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The heat shock response in yeast: differential regulations and contributions of the Msn2p/Msn4p and Hsf1p regulons

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Summary

The heat shock transcription factor Hsf1p and the stress-responsive transcription factors Msn2p and Msn4p are activated by heat shock in the yeast *Saccharomyces cerevisiae*. Their respective contributions to heat shock protein induction have been analysed by comparison of mutants and wild-type strains using [^{35}S]-methionine labelling and two-dimensional gel electrophoresis. Among 52 proteins induced by a shift from 25°C to 38°C, half of them were found to be dependent upon Msn2p and/or Msn4p (including mostly antioxidants and enzymes involved in carbon metabolism), while the other half (including mostly chaperones and associated proteins) were dependent upon Hsf1p. The two sets of proteins overlapped only slightly. Three proteins were induced independently of these transcription factors, suggesting the involvement of other transcription factor(s). The Ras/cAMP/PKA signalling pathway cAMP had a negative effect on the induction of the Msn2p/Msn4p regulon, but did not affect the Hsf1p regulon. Thus, the two types of transcription factor are regulated differently and control two sets of functionally distinct proteins, suggesting two different physiological roles in the heat shock cellular response.

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Introduction

When shifted to high temperatures, yeast cells induce the synthesis of heat shock proteins, some of which are chaperones and associated proteins involved in the care of damaged proteins. In *Saccharomyces cerevisiae*, this response is controlled by the heat shock transcription factor (Hsf1p), which binds the heat shock-responsive element (HSE) (Wu, 1995). The heat shock response also involves the synthesis of metabolic enzymes and antioxidant defence proteins (Boucherie *et al*., 1996). Several of these proteins are also induced at the diauxic transition by Msn2p and/or Msn4p transcription factors (Msn2/4p) (Boy-Marcotte *et al*., 1998). The genes encoding these proteins generally contain stress response elements (STRE: CCCCT) in their regulatory region, a sequence that mediates the response to many environmental changes, including heat shock, osmotic stress and the diauxic transition (Marchler *et al*., 1993). In response to various stresses, the Msn2/4p transcriptional factors translocate to the nucleus and activate transcription of target genes by binding to STREs (Martinez-Pastor *et al*., 1996; Görner *et al*., 1998). The Msn2/4p-dependent gene induction through the STREs is sensitive to the cAMP-dependent protein kinase (PKA), which is under the control of the Ras/cAMP signalling pathway (Marchler *et al*., 1993; Martinez-Pastor *et al*., 1996; Boy-Marcotte *et al*., 1998). One role of this pathway is to control the nuclear localization of Msn2/4p negatively (Görner *et al*., 1998).

We have analysed the contribution of Hsf1p and Msn2/4p to the yeast heat shock responses, using a global approach for the analysis of gene expression based on two-dimensional gel electrophoresis. We compared the heat shock response between wild-type cells and cells lacking either a functional *HSF1* gene or *MSN2* and *MSN4* genes. We observed that, whereas Msn2/4p controls the expression of most of the carbon metabolic enzymes and antioxidant defence proteins of the heat shock response, Hsf1p is very specific for the induction of the chaperones and chaperone-associated heat shock proteins.

Results

The heat shock response stimulon

The heat shock response was obtained by a temperature

shift from 25°C to 38°C. The proteins were labelled with [^{35}S]-methionine 15 min after the shift (cf. *Experimental procedures*), as it has been reported previously that the induction of heat shock proteins is transient, with a maximum expression at 15–20 min (Smith and Yaffe, 1991a; Martinez-Pastor *et al*., 1996). This response was analysed in three different wild-type strains, MYY290, W303-1A and OL526, by comparative two-dimensional gel electrophoresis. This two-dimensional gel analysis allows the detection of about 1000 soluble proteins, of which more than 350 have been identified (Boucherie *et al*., 1996). We detected 52 proteins (Fig. 1) whose synthesis was induced by more than twofold in at least two of the three genetic backgrounds. The results obtained in the OL526 strain are presented in Fig. 2. Forty-one of these proteins are the products of known genes, of which 13 had not been identified previously as heat shock proteins: these are products of *ALD2/5*, *ALD7*, *DAK1*, *DNM1*, *GAL1*, *TFS1*, *TKL2*, *YBR149w*, *YGL037c*, *YLR206w*, *YNL274c*, *YOR021c* and *WTM1*. The 11 other proteins were not identified as gene products. Numerous proteins were also repressed in these conditions; they involved essentially

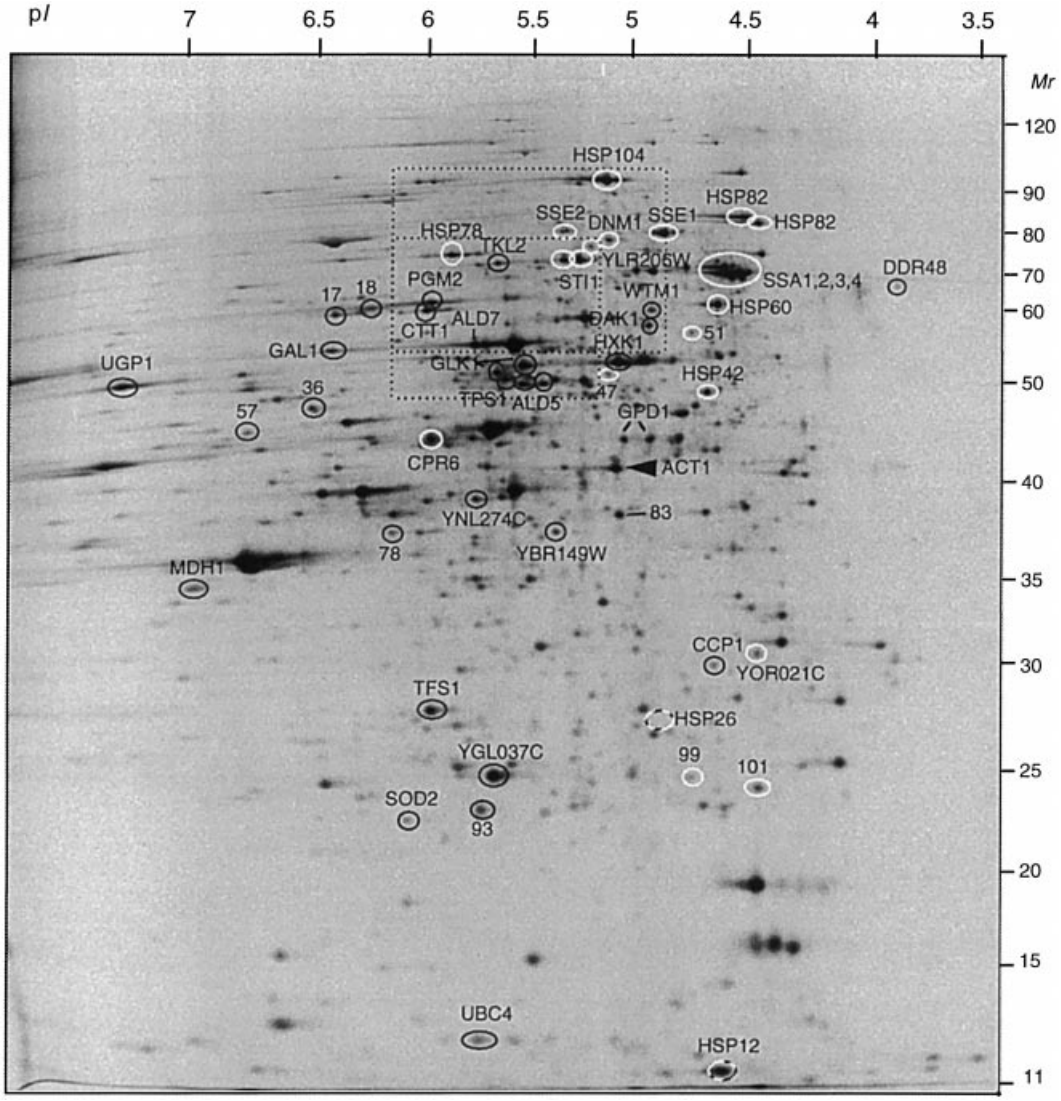

Fig. 1. Two-dimensional gel electrophoresis of total yeast proteins expressed under heat shock conditions. The wild-type strain W303-1A was labelled with [^{35}S]-methionine after transfer from 25°C to 38°C, and two-dimensional gel electrophoresis was performed as described in *Experimental procedures*. All proteins induced by heat shock and Act1p (used as internal standard) are indicated on the map. The unidentified spots are defined by a number. The protein spots are designated with: a white circle when they are less induced in the MYY385 *hsf1-m3* mutant (class 1); a black circle when they are less induced in the *Wmsn2msn4* mutant (class 2); a white and black circle when they are less induced in *hsf1-m3* mutant and in *msn2msn4* mutant; a bar when they are normally induced in the two mutants (class 3). The position of the HSP26 gene product is also indicated, although not detectable as ^{35}S-labelled protein in W303-1A (Fig. 2). The two framed regions are enlarged in Fig. 3.

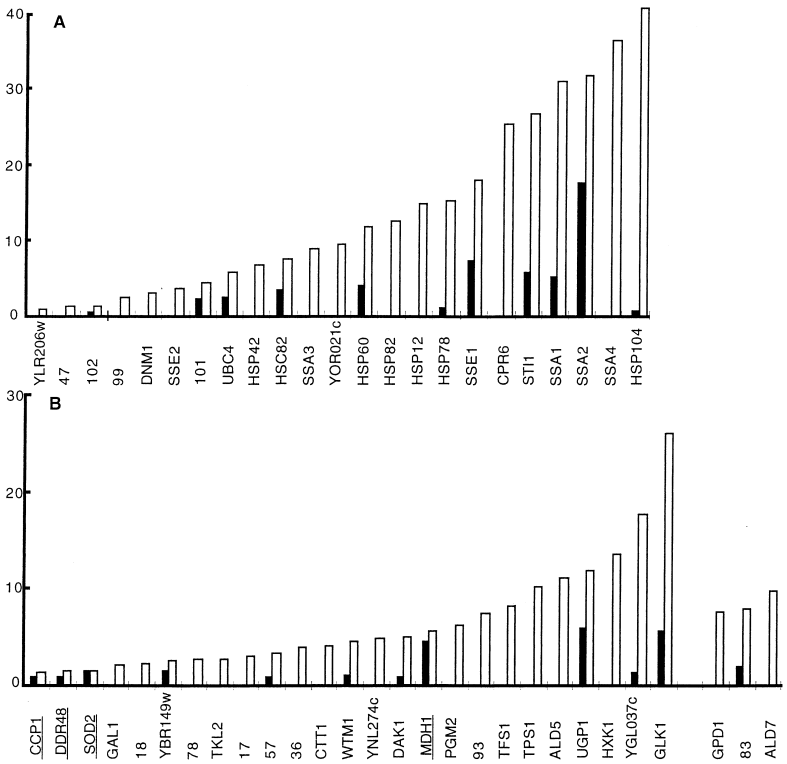

Fig. 2. Induction of heat shock proteins in OL526 strain. [^{35}S]-methionine-labelled extracts of strain OL526 under standard and heat shock conditions were subjected to two-dimensional gel electrophoresis, and the 52 induced proteins were quantified as described in *Experimental procedures*. The values (in pixel units) were normalized to Act1p spot. Expression level is represented by a black bar under standard conditions and by a white bar under heat shock conditions. Experiments have been repeated twice, and the standard deviation ranged from 20% to 30%. Similar results were obtained in W303-1A and MYY290 strains (data not shown). Underlined gene names correspond to gene products that were not significantly induced in OL 526, but were induced more than twofold in the two other genetic backgrounds. In the case of Hsp26p, quantitative data with [^{35}S]-methionine were obtained only in the MYY290 background. In the other backgrounds, Hsp26 does not contain methionine or cysteine residues, and the induction was qualitatively observed by Coomassie blue coloration.
A. Class 1 gene products.
B. Class 2 and 3 gene products, as referred to in Fig. 1 and Table 1.

amino acid biosynthetic enzymes, ribosomal proteins and translation factors (data not shown).

Genes dependent upon Hsf1p

To identify among the 52 induced proteins those that are dependent upon Hsf1p, we used a mutant strain (MYY385) containing the thermosensitive allele *hsf1-m3*. The heat shock response was compared between the mutant strain MYY385 and the corresponding isogenic wild-type strain MYY290. An effect of the Hsf1p defect was considered as significant when the induction was reduced at least twofold in the mutant compared with the wild type. The heat shock induction of 24 proteins was undetectable or significantly reduced in the *hsf1-m3* background (Fig. 1, Fig. 3A for details). Twenty of these proteins are known, most of them being chaperones and associated proteins (Table 1, class 1). In addition to some heat shock proteins described previously as regulated by Hsf1p (Ssa1p, 2, 3 and 4, Hsp104, Hsc82 and Hsp82, Hsp78, Hsp26, Hsp12, Sti1p; Nicolet and Craig, 1989; Smith and Yaffe, 1991a,b; Lindquist and Kim, 1996; Zarzov *et al*., 1997; Treger *et al*., 1998), we found new proteins belonging to this regulon: the Hsp70 chaperones Sse1p and Sse2p, the mitochondrial Hsp60 chaperonin (a homologue of *Escherichia coli* GroE), the ubiquitin-conjugating enzyme Ubc4p, the dynamin homologue Dnm1p and Hsp42. It is noteworthy that the proteins that were the most expressed at high temperature (i.e. Hsp104 and the Ssap family) belong to this class (Fig. 2). The effect of the *hsf1-m3* thermosensitive mutation was variable depending on the gene; some proteins were not induced at a detectable level in the mutant, and others were induced but at a lower level than in the wild-type strain (Table 1, class 1).

Genes dependent upon Msn2/4p

Msn2/4p are redundant transcription factors (Martinez-Pastor *et al*., 1996). Therefore, we analysed the effect of the null deletion of both genes on the heat shock response in the W303-1A genetic context. Twenty-seven proteins of the 52 analysed were undetectable or have at least a twofold reduced expression at 38°C compared with the wild type (Fig. 1 and Fig. 3B for details). Among them, 21 are the products of known genes (Table 1, class 2). Most of them are involved in carbon metabolism (*PGM2*, *UGP1*, *TPS1*, *HXK1*, *GLK1*, *GAL1*, *TKL2*, *YBR149w*, *DAK1*, *ALD2/5* and *MDH1*) or in response to oxidative stress (*CTT1*, *SOD2* and *CCP1*). We also found some gene products with diverse functions (*DDR48*, *TFS1* and *WTM1*/*YOR230w*). Msn2/4p also control the induction of Hsp12 and Hsp26. These data are consistent with previous results showing that *CTT1*, *HSP12*, *HSP26*, *PGM2* and *TPS1* are regulated by Msn2/4p in response to heat shock

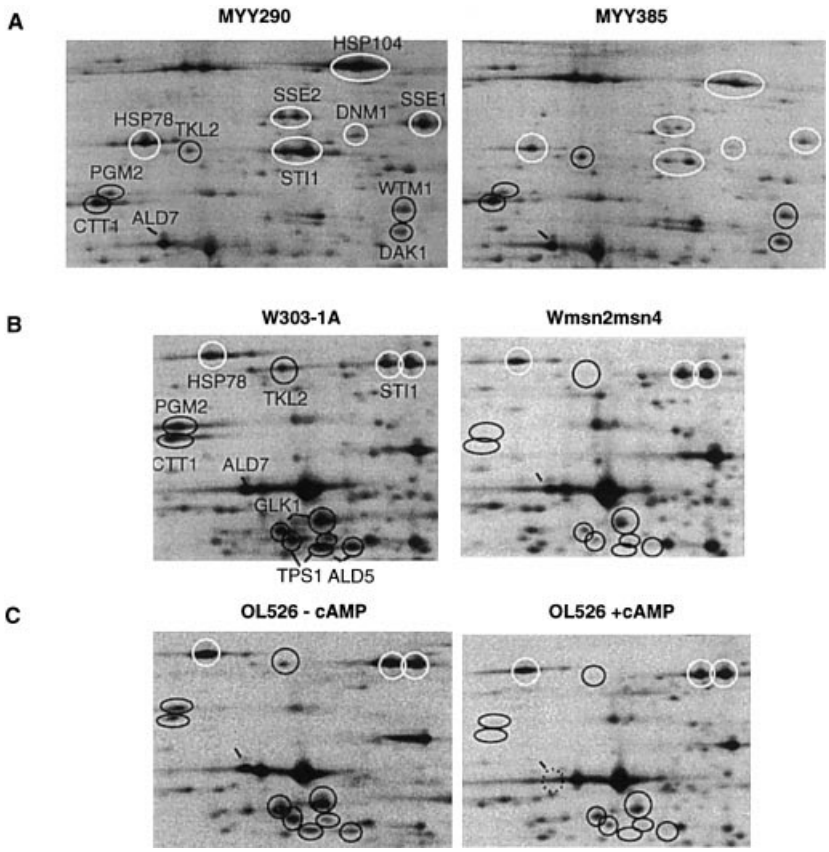

Fig. 3. Two-dimensional gel electrophoresis of wild-type and mutant extracts under heat shock conditions. Enlargement of the two regions framed in Fig. 1: Strains were labelled with [^{35}S]-methionine after transfer from 25°C to 38°C.
A. Strains MYY290 and MYY385 (*hsf1-m3*).
B. Strains W3031A, Wmsn2msn4.
C. OL526 grown without and with 3 mM cAMP.
(B) and (C) are enlargements of the same region. Designations of induced proteins are the same as in Fig. 1. The expected position of the Ald7p, which is absent in the map OL526 + cAMP, is indicated with a dotted circle.

(Martinez-Pastor *et al*., 1996; Schmitt and McEntee, 1996; Winderickx *et al*., 1996; Treger *et al*., 1998).

The overlap observed between the Hsf1p and the Msn2/4p regulons is very small: only Hsp12 and Hsp26 are controlled by both factors. Most of the heat shock-induced proteins were dependent upon Hsf1p or Msn2/4p. However, the induction of three proteins (including Gpd1p and Ald7p) by heat shock was not affected by the *hsf1-m3* or *msn2msn4* mutations (Table 1, class 3). Interestingly, the set of repressed proteins was also independent of both factors (data not shown).

The cAMP effect on the heat shock response

We analysed the effect of cAMP on the heat shock response in strain OL526 grown in the presence or absence of cAMP. In this strain, the *rca1* mutated allele of the *PDE2* gene causes a defect in the high-affinity phosphodiesterase and allows the manipulation of the intracellular cAMP level by adding cAMP to the culture medium (Boy-Marcotte *et al*., 1996). All the Msn2/4p-dependent proteins had their induction reduced twofold or more by cAMP (Table 1, class 2 and Fig. 3C for details). There was a good correlation between the effect of the *msn2msn4* deletion and the cAMP effect for an important fraction of the gene products analysed. This correlation has been described previously during the diauxic transition (Boy-Marcotte *et al*., 1998). Only a few class 1 proteins (Hsp104p, Ssa3p and Ubc4) and class 3 proteins (Ald7p and Gpd1p) had their induction significantly affected by cAMP (Table 1).

Distribution of the STRE and HSE sites in the promoter regions

HSE and STRE sequences were searched in the 700 bp upstream region of the Hsf1p- and Msn2/4p-dependent genes (Fig. 4). The distribution of the STRE sites in the Msn2/4p-dependent genes is very different from that in the Hsf1p-dependent genes: STRE elements are present at a frequency of 2.7 sites in the upstream region of the genes controlled by Mn2/4p and at a frequency of 1.3 in the Hsf1p-dependent genes. The fraction of genes with more than three STRE sites is 38% for the Msn2/4p-dependent and 10% for the Hsf1p-dependent genes. It is also remarkable that a large fraction of these sites are clustered in less than 60 bp. We also observed that the intensity of Msn2/4p-dependent expression measured by the differential expression at 38°C between the wild-type W303-1A and the mutant *msn2msn4* is correlated with the number of STRE sites (Fig. 4B), suggesting an additive

Table 1. Effect of Msn2/4p, Hsf1p and cAMP on the induction of the heat shock proteins.

Gene name or spot number	Protein function[a]	Ratio of expression level *hsf1-m3*/WT	*msn2msn4*/WT	+/− cAMP
Class 1				
SSA3	Chaperone hsp70	0.00	0.74	0.33
47		0.00	1.47	1.46
HSP26[c,d]	Small hsp	0.07	<0.5[c]	<0.5[c]
HSP82	Hsp70-associated protein	0.08	1.09	1.28
SSA4	Chaperone hsp70	0.11	0.9	0.70
SSE2	Chaperone hsp70	0.16	0.83	0.93
CPR6	Peptidyl proline isomerase like*	0.17	1.50	0.67
99		0.18	1.17	0.88
YOR021c	Unknown*	0.22	2.08	0.54
SSA1	Chaperone hsp70	0.21	0.68	0.73
HSP104	Chaperone	0.22	0.80	0.47
STI1	Hsc70–Hsp90 complex component	0.22	1.26	0.97
HSC82	Hsp70-associated protein	0.25	NT[b]	0.72
51		0.26	0.93	0.67
HSP12[d]	Small hsp*	0.26	0.23	0.00
HSP78	Chaperone*	0.30	0.84	0.71
YLR206w	Unknown*	0.31	0.82	1.29
SSE1/MSI3	Chaperone hsp70	0.36	1.14	0.99
101		0.38	NT[b]	0.91
HSP60	Mitochondrial chaperonin	0.45	1.33	0.92
SSA2	Chaperone hsp70	0.48	1.06	0.83
DNM1	Dynamin like*	0.56	1.02	0.88
HSP42	Actin cytoskeleton maintenance*	0.59	0.90	0.63
UBC4	Ubiquitin-conjugating enzyme*	0.56	NT[b]	0.52
Class 2				
93		0.77	0.00	0.00
ALD2/5	Aldehyde dehydrogenase like*	0.88	0.00	0.00
PGM2	Phosphoglucomutase	1.01	0.00	0.00
CTT1	Catalase T*	1.12	0.00	0.00
18		1.61	0.00	0.00
36		1.87	0.00	0.43
DDR48	DNA damage response protein	1.51	0.00	0.51
HXK1	Hexokinase I	0.89	0.05	0.16
TES1	Suppressor of cdc25 mutation*	1.05	0.09	0.13
TPS1	Trehalose-6-phosphate synthase	1.29	0.11	0.15
TKL2	Transketolase 2*	0.98	0.17	0.00
YNL274c	Aketo-isocaproate reductase like**	0.86	0.17	0.34
17		2.10	0.18	0.28
HSP12[d]	Small hsp*	0.26	0.23	0.00
UGP1	UDP-glucose pyrophosphorylase	1.35	0.31	0.24
MDH1	Malate dehydrogenase mitochondrial	1.69	0.34	0.42
GAL1	Galactokinase**	2.05	0.35	0.00
GLK1	Glucokinase	1.14	0.36	0.33
YGL037c	Unknown*	0.86	0.37	0.29
78		1.32	0.37	0.00
CCP1	Cytochrome-*c* peroxidase	1.73	0.41	0.35
SOD2	Manganese superoxide dismutase	1.84	0.41	NT[b]
WTM1/YOR230w	Transcriptional modulator*	1.39	0.48	0.46
HSP26[c,d]	Small hsp	0.07	<0.5[c]	<0.5[c]
YBR149w	Glycerol dehydrogenase	1.25	0.57	0.49
DAK1	Dihydroxyacetone kinase like	1.03	0.67	0.57
57		1.01	0.67	0.44
Class 3				
ALD7/YOR374w	Aldehyde hydrogenase like**	0.71	0.81	0.00
GPD1	Glycerol-3-phosphate dehydrogenase	2.01	0.90	0.43
83		2.21	0.98	0.88

For each protein, the ratio of their spot intensities is indicated (ratio of expression level) in two heat-shocked strains at 38°C: These strains are: MYY385 and MYY290 (*hsf1-m3*/WT); Wmsn2msn4 and W303-1A (*msn2msn4*/WT); OL526 grown with and without 3 mM cAMP (± cAMP). Experiments have been repeated twice, and the same qualitative results have been obtained. Classes 1, 2 and 3 are defined in the *Results*.

a. Proteins annotated with one asterisk were identified by double labelling (Godon *et al*., 1998). Proteins annotated with a double asterisk were identified by mass spectrometry (this paper). The other proteins have already been identified as described previously (Boucherie *et al*., 1996).

b. Not tested because no induction was observed in the corresponding wild-type strain.

c. In the case of Hsp26p, which is not labelled with [^{35}S]-methionine, the amount of protein was estimated by Coomassie blue staining.

d. Hsp12 and Hsp26 belong to class 1 and class 2.

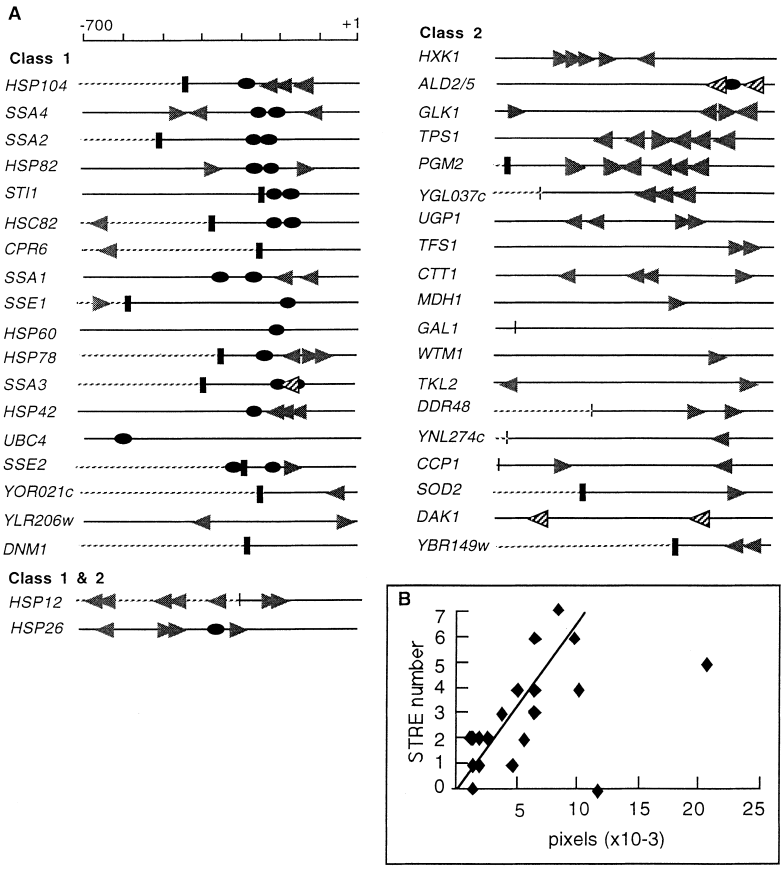

Fig. 4. STRE and HSE consensus sites found in the promoter regions of Msn2/4p- and Hsf1p-dependent genes.
A. STRE sites (CCCCT, grey arrows) and HSE sites (NGAANNTTC repeated more than once, black circles) were searched in the promoter region (700 bp upstream of the ATG) of each heat shock-induced gene. For this search, yeast genomic DNA sequences were obtained from the *Saccharomyces* Genome Database (SGD). PDS sites (TCCCT) present in promoter regions of Msn2/4p-dependent genes with no STRE sites are indicated by striped arrows. Intergenic regions and ORFs are represented, respectively, by a plain line and a dotted line. Vertical bars indicate the border lines between intergenic regions and ORFs (thin bar, ATG; and large bar, terminator).
B. The level of Msn2/4p-dependent activation is correlated with the number of STRE motifs in Msn2/4p target genes: the number of STREs in the promoter region of each Msn2/4p target gene was plotted against the difference in protein expression (in pixel units) between the strains W303-1A and Wmsn2msn4 under heat shock conditions.

contribution of these sites to Msn2/4p-dependent expression. as in the *HSP12* promoter (Varela *et al*., 1995). In the case of *ALD2/5*, *DAK1*, *GAL1* and *SSA3*, no STRE motif was found in their promoter regions. However, the post-diauxic shift (PDS) sites present in the *ALD2/5*, *DAK1* and *SSA3* promoter regions could be responsive to Msn2/4p (Marchler *et al*., 1993).

Fifteen out of 20 of the Hsf1p-dependent genes contain HSE motifs in their promoter region, whereas one gene out of the 19 genes only controlled by Msn2/4p contains an HSE (Fig. 4A). In the case of *HSP26*, *HSC82*, *SSA1*, *SSA3* and *SSA4*, the HSE motifs have been shown to be important for Hsf1p-dependent induction (Boorstein and Craig, 1990a,b; Stone and Craig, 1990; Chen and Pederson, 1993; Erkine *et al*., 1996).

The presence of HSE motifs in the promoter regions of the Hsf1p-dependent genes, but not in those dependent on Msn2/4p, and the significant excess of the STRE motifs in the Msn2/4p-dependent genes suggest that many of these genes are direct targets for Hsf1p or Msn2/4p. The absence of HSE and STRE motifs in some genes may indicate an indirect role for Hsf1p and Msn2/4p in the control of these genes. However, a direct effect via degenerated sites cannot be ruled out.

Acquired thermotolerance is not reduced in the msn2msn4 *mutant*

One property of cells treated with a mild heat shock is to acquire thermotolerance to an otherwise lethal heat stress. As Msn2/4p controls a large fraction of the proteins induced by mild heat shock, we analysed whether the thermotolerance is controlled by Msn2/4p. The *msn2msn4* mutant was more sensitive to a shift to 50°C than the wild type, as described previously (Martinez-Pastor *et al*., 1996) (Fig. 5). A 30 min pretreatment at 38°C restored the resistance of both mutant and wild-type strains to a subsequent shift to 50°C. Thus, the Msn2/4p regulon is involved in the capacity of the cell to resist high temperatures but is not required for the thermotolerance acquired after a mild heat shock.

Discussion

Although not exhaustive, this genome-wide approach based on two-dimensional gel electrophoresis allowed us to establish that heat shock-induced proteins are mainly controlled by the transcriptional activators Hsf1p and Msn2/4p. The Hsf1p regulon is mostly composed of

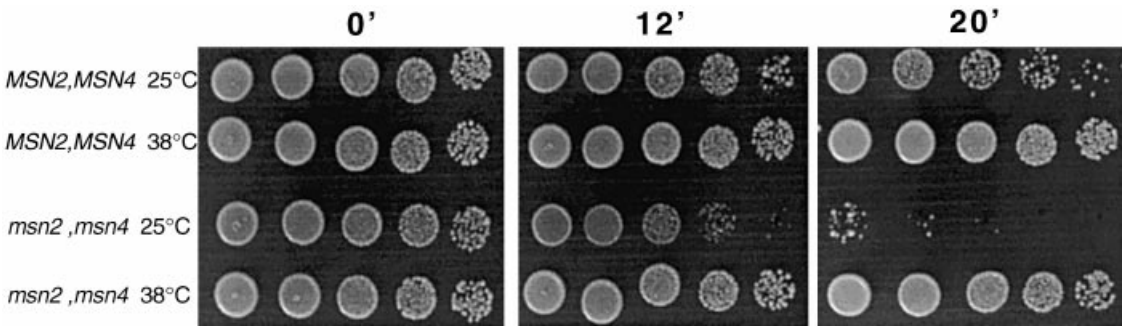

Fig. 5. The acquisition of thermotolerance is not impaired in the *msn2msn4* mutant. W303-1A and Wmsn2msn4 strains were grown in YPD at 25°C. When the OD_{710} reached 1.3, two aliquots of each culture were either transferred to 38°C or maintained at 25°C. After 30 min, samples were transferred to 50°C. After 0, 12 and 20 min, aliquots were placed on ice and serially diluted (fivefold at each step). Five microlitres was spotted onto YPD plates and incubated at 25°C to measure colony-forming units.

chaperones, and the Msn2/4p regulon includes enzymes of carbon metabolism, indicating that the two regulons have distinct physiological functions in the heat shock response. Moreover, whereas the Msn2/4p regulon is repressed by the Ras/cAMP/PKA pathway, the Hsf1p regulon is not under this control.

The Msn2/4p factors mainly control a metabolic response of the cell during heat shock

The proteins of the Msn2/4p regulon consist mainly of carbon metabolism and antioxidant enzymes. It is remarkable that a similar (if not identical) set of proteins is also induced by Msn2/4p at the diauxic transition (Boy-Marcotte *et al*., 1998) and by oxidative stress (M. Toledano and J. Labarre, unpublished results). Their physiological role in the heat shock and other stress responses is unclear. Although speculative, considerations for some of these enzymes are discussed here.

Trehalose metabolism. Some enzymes of the trehalose metabolism were found to be induced by Msn2/4p in this work (i.e. Pgm2p, Ugp1p and Tps1p). It has also been shown that the expression of *TPS2* is Msn2p dependent (Schmitt and McEntee, 1996), as well as that of *TPS3* (D. Tadi, unpublished data). Consistently, the trehalose accumulation in response to a mild heat shock is defective in the *msn2msn4* mutant (Parrou *et al*., 1997). The trehalose could contribute to thermoresistance, as this compound increases the thermal stability of proteins *in vitro* (Hottiger *et al*., 1994) and strains unable to produce trehalose (*tps1*, *tps2* and *msn2msn4* null strains) are sensitive to heat shock (De Virgilio *et al*., 1994; this work). Trehalose is also important for thermotolerance when pretreatment is performed at temperatures higher than 40°C (Ribeiro *et al*., 1997). Accordingly, in our experimental conditions (pretreatment at 37°C), thermotolerance is not impaired in the mutant strain. However, it should be noted that the accumulation of trehalose as a result of heat shock is limited, as the neutral trehalose Nth1p is also induced under these conditions (Parrou, 1997), suggesting a recycling of trehalose in response to stress.

Hexokinase regulation. One function of this futile cycling of trehalose may be to increase the intracellular level of trehalose-6-phosphate, which is a potent inhibitor of the main hexokinase Hxk2p (Blazquez *et al*., 1993; Teusink *et al*., 1998). This enzyme could be replaced by the Glk1p and Hxk1p hexokinases, which are induced under heat shock conditions (this work) and are, respectively, not and less inhibited by trehalose-6-phosphate (Blazquez *et al*., 1993). This replacement may have a role in the control of glycolytic flux and/or alternative pathways (trehalose cycle and pentose phosphate pathway).

Antioxidant defence and NADPH regeneration. Msn2/4p induce Ctt1p, Sod2p and Ccp1p enzymes that have antioxidant activities. These activities could enhance the cellular defence towards the oxidative stress, which is thought to be associated with heat shock (Davidson *et al*., 1996). In addition, an increase in the regeneration of NADPH, an important cofactor in the oxidative stress response, can also be postulated from the induction of Tkl2p and could participate in the antioxidant defence, as suggested previously (Godon *et al*., 1998). The *YBR149w* gene product and Dak1p could also contribute to this NADPH regeneration (Godon *et al*., 1998), as well as the aldehyde dehydrogenases Ald2/5p and Ald7p.

All these metabolic effects should act in concert with the transient arrest of the cell cycle in G_1 observed in response to various stresses (Rowley *et al*., 1993; Lee *et al*., 1996). This cell cycle arrest could be caused by Msn2/4p, which may antagonize the PKA-dependent growth by stimulating the expression of genes, such as *YAK1*, that inhibit growth (Smith *et al*., 1998). The role of the Msn2/4p regulon is not essential but is very general,

as it is induced under a wide range of stress conditions and environmental changes (Martinez-Pastor *et al*., 1996). By a transient modification of the cellular metabolism, the Msn2/4p regulon could facilitate proper setting of a new protein machinery and the adaptation of the cell to the new conditions.

On the contrary, the Hsf1p regulon has an essential and specific role in the heat shock cellular response (Smith and Yaffe, 1991a; Lindquist and Kim, 1996). Indeed, it is mostly composed of chaperones and associated proteins that must be involved in the care and management of proteins denatured by the heat shock. The *UBC4* gene product also belongs to this regulon. This ubiquitin-conjugating enzyme contributes to a major portion of the ubiquitin-dependent protein degradation in stressed cells (Seufert and Jentsch, 1990). Two gene products of unknown function (*YOR021c* and *YLR206w*) were also found to be induced by Hsf1p and could also have chaperone-like functions.

The metabolic response to heat shock is under the control of the Ras/cAMP pathway

The Ras/cAMP pathway controls STRE motifs and acts on Msn2/4p, at least on their nuclear localization induced by heat shock (Marchler *et al*., 1993; Görner *et al*., 1998). Accordingly, we found that all the genes induced by Msn2/4p under heat shock conditions were repressed by an excess of cAMP. The same correlation was also observed during the diauxic transition (Boy-Marcotte *et al*., 1998). A control of the cAMP intracellular level must be part of the cellular response to heat shock and could be achieved through Ssa1p, which is complexed with the guanine nucleotide exchange factor Cdc25p, the main activator of cAMP synthesis (Geymonat *et al*., 1998). In addition to the Msn2/4p genes, a few others were significantly repressed by cAMP. It is possible that the cAMP pathway exerts its effect through other regulators in addition to Msn2/4p, as observed at the diauxic transition (Boy-Marcotte *et al*., 1998). Most of the Hsf1p-dependent genes are insensitive to cAMP, consistent with the absence of control by the Ras/cAMP pathway over HSE heat shock-induced transcription (Marchler *et al*., 1993; Engelberg *et al*., 1994).

Hsf1p and Msn2/4p are the two main transcriptional activators in heat shock response

Most of the heat shock proteins belong either to the Hsf1p regulon or to the Msn2/4p regulon, indicating the prominent role of these two factors in the heat shock response. The expression of only three proteins is not impaired in the absence of Hsf1p or Msn2/4p. It is possible that some other transcriptional activators are involved in the heat shock response (Kamada *et al*., 1995). However, a residual activity of Hsf1p (Lindquist and Kim, 1996) and a different sensitivity of the different Hsf1p-regulated genes to the particular defect of the *hsf1-m3* thermosensitive mutation cannot be excluded. This hypothesis is supported by the phenotype of the *hsf1-82* thermosensitive mutant, which specifically reduces the transcription of Hsp82 and Hsc82p (Zarzov *et al*., 1997).

The overlap between the two heat shock regulons is limited to Hsp12 and Hsp26. Ssa3p and Hsp104 could also be added to this group, as a small effect of the *msn2msn4* mutation is correlated with a significant cAMP effect. It is also possible that other genes are redundantly induced by Hsf1p and Msn2/4p, but that compensatory effects lead to a normal heat shock induction in one or both mutants. Such an effect has been described for *HSP104* and *HSP78*, whose induction is only impaired in the triple mutant *hsf1-m3,msn2,msn4* (Treger *et al*., 1998). Therefore, the number of genes controlled by both factors is probably underestimated in our analysis. Nevertheless, most of the Hsf1p-independent genes described in this work do not contain an HSE site and, thus, it is very improbable that they are actually controlled by Hsf1p.

Two-dimensional gel and yeast genome microarray analysis

Using DNA microarrays to analyse the gene expression of 2479 open reading frames (ORFs) of *S. cerevisiae*, 17 genes were found to be induced by heat shock (Lashkari *et al*., 1997). Some of them belong to the Hsf1p regulon defined in this work. On the contrary, none of the Msn2/4p-dependent genes was found. This difference may result from the difference in kinetics of the two procedures: whereas the analysis of the mRNA by hybridization of the DNA microarrays was performed 1 h after the heat shock, we analysed the heat shock protein induction 15 min after the temperature shift. After 1 h, it is possible that a large part of the transient response is not detectable any more, as observed for the heat shock-induced genes *SSA1* and *CTT1* (Smith and Yaffe, 1991a; Martinez-Pastor *et al*., 1996). Nevertheless, a difference resulting from unknown post-transcriptional regulation cannot be excluded, as the DNA microarrays analyse mRNA levels, whereas two-dimensional gel analysis measures protein synthesis rate. This DNA microarray analysis allowed the detection of heat shock-responsive genes whose products are not detectable on two-dimensional gels as membrane proteins (for example Hsp30p), basic proteins (i.e. most of the ribosomal proteins) or proteins expressed at a low level. An exhaustive analysis with the yeast genome microarrays should complete the description of the heat shock regulons in yeast.

Experimental procedures

Yeast strains and growth conditions

OL526 (α *rca1, leu2, ura3, his3, trp1*, this work). W303-1A (*a, ade2, his3, leu2, trp1, ura3*) and Wmsn2-msn4 (*a, ade2, his3, leu2, trp,1 ura,3 msn2-Δ3::HIS3, msn4–1::TRP1*) are isogenic strains (Estruch and Carlson, 1993). MYY290 (*a, leu2, his3, ura3, phoc, phoE*) and MYY385 (*a leu2, his3, ura3, phoC, phoE, hsf1-m3*) are isogenic strains (Smith and Yaffe, 1991a). Strains were grown in the glucose medium YNBS described previously (Boy-Marcotte *et al*., 1996), supplemented with the required amino acids. Heat shock experiments were performed as follows: cells growing exponentially at 25°C (OD_{710} of 0.3 in a Jenway 6061 colorimeter) were shifted to 38°C in a prewarmed flask.

Protein synthesis analysis

For protein labelling, 5 ml of log-phase culture (OD_{710} of 0.3) was labelled for 15 min with 5.5×10^6 mBq of [^{35}S]-methionine ($> 3.7 \times 10^{13}$ mBq mmol^{-1}). In the case of heat shock, [^{35}S]-methionine was added 15 min after the transfer to 38°C. The control labelling was performed at 25°C. The temperature of 38°C was chosen as it was reported that, above 40°C, the induction of some STRE-regulated genes was abolished (Parrou *et al.*, 1997). Preparation of cell extracts and two-dimensional gel electrophoresis were performed as described previously (Maillet *et al*., 1996). Quantitative analysis of the synthesis of the polypeptides separated on the two-dimensional gel was performed as follows: after drying, gels were exposed to phosphor-screens scanned in a Molecular Dynamics PhosphorImager. Image files were then exported into the BIOIMAGE software for image analysis and spot quantification. The spot intensities were obtained in pixel units and normalized to the value of Act1p spot. For proteins that are present as several distinct polypeptides of different pI values, the spot intensities were added.

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