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A *Saccharomyces cerevisiae* UAS element controlled by protein kinase A activates transcription in response to a variety of stress conditions

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Transcription of the *Saccharomyces cerevisiae CTT1* gene encoding the cytosolic catalase T is activated by a variety of stress conditions: it is derepressed by nitrogen starvation and induced by heat shock. Furthermore, it is activated by osmotic and oxidative stress. This study shows that a *CTT1* upstream region previously found to be involved in nitrogen, cAMP and heat control (base pairs −382 to −325) contains a UAS element (STRE, −368 to −356), which is sufficient for the activation of a reporter gene by all types of stress acting on *CTT1*. Gel retardation experiments demonstrated the existence of a factor specifically binding to STRE, but to a lesser extent to mutated elements having partly or entirely lost the ability to mediate stress control. Heat activation of STRE, but not of a canonical heat shock element, is enhanced by a *ras2* defect mutation, which enhances thermotolerance, and is dramatically reduced by a *bcy1* disruption mutation, which decreases thermotolerance. It can be hypothesized, therefore, that the novel stress control element is important for the establishment of induced stress tolerance.

Key words: *CTT1*/heat shock/nutrient stress/osmotic stress/oxidative stress

Introduction

It is well established that, like in many or probably all types of organisms, tolerance of a wide variety of types of stress can be induced in the yeast *Saccharomyces cerevisiae* (Lindquist and Craig, 1988; Collinson and Dawes, 1992; Sanchez *et al.*, 1992; Varela *et al.*, 1992). Examples for this include the phenomenon of thermotolerance induced by a mild heat shock or the resistance to long periods of nutrient deprivation, which is induced in wild-type yeast cells during the transition to stationary phase. It has been observed that mild heat shock also induces increased resistance to nutrient starvation, and that transition to stationary phase dramatically increases heat resistance of yeast cells. This phenomenon of cross-protection could be explained by the existence of one mechanism or, more likely, a limited number of mechanisms protecting cells against a wide variety of types of stress. Such (a) system(s) could be alternatively or synergistically induced by different stress signals. Evidence for the involvement of the protein kinase A signal pathway in the induction of various types of stress responses has been obtained, but the findings of Cameron *et al.* (1988) indicate that either alternative signalling mechanisms can replace a signal transmitted by control of activity of protein kinase A or that this enzyme acts only as a rather general modulator of stress signals transmitted by other means.

It has generally been assumed that heat shock proteins are important components of the system protecting cells against various types of stress, and that their induction mediated by heat shock transcription factor binding to heat shock elements plays an important, if not essential role in the induction of stress tolerance (Lindquist and Craig, 1988). While this assumption might be at least partly correct, a number of findings demonstrate that the actual situation is much more complicated. Only a limited number of heat shock proteins, particularly hsp104 (Sanchez and Lindquist, 1990) and hsp70 (Li *et al.*, 1992), have been demonstrated to play a role in the induction of stress tolerance. It has recently been shown by Smith and Yaffe (1991) that full thermotolerance can be induced by a mild heat shock in a mutant producing a temperature-sensitive heat shock transcription factor which does not mediate induction of the major heat shock proteins during heat shock treatment. Furthermore, recent studies (Kobayashi and McEntee, 1990, 1993; Wieser *et al.*, 1991) have demonstrated that at least some proteins that are or may be involved in stress protection can be induced by heat via a heat shock factor-independent mechanism. One of these proteins, the product of the *DDR2* gene (previously called *DDRA2*), is also induced by DNA damage (McClanahan and McEntee, 1986), and might have a function in the control of negative effects caused by such lesions. Transcription of the *CTT1* gene encoding the cytosolic catalase T of *S.cerevisiae* is induced by oxygen, heme, nutrient starvation and heat shock (Hörtner *et al.*, 1982; Bissinger *et al.*, 1989; Wieser *et al.*, 1991). This enzyme plays a protective role in connection with oxidative and heat stress (Wieser *et al.*, 1991).

This study shows that the *CTT1* gene is induced by a broad variety of types of stress. All these stress signals, including heat and nutrient stress, are mediated by a 13 bp DNA element, which also appears to be present in the promoter regions of a number of other genes encoding yeast stress proteins.

Results

Localization of a cAMP-responsive nutrient control element of CTT1

Previous work from our group has demonstrated that the upstream region of the *CTT1* gene contains a UAS element mediating derepression by nutrient (nitrogen) starvation (Belazzi *et al.*, 1991). In these earlier studies, expression of a *LEU2−lacZ* fusion gene driven by the DNA region containing this element (base pairs −382/−325; construct AW1N) was used to assay for UAS activity. Derepression

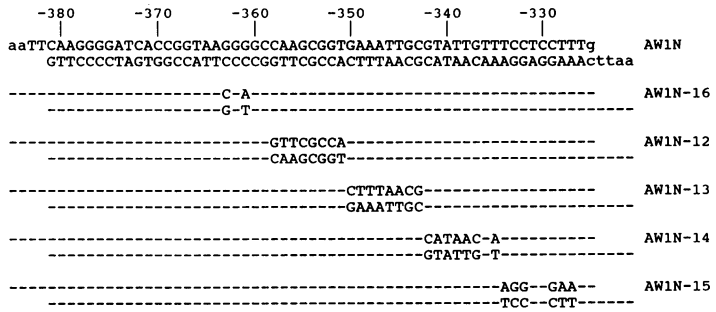

Fig. 1. Sequences of mutated versions of construct AW1N. Base positions are those of the *CTT1* wild-type promoter. Only bases mutated are printed for constructs AW1N-12 to 16. Bases introduced for cloning into vector pLS9 are presented in lower case letters.

of expression of this construct in a *ras2* mutant compared to an isogenic wild-type strain was used as a criterion for negative control of this element by cAMP *in vivo*. Since the DNA region demonstrated by these experiments to be sufficient for mediating control was fairly large a more precise localization of the DNA element(s) involved in control was necessary.

According to results of a deletion analysis a DNA sequence (−364 to −340) contained within this cAMP-responsive region is important for both UAS activity and control by cAMP. It was therefore tested which base substitutions within this region or in other regions of the insert of construct AW1N cause a (partial) loss of activity of the control element. The mutations introduced are summarized in Figure 1. Like the wild-type element (Belazzi *et al.*, 1991), the mutant elements were cloned upstream of a *LEU2−lacZ* fusion gene into the *Eco*RI site of the integrative vector pLS9 (Sarokin and Carlson, 1986). Only constructs containing a single copy of the element in the orientation found in the *CTT1* upstream region were used for further experiments. They were integrated into the chromosomal *URA3* locus of strain WS17-5D, transformants were tested by Southern analysis and β-galactosidase levels were assayed in single-copy integrants. The results of such assays are summarized in Table I. They demonstrate that two regions characterized by mutations around base pair −362 and around base pair −340 are important for expression. This observation may be explained by the existence of one fairly extended positive DNA element or by the presence of two elements, which are important for derepression by N-starvation as well as for response to the *ras2* mutation. These two elements would be defined by the bases mutated in constructs AW1N-16 and AW1N-14, respectively. One of these regions (TAAGGGG; −366 to −360) is similar in sequence to an element (sequence: TTAGGGA; base pairs −179 to −173) of the *SSA3* gene, which encodes one of the hsp70 proteins of yeast. This element has been demonstrated by Boorstein and Craig (1990) to be responsive to cAMP and to mediate derepression during postdiauxic shift. The other region (−341 to −332) coincides with a sequence identical to base pairs −390 to −381 of the yeast polyubiquitin (*UBI4*) gene (Özkaynak *et al.*, 1987). No function has yet been assigned to the *UBI4* sequence, which flanks a canonical heat shock element.

To analyse the function of the two subelements of the insert of AW1N in more detail, oligonucleotides corresponding to them were cloned into the *Eco*RI site of pLS9, and it was assayed whether they have UAS activity and whether they are sufficient to confer control by cAMP and/or nitrogen starvation to the *LEU2* promoter. The results obtained are summarized in Figure 2. They show that a 13 bp element (−368 to −356) of *CTT1* is sufficient for conferring cAMP and N-starvation control to the *LEU2* promoter. With an oligonucleotide corresponding to the second element localized by mutagenesis of the region no UAS activity was observed when it was cloned in two copies in front of the *LEU2* promoter (data not shown). However, UAS activity was observed with higher copy numbers (Figure 2). This activity was not significantly affected by the *ras2* mutation or by N-starvation. In a further experiment it was tested whether the UAS activity of the element located around base

Table I. Mutational analysis of cAMP-responsive nutrient control element of *CTT1* gene

pLS9 derivative	β-Galactosidase activity (nmol/min/mg of protein)		
	RAS2$^+$ YPD	*ras2* YPD	*RAS2*$^+$ STMD
AW1N	7.5	29.6	77.4
AW1N-16	3.1	4.5	26.7
AW1N-12	5.7	40.1	94.5
AW1N-13	4.4	40.9	75.3
AW1N-14	1.8	3.4	7.0
AW1N-15	3.3	13.5	87.0

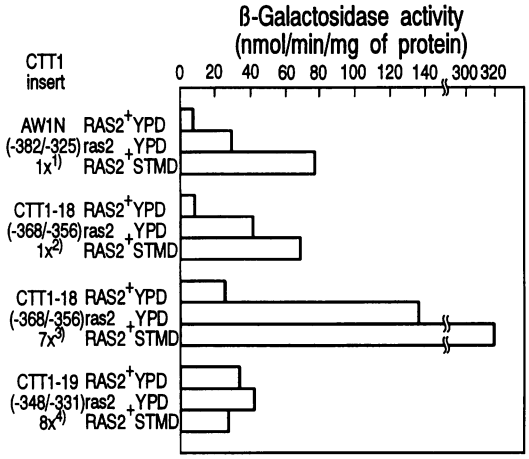

Fig. 2. Derepression by *ras2* mutation and on N-starvation medium (STMD) mediated by *CTT1* upstream elements. Cells of strain WS17-5D transformed with pLS9 derivatives were grown on YPD and transferred to STMD for 18 h. [1)]Insert orientation corresponding to that in *CTT1* upstream region. [2)]Actual insert sequence: 5′-GGTAAGGGGCCTTACC-3′. [3)]Orientation of insert copies: →→→→←←←. [4)]Orientation of insert copies: ←→→←→→→←.

Table II. Effect of *bcy1* mutation on expression driven by cAMP-responsive control element (base pairs −368/−356) of *CTT1*

Relevant genotype	β-Galactosidase activity (nmol/min/mg of protein)		Catalase T activity (μmol/min/mg protein)
	YPD	STMD	STMD
Wild-type	21.1	143.0	121.1
bcy1	0.6	1.2	<0.2

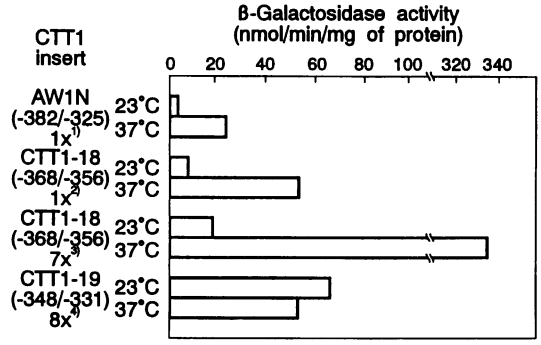

Fig. 3. Heat shock control by *CTT1* upstream elements. Cells of strain WS17-5D transformed with pLS9 derivatives were grown on YPD at 23°C and transferred to 37°C for 1 h. [1]Insert orientation corresponding to that in *CTT1* upstream region. [2]Actual insert sequence: 5′-GGTAAGGGGCCTTACC-3′. [3]Orientation of insert copies: →→→←←←. [4]Orientation of insert copies: ←→→←→→→←.

pair −362 is also affected by a mutation causing loss of the regulatory protein kinase A subunit (*bcy1*). As Table II demonstrates, this mutation causes an almost complete loss of UAS activity and of derepression by N-starvation. This effect is very similar to the response of the wild-type *CTT1* promoter to high constitutive protein kinase A activity [see catalase T activity in Table II and Bissinger *et al.* (1989)].

Heat shock control mediated by nutrient control element

We have previously demonstrated that heat shock control of *CTT1* transcription does not involve a canonical heat shock element, and mutational analysis has provided evidence for the importance of the sequence between base pairs −365 and −361 as well as of the region between base pairs −341 and −335 for heat shock activation of transcription (Wieser *et al.*, 1991). In the course of our further characterization of this new type of heat stress control element, it was investigated whether any one of the two regions important for heat control defines an element sufficient for mediating the heat shock effect. For this purpose, the constructs used for the localization of the cAMP-responsive nutrient control element were studied again. Yeast strains containing single chromosomal copies of *LEU2−lacZ* fusion genes activated by *CTT1* elements were grown at 23°C and subsequently transferred to 37°C. Figure 3 presents a comparison of β-galactosidase levels of extracts from cells kept at 23°C with those from heat-shocked cells. The data obtained are remarkably similar to the results of N-starvation experiments. The UAS element located around base pair −362 is sufficient for conferring heat control to a yeast promoter not regulated by heat shock. The region between base pairs −348 and −331 exhibits UAS activity when present in multiple copies, but is not sufficient for heat shock control in spite of the fact that its mutation in construct AW1N causes a reduction of heat shock control at least as dramatic as that caused by a mutation of the element located near base pair −362 (Wieser *et al.*, 1991). While it cannot be excluded from the data obtained that a negative element repressing the UAS element at base pair −362 has been artificially created by introducing the point mutations present in construct AW1N-14, it seems more likely to us that the down effect on expression observed is caused by disruption of a second UAS element located around base pair −340.

A pronounced synergism between heat shock and protein kinase A effects on control of *CTT1* expression has previously been noted (Belazzi *et al.*, 1991). It is further known that logarithmic cells (Pringle and Hartwell, 1982) or *bcy1* mutants (Cannon and Tatchell, 1987; Shin *et al.*, 1987; Toda *et al.*, 1987) are dramatically less thermotolerant than stationary phase wild-type cells or than cells with reduced adenylate cyclase activity (Iida, 1988). To obtain evidence concerning the importance of the protein kinase A-regulated heat shock element at base pair −362 for heat stress protection, heat shock activation of the *LEU2−lacZ* gene expression driven by this element was studied in wild-type, *ras2*, and *bcy1* mutant cells. This expression was compared to that driven by a synthetic canonical heat shock element (Sorger and Pelham, 1987; Wieser *et al.*, 1991), which is activated by heat shock transcription factor. The kinetics of heat shock induction of the two constructs are presented in Figure 4. As Figure 4A shows, heat shock transcription factor-dependent activation is hardly affected by mutations in the RAS-protein kinase A pathway. On the other hand, the dramatic synergism of heat shock and protein kinase A control previously observed with the complete *CTT1* promoter is entirely preserved in constructs activated only via the UAS element located at base pair −362 (Figure 4B).

The enhancement of heat induction of transcription via the *CTT1* heat stress element by a mutation reducing protein kinase A activity *in vivo*, and the virtual abolishment of this induction in strains with high constitutive protein kinase A activity, might be explained by a direct role of the protein kinase A pathway in the transmission of heat stress signals. This hypothesis was further tested by studying β-galactosidase formation mediated by the cAMP-responsive heat stress element in a mutant strain, which has low protein kinase A activity (*tpk1^w tpk2 tpk3*) and lacks a functional regulatory subunit of protein kinase A (*bcy1*). As expected, such a strain exhibits fairly high β-galactosidase activity when grown at 30°C on complete medium and, in addition, shows only 2-fold stimulation of β-galactosidase production by N-starvation (Figure 5). Like in previous experiments, heat shock control parallels control by nitrogen levels since expression is high at 23°C and the temperature dependence of β-galactosidase expression is dramatically reduced in the mutant strain. In contrast to this finding, a *LEU2−lacZ* construct activated by a canonical heat shock element (construct AW3) exhibited normal heat shock induction in the *bcy1 tpk1^w tpk2 tpk3* mutant strain (data not shown).

Activation of the CTT1 promoter and of the cAMP-responsive heat stress element by other types of stress

Since it should be the obvious immediate function of a catalase protein to protect cells against deleterious effects

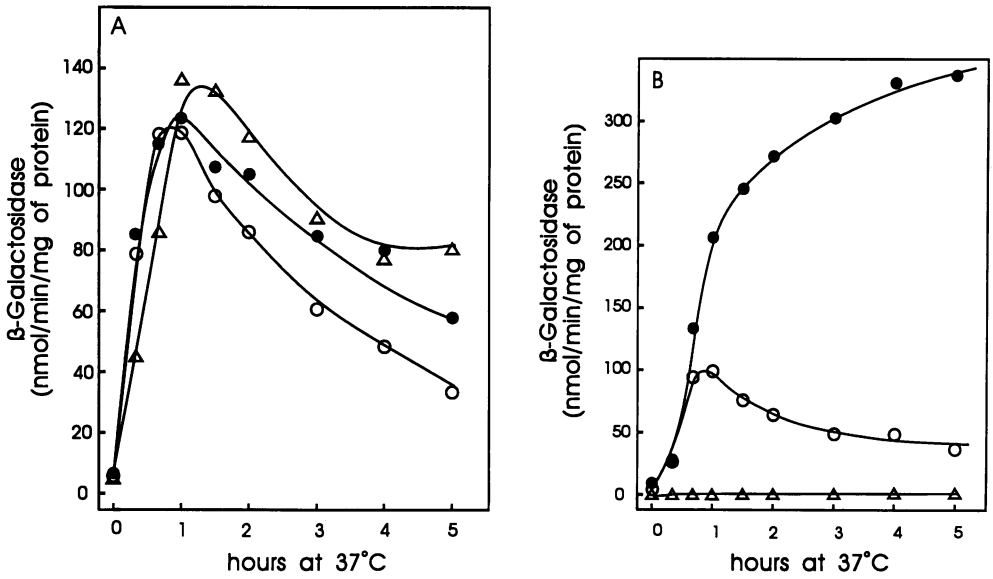

Fig. 4. Kinetics of heat shock activation mediated by canonical heat shock element (**A**) and by cAMP-responsive heat stress element CTT1-18 (−368/−356)1x (**B**). Cells of strain WS17-5D, and of isogenic *ras2* and *bcy1* disruption mutants transformed with construct AW3 containing a canonical heat shock element or with construct CTT1-18 cloned into vector pLS9, were grown on YPD at 23°C and were transferred to 37°C for various time periods. Every hour the cultures were diluted with fresh YPD to an OD_{600} of 1. Open circles: wild-type strains; closed circles: *ras2* mutants; triangles: *bcy1* mutants.

of hydrogen peroxide, it appeared necessary to test the potential of H_2O_2 to induce the formation of catalase T or to activate the formation of β-galactosidase via the stress control element identified in our studies. Furthermore, studies by Varela *et al.* (1992) have demonstrated that a number of yeast proteins are induced by osmotic stress. Among these proteins are the heat shock proteins hsp12 and hsp26 which, at least according to their expression patterns, could be controlled at the transcriptional level by an element related to the stress control element of *CTT1* (Praekelt and Meacock, 1990; Susek and Lindquist, 1989). These considerations suggested that catalase T might be induced by a broader variety of stress signals. Its induction by osmotic stress (0.3 M NaCl, 0.4 M sorbitol) and by oxidative stress (0.3 mM H_2O_2) was therefore studied. As the results summarized in Table III demonstrate, catalase T formed by expression of the wild-type *CTT1* gene is rapidly induced in cells growing logarithmically on YPD medium by 0.3 M NaCl as well as by 0.4 M sorbitol. Higher concentrations of NaCl have similar effects after a lag period, which appears to depend on the concentration used, other salts (LiCl, KCl) have a similar potential to induce catalase T (data not shown). Whereas the induction of catalase T observed in our experiments with exogenous H_2O_2 is considerably less pronounced than that triggered by osmotic stress, the effects observed are nevertheless highly significant. It should be emphasized that the effects caused by this agent appear to depend on minor details of experimental conditions and on the genetic background of strains used (endogenous levels of various antioxidant enzymes?). In spite of the fact that some background expression was observed with the *CTT1−LEU2−lacZ* construct containing the minimal *CTT1* upstream sequence observed in our studies to act as a cAMP-responsive heat stress element, it is apparent from the data presented in Table III that this element responds to osmotic and oxidative stress, and that the kinetics of induction of β-galactosidase are at least similar to those observed with

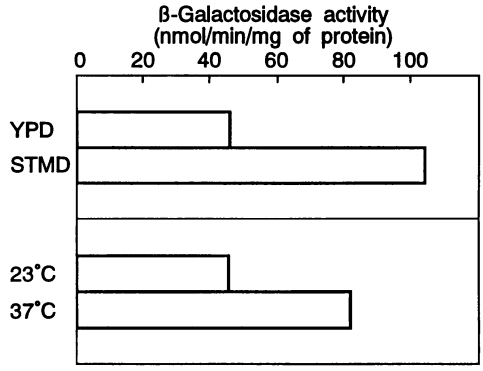

Fig. 5. Derepression by N-starvation medium (STMD) and activation by heat shock mediated by cAMP-responsive heat stress element CTT1-18 (−368/−356)7x in strain S13-58ArA (*bcy1 tpk1^w tpk2 tpk3*). To assay for derepression by N-starvation, cells transformed with pLS9 derivative CTT1-18 were grown on YPD and transferred to STMD for 24 h, to assay for heat shock activation they were grown at 23°C and were transferred to 37°C for 1 h.

catalase T. These data indicate that the UAS element located around base pair −362 mediates the transcriptional response to a variety of stress factors. It is therefore at least tentatively suggested to name this UAS element stress response element (STRE).

Crude yeast protein extracts contain a factor binding specifically to STRE

Gel retardation experiments were carried out with labelled oligonucleotide CTT1-20 (−382/−355; see Figure 6), which contains the STRE located around base pair −362 and an element of similar sequence (base pairs −375 to −381). As illustrated in Figure 7, formation of one major specific complex was observed (arrow) with crude protein extracts isolated from cells grown to stationary phase. Similar binding activities were observed in experiments with protein

Table III. Induction of catalase T and of a *CTT1*(−368/−356)−*LEU2*−*lacZ* fusion gene by osmotic and oxidative stress

Additions	Catalase T activity (μmol/min/mg protein) after			β-Galactosidase activity (nmol/min/mg protein) after		
	0 h	0.5 h	1 h	0 h	0.5 h	1 h
−	< 0.5	< 0.5	< 0.5	32.4	43.3	55.0
0.3 M NaCl	< 0.5	40.1	48.8	32.4	358.3	527.3
0.4 M sorbitol	< 0.5	45.2	47.6	32.4	465.3	563.3
0.3 mM H_2O_2	< 0.5	1.2	4.1	32.4	90.3	155.4

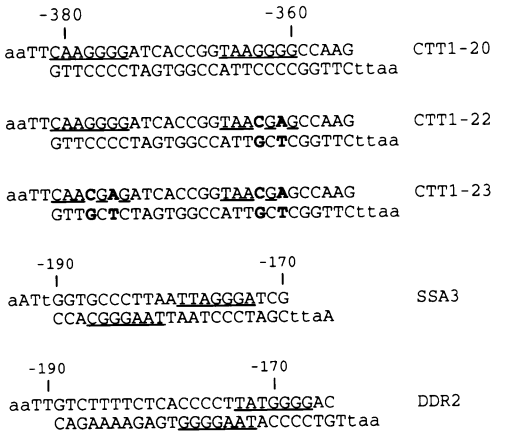

Fig. 6. Sequences of oligonucleotides used in gel retardation experiments and in experiments summarized in Table IV. Sequences similar to the sequence 5′-TAAGGGG-3′ are underlined, bases mutated in oligonucleotides CTT1-22 and CTT1-23 are depicted in bold letters. Bases introduced for cloning into vector pLS9 are presented in lower case letters.

extracts prepared from early logarithmic cells, from cells grown at 23°C and from heat-shocked cells (not shown).

To correlate the binding activity observed with regulatory capacity, two mutant oligonucleotides with two point mutations in the STRE element previously shown to be functional (CTT1-22) or in both STRE-like elements (CTT1-23) were prepared (see Figure 6). These oligonucleotides were cloned into vector pLS9 and the UAS activities of the various constructs obtained were tested as usual (Table IV). As expected, oligonucleotide CTT1-20 mediates derepression by N-starvation as well as by heat shock in both orientations. A single copy of oligonucleotide CTT1-22, which is a shortened version of mutant construct AW1N-16, exhibits significantly reduced regulatory activity. Two tandem copies of this oligonucleotide, together containing two copies of the CAAGGGG sequence, exhibit regulatory activity which is close to that of one copy of wild-type construct CTT1-20. In contrast, constructs containing one or two tandem copies of oligonucleotide CTT1-23 have entirely lost regulatory activity. It can be concluded from these results that the STRE-like element located around base pair −380 does indeed have STRE activity. It should be emphasized that UAS activities observed under N-starvation and under heat shock conditions correlate as well in these experiments as in all previous experiments using different wild-type or mutant constructs.

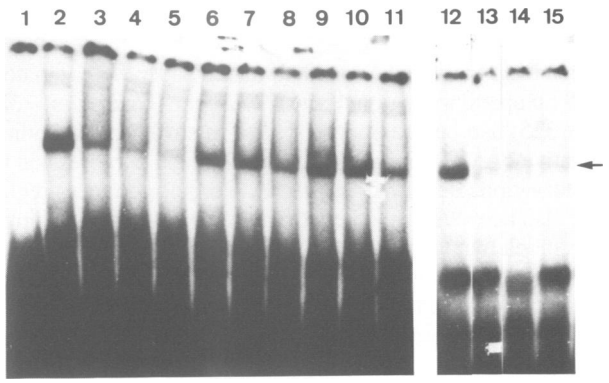

Fig. 7. Gel retardation experiments with ^{32}P-labelled oligonucleotide CTT1-20 and crude yeast protein extract isolated from stationary phase cells. Lane 1: ^{32}P-labelled CTT1-20 (−382/−355); lanes 2−15: plus 40 mg of protein extract; lane 3: plus 25-fold molar excess of unlabelled CTT1-20; lane 4: plus 50-fold excess of CTT1-20; lane 5: plus 100-fold excess of CTT1-20; lanes 6−8: plus 20-, 40- and 80-fold excess of unlabelled CTT1-22; lanes 9−11: plus 20-, 40- and 80-fold excess of CTT1-23; lane 12: no competitor added; lane 13: plus 50-fold excess of unlabelled CTT1-20; lane 14: plus 50-fold excess *DDR2* heat shock factor-independent heat shock element (Kobayashi and McEntee, 1990, 1993) (−191/-164); lane 15: plus 50-fold excess *SSA3* PDS element (Boorstein and Craig, 1990) (−190/-170). Arrow: STRE-specific complex

Table IV. STRE activities of wild-type and mutant *CTT1* oligonucleotides (−383/−355) in *CTT1*−*LEU2*−*lacZ* fusion genes

CTT1 insert	β-Galactosidase activity (nmol/min/mg protein)			
	YPD	STMD	23°C	37°C
CTT1-20	9.5	74.6	9.7	55.7
CTT1-20R[a]	11.4	57.1	14.1	60.0
CTT1-22	5.7	10.1	5.4	12.2
CTT1-22-2X[b]	6.2	33.5	9.7	44.6
CTT1-23R[a]	4.9	4.9	5.4	6.4
CTT1-23R[a]-2X[b]	11.4	10.1	–	–

[a]Reverted orientation.
[b]Two tandem copies of insert.

Competition experiments with mutant oligonucleotides CTT1-22 and CTT1-23 demonstrate that UAS activities correlate rather well with competitor activities of the oligonucleotides, at least with lower levels of competitors. Under these conditions, CTT1-22, which still contains one functional STRE element, is less active as a competitor than CTT1-20. Low concentrations of CTT1-23 with two mutated STREs are almost entirely inactive as competitor. To test whether the factor binding to the STRE of the *CTT1* gene can also bind to similar elements of other yeast promoters, competition experiments were carried out with oligonucleotides corresponding to the PDS element of the *SSA3* gene, which has been demonstrated to be under negative control by protein kinae A (Boorstein and Craig, 1990), and to the heat shock factor-independent heat shock element of the *DDR2* gene (Kobayashi and McEntee, 1990, 1993) (see sequences in Figure 6). Both oligonucleotides were found to be as efficient competitors as the *CTT1* element used in comparable concentration.

Discussion

When the experiments described in this paper were initiated, a 58 bp upstream region of the *CTT1* gene (base pairs −382 to −325) had been demonstrated to be sufficient to mediate negative cAMP control of transcription and derepression by N-starvation (Belazzi *et al.*, 1991). Furthermore, this region had also been shown to be sufficient for heat stress control, two subelements important for this type of control had been identified by mutational analysis of the 58 bp fragment, and it had been demonstrated that this activation did not involve a functional canonical heat shock element (Wieser *et al.*, 1991).

The results presented in this paper go considerably beyond these previous data. They demonstrate that the same 13 bp element (−368 to −356) is sufficient for response to cAMP, for control by N-starvation and for heat shock control. Point mutations within this element and within an adjacent similar element eliminate UAS activity under both heat shock and N-starvation conditions. Therefore, some of the bases essential for both types of response have been defined and shown to be identical. Gel retardation experiments have demonstrated the existence of a factor binding specifically to both these elements. Point mutations eliminating UAS activity have a negative effect on binding of this factor, which might therefore play a role in control mediated by the element. Furthermore, competition experiments have demonstrated that this factor also binds to the cAMP-responsive PDS element of the *SSA3* gene (Boorstein and Craig, 1990) and to the heat shock transcription factor-independent heat shock element of the *DDR2* gene (Kobayashi and McEntee, 1990, 1993). This finding is consistent with the assumption that transcriptional control mediated by the *CTT1*, *SSA3* and *DDR2* elements shares at least one *trans*-acting regulator.

Our results show that the transcription of the *CTT1* gene is also activated by at least two other types of stress, osmotic stress caused either by high salt or by sorbitol, and by oxidative stress caused by exogenous hydrogen peroxide. Stress caused by heat, nutrient starvation as well as oxidative and osmotic stress can all be mediated by the same 13 bp cAMP-responsive DNA element. The designation stress response element (STRE) is therefore proposed to characterize the function of this element.

Whereas there is clearly some functional overlap between canonical heat shock elements (HSEs), which are activated by heat shock transcription factor, and the STRE characterized by our studies, these elements seem to have distinct overall functions. As demonstrated by our and other studies, the activity of HSEs is not significantly affected by nutrient starvation or by protein kinase A. On the other hand, the STRE of the *CTT1* gene does not respond to arsenite (C.Schüller, unpublished data). A more systematic comparison of the responses of STREs, HSEs, the PDS element of the *SSA3* gene and the heat stress element of the *DDR2* gene would undoubtedly be important to clarify the extent of functional overlap between these elements. Cloning of transcription factors binding to these elements and a detailed analysis of the function of these factors will obviously also be necessary for a more detailed understanding of mechanisms of transcriptional control by stress. To mention just three hypothetical models, it could be suggested that all the genes mentioned above interact with one type of factor receiving all those stress signals shown to activate the STRE of the *CTT1* gene. Alternatively, one factor binding to all elements could interact with a number of other factors, which are specific for certain stress signals and for a subset of genes controlled by stress, or elements which are similar in sequence could interact *in vivo* with a family of factors which have different, but overlapping, specificities for various stress signals. Beyond such mechanisms, alternative signal pathways mediating only the response to one type of stress might well exist. In this context, it would be interesting to test whether the salt-induced HAL1 protein (Gaxiola *et al.*, 1992), which appears to have a specific function in connection with NaCl stress, can be induced by other types of stress. Specific mechanisms might also explain the observation that salt tolerance can be induced by previous salt treatment, but not by a previous heat shock (Varela *et al.*, 1992), or the finding that induction of hydrogen peroxide resistance does not induce thermotolerance (Collinson and Dawes, 1992), whereas a mild heat shock can induce tolerance to oxidative damage by H_2O_2 (Watson, 1990; Wieser *et al.*, 1991).

The relative importance of canonical heat shock elements activated by heat shock transcription factor, of STREs and of other, post-transcriptional mechanisms for the establishment of stress tolerance remains to be investigated. Heat shock proteins like hsp70 (see e.g. Li *et al.*, 1992) or hsp104 (Sanchez and Lindquist, 1990), which are induced via heat shock transcription factor, have been shown to contribute to thermotolerance and hsp104 has been recently demonstrated to have a protective function in connection with other types of stress (Sanchez *et al.*, 1992). However, levels of heat shock proteins and thermotolerance do not always correlate, and Smith and Yaffe (1991) have recently reported that a mutant yeast strain defective in the activation of heat shock factor and in heat shock induction mediated by this factor exhibits normal induction of thermotolerance by heat treatment. It has been suggested therefore that modification of pre-existing factors or post-translational events like the accumulation of trehalose (De Virgilio *et al.*, 1991) are more important for the induction of thermotolerance than activation of transcription mediated by heat shock factor. Alternatively, or in addition, induction of transcription by stress via STREs should play a role in the establishment of induced stress tolerance. It is presently unclear whether the cAMP-protein kinase A pathway is directly involved in the transduction of stress signals to STREs, whether nitrogen levels (and perhaps heat shock) regulate protein kinase A activity by a cAMP-independent mechanism (Thevelein, 1991) or whether protein kinase A is a powerful modulator of STRE activity, but not directly involved in the transduction of stress signals. Nevertheless, the strong correlation between reduced adenylate cyclase or protein kinase A activity, the induction of transcription mediated by STREs and between enhanced thermotolerance of yeast cells suggests a functional connection. As our studies show, no such correlation exists in the case of transcription mediated by canonical heat shock elements. Furthermore, the product of the *CTT1* gene, catalase T, has been shown to be beneficial to yeast cells under heat stress conditions (Wieser *et al.*, 1991), but is obviously only one, perhaps minor factor among a number of components of the complex cellular system necessary for efficient stress protection. While it cannot be entirely excluded at the moment that this complex system is predominantly controlled by one mechanism, it seems more likely that several types of mechanisms contribute to the

phenomenon of stress tolerance of yeast and of other eukaryotes.

Materials and methods

Yeast strains and media

The *S.cerevisiae* strains WS17-5D (MATα *leu2 trp1 ura3 arg1*) (Spevak *et al.*, 1986) GA74-6A (MAT**a** *leu2 ura3 his3 trp1 ade8 cta1-2*) and S13-58Ara (MAT**a** *leu2 ura3 his3 trp1 ade8 bcy1::LEU2 tpk1^{w} tpk2::HIS3 tpk3::TRP1*) (obtained from C.Denis) were used for integrative transformation with derivatives of plasmid pLS9 (Sarokin and Carlson, 1986). Strains were routinely grown on YPD medium (Fink, 1970) at 30°C and were shifted to nitrogen starvation medium (STMD) as described previously (Belazzi *et al.*, 1991). In heat shock experiments, cells were usually grown at 23°C to an OD_{600} of 2–3, and cultures were then shifted to 37°C. In experiments involving osmotic or oxidative stress, cells were diluted from an overnight culture in YPD medium at an OD_{600} of 1. Concentrated solutions of NaCl, sorbitol or H_2O_2 (< 10% of culture volume) were added to the final concentrations desired, and equal volumes of water were added for control experiments. Cultures were then incubated at 30°C for the times indicated in connection with individual experiments.

Plasmids, DNA constructions, oligonucleotides

Vector pLS9 (Sarokin and Carlson, 1986) was kindly supplied by M.Carlson. Derivatives containing *CTT1* upstream sequences or a synthetic heat shock element were produced by cutting pLS9 with *Eco*RI, followed by ligation with appropriate fragments or synthetic oligonucleotides. Orientation and number of elements inserted was assayed by DNA sequencing. Construct AW1N (base pairs −382 to −325 of *CTT1*) has been described previously (Belazzi *et al.*, 1991). Sequences of synthetic oligonucleotides representing mutated versions of the AW1N insert are summarized in Figure 1. The following two oligonucleotides were hybridized to produce the insert of construct CTT1-18 (−368 to −356): 5′AATTGGTAAGGGGCC3′ and 5′AATTGGCCCCTTACC3′. The insert of construct CTT1-19 (−348 to −331) was produced by hybridization of oligonucleotides 5′AATTGCGTATTGTTTCCT3′ and 5′AATTAGGAAACAATACGC3′. The sequences of the double-stranded oligonucleotides CTT1-20, CTT1-22, CTT1-23, SSA3 (Boorstein and Craig, 1990) and DDR2 (Kobayashi and McEntee, 1990) are summarized in Figure 6. AW3, a construct containing a synthetic canonical heat shock element, has been described previously (Wieser *et al.*, 1991). A 5.8 kb *Hin*dIII–*Xba*I fragment isolated from plasmid pRa530 (Tatchell *et al.*, 1984), which was donated by K.Tatchell, was used for *ras2::LEU2* gene disruption. A 6.5 kb *Bam*HI fragment used for *bcy1::LEU2* disruption was isolated from a plasmid produced from a *bcy1::URA3* disruption plasmid (Cannon and Tatchell, 1987) by exchanging the *URA3* gene with a *LEU2* gene. Synthetic oligonucleotides were synthesized by G.Schaffner, Institute of Molecular Pathology, Vienna.

Gel retardation experiments

Gel retardation experiments were performed with double-stranded synthetic oligonucleotides. DNAs used were labelled with [γ-^{32}P]ATP using T7 polynucleotide kinase (Boehringer Mannheim). Protein extracts were prepared from strain GA74-6A. Cells were grown at 30°C on YPD to stationary phase (OD_{600} of 7.5). Protein extracts were prepared essentially as previously described (Winkler *et al.*, 1988), omitting the ammonium sulphate precipitation step. Labelled fragments (1 ng double-stranded oligonucleotide/lane) were incubated with protein extract (40 μg) for 30 min at 4°C in buffer containing 50 mM Tris–HCl (pH 8.0), 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA and 12% glycerol. Samples were subjected to gel electrophoresis on 5% polyacrylamide gels in 45 mM Tris, 45 mM borate, 1.25 mM EDTA, 4 % glycerol (pH 8.0).

Yeast transformation

Transformation of yeast strains was carried out according to Beggs (1978) or as described by Ito *et al.* (1983). Linearized pLS9 derivatives were integrated into the *URA3* locus (Belazzi *et al.*, 1991), their mode of integration was tested by Southern analysis (Southern, 1975) and only single-copy integrants were used for further studies. Gene disruptions were carried out with the fragments listed above.

Enzyme activities

Catalase activity of cell extracts was assayed spectrophotometrically at 240 nm (Beers and Sizer, 1952). β-Galactosidase activity was assayed using *o*-nitrophenyl-β-D-galactoside as substrate (Miller, 1972). Protein was determined by the method of Bradford (1976). All specific catalase or β-galactosidase activities reported are averages of at least three independent experiments.

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