *leu2 his3 ura3 lys2 RAS2*$^+$	This study
BP30-6B	**a** *leu2 his3 lys2 ras2-530::LEU2 ura3::CTT1-lacZ*	This study
BP30-6C	**a** *leu2 his3 RAS2*$^+$ *ura3::CTT1-lacZ*	This study
BP30-6D	α *leu2 his3 ura3 ras2-530::LEU2*	This study
1169	**a** *bar1::URA3*	K. Nasmyth

[a] *ctn1* was demonstrated to be allelic to *CDC25* (see Results).
[b] *ctn5* was demonstrated to be allelic to *RAS2* (see Results).

were detected by X-Gal staining of colonies, using a filter method described by Breeden and Nasmyth (4). Standard media and standard procedures were used for complementation and segregation analysis of mutants (15).

RESULTS

Control of *CTT1* gene expression by availability of nutrients. β-Galactosidase was assayed in extracts from yeast strains containing a single-copy *CTT1-Escherichia coli lacZ* fusion gene (Table 2). A strain (A2-200) carrying a gene with a wild-type promoter and another strain (A2-201) with a deletion derivative of the promoter designed for identification of regulatory mutants (see below) were tested. The fusion genes used and their mode of integration into the genome, which was controlled by Southern hybridization (not documented), are illustrated in Fig. 1. They were integrated into *URA3* and not into the *CTT1* locus. Thus, any potential regulatory mutations identified by an increase or decrease of β-galactosidase activity can be directly tested for their effect on the wild-type *CTT1* promoter present in the strains, and mutations affecting *CTT1* expression in *trans* can easily be distinguished from other effects. Qualitatively, a similar response to nutrient conditions was observed with both

TABLE 2. β-Galactosidase activities of *CTT1-lacZ* transformant

Growth medium	β-Galactosidase activity[a] of strain:	
	A2-200	A2-201
YPD2%	1.8	0.2
YNBCD2%	8.1	0.8
STMD2%	34.5	4.2
SM (2% glucose)	1.5	–[b]
SM minus nitrogen	28.9	–
SM minus phosphate	17.5	–
SM minus sulfate	19.8	–
YPD10%	0.3	0.2
YPGE	10.5	0.5
YNBCD10%	7.5	2.1
YNBCGE	12.0	2.2

[a] Nanomoles of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) hydrolyzed per minute per milligram of protein (26).
[b] –, Not tested.

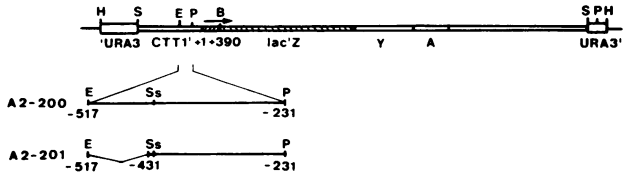

FIG. 1. *CTT1-lacZ* fusion genes in strains A2-200 and A2-201. A *CTT1-lacZ* fusion derived from a fusion gene previously described (36) and a mutant version of this fusion lacking one of the HAP1-binding sites of the *CTT1* upstream region (44) were inserted into an *S. cerevisiae URA3* gene after conversion of an *Eco*RV site of this gene into a *Sal*I site. Linear *Hin*dIII fragments (9.2 kilobases) containing the *URA3* gene disrupted by one of the *CTT1-lacZ* fusions were integrated at the chromosomal *URA3* locus by cotransformation with plasmid YEp13 (5) followed by loss of this plasmid on complete medium. Abbreviations: H, *Hin*dIII; S, *Sal*I; E, *Eco*RI; P, *Pst*I; B, *Bam*HI; Ss, *Sst*II.

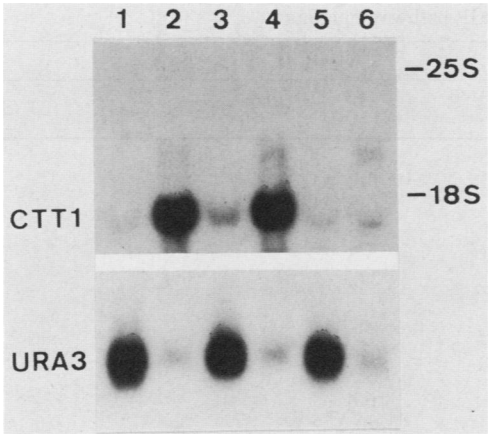

FIG. 2. *CTT1* mRNA levels in strain JC482 (*RAS2*$^+$) (lanes 1 and 2), JC302-26B (*ras2*) (lanes 3 and 4), and JC303-79 (*ras2 sra1-13*) (lanes 5 and 6). The strains were grown on YPD2% (lanes 1, 3, and 5) or STMD2% (lanes 2, 4, and 6). Equal amounts of poly(A)$^+$ RNAs were applied and separated electrophoretically on 1.1% agarose gels after denaturation with glutaraldehyde (41). Blots were probed with a 1.15-kilobase *Eco*RI fragment containing the 3′ part of the *CTT1* coding region (17) and with a 1.1-kilobase *Hin*dIII *URA3* gene fragment. Probes were labeled by random-primed labeling (14).

strains. Compared with expression of the gene in cells grown on a rich medium (YPD2%), an induction occurred after growth on yeast nitrogen base medium (YNBCD2%). The most likely explanation for this phenomenon appears to be limitation of some nutrient, since a similar but more pronounced derepression was observed when cells were incubated on a starvation medium (STMD2%) containing extremely limiting amounts of nitrogen. Incubation of cells in a synthetic complete medium not derived from yeast nitrogen base (SM) shows that derepression is not a consequence of a deficiency of all standard synthetic media. The component lacking or limiting in yeast nitrogen base medium has not been identified. However, we tested whether omission of standard nutrients from SM has similar effects on *CTT1* gene expression, although these are obviously not the components that are limiting in YNBCD2%. The results (Table 2) show that derepression can be caused by deficiency in either nitrogen, phosphate, or sulfate. Therefore, the regulatory mechanism involved appears to be connected to a more general type of nutritional control. Cells were arrested at the early G1 phase under all these conditions. Omission of the carbon source or incubation at low cell density in medium containing 0.05% glucose did not allow derepression of the *CTT1* gene (data not shown). Although differences in expression between YP media containing 10% glucose or glycerol-ethanol as carbon sources could be interpreted as glucose repression, most of this difference between the two carbon sources disappears when cells are grown on yeast nitrogen base media.

Involvement of *RAS*-adenylate cyclase pathway. The *RAS*-adenylate cyclase signal pathway of *S. cerevisiae* (22) has been postulated to mediate a pleiotropic response to nutrient starvation (2, 39, 40), e.g., accumulation of storage carbohydrates, early G1 arrest, and sporulation of **a**/α diploids (13, 21, 28). To find whether the nutrient starvation effect on *CTT1* gene expression might be mediated by this pathway, we tested various mutants (Fig. 2; Tables 3 and 4). Northern hybridization experiments were carried out (Fig. 2) with RNA preparations from an isogenic set of three strains. One of them is a *RAS2* wild-type strain, the second is a *ras2* gene disruption mutant, and the third is a *ras2 sra1-13* double mutant. The *sra1* mutation has been described (8) as a *ras2* suppressor allele of the *BCY1* gene, which codes for the regulatory subunit of *S. cerevisiae* cAMP-dependent protein kinases (9, 42). Results with RNA from wild-type cells grown on complete medium or starved for nitrogen show that the starvation effect observed by assaying β-galactosidase expression is also manifest when *CTT1* mRNA (35) levels are compared. In the *ras2* mutant grown on complete medium, levels of *CTT1* mRNA were elevated, but not to the extent observed in starved wild-type cells. The effect of nitrogen starvation on the *CTT1* mRNA level was virtually completely suppressed by the *sra1-13* allele. A control experiment with a *URA3* probe shows that mutations in the *RAS*-cAMP pathway have no effect on the level of the corresponding mRNA and that its response to nitrogen starvation is opposite to that of *CTT1* mRNA.

Tables 3 and 4 summarize the results of a more detailed and more quantitative investigation of the effects of mutations in the *RAS*-adenylate cyclase pathway on *CTT1* expression. In most cases tested, levels of *CTT1* mRNA estimated by hybridization, activity of β-galactosidase produced from the *CTT1-lacZ* fusion gene, and activity of catalase T produced by expression of the wild-type *CTT1* gene correlated fairly well (see Discussion for exceptions). Therefore, catalase T activity was used in the majority of the experiments to assay for *CTT1* gene expression. Activity of catalase A, which is at least much less sensitive to nutrient levels, was determined as an internal control. The results obtained with cells grown on rich (YPD) medium show that high catalase T (Table 3) or β-galactosidase (Table 4) levels are always associated with the *ras2* genotype. In contrast, no significant effect of a *ras1* mutation was observed (data not shown). A high level of catalase T on rich medium was also observed with a *cdc25* mutant at the restrictive temperature (Table 3), whereas the *cdc28* allele tested had no significant effect. This is in good agreement with experiments showing that the *CDC25* gene product (6, 7, 12), but not the *CDC28* protein kinase (25), controls the activity of the *RAS*-adenylate cyclase pathway of *S. cerevisiae*. A low catalase T level in the *cdc28* mutant at the restrictive temperature and lack of catalase T induction by mating-factor arrest demonstrate that inhibition of mitotic division is not in itself sufficient to trigger expression of the gene. The fact that α-factor prevents *CTT1* induction by starvation medium (at least 90% of cells were arrested as shmoos under the conditions used) deserves further investigation.

Independent of the nutritional status of cells, the effect of the *ras2* mutation was reversed or at least partly compensated not only by the *sra1-13* mutation, but also by other mutations known to increase the activities of adenylate cyclase or protein kinases of the *RAS*-cAMP pathway (Table 3). Such an effect was observed with a *RAS2*$^{Val\text{-}19}$ mutant strain, which contains the version of the *S. cerevisiae RAS2* gene equivalent to the mammalian *ras* oncogene (20) in place of the wild-type gene. Further, three more *sra* mutants (8) were tested. Mutant allele *SRA4-5*, like *sra1-13*, is a very efficient suppressor of the effect of the *ras2* mutation on *CTT1* expression. It has been described (8) to be an allele of the *CYR1* gene, which codes for adenylate cyclase. Mutant alleles *sra5-5* and *sra6-15*, which are less efficient suppressors, have been reported to affect cAMP phosphodiesterase activity (43) and *RAS1* expression (8), respectively. The quantitative effect of the different *sra* mutations is in good agreement with the effectiveness of these alleles in suppressing other phenotypes of *ras2* mutants (8). All genetic data

TABLE 3. Catalase activities of *RAS*-cAMP pathway mutants

Strain	Relevant genotype	Special culture conditions	Activity[a] of catalase			
			A (YPD2%)	T (YPD2%)	A (STMD2%)	T (STMD2%)
EG81-40A	*RAS2*$^+$	–	0.8	2.1	2.7	28.3
EG81-40B	*ras2*	–	3.2	19.8	6.1	32.7
EG81-40C	*ras2*	–	2.9	18.9	5.8	27.2
EG81-40D	*RAS2*$^+$	–	1.2	2.3	3.2	24.8
KT308[b]	*RAS2*$^+$	–	2.9	3.3	4.2	38.0
KT131[b]	*ras2*	–	3.1	29.3	6.9	42.5
OL86	*cdc25-5*	23°C	–	10.3	–	37.5
		35°C	–	63.0	–	62.5
185-3-4	*cdc28-1*	23°C	–	3.3	–	21.1
		35°C	–	6.8	–	12.7
EG81-40D	Wild type	23°C	–	<0.2	–	10.9
		35°C	–	<0.2	–	11.3
1169	**a** *bar1*	–	–	<0.2	–	10.5
		Plus α-factor	–	<0.2	–	<0.2
KT301[b]	*RAS2*$^{Val-19}$	–	0.5	0.8	0.9	1.2
JC482[c]	*RAS2*$^+$	–	0.8	3.4	3.5	35.8
JC302-26B[c]	*ras2*	–	1.0	26.6	3.2	34.4
JC303-79[c]	*ras2 sra1-13*	–	0.3	0.3	0.5	0.6
JC303-31[c]	*ras2 SRA4-5*	–	0.4	0.3	0.5	0.8
JC303-18[c]	*ras2 sra5-5*	–	0.7	4.1	0.1	7.0
JC303-46[c]	*ras2 sra6-15*	–	0.9	19.9	0.6	12.9
OL170-4B	*rca1*	–	–	7.7	10.5	223.4
OL170-4B	*rca1*	10^{-5} M cAMP	–	–	8.6	210.3
OL170-4B	*rca1*	10^{-4} M cAMP	–	0	9.5	146.4
OL170-4B	*rca1*	10^{-3} M cAMP	–	0	10.5	91.2
OL170-4B	*rca1*	10^{-2} M cAMP	–	0	7.3	48.6

[a] Micromoles of H_2O_2 decomposed per minute per milligram of protein (37).
[b] Isogenic strains.
[c] Isogenic strains.

obtained are in line with the assumption that *CTT1* expression is under negative control by a cAMP-dependent protein kinase.

In at least qualitative agreement with the results presented in Fig. 2, *ras2* mutants showed a less pronounced effect of starvation conditions (STMD2%) on *CTT1* expression, and induction of this gene by starvation was completely prevented by the *RAS2*$^{Val-19}$ mutation and by the two more effective *ras2* suppressor alleles tested, *sra1-13* and *SRA4-5* (Table 3). These observations show that the starvation effect on *CTT1* expression is mediated by the *RAS*-cAMP pathway.

Biochemical evidence for the role of cAMP was obtained by starving a *rca1* mutant in the presence of different concentrations of cAMP. The *rca1* mutation has been shown to allow adenylate cyclase-deficient mutants to divide in the presence of exogenous cAMP (2). In the absence of cAMP, the *rca1* mutant tested exhibits significantly higher catalase T activity on starvation medium than do the other strains used in this study. However, catalase T activities vary at least by a factor of 20 among different wild-type strains of *S. cerevisiae* (H. S. Cross and H. Ruis, unpublished results), and the activity of the mutant is within the range observed for such strains. Simultaneously with a reduction of the number of cells arrested in the early G1 phase (data not shown), which has also been previously reported to occur in the mutant in the presence of exogenous cAMP (2), a significant reduction of the level of catalase T, but not of catalase A, by cAMP was observed (Table 3). We also used Northern hybridization to test whether cAMP depletion in a *cyr1* mutant caused an increase in the *CTT1* transcript level in the absence of protein synthesis, which was inhibited by cycloheximide. At least some effect of cAMP was observed under such conditions, but interpretation of the result is not straightforward, because of a very strong induction of *CTT1* mRNA by cycloheximide in the presence as well as in the absence of cAMP. The experiment is therefore not documented in detail. The cycloheximide effect observed is comparable to but more pronounced than that on the level of yeast *UBI4* mRNA, which is also controlled by cAMP (38).

TABLE 4. Genetic segregation of high-level expression of *CTT1-lacZ* fusion with *ras2*

Strain	Relevant genotype	β-Galactosidase activity[a]
BP30-6A	*RAS2*$^+$	0
BP30-6B	*ras2 ura3::CTT1-lacZ*	13.2
BP30-6C	*RAS2*$^+$ *ura3::CTT1-lacZ*	1.2
BP30-6D	*ras2*	0

[a] Nanomoles of ONPG hydrolyzed per minute per milligram of protein (26). Strains were grown on YPD2%.

Isolation and characterization of mutants with increased *CTT1* expression on complete medium. From the results described above, we concluded that it should be possible to detect mutations affecting the *RAS*-cAMP pathway by monitoring the expression of chromosomally integrated *CTT1-lacZ* fusion genes by staining colonies with X-Gal. An initial

TABLE 5. Phenotypes of catalase T nutritional response mutants

Strain	Relevant genotype	β-Galactosidase activity[a] on:		Catalase T activity[b] on YPD2%	Accumulation of:	
		YPD2%	STMD2%		Glycogen[c]	Trehalose[d]
A2-201	Wild type	0.2	2.3	2.8	+	7
PM57	*ctn1-1*	1.3	5.6	16.9	+++	66
PM116	*ctn2-1*	1.7	58.3	8.9	+	21
PM416	*ctn3-1*	7.1	11.3	19.8	+	10
PY1	Wild type	0.2	13.1	3.0	+	10
PYM801	*ctn4-1*	1.2	3.3	13.3	+	35
PYM864	*ctn5-1*	6.4	6.7	14.2	+++	88

[a] Nanomoles of ONPG hydrolyzed per minute per milligram of protein (26).
[b] Micromoles of H_2O_2 decomposed per minute per milligram of protein (37).
[c] Assayed in colonies by iodine staining (10).
[d] Micrograms of trehalose per milligram (dry weight); assayed at stationary phase on YPD2% (21).

account of experiments to characterize genes involved in this pathway, not described previously, is given here to illustrate the usefulness of this system. Mutants were isolated after mutagenesis of strains A2-201 and PY1, which contain a low-level expression version of the *CTT1-lacZ* fusion gene (Fig. 1), which has, however, retained the ability to respond to nutrient supply (Table 2). In our initial experiments, a total of 48 mutants with increased expression of the *CTT1-lacZ* fusion gene were detected by X-Gal staining of colonies. Some of those mutants, exhibiting a reasonably stable phenotype and showing increased catalase T activity compared with the parent strains (see below), were characterized in more detail. No screening for mutants temperature sensitive for growth was carried out in this initial study, and none of the mutants isolated at 30°C showed a pronounced growth defect at elevated temperature.

When the mutants obtained were mated with wild-type strains (PY1 for mutants derived from A2-201, and A2-201 for those obtained from PY1), most of the mutations (all of those described here in detail) turned out to be recessive. After sporulation of heterozygous diploids, all the mutants described below exhibited 2+:2− segregation. Complementation analysis showed that the mutants studied belong to five complementation groups. Preliminary designations for the genes probably corresponding to these complementation groups are *CTN1* to *CTN5* (where *CTN* stands for catalase T nutrition). One member of each complementation group (mutants PM57, PM116, and PM416 derived from wild-type strain A2-201 and mutants PYM801 and PYM864 derived from strain PY1) were further characterized in this initial analysis.

The main results of a characterization of mutant phenotypes are summarized in Table 5. As expected from the β-galactosidase assays carried out with colonies, crude extracts of all mutants grown on YPD medium exhibited higher β-galactosidase activities than did the corresponding wild-type strains. Catalase T activities were also elevated in all mutant strains. Since the *CTT1-lacZ* fusion gene in the strains is integrated at the *URA3* locus, this finding demonstrates that the mutations characterized are acting in *trans* and are truly affecting *CTT1* expression, and that their phenotype is in no way dependent on the presence of the *lacZ* part of the fusion gene. With the exception of mutant PM116, all mutants exhibit a reduced stimulation of *CTT1* expression by starvation conditions. Although the phenotypic characteristics outlined above have been consistently observed in a number of experiments, the significance of quantitative differences between mutants should not be overestimated, since our experiments have shown that expression levels are highly sensitive to minor changes in physiological conditions. All the genes mutated seem to exert their effects at the transcript level, since similar results to those described above were obtained in Northern experiments (results not shown). Like some of the *RAS*-cAMP pathway mutants, at least two of the mutants characterized (PM57 and PYM864) and some other mutants not described here in detail hyperaccumulate glycogen and trehalose.

Complementation of the mutants with *RAS*-cAMP pathway mutants hyperaccumulating glycogen, *cdc25* and *ras2* mutants, was therefore tested. No complementation of the β-galactosidase phenotype of the mutants was observed when mutant PM57 (*ctn1*) was mated with strain 321 (*cdc25-1*) or in matings of the *ras2* mutant strains EG81-40B and JC302-26B with strain PYM864 (*ctn5-1*). In tetrad analysis, no recombinants were detected in the cross between the *ctn1* and *cdc25* mutants among 29 complete tetrads. An analogous result was obtained in the cross of the *ctn5* mutant with the *ras2* mutant (39 complete tetrads were tested). The results obtained are consistent with the interpretation that *ctn1-1* is allelic to *CDC25* and that *ctn5-1* is a *RAS2* allele.

DISCUSSION

cAMP has been proposed to be involved in cell cycle control in *S. cerevisiae* and is known to affect the activity of enzymes like trehalase and glycogen synthetase (22). However, with the exception of some heat shock genes (34, 38), little information is available concerning its role in the control of gene expression. The results of this investigation show that the expression of the *S. cerevisiae CTT1* gene, which codes for a cytoplasmic catalase, is controlled by the supply of essential nutrients available to the cell. On rich, complete media, expression of the gene is low. When nitrogen, sulfur, or phosphorus sources become limiting, levels of the gene product are increased. Furthermore, our results make it very likely that regulation of *CTT1* expression by the quality of the carbon source is not due to typical glucose (carbon catabolite) repression, but is mediated by the more general mechanism involved in nutrient control of the gene. Nutrient levels are signalled to control regions of the *CTT1* gene by a system consisting of the CDC25 protein, the RAS2 protein, adenylate cyclase, and one or more cAMP-dependent protein kinases. Quantitative differences observed in hybridization experiments (Fig. 2) between effects of nutritional conditions and mutations may be explained by the leakiness of mutations and/or the existence of second genes involved in signal transduction (*RAS1* in *ras2* mutants). Quantitative differences between effects of nutri-

tional starvation on the level of *CTT1* mRNA (Fig. 2) and on activities of the final gene product (β-galactosidase or catalase T [Tables 2 and 3]) are most probably explained by the specific control of yeast catalase mRNA translation by hemin that was reported earlier (16). DNA sequences mediating nutrient control have been tentatively located in the 5′ upstream region of the *CTT1* gene (T. Belatti, M. Schant, and H. Ruis, unpublished results). Our results show that *CTT1* expression is repressed by the action of a cAMP-dependent protein kinase. The substrate of this protein kinase is unknown. It could be either a *CTT1* positive regulator inactivated by phosphorylation or a negative regulator activated by this modification. Although, for a number of reasons, we consider it more likely that cAMP-dependent protein phosphorylation affects *CTT1* gene transcription in a fairly direct manner, the data presented in this paper do not exclude a mechanism by which cAMP prevents cells from entering a G0-like state, which could be a prerequisite for high-level transcription of *CTT1*. Further experiments that should clarify this point are in progress.

In any case, the results of this investigation have provided a convenient assay for effects of nutrient limitation and for the activity of the *RAS*-adenylate cyclase pathway in *S. cerevisiae*, since colonies and extracts of strains bearing a chromosomally integrated *CTT1-lacZ* fusion gene can be easily tested for β-galactosidase. Our results show that this assay can be conveniently used to isolate regulatory mutants. At least some of the mutations detected affect genes involved in signalling nutrient levels to control regions of the *CTT1* gene and probably of other genes controlled by cAMP.

The fact that *RAS2* and *CDC25* alleles are among the mutations detected demonstrates that the approach used for mutant isolation is valid and provides additional evidence for the role of these genes in the regulation of *CTT1* gene transcription. To obtain new insights into signal transduction mechanisms, the mutants obtained must be studied in more detail and more mutants must be isolated and characterized. Among the mutants obtained by our assay, those affecting glycogen accumulation as well as *CTT1* expression should be located in genes whose products participate in signal transduction between nutrients and cAMP-dependent protein kinases. A second class of mutants also detected in this study is affected in *CTT1* expression but not in accumulation of storage carbohydrates. Either the corresponding mutations could be located in genes involved in some aspect of catalase regulation not connected to cAMP or, if the mutations are epistatic to suitable mutations in the *RAS*-cAMP pathway, they could identify genes involved in signal transmission between cAMP-dependent protein kinases and control regions of yeast genes regulated by cAMP.

The results of this study provide a hypothetical explanation for the function of a cytoplasmic catalase in connection with nutritional stress. Until recently, even the existence of nonperoxisomal, cytoplasmic catalases has been controversial (31), and their detailed function is unclear. This study shows that the cytoplasmic catalase T of *S. cerevisiae* is induced by nutrient stress. This suggests a protective but nonessential function of this antioxidant enzyme under conditions of suboptimal nutrient supply, in which reactions of, e.g., DNA or membrane lipids with oxygen metabolites might do more damage to a population of cells than in a rapidly dividing culture.

ACKNOWLEDGMENTS

We thank M. Eichler for carrying out trehalose assays and M. Breitenbach, K. Nasmyth, and P. Nurse for valuable discussions.

Financial support by grants from the Fonds zur Förderung der wissenschaftlichen Forschung, Vienna, and from the Hochschuljubiläumsstiftung der Stadt Wien is gratefully acknowledged.

LITERATURE CITED

1. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA **69**:1408–1412.
2. **Boy-Marcotte, E., H. Gareau, and M. Jacquet.** 1987. Cyclic AMP controls the switch between division cycle and resting state programs in response to ammonium availability in *Saccharomyces cerevisiae*. Yeast **3**:85–93.
3. **Bradford, M. M.** 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
4. **Breeden, L., and K. Nasmyth.** 1985. Regulation of the yeast HO gene. Cold Spring Harbor Symp. Quant. Biol. **50**:643–650.
5. **Broach, J. R., J. N. Strathern, and J .B. Hicks.** 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. Gene **8**:121–133.
6. **Broek, D., T. Toda, T. Michaeli, L. Levin, C. Birchmeier, M. Zoller, S. Powers, and M. Wigler.** 1987. The S. cerevisiae *CDC25* gene product regulates the *RAS*/adenylate cyclase pathway. Cell **48**:789–799.
7. **Camonis, J. H., M. Kalékine, B. Goudré, H. Garreau, E. Boy-Marcotte, and M. Jacquet.** 1986. Characterization, cloning and sequence analysis of the *CDC25* gene which controls the cyclic AMP level of *Saccharomyces cerevisiae*. EMBO J. **5**:375–380.
8. **Cannon, J. F., J. B. Gibbs, and K. Tatchell.** 1986. Suppressors of the *ras2* mutation of *Saccharomyces cerevisiae*. Genetics **113**:247–264.
9. **Cannon, J. F., and K. Tatchell.** 1987. Characterization of *Saccharomyces cerevisiae* genes encoding subunits of cyclic AMP-dependent protein kinase. Mol. Cell. Biol. **7**:2653–2663.
10. **Chvojka, A., M. Barlas, H. Ruis, G. R. C. B. Padrao, A. D. Panek, and J. R. Mattoon.** 1981. A regulatory mutation in yeast which affects catalase T formation and metabolism of carbohydrate reserves. Curr. Genet. **4**:47–50.
11. **Cohen, G., F. Fessl, A. Traczyk, J. Rytka, and H. Ruis.** 1985. Isolation of the catalase A gene of *Saccharomyces cerevisiae* by complementation of the *cta1* mutation. Mol. Gen. Genet. **200**: 74–79.
12. **Daniel, J., J. M. Becker, E. Enari, and A. Levitzki.** 1987. The activation of adenylate cyclase by guanyl nucleotides in *Saccharomyces cerevisiae* is controlled by the *CDC25* start gene product. Mol. Cell. Biol. **7**:3857–3861.
13. **Esposito, R. E., and S. Klapholz.** 1981. Meiosis and ascospore development, p. 211–287. *In* J. Strathern, E. Jones, and J. Broach (ed.), The molecular biology of the yeast *Saccharomyces*—life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
15. **Fink, G. R.** 1970. The biochemical genetics of yeast. Methods Enzymol. **17A**:59–78.
16. **Hamilton, B., R. Hofbauer, and H. Ruis.** 1982. Translational control of catalase synthesis by hemin in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **79**:7609–7613.
17. **Hartig, A., and H. Ruis.** 1986. Nucleotide sequence of the *Saccharomyces cerevisiae CTT1* gene and deduced amino-acid sequence of yeast catalase T. Eur. J. Biochem. **160**:487–490.
18. **Hörtner, H., G. Ammerer, E. Hartter, B. Hamilton, J. Rytka, T. Bilinski, and H. Ruis.** 1982. Regulation of synthesis of catalases and iso-1-cytochrome c in *Saccharomyces cerevisiae* by glucose, oxygen and heme. Eur. J. Biochem. **128**:179–184.
19. **Johnston, G. C., J. R. Pringle, and L. H. Hartwell.** 1977. Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. Exp. Cell Res. **105**:79–98.
20. **Kataoka, T., S. Powers, C. McGill, O. Fasano, J. Strathern, J. Broach, and M. Wigler.** 1984. Genetic analysis of yeast *RAS1* and *RAS2* genes. Cell **37**:437–445.

21. **Lillie, S. H., and J. R. Pringle.** 1980. Reverse carbohydrate metabolism in *Saccharomyces cerevisiae*: responses to nutrient limitation. J. Bacteriol. **143**:1384–1394.
22. **Matsumoto, K., I. Uno, and T. Ishikawa.** 1985. Genetic analysis of the role of cAMP in yeast. Yeast **1**:15–24.
23. **Matsumoto, K., I. Uno, T. Ishikawa, and Y. Oshima.** 1983. Cyclic AMP may not be involved in catabolite repression in *Saccharomyces cerevisiae:* evidence from mutants unable to synthesize it. J. Bacteriol. **156**:898–900.
24. **Matsumoto, K., I. Uno, K. Kato, and T. Ishikawa.** 1985. Isolation and characterization of a phosphoprotein phosphatase-deficient mutant in yeast. Yeast **1**:25–38.
25. **Mendenhall, M. D., C. A. Jones, and S. I. Reed.** 1987. Dual regulation of the yeast CDC28-p40 protein kinase complex: cell cycle, pheromone, and nutrient limitation effects. Cell **50**: 927–935.
26. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Pfeifer, K., T. Prezant, and L. Guarente.** 1987. Yeast HAP1 activator binds to two upstream activation sites of different sequence. Cell **49**:19–27.
28. **Pringle, J. R., and L. H. Hartwell.** 1982. The *Saccharomyces cerevisiae* cell cycle, p. 97–142. *In* J. Strathern, E. Jones, and J. Broach (ed.), The molecular biology of the yeast *Saccharomyces*—life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Rose, M., and D. Botstein.** 1983. Construction and use of gene fusions to lacZ (β-galactosidase) that are expressed in yeast. Methods Enzymol. **101**:167–180.
30. **Rothman-Denes, L. B., and E. Cabib.** 1970. Two forms of yeast glycogen synthetase and their role in glycogen accumulation. Proc. Natl. Acad. Sci. USA **66**:967–974.
31. **Ruis, H.** 1979. The biosynthesis of catalase. Can. J. Biochem. **57**:1122–1130.
32. **Seah, T. C. M., A. R. Bhatti, and J. G. Kaplan.** 1973. Novel catalatic proteins of bakers' yeast. I. An atypical catalase. Can. J. Biochem. **51**:1551–1555.
33. **Seah, T. C. M., and J. G. Kaplan.** 1973. Purification and properties of the catalase of bakers' yeast. J. Biol. Chem. **248**:2889–2893.
34. **Shin, D.-Y., K. Matsumoto, H. Iida, I. Uno, and T. Ishikawa.** 1987. Heat shock response of *Saccharomyces cerevisiae* mutants altered in cyclic AMP-dependent protein phosphorylation. Mol. Cell. Biol. **7**:244–250.
35. **Spevak, W., F. Fessl, J. Rytka, A. Traczyk, M. Skoneczny, and H. Ruis.** 1983. Isolation of the catalase T structural gene of *Saccharomyces cerevisiae* by functional complementation. Mol. Cell. Biol. **3**:1545–1551.
36. **Spevak, W., A. Hartig, P. Meindl, and H. Ruis.** 1986. Heme control region of the catalase T gene of the yeast *Saccharomyces cerevisiae*. Mol. Gen. Genet. **203**:73–78.
37. **Susani, M., P. Zimniak, F. Fessl, and H. Ruis.** 1976. Localization of catalase A in vacuoles of *Saccharomyces cerevisiae*: evidence for the vacuolar nature of isolated "yeast peroxisomes." Hoppe-Seyler's Z. Physiol. Chem. **357**:961–970.
38. **Tanaka, K., K. Matsumoto, and A. Toh-e.** 1988. Dual regulation of the expression of the polyubiquitin gene by cyclic AMP and heat shock in yeast. EMBO J. **7**:495–502.
39. **Tatchell, K.** 1986. *RAS* genes and growth control in *Saccharomyces cerevisiae*. J. Bacteriol. **166**:364–367.
40. **Tatchell, K., L. C. Robinson, and M. Breitenbach.** 1985. *RAS2* of *Saccharomyces cerevisiae* is required for gluconeogenic growth and proper response to nutrient limitation. Proc. Natl. Acad. Sci. USA **82**:3785–3789.
41. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
42. **Toda, T., S. Cameron, P. Sass, M. Zoller, J. D. Scott, B. McMullen, M. Hurwitz, E. G. Krebs, and M. Wigler.** 1987. Cloning and characterization of *BCY1*, a locus encoding the regulatory subunit of the cyclic AMP-dependent protein kinase in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:1371–1377.
43. **Wilson, R. B., and K. Tatchell.** 1988. *SRA5* encodes the low-K_m cyclic AMP phosphodiesterase of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8**:505–510.
44. **Winkler, H., G. Adam, E. Mattes, M. Schanz, A. Hartig, and H. Ruis.** 1988. Co-ordinate control of synthesis of mitochondrial and non-mitochondrial hemoproteins: a binding site for the HAP1 (CYP1) protein in the UAS region of the yeast catalase T gene (*CTT1*). EMBO J. **7**:1799–1804.