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Heat Shock Factor-independent Heat Control of Transcription of the *CTT1* Gene Encoding the Cytosolic Catalase T of *Saccharomyces cerevisiae**

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Transcription of the *Saccharomyces cerevisiae CTT1* gene encoding the cytosolic catalase T has been previously shown to be derepressed by nutrient stress. To investigate whether expression of this gene is also affected by other types of stress, the influence of heat shock on *CTT1* expression was studied. The results obtained show that expression of the gene is low at 23 °C and is induced rapidly at 37 °C. By deletion analysis, a promoter element necessary for high level induction by heat shock was located between base pairs −340 and −364 upstream of the translation start codon. This region was demonstrated to be sufficient for heat shock control by placing it upstream of a *S. cerevisiae LEU2-lacZ* fusion gene. Mutagenesis of the region showed that the response to heat shock is not mediated by a sequence similar to canonical heat shock elements, but by DNA elements also involved in nutrient control of transcription. Catalase T appears to have a function in protecting yeast cells against oxidative damage under stress conditions. Catalase T-containing strains are less sensitive to exposure to 50 °C ("lethal heat shock") than isogenic catalase T-deficient mutants, and catalase T-containing strains pretreated by incubation at 37 °C are less sensitive to H_2O_2 than pretreated catalase-deficient mutants.

CTA1 (1) and *CTT1* (2), the catalase genes of the yeast *Saccharomyces cerevisiae*, encode a peroxisomal protein, catalase A (3), and a cytosolic protein, catalase T (4). Expression of these two genes has been demonstrated to be under positive control by heme (1, 2, 5, 6). In *S. cerevisiae*, heme synthesis requires molecular oxygen. Heme has been postulated to signal the presence of oxygen to control regions of genes encoding those hemoproteins, which have functions in electron transport to oxygen (cytochromes) or as components of a system protecting cells against toxic oxygen metabolites (*e.g.* catalases) (7, 8).

While this type of control is common to both catalase genes they respond differently to other regulatory signals and are therefore likely to have distinct physiological functions. Catalase A is induced by fatty acids and appears to have a function connected to peroxisomal fatty acid β-oxidation (9). The *CTT1* gene is under negative control by cAMP (10). In *S. cerevisiae*, this second messenger is involved in intracellular signaling of nutrient levels (11). Thus, catalase T levels are low while cells are grown on complete medium and rise during nutrient starvation. A similar response has been reported for several heat shock proteins of *S. cerevisiae* (12–15). The combination of positive control by heat shock and negative cAMP control might therefore be characteristic for a class of stress proteins needed by yeast cells under conditions of stress caused by heat and/or by nutrient starvation.

We have tested therefore whether catalase T belongs to this class of proteins. Our results described in this paper show that *CTT1* transcription is positively controlled by heat shock via a heat shock transcription factor-independent mechanism. This finding suggests that the cytosolic catalase encoded by this gene is mainly needed when oxidative stress caused by hydrogen peroxide is combined with nutrient starvation or heat stress. Data obtained in the course of this investigation are consistent with such a function.

EXPERIMENTAL PROCEDURES

Yeast Strains, Media, Growth Conditions—The *S. cerevisiae* strains used in this study are listed in Table I. Strains were routinely grown on YPD medium (16) at 30 °C. In heat shock experiments, cells were grown at 23 °C to an optical density at 600 nm of 3, and cultures were subsequently shifted to 37 °C for the time periods indicated for individual experiments.

"Lethal Heat Shock" Treatment, H_2O_2 Resistance—To test resistance of cells under a "lethal heat shock" regime, logarithmic cultures were grown to an optical density at 600 nm of 1; stationary phase cultures were grown for 2 days. Cells were suspended in fresh medium at an optical density of 1, heated to 50 °C for 20 min, and plated after serial dilutions to assay for survivors. To test for H_2O_2 resistance, logarithmic cultures grown at 23 °C were divided, one-half of each culture was subjected to heat shock, and heat-shocked and non-heat-shocked cells were suspended at a density of 10^5 cells/ml in various concentrations of H_2O_2 in phosphate buffer, pH 6.8. Cells were incubated for 15 min, and serial dilutions were subsequently plated to assay for survivors.

Plasmids, Yeast Transformation—Plasmid pTB3 used for single copy integration of a *CTT1-lacZ* fusion gene into the chromosomal *URA3* locus and its derivatives bearing deletions in the region upstream of the *CTT1* gene are described elsewhere (17). The *CTT1* portion of the *CTT1-lacZ* fusion genes used in this study extends to base pair +390 of *CTT1* (10). The yeast integration vector pLS9 (18) bearing a *LEU2-lacZ* fusion gene lacking the UAS region of the *LEU2* gene and containing a unique *Eco*RI site suitable for integration of upstream fragments of other genes was donated by M. Carlson (Department of Genetics and Development, Columbia University, New York). The insertion of *CTT1* upstream elements or synthetic oligonucleotides into this vector is described elsewhere (17). A plasmid containing a synthetic heat shock element, which was used

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as one of the pLS9 inserts (construct AW3; insert sequence: 5′AATTCGTGCAGGTCG-ACTCTAGAAGCTTCTAGAGTCGAC CTGCACGAATTCGTGC-AGGTCGACTCTAGAAGCTTCTAGA GGATCCCCGGGTACC-GAGCTCG3′; canonical heat shock elements (19) are underlined), was donated by P. Sorger (Department of Microbiology and Immunology, University of California, San Francisco).

Linear DNA fragments produced by cutting the plasmids at a unique *Nco*I site within their *URA3* gene were used for yeast transformation (20). Genomic DNA of transformants was analyzed by hybridization according to Southern (21), and single copy integrants were used for further analysis. Oligonucleotides used were synthesized by G. Schaffner, Institute of Molecular Pathology, Vienna.

Enzyme Activities of Crude Cell Extracts—β-Galactosidase activity of extracts prepared by breakage of cells with glass beads (22) was assayed spectrophotometrically using *o*-nitrophenyl-β-D-galactoside as substrate (23). Under the conditions used, β-galactosidase was detectable down to a level of 0.1 nmol of substrate hydrolyzed per min per mg of protein. Catalase activity was assayed spectrophotometrically by following disappearance of H_2O_2 at 240 nm (24). Protein concentrations of extracts were assayed as described by Bradford (25). All values reported are averages of at least three independent experiments.

DNA-RNA Hybridization—Yeast poly(A)$^+$ RNAs were isolated as described previously (5). They were separated electrophoretically, blotted, and hybridized essentially as described by Thomas (26).

Gel Retardation Assay—Gel retradation experiments using purified heat shock transcription factor kindly donated by P. Sorger were carried out as described by Sorger and Pelham (27).

RESULTS

Expression of the CTT1 Gene Is Induced by Heat Shock—To investigate whether the *CTT1* gene is under control by heat shock, its expression was examined at the mRNA level, by assaying the dependence of catalase T activity on heat treatment of cells, and by following the expression of a *CTT1-lacZ* fusion gene during heat shock. Fig. 1 demonstrates that the level of mRNA transcribed from the endogenous *CTT1* gene is significantly enhanced when yeast cells grown at 23 °C are exposed to a mild heat shock (37 °C). In agreement with this observation, 5–10-fold induction of catalase T activity and of β-galactosidase produced by expression of a *CTT1-lacZ* fusion gene by heat shock is observed (Fig. 2). In contrast to catalase T, the peroxisomal catalase A is not induced by heat shock.

Localization of CTT1 Upstream Region Mediating Heat Shock Induction—The upstream region of the *CTT1* gene contains no elements conforming well to the canonical heat shock consensus (5′CNNGAANNTTCNNG3′) (19) or to the sequences more recently defined as functional heat shock elements of *Drosophila melanogaster* (three repeats of GAA

TABLE I
S. cerevisiae strains used

Strain	Genotype	Origin
WS17-5D	*Matα leu2 trp1 ura3 arg1*	(30)
GA74-1A	*Mat***a** *leu2 his3 trp1 ade8 cta1-2 ura3::CTT1-lacZ*	This work
GA74T-1A	*Mat***a** *leu2 his3 trp1 ade8 cta1-2 ura3::CTT1-lacZ ctt1::URA3*	This work
A2-200	*Matα leu2 his3 ura3::CTT1-lacZ can1*	(10)
SP4	*Matα leu1 arg4*	(42)
A50	*Mat***a** *leu1 arg4 ctt1-1 cta1-9*	(1)
DCT1-4B	*Matα leu1 arg4 ctt1-1*	(43)
JC482	*Matα leu2 ura3 his4*	(44)
JC482-T1	*Matα leu2 ura3 his4 ctt1::URA3*	This work
JC302-26B	*Matα leu2 ura3 his4 ras2::LEU2*	(45)
JC302-26B-T1	*Matα leu2 ura3 his4 ras2::LEU2 ctt1::URA3*	This work
JC303-79	*Matα leu2 ura3 his4 ras2::LEU2 sra1*	(45)
JC303-79-T1	*Matα leu2 ura3 his4 ras2::LEU2 sra1 ctt1::URA3*	This work

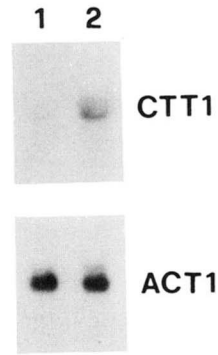

FIG. 1. **Heat shock induction of *CTT1* mRNA.** Poly(A)$^+$ RNA from strain A2–200 grown at 23 °C (*lane 1*) and from cells heat shocked at 37 °C for 50 min (*lane 2*) was probed with a *CTT1 Eco*RI fragment (+896 to +2014) (41) and an actin (*ACT1*) gene fragment after electrophoretic separation and transfer to nylon membranes.

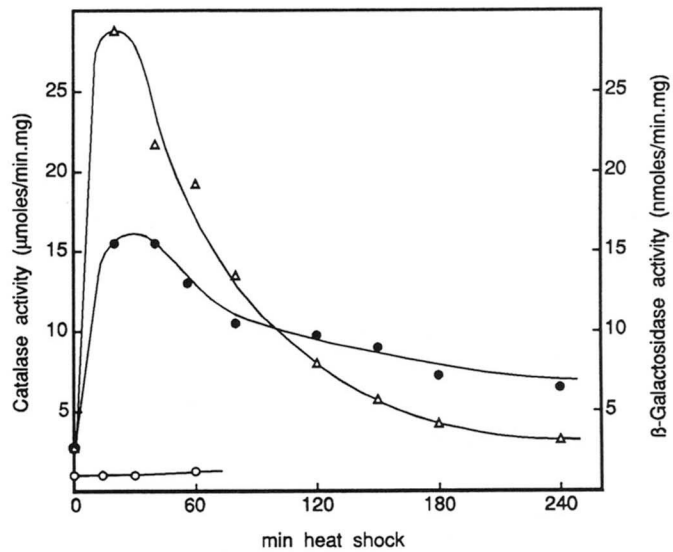

FIG. 2. **Kinetics of heat shock induction of *CTT1* expression.** Cells of strain GA74–1A (*CTT1$^+$ cta1 ura3::CTT1-lacZ*) and of strain DCT1–4B (*ctt1 CTA1$^+$*) were grown at 23 °C to logarithmic growth phase and transferred to 37 °C (0 min). Samples were taken, and specific catalase T (*full circles*) and β-galactosidase activity (*triangles*) was assayed in extracts from strain GA74–1A, catalase A activity (*open circles*) in extracts of strain DCT1–4B.

modules in alternating orientation punctuated by two bases of lower conservation) (28, 29). A set of internal deletions (17) covering the region previously shown to be important for activity of the *CTT1* promoter (30) was therefore tested for heat shock element activity. Preliminary experiments had shown that the *CTT1* promoter has only low basal activity at 23 °C (see also Fig. 1) and that minimal heat stress, which is provided during routine growth of cells at 30 °C, is necessary for its induction under all physiological conditions tested. It was possible therefore to limit the analysis for heat shock element activity to those regions, which had previously been shown to contain positive promoter elements (17, 30). When corresponding deletions are tested for their effect on heat shock control of a *CTT1-lacZ* fusion gene (Table II), it is evident that all those deletions causing a pronounced decrease in heat shock control (7–10-fold) lack a DNA sequence between base pairs −340 and −364. This region should therefore contain or at least overlap with an element important for heat shock control. To test whether the region containing the element defined by deletion analysis is sufficient for heat shock control, promoter fragments or synthetic oligonucleo-

TABLE II

Heat shock control of expression of CTT1-lacZ fusion genes

Cells of strain WS17-5D were transformed with *CTT1-lacZ* fusion genes (single copy chromosomal integrants) with wild type promoter or with the deletions indicated. They were grown at 23 °C to an optical density at 600 nm of 3. One half of the culture was heat shocked at 37 °C for 60 min, and the other half was kept at 23 °C. β-Galactosidase (nmoles of *o*-nitrophenyl-β-D-galactoside hydrolyzed per min per mg of protein (23)) was assayed in crude extracts.

Gene construct	Deletion	β-Galactosidase activity of cells incubated at		37 °C / 23 °C
		23 °C	37 °C	
TB3		0.2	7.0	35
TB301	−497/−455	0.1	4.3	43
TB314	−379/−260	2.4	11	4.7
TB347	−379/−331	0.1	0.3	3.0
TB350	−364/−340	0.1	0.5	5.0
TB354	−335/−289	0.7	10	14
TB310	−317/−231	1.2	22	18

TABLE III

Heat shock element activity of CTT1 upstream fragments

Cells of strain WS17-5D were transformed with plasmid pLS9 (18) or one of its derivatives (single copy chromosomal integrants) bearing the inserts indicated in the unique *Eco*RI site upstream of a *LEU2-lacZ* fusion gene. Transformants were grown at 23 °C to an optical density at 600 nm of 3. One half of the culture was heat shocked at 37 °C for 60 min, and the other half was kept at 23 °C. β-Galactosidase (nmoles of *o*-nitrophenyl-β-D-galactoside hydrolyzed per min per mg of protein (23)) was assayed in crude extracts.

Gene construct	CTT1 insert	β-Galactosidase activity of cells incubated at		37 °C / 23 °C
		23 °C	37 °C	
LS9		1.3	1.8	1.4
MS903	−522/−142	2.6	20	7.7
MS906	−522/−369	20	34	1.7
MS909	−480/−400	1.8	3.5	1.9
AW1N	−382/−325	2.1	25	12
AW1I	−325/−382[a]	1.5	13	8.7
AW2X	−382/−325[b]	3.2	47	15
AW3	Synthetic heat shock element	2.7	56	22

[a] Reverse orientation.
[b] Two tandem copies.

tides with corresponding sequences were tested for their ability to confer heat shock control to the yeast *LEU2* promoter lacking its own UAS element (Table III). The results obtained show that upstream fragments containing the region deleted in TB350 have heat shock element activity. They are functional in both orientations, and two copies of the region are comparable in their activity to a synthetic canonical heat shock element tested in combination with the *LEU2* promoter (AW3).

To localize the element mediating heat shock control more precisely, blocks of point mutations were introduced into construct AW1N (Fig. 3), and the mutant sequences were again tested for heat shock element activity. As shown in Table IV, mutation of the sequence similar to a canonical heat shock element (AW1N-13) has no significant effect on heat shock control. However, mutation of the sequence similar to the cAMP responsive elements of the *SSA3* promoter (31) (consensus sequence: 5′TA/TAGGGAT3′) significantly reduces response to heat shock (AW1N-11). A similar effect is observed with construct AW1N-14, but in this case, both basal promoter function and response to heat shock are affected. The sequence mutated in this construct (5′GTATTGTTTC3′) is also found in the upstream region

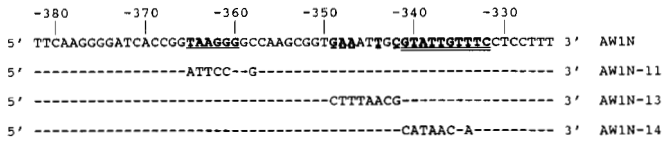

FIG. 3. **Mutations introduced into construct AW1N.** Identities to the cAMP responsive element of the yeast *SSA3* gene (31) (consensus: 5′TA/TAGGGAT3′; *solid line*), to the canonical heat shock consensus (19) (5′CNNGAANNTTCNNG3′; *broken line*) or to a sequence of the upstream region of the yeast *UBI4* gene (32) (5′GTATTGTTTC3′; *double line*) are indicated by *bold letters* in the sequence of AW1N. Base positions given are those of the *CTT1* wild-type promoter. Only bases mutated are printed for constructs AW1N-11, AW1N-13, and AW1N-14.

TABLE IV

Mutational analysis of heat shock control region of CTT1

Cells of strain WS17-5D were transformed with plasmid AW1N or one of its mutant versions (see Fig. 3). Transformants were grown at 23 °C to an optical density at 600 nm of 3. One half of the culture was heat-shocked at 37 °C for 60 min, the other half was kept at 23 °C. β-Galactosidase (nmoles of *o*-nitrophenyl-β-D-galactoside hydrolyzed per minute per mg of protein (23)) was assayed in crude extracts. The data presented are means ± S.D. of at least three independent experiments.

Gene construct	β-Galactosidase activity of cells incubated at		37 °C / 23 °C
	23 °C	37 °C	
AW1N	1.3 ± 0.2	11.6 ± 0.7	8.9
AW1N-11	1.4 ± 0.1	3.8 ± 0.2	2.7
AW1N-13	1.6 ± 0.2	9.7 ± 1.8	6.1
AW1N-14	0.7 ± 0.1	1.8 ± 0.3	2.6

(base pairs −390 to −381) of the *S. cerevisiae UBI4* gene (32), which has been reported to be under control by heat shock and cAMP (12). The response of the promoter to nitrogen limitation is also considerably reduced by both mutations affecting heat shock control.[1] Other mutations, which, together with AW1N-11, AW1N-13, and AW1N-14 cover the entire region between base pairs −365 and −326, have no significant effect on heat shock control (data not shown). In gel retardation experiments, no binding *in vitro* of purified heat shock transcription factor to oligonucleotides covering the region corresponding to base pairs −382 to −325, and no competition of this sequence for binding of heat shock transcription factor with a canonical heat shock sequence was observed (Fig. 4). It can be concluded from these results that the two DNA sequences mutated in constructs AW1N-11 and AW1N-14 are important for heat shock control of the promoter, and that the sequence similar to a canonical heat shock element, which is mutated in construct AW1N-13, is not involved in control of *CTT1* transcription.

Functional Relevance of Heat Shock Induction of the Cytosolic Catalase T—Although it is generally assumed that catalase protects cells against hydrogen peroxide or perhaps other peroxides, the function of this well characterized enzyme is not really sufficiently understood in detail. There is even less information concerning the relevance of extraperoxisomal catalases. The finding that expression of the gene encoding catalase T is controlled by heat shock suggests a function of this protein under heat stress conditions. We have attempted to obtain evidence for such a function by testing the heat shock resistance of various yeast strains producing catalase T or lacking it because of a disruption of the *CTT1* gene. The results obtained are summarized in Table V. The fact that stationary phase cells are much more resistant to heat shock

[1] C. Schüller and G. Marchler, unpublished results.

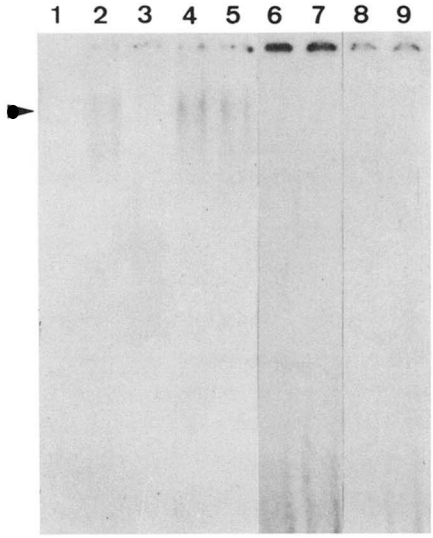

FIG. 4. **Gel retardation assay for binding *in vitro* of heat shock transcription factor to *CTT1* sequences mediating heat shock control and to a canonical heat shock element.** Labeled DNA fragments: *lanes 1–5*, HSE2 (27) (5′GGTCGACTCTAGAAGC TTCTAGAAGC-TTCTAGAGGATCCCCGGGTACCGAGCTCGA ATT3′); *lanes 6* and *7*, CTT1–11 (5′AATTCAGGGGATCACCGGA TTCCGGGCA-AGCGGTGAAATTGCGTATTGTTTCCTCCTTT GAATT3′); *lanes 8* and *9*, CTT1–10 (5′AATTCAAGGGGATCACC GGTAAGG-GGCCAAGCGGTAGAATTGCGTATTGTTTCCTCC TTTGA-ATT3′). Similarities to canonical heat shock elements (19) are *underlined*; because of slow migration of heat shock factor-heat shock element complex (27) (position: *arrow*) free DNAs have migrated out of the gel and are therefore not visible. Additions: *lanes 1*, *6*, and *8*, none; *lanes 2–5*, *7*, and *9*, plus yeast heat shock factor; *lane 3*, plus 10-fold excess unlabeled HSE2; *lane 4*, plus 200-fold excess unlabelled CTT1–11; *lane 5*, plus 200-fold excess CTT1–10.

TABLE V

Resistance of catalase T-positive strains and of catalase T-deficient mutants to "lethal heat shock"

Cells were grown to logarithmic or stationary phase, incubated at 50 °C for 20 min, and plated to assay for survivors. Means ± S.D. of three independent experiments are presented for the ratios of survivors in the pairs of isogenic *CTT1*$^+$ and *ctt1* mutant strains.

Strain	Relevant genotype	Growth phase	Survivors	$\frac{\text{Survivors}_{CTT1}}{\text{survivors}_{ctt1}}$
			%	
GA74-1A[a]	*CTT1*$^+$	Logarithmic	0.022	
GA74T-1A[a]	*ctt1*	Logarithmic	0.007	3.1 ± 1.0
GA74-1A[a]	*CTT1*$^+$	Stationary	26	
GA74T-1A[a]	*ctt1*	Stationary	11	2.4 ± 0.9
JC482[b]	*CTT1*$^+$	Logarithmic	0.033	
JC482-T1[b]	*ctt1*	Logarithmic	0.014	2.4 ± 0.6
JC302-26B[b]	*CTT1*$^+$ *ras2*	Logarithmic	1.44	
JC302-26B-T1[b]	*ctt1 ras2*	Logarithmic	0.39	3.7 ± 1.6
JC303-79[b]	*CTT1*$^+$ *ras2 sra1*	Logarithmic	0.014	
JC303-79-T1[b]	*ctt1 ras2 sra1*	Logarithmic	0.015	0.9 ± 0.2

[a] Isogenic strains.
[b] Isogenic strains.

than logarithmic cells and that mutations in the RAS-cAMP nutrient signaling pathway cause alterations in heat resistance is well established (see *e.g.* Refs. 33, 34). Further, it cannot be doubted that multiple factors, most of them not yet identified, contribute to heat resistance. The results presented in Table V establish the cellular catalase T level as one of these factors. In two different isogenic sets of strains, the *ctt1* mutants, which lack catalase T, exhibited significantly lower heat resistance than catalase T-containing *CTT1* wild-type cells. This effect was observed in logarithmic and in stationary phase wild-type cells and in *ras2* mutant strains. The only exception to this observation were the pair of *sra1* (*bcy1*) mutants tested, which lack the protein kinase A regulatory subunit. This latter result is also consistent with a role of catalase T in heat resistance since we have observed that *sra1* mutants do not show heat shock induction of catalase T and that their catalase T level is similar to that of *ctt1* mutants (17).

The observation that catalase T contributes to heat resistance of yeast cells provides a functional reason for its inducibility by heat shock. It is likely that it is the function of catalase T to protect cells against oxidative damage by H_2O_2, which might be more dangerous to yeast during heat stress than at lower temperatures. The results summarized in Fig. 5 show that pretreatment of yeast cells by a mild heat shock (37 °C) for 30 min dramatically increases their resistance to H_2O_2. This increase is much more pronounced in the catalase-positive strain which induces catalase T by the heat shock treatment than in the catalase-deficient mutant studied. A similar result was also obtained when strain GA74–1A (*CTT1*$^+$ *cta1*) was compared with the isogenic *ctt1* disruption mutant GA74T-1A (data not shown).

DISCUSSION

The results of this investigation demonstrate that transcription of the *CTT1* gene, which encodes the cytosolic catalase T of *S. cerevisiae*, is controlled by heat shock. This finding increases our understanding of the complex mode of regulation of this gene, which has previously been shown to be controlled by oxygen via heme and by nutrient availability via cAMP. The control of expression of *CTT1* is likely to reflect functional aspects. It suggests that the cytosolic catalase of *S. cerevisiae* is needed when oxidative stress caused by oxygen metabolites is combined with other types of stress (heat, nutrient starvation). Results obtained in the course of this investigation are consistent with this conclusion. They demonstrate that catalase T is not only induced by heat shock, but also has a protective effect at high temperatures. It is obvious from our results as well as from previous information that catalase is only one of a number of factors important for heat stress protection. Our demonstration of a protective

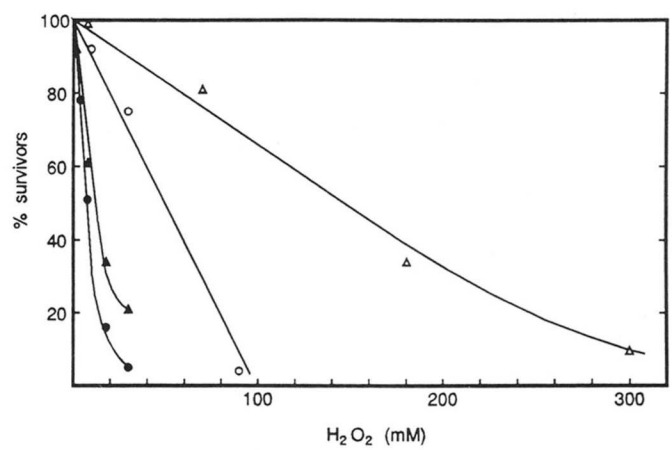

FIG. 5. **Hydrogen peroxide resistance of heat shocked and noninduced cells.** Strains were grown at 22 °C to logarithmic phase. A part of the cultures was subjected to heat shock at 37 °C for 30 min, and heat shocked and noninduced cells were suspended at various concentrations of H_2O_2 in phosphate buffer, pH 6.8, at a density of 10^5 cells/ml, incubated for 15 min at 22 °C, and plated to assay for survivors. *Closed symbols*, cells kept at 22 °C; *open symbols*, cells pretreated by heat shock; *circles*, strain A50 (*ctt1 cta1*); *triangles*, strain SP4 (*CTT1*$^+$ *CTA1*$^+$).

effect of a mild heat shock against toxic effects of hydrogen peroxide is consistent with the plausible role of catalase T as a peroxide scavenging agent under stress conditions. However, some questions connected to this function remain to be answered. One of these questions concerns the nature of the main cellular targets of hydrogen peroxide. It should be possible to identify these targets with the help of catalase null mutants of *S. cerevisiae* obtained by gene disruption.

Cross-protection by different types of stress is a phenomenon already known from prokaryotic systems. In *Salmonella typhimurium*, an overlapping set of proteins induced by oxidative stress and heat shock has been reported (35). Starvation-induced cross-protection against hydrogen peroxide has been observed in *Escherichia coli* (36). One of the catalases of *E. coli*, hydroperoxidase II, the product of the *katE* gene, is induced at the level of transcription in cells entering stationary phase (37). In contrast to *katE*, *katG*, which encodes hydroperoxidase I, is induced by oxidative stress via the oxyR sensor (38).

The localization of the positive promoter element mediating heat shock control of *CTT1* should be an important basis for further insights into cellular mechanisms of stress protection. Our data show that heat shock induction of *CTT1* transcription is possible without involvement of heat shock transcription factor. A similar finding has recently been reported for the *S. cerevisiae DDRA2* gene, which is activated by heat shock and by DNA damage (39). It is likely that heat shock control of *DDRA2* and *CTT1* is mediated by the same factor(s) since the *DDRA2* region reported to be sufficient for heat shock induction (39) contains sequences similar to both types of elements identified in this study. Coordinate control of expression of a catalase gene and of a gene transcribed in response to DNA damage is not surprising, since DNA damage is thought to be the main cause of toxicity of oxygen radicals (40).

Our results further demonstrate that induction of *CTT1* transcription by heat shock and by nitrogen starvation occurs via the same DNA elements. It is consistent with this notion that both effects have been shown to be antagonized by cAMP-dependent protein phosphorylation, and that no significant derepression of *CTT1* transcription by a low cAMP level was detected in cells grown at 23 °C (10, 17). An element of the *SSA3* gene encoding one of the HSP70 proteins of *S. cerevisiae* has recently been reported to be important for induction of transcription by nutrient limitation, and to be under negative control by cAMP (31), but a role of this element in heat shock control was not recognized in this study. This element is similar in sequence to one of the two *CTT1* regions important for nutrient and heat shock control (sequence 5′TAAGGG3′; see Fig. 3). The individual functions of the two subelements important for control by heat shock and by nutrients (cAMP) have to be investigated in further experiments. In principle, either both elements might be targets of heat shock and cAMP signals or one of the two sequences could be a nutrient (cAMP) control element and the other one a heat shock element. In the latter case, synergistic interaction of both elements would be necessary for full response to heat shock and nutrient levels. The fact that synergism between the separate heme (HAP1) control elements of the promoter with heat shock and nutrient control elements has been observed (17) demonstrates that this assumption is reasonable.

It appears likely that the combination of positive control by heat shock with negative cAMP control will be observed in a major subgroup of genes encoding stress proteins. Sequences similar to one of the elements identified in our study (5′TAAGGG3′) are present in the upstream regions of all genes reported to be under combined control by these two mechanisms (*UBI4* (12, 32) and *HSP12* (14) in addition to *CTT1* and *SSA3*). It should be pointed out, however, that, with the exception of *DDRA2* and *CTT1*, the heat shock response of the genes mentioned is at least partly mediated by heat shock transcription factor. It remains to be investigated whether these newly discovered control elements respond to other types of stress, how the two sequences important for derepression by heat shock and nutrient stress interact, and which factors are involved in the integration of different types of signals like heat stress and nutrient availability.

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