

Transcriptional Factor Mutations Reveal Regulatory Complexities of Heat Shock and Newly Identified Stress Genes in *Saccharomyces cerevisiae**

(Received for publication, February 23, 1998, and in revised form, August 6, 1998)

Janet M. Treger, Anthony P. Schmitt‡, John R. Simon§, and Kevin McEntee¶

From the Department of Biological Chemistry, UCLA School of Medicine, Center of Health Sciences, University of California, Los Angeles, California 90095

A computer-aided pattern search of the entire yeast genome was designed and used to identify 186 putative stress response element-regulated genes in *Saccharomyces cerevisiae*. Transcript levels of eight of these candidate genes were examined, and three (37%) were shown to be heat shock- and DNA damage-inducible and to require the Msn2p and Msn4p transcriptional activators for stress regulation. Significantly, several heat shock protein (HSP) genes were identified in this computer search. Using a series of single and multiple regulatory mutants, we demonstrate unexpected regulatory complexities among the HSP genes from *S. cerevisiae* following heat shock.

Alterations in the patterns of gene expression occur in cells in response to environmental challenges or stresses. In *Saccharomyces cerevisiae*, response to a diverse spectrum of stresses is mediated via a pentanucleotide element, STRE¹ (Stress Response Element; C₄T), and the transcriptional activator proteins, Msn2p and Msn4p (1, 2). Several genes have been shown to be regulated via the Msn2p/Msn4p/STRE pathway. Molecular analyses of the upstream region of the *DDR2* has shown that stress regulation occurred exclusively via the STRE (3, 4). Deletion studies of the promoters of the *CTT1*, *HSP12*, *TPS2*, and *GSY2* genes have shown that following heat shock, osmotic shock, post-diauxic shift growth, and nitrogen starvation (5–9) transcription induction is mediated by sequences containing STREs. The promoter regions of these genes contain multiple STREs, which function independently of their orientation relative to the TATA box (3). Recently, we have demonstrated that the STREs are not only necessary but are sufficient for mediating the multistress response. A synthetic STRE-containing oligonucleotide with altered spacing between two STREs and changes in the flanking sequence conferred multistress control to an *Escherichia coli galK* reporter gene following 10 different stress conditions. The pattern of stress responses was similar to the response of oligonucleotide 31/32 derived from the *DDR2*

gene (4).

We have used this structural and functional information as the basis for a computer search of the entire yeast genome and have generated a list of 186 potential STRE-regulated genes. Using Northern hybridization to analyze the regulation of eight of these genes, we show that three of these loci are stress-regulated and require Msn2p/Msn4p for their activation. Among the genes identified in the STRE pattern search were several yeast heat shock protein (HSP) genes assumed to be regulated exclusively via the heat shock factor/heat shock element (HSF/HSE) pathway. Using a series of multiple regulatory mutants, we demonstrate that several HSP genes are redundantly controlled by both the Msn2p (Msn4p)/STRE and HSF/HSE pathways following heat shock.

MATERIALS AND METHODS

Strains, Growth Medium, and Chemicals—Yeast strains used in this study are listed in Table I. Phenotypes were determined by growing cells on SC drop mix minimal plates (10). Cells were grown in YPD (10) for stress treatments.

Construction of Double and Triple Mutant Strains—Strains MCY2144 (*msn2Δ*) and MCY2146 (*msn4Δ*) were mated to create the diploid strain JT615, which was sporulated as described previously (11). The sporulated culture was treated exhaustively with glutalase to disrupt the asci and vortexed with glass beads to separate the spores (10). The spores were plated onto SC his⁺, SC ura⁺, and SC his⁺ ura⁺ plates to recover *msn2Δ*, *msn4Δ*, and *msn2Δ msn4Δ* haploid strains, respectively. From each mating and subsequent sporulation, 20 red ade⁺ haploid colonies were isolated, restreaked to isolate individual colonies, and patched to SC plates lacking one of several nutrients to determine their phenotypes. The resulting strains, JT616 (*msn2Δ*), JT620 (*msn4Δ*), and JT624 (*msn2Δ msn4Δ*), were subsequently mated to strain MYY385 (*mas3*) to generate diploid strains JT628, JT629, and JT632, respectively. These three strains were sporulated and haploid ade⁺ colonies containing the appropriate markers were identified as described above. The presence of the disrupted *msn2Δ* and *msn4Δ* alleles was confirmed by Southern hybridization analysis of genomic DNA. Temperature-sensitive growth at 37 °C and the loss of heat shock induction of the *SSA3* transcript were scored to confirm the presence of the *mas3* mutation.

Stress Treatments—Cells were subjected to stress treatment as described previously (4). Briefly, cells were exposed to 0.07% methyl methanesulfonate (MMS) for 60 min. For post-diauxic shift (PDS), cells were harvested 1–2 h after the diauxic shift from fermentative to respiratory growth. The diauxic shift was determined by monitoring optical density of the culture and was reached approximately 12–14 h after the control sample was harvested at a cell density of $\sim 7 \times 10^6$ cells/ml. For heat shock (HS) treatment, cultures were grown at 23 °C, and an aliquot was shifted to 37 °C for 20 min.

RNA Preparation and Northern Hybridization—Total RNA was prepared as described previously (11). Denatured RNA (25 µg) was electrophoresed in formaldehyde-agarose gels and transferred to nylon membranes. RNA blots were UV-cross-linked and hybridized with [α -³²P]dCTP-radiolabeled probes and washed as described (4). The Northern blots were exposed to x-ray film and quantitated using an Ambis radio-imager system (Scanalytics). The probes used for hybridization were the 1.45-kb *Hind*III fragment of *DDR2* from plasmid

* This work was supported by Research Grant GM38456 from the National Institutes of Health (to K. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208.

§ Present address: Dept. of Medicine, Division of Hematology, University of Utah Health Sciences Center, Salt Lake City, Utah 84312

¶ To whom correspondence should be addressed. Tel.: 310-825-5251; Fax: 310-206-5272; E-mail: mcentee@mednet.ucla.edu.

¹ The abbreviations used are: STRE, stress response element; HS, heat shock; HSE, heat shock element; HSF, heat shock transcription; HSP, heat shock protein; MMS, methyl methanesulfonate; PDS, post-diauxic shift; kb, kilobase pair(s); bp, base pair(s).

TABLE I
Strains used

Strain	Genotype	Source
MCY2144	<i>Mata ade2-101 ura3-52 lys2-801 his3-Δ200 msn2-Δ3::HIS3</i>	Carlson
MCY2144/pEY32H	<i>Mata ade2-101 ura3-52 lys2-801 his3-Δ200 msn2-Δ3::HIS3/pEY32H</i>	Ref. 2
MCY2146	<i>Mata trp1-Δ1 lys2-801 his3-Δ200 ura3-52 msn4-Δ2::URA3</i>	Carlson
MCY2150	<i>Mata lys2-801 his3-Δ200 msn2-Δ1::HIS3 msn4-Δ2::URA3 ura3-52</i>	Carlson
S288C	<i>Mata SUC2 mal mel gal2 CUP1</i>	Ref. 2
MY3385	<i>Mata mas3 his3 ura3 leu2 phoC phoE</i>	Ref. 22
JT615	<i>Mata/α ade2-101/+ lys2-801/lys2-801 trp1-Δ1/+ his3-Δ200/his3-Δ200 msn2-Δ3::HIS3/+ ura3-52/+ msn4-Δ2::URA3/+</i>	This study
JT628	<i>Mata/α ade2-101/+ lys2-801/+ ura3-52/ura3 trp1Δ1/+ +/leu2 his3-Δ200/his3 msn2Δ3::HIS3/+ +/mas3 +/phoC +/phoE</i>	This study
JT629	<i>Mata/α ade2-101/+ lys2-801/+ ura3-52/ura3 trp1Δ1/+ +/leu2 his3Δ200/his3 msn4Δ2::URA3/+ +/mas3 +/phoC +/phoE</i>	This study
JT632	<i>Mata/α ade2-101/+ lys2-801/+ ura3-52/ura3 trp1Δ1/+ +/leu2 his3-Δ200/his3 msn2-Δ3::HIS3/+ msn4Δ2::URA3/+ +/mas3 +/phoC +/phoE</i>	This study
JT616	<i>Mata ade2-101 lys2-801 ura3-52 trp1Δ1 msn2-Δ3::HIS3 his3-Δ200</i>	This study
JT620	<i>Mata ade2-101 lys2-801 ura3-52 trp1Δ1 msn4Δ-2::URA3 his3-Δ200</i>	This study
JT624	<i>Mata ade2-101 lys2-801 ura3-52 msn2-Δ3::HIS3 msn4-Δ2::URA3 his3-Δ200</i>	This study
JT638	<i>Mata ade2-101 ura3 trp1-Δ1 leu2 his3 msn2-Δ3::HIS3 mas3</i>	This study
JT641	<i>Mata ade2-101 lys2-801 ura3 trp1Δ1 msn2-Δ3::HIS3</i>	This study
JT643	<i>Mata ade2-101 ura3 trp1-Δ1 his3 msn4-2::URA3 mas3</i>	This study
JT646	<i>Mata ade2-101 ura3 trp1-Δ1 his3 msn4-Δ2::URA3</i>	This study
JT649	<i>Mata ade2-101 lys2-801 ura3 leu2 his3 msn2-Δ3::HIS3 msn4-Δ2::URA3 mas3</i>	This study
JT652	<i>Mata ade2-101 ura3 leu2 his3 msn-2Δ3::HIS3 msn4-Δ2::URA3</i>	This study
JT655	<i>Mata ade2-101 lys2-801 ura3 trp1Δ1 leu2 his3 mas3</i>	This study
JT659	<i>Mata ade2-101 ura3 trp1Δ1 his3</i>	This study
M12B	<i>Mata trp1-289 ura3-52 gal2</i>	Ref. 12

TABLE II
Genes examined for STRE-regulation and the location of the STREs in the promoter

Candidate	STREs ^a	Ref.
<i>PDE2</i> (3',5'-cyclic nucleotide phosphodiesterase)	-135, -289, -336	26
<i>PGM2</i> (phosphoglucosyltransferase, major isoform)	-216, -259, -305, -359, -406	27
<i>RAS2</i> (guanine-nucleotide binding protein)	-318, -427, -439, -447, -469	28
<i>RPA14</i> (RNA polymerase I subunit a14)	-178, -184, -200	29
<i>RPB4</i> (RNA polymerase II subunit b32)	-326, -353, -369	30
<i>SMC2</i> (segregation of mitotic chromosomes)	-182, -206, -214	31
YGR067C (similarity to yjeF protein family)	-306, -451, -493	<i>Saccharomyces</i> genome data base
YKL151C (C ₂ H ₂ zinc finger motif protein)	-310, -350	<i>Saccharomyces</i> genome data base

^a Position of the STRE relative to the translation start site.

pBRA2 (12), the 1.3-kb *Pst*I-BglIII fragment of *HSP26* from plasmid pS26-1 (S. Lindquist); the 3.2-kb *Hind*III fragment of *UBI4* from plasmid pUC-UBI4, the 1.3-kb *Hind*III fragment of *HSP78* from pLS12 (T. Mason), and the 1.4-kb *Hind*III-*Sst*I fragment of *HSP104* from plasmid pYS104 (13). The remaining probes were polymerase chain reaction-amplified from selected coding regions of appropriate genes using genomic DNA from individual M12B colonies as templates. The regions amplified were: nucleotides +1 to +714 of *ACT1*, +142 to +768 of *PGM2*, +599 to +1120 of *PDE2*, +249 to +694 of YKL151C, +44 to +501 of *RPB4*, +1558 to +1930 of *SSA3*, +1 to +326 of *HSP12*, and +1 to +424 of *HSP82*.

Computer-aided Pattern Matching—The PatScan pattern matcher, maintained at Argonne National Laboratory (<http://www-c.mcs.anl.gov/home/overbeek/PatScan/HTML/patscan.html>), was used to search the yeast genome for candidate STRE-regulated genes. The search was performed against both strands of the entire genomic sequence of *S. cerevisiae* accessed from the EBI nucleotide sequence data base (Release 49). The following pattern was used for the search: CCCCT 1 . . . 200 CCCCT 1 . . . 300 TATA in which letters represent direct matches to nucleotides and numerals represent an interval between *x* and *y* nucleotides.

RESULTS AND DISCUSSION

Identification of Novel STRE-regulated Yeast Genes by Computer-aided Pattern Matching—We demonstrated previously that C₄T pentanucleotides function as stress response elements independently of nucleotide sequence context or spacing (4). This observation suggested that we could identify stress-controlled genes based upon the presence of multiple upstream STREs. Using a computer-aided pattern matching strategy, we searched the fully sequenced *S. cerevisiae* genome for candidate genes containing two or more STREs separated by up to 200 bp

and lying within 300 bp of a TATA sequence. The search returned approximately 350 matches and of these, 186 matches were found to define groups of STREs located immediately upstream of characterized genes or predicted open reading frames (within 500 bp of the translation start). Eighty-three of these candidates corresponded to known yeast genes, while the remaining 103 sequences represented uncharacterized open reading frames. A diverse group of genes was identified, including those encoding mitochondrially localized gene products (*CIT1*, *MCR1*, and *MDH1*), known or predicted transporter proteins (*GAL2*, *MEP1*, *PUT4*, and YGR224W), known or predicted proteases (*PAI3*, *PRC1*, *STE23*, and YBR139W), homologs of *DnaJ* (*MDJ1*, YNL077W, and YPR061C) and several HSP genes (*HSP12*, *HSP26*, *HSP42*, *HSP78*, and *HSP104*).

Eight candidates (*PDE2*, *PGM2*, *RAS2*, *RPA14*, *RPB4*, *SMC2*, YGR067C, and YKL151C; see Table II) were selected and tested for STRE control by Northern hybridization analysis. Genes under STRE control exhibit the following characteristics: (i) transcript levels are elevated after heat shock, DNA damage, or other stresses; (ii) stress induction is curtailed or greatly reduced in strains that lack the STRE-binding transcription factors, Msn2p and Msn4p; and (iii) basal transcript levels are significantly elevated in a strain overexpressing Msn2p (2). Based on these criteria, three of the eight candidates tested (*PDE2*, *PGM2*, and YKL151C) were found to be STRE-regulated (Fig. 1).

Recently, using two-dimensional protein gels, Boy-Marcotte *et al.* (14) have shown that 39 proteins induced during diauxic

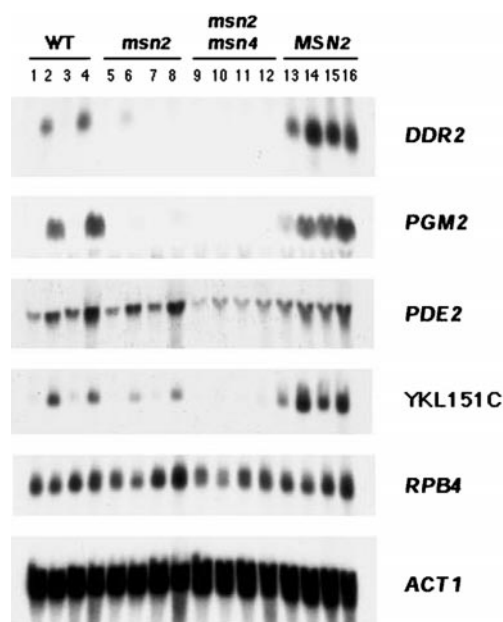


FIG. 1. Identification of novel STRE-regulated genes. RNA (25 μ g) isolated from control and stressed cells (heat shock or MMS) was analyzed by Northern hybridization. The blots were hybridized with the *DDR2*, *PGM2*, *PDE2*, *YKL151C*, *RPB4*, and *ACT1* DNA fragments. Lanes 1, 5, 9, and 13, control 23 $^{\circ}$ C; lanes 2, 6, 10, and 14, heat shock; lanes 3, 7, 11, and 15, control 30 $^{\circ}$ C; and lanes 4, 8, 12, and 16, MMS. The strains used were S288C (wild-type), MCY2144 (*msn2* Δ), MCY2150 (*msn2* Δ , *msn4* Δ), and MCY2144/pEY32H (*MSN2* overexpression).

shift are dependent upon Msn2p/Msn4p for their expression. Among the genes identified are several that were found in our pattern search, including *HSP104*, *HXK1*, *PGM2*, *TPS1*, and *YBR149W*. These results reinforce the notion of a large family of yeast stress genes that are co-regulated via conserved STREs.

Transcription of the *PDE2*, *PGM2*, and *YKL151C* genes was stress-inducible in the wild-type strain, but no transcripts accumulated in the *msn2* Δ *msn4* Δ double mutant strain following stress. However, each STRE-controlled gene exhibited different characteristics with respect to stress induction in the various yeast mutants examined. Stress induction of *PGM2* transcripts was completely abolished, even in the *msn2* Δ single mutant strain. This result contrasted with the results observed for other genes, which showed residual levels of induction in the *msn2* Δ mutant strain. *DDR2*, the positive control, exhibited residual heat shock induction in the *msn2* Δ mutant strain, a result that agrees with earlier studies (2). *YKL151C* transcript induction appeared more complex. Stress induction was clearly reduced in the *msn2* Δ strain, but was not abolished unless both *MSN2* and *MSN4* genes were disrupted. The *PDE2* gene exhibited a behavior that was unique among all STRE-controlled genes studied. Stress induction of its transcripts appeared completely refractory to the *msn2* Δ disruption, while accumulation was abolished in the *msn2* Δ *msn4* Δ double mutant strain. Previous studies showed that overexpression of Msn4p partially suppressed the defect in transcript induction occurring in an *msn2* Δ strain (2). However, the dominance of Msn4p in the regulation of *PDE2* demonstrates the role of the *MSN4* gene in STRE-regulated gene expression when it is present as a single chromosomal copy. Thus, there appears to be substantial variation in the relative contribution of Msn2p versus Msn4p in the transcriptional control of different STRE-regulated genes. Perhaps with the characterization of more STRE-controlled genes from the pool of candidates, regulatory patterns will emerge that will allow prediction of *MSN2* versus *MSN4* con-

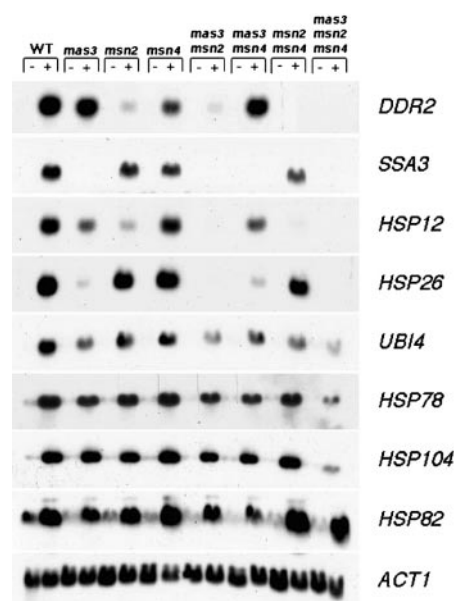


FIG. 2. Contribution of STREs and HSEs to the heat shock induction of different yeast HSP transcripts. RNA was isolated from control (–) and heat shock-treated (+) cells. RNA (25 μ g) was analyzed by Northern hybridization. Blots were hybridized with probes for each gene listed. The strains used were JT659 (wild-type), JT655 (*mas3*), JT641 (*msn2* Δ), JT646 (*msn4* Δ), JT638 (*mas3*, *msn2* Δ), JT643 (*mas3*, *msn4* Δ), JT652 (*msn2* Δ , *msn4* Δ), and JT649 (*mas3*, *msn2* Δ , *msn4* Δ).

trol based on sequence characteristics among and around the STRE elements.

Contribution of STREs in Heat Shock Regulation of Yeast Heat Shock Proteins—Five genes encoding known heat shock proteins were identified in the pattern search for STRE-controlled genes (*HSP12*, *HSP26*, *HSP42*, *HSP78*, and *HSP104*). Significantly, the promoters of these genes contain HSEs, which have been shown to be required for heat shock activation of several genes in yeast and higher eukaryotes (15–18). To evaluate the contribution of the STREs to HSP gene regulation, we constructed a series of regulatory mutant strains containing the *msn2* Δ , *msn4* Δ , and *mas3* (*hsf1*) mutations, singly or in combination. As reported previously (2), heat shock induction of *DDR2* transcription was solely regulated by the STRE pathway as judged by Northern blots of RNAs from these different regulatory mutants (Fig. 2; Table III). For the *SSA3* gene, encoding an Hsp70 cognate, regulation by heat shock was mediated exclusively through a functional HSF gene and showed no requirement for Msn2p or Msn4p. This result is consistent with earlier studies (19, 20). The two small HSPs, *HSP12* and *HSP26*, showed differential requirements for the STRE and HSE pathways. *HSP12* was predominantly regulated via Msn2p/STRE after heat shock with very little heat shock induction attributable to the HSF/HSE pathway. Conversely, regulation of *HSP26* expression after heat shock occurred predominantly via HSF/HSE with little contribution to heat shock regulation through its STREs. In each case, however, transcript accumulation was completely abolished following heat shock exposure of the triple mutant strain (*msn2* Δ , *msn4* Δ , *mas3*; Fig. 2; Table III). Thus, these two pathways accounted for all the heat shock induction of transcripts from these two genes.

For two HSP genes, *HSP78* and *HSP104*, and the stress-regulated polyubiquitin gene, *UBI4*, we observed that heat shock induction was redundantly regulated by the two pathways. As shown in Fig. 2, each pathway alone was capable of activating transcription to nearly wild-type levels. When both

TABLE III
Quantitation of the transcript levels of HSP genes following heat shock induction in mutant strains

	Wild-type	<i>mas3</i>	<i>msn2Δ</i>	<i>msn4Δ</i>	<i>mas3 msn2Δ</i>	<i>mas3 msn4Δ</i>	<i>msn2Δ msn4Δ</i>	<i>mas3 msn2Δ msn4Δ</i>
<i>DDR2</i>	100 ^a	88	19	75	18	96	1	2
<i>SSA3</i>	100	2	70	92	1	3	56	2
<i>HSP12</i>	100	53	29	113	7	66	10	1
<i>HSP26</i>	100	7	54	95	4	13	57	1
<i>UBI4</i>	100	59	72	92	53	58	69	46
<i>HSP78</i>	100	66	81	161	68	70	76	32
<i>HSP104</i>	100	82	71	137	57	63	67	17
<i>HSP82</i>	100	59	73	132	61	40	83	80

^a The Northern blots used in Fig. 2 were quantified using an Ambis radioimager. The amount of message for each gene was normalized to the amount of actin message present in each lane. The normalized amount of message remaining in each lane was expressed as a percentage of the wild-type set as 100%.

pathways were inactivated in the triple mutant strain, however, there was a substantial decrease in the accumulation of these transcripts following heat shock. However, for each of these genes, unlike *HSP12* and *HSP26*, there was residual transcript accumulation in the triple mutant suggesting that additional transcriptional activators or pathways are involved in stress activation of these genes (Fig. 2; Table III).

Surprisingly, for the *HSP82* gene, inactivation of both the Msn2p(Msn4p)/STRE and HSF/HSE pathways had relatively little effect on transcript accumulation following heat shock (Fig. 2; Table III). There was negligible contribution to the heat shock regulation of the *HSP82* gene by the Msn2p(Msn4p)/STRE pathway, while the *mas3* mutation reduced *HSP82* heat shock-induced transcript levels by about 40%. Thus, it appears that the Msn2p(Msn4p)/STRE pathway plays no role in heat shock induction of the *HSP82* gene and that the HSF/HSE pathway plays a less significant role in *HSP82* regulation following heat shock than previously thought (21).

The *mas3* mutation, originally isolated by Smith and Yaffe (22) based upon its temperature-sensitive defect in mitochondrial protein import, is an amber mutation located in the portion of the gene encoding the trimerization domain of HSF. This mutation is suppressed in yeast strain MYY385 by an uncharacterized nonsense suppressor, which renders the protein thermolabile. The temperature-sensitive phenotype can be recapitulated by site-directed mutagenesis of the position corresponding to the *mas3* amber mutation within the HSF trimerization domain. Moreover, HSF protein levels remain unchanged after heat shock of the *mas3* mutant.² These results indicate that the temperature sensitivity associated with the *mas3* allele is due to an intrinsic thermolability of this mutant transcription factor.

The residual accumulation of transcripts following heat shock of the *mas3* mutant strains is not likely to result from incomplete inactivation of HSF upon the temperature shift. HSF contains two regions responsible for transcription activation following heat shock stress (23). Thiele and co-workers (24) have shown that *CUP1* transcription is induced by heat shock and requires a carboxyl-terminal activation domain in HSF, while heat shock activation of *SSA3* gene expression requires an amino-terminal activation domain (23, 24). Our data indicate that the *mas3* mutation inactivates both domains as shown by the failure to induce *SSA3* transcription following heat shock (Fig. 2) and our finding that the *mas3* allele blocks *CUP1* expression at 39 °C (data not shown). Taken together, these results argue that the transcript accumulation observed in the *mas3* mutants was not due to residual HSF activity but

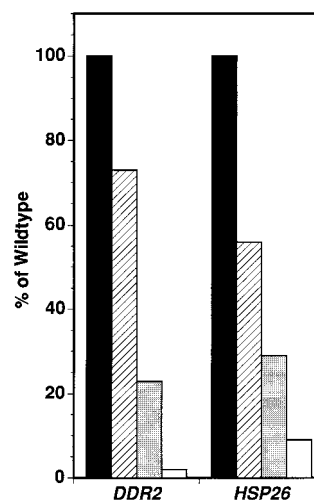


FIG. 3. Effect of *msn2Δ* and *msn4Δ* mutations on PDS induction. RNA (25 μg) from control and PDS grown cells was analyzed by Northern hybridization. After washing, the hybridization was quantified using an Ambis radio-imager as described in Table III. Black bars, JT659 (wild-type); striped bars, JT641 (*msn2Δ*); stippled bars, JT646 (*msn4Δ*); and open bars, JT652 (*msn2Δ msn4Δ*).

was due to a distinct regulatory mechanism(s).

The redundant nature of HSP gene regulation following heat shock was demonstrated using the multiple regulatory mutant strains we constructed. Indeed, there were several HSP genes where inactivation of all three transcription factors (HSF, Msn2p, and Msn4p) did not completely abolish transcript induction. Several explanations for the residual accumulation of transcripts in the *msn2Δ msn4Δ mas3* triple mutant are possible. First, a third independent stress regulatory pathway might account for heat shock induced expression of these genes. However, sequence comparisons of the upstream region of several of these genes did not reveal any consensus elements, other than the HSEs and STREs, which might identify this hypothetical pathway (data not shown). Second, it remains possible that the observed transcript levels increased because of transcript stabilization rather than increased transcriptional initiation. Such a mechanism might be expected to produce kinetic differences in the rates of transcript accumulation between mutant and wild-type cells. Our preliminary results indicate that there were no meaningful differences in the kinetics of transcript accumulation between the *mas3* mutant and wild-type (data not shown). Last, residual transcripts may result from the action of another transcription factor acting through the STRE. Consistent with this explanation, we have obtained preliminary evidence that all HSF-independent heat shock-induced transcription of the *UBI4* gene requires at least one intact STRE (data not shown). One candidate for this putative transcription factor (YER169W) was identified during an expression library screening using an STRE-containing oligonucleotide (2). This factor bound STREs specifically but is structurally unrelated to Msn2p except for the presence of a zinc finger domain. However, disruption of YER169W, singly or in combination with mutations in the *HSF1*, *MSN2*, and *MSN4* genes, had little or no effect on heat shock induction of several STRE-containing genes (data not shown). Nevertheless, we cannot rule out the possibility that one or more additional zinc finger transcription factors act to control residual-induced expression of these stress response genes.

STREs May Function for a Subset of Stresses for Some Genes—Transcription of five candidate genes identified from the computer pattern search was noninducible following heat shock or MMS and showed no dependence upon *MSN2* or *MSN4* for their expression (data not shown). Additionally, the

² M. P. Yaffe, personal communication.

STREs in the *HSP26* promoter were not functional following heat shock. To determine whether the STREs might function in response to different stress conditions, we tested the requirement for Msn2p and Msn4p for transcriptional activation of the *HSP26* gene during PDS growth. As seen in Fig. 3, the transcriptional induction of the *HSP26* gene during PDS was dependent on the Msn2p and Msn4p transcriptional activators. Furthermore, carbon source starvation induction of *HSP26* required the Msn2p (Msn4p)/STRE pathway (1). Similarly, Inoue *et al.* (25) have shown that *GLO1-lacZ* expression was controlled via the Msn2p/Msn4p pathway only following osmotic shock. There was no inducible expression of the same *GLO1-lacZ* fusion gene by other stresses such as heat shock, ethanol, or hydrogen peroxide, which have been shown to mediate their effects on the transcription of the *CTT1* and *DDR2* genes via upstream STREs (1, 2, 4). Thus, for some genes, such as *DDR2*, transcriptional control following all stress conditions tested is under the regulation of the STREs and Msn2p/Msn4p (4). However, function of STREs in the promoters of other genes such as *HSP26* may be more limited. Presently, we do not understand the reason for the "inactivity" of STREs in the upstream regions of some genes following specific stresses. It is possible that the binding of additional regulatory factors to neighboring elements in the promoters of some genes, such as *HSP26*, may interfere with or modulate the binding of Msn2p or Msn4p to the STREs. Analysis of the STREs and their possible interactions with other promoter elements or regulatory factors should help us understand the relationship between STRE and dependence upon the Msn2p and Msn4p transcriptional regulators following stress.

Acknowledgments—We thank Dr. Thomas Mason and Dr. Susan Lindquist for plasmids; Dr. Marian Carlson for yeast strains MCY2144, MCY2146, and MCY2150; and Dr. Michael Yaffe for strain MYY385.

APPENDIX

The following genes and putative open reading frames were identified using the computer generated pattern search of the yeast genome: *ACH1*, *ADE16*, *ALD4*, *CIT1*, *CKA2*, *CLG1*, *CYC7*, *CRZ1*, *DDR2*, *DDR48*, *DIT1*, *DIT2*, *END3*, *ERR1*, *ERR2*, *FUN14*, *GAL2*, *GAL80*, *GLC3*, *GLO1*, *GND2*, *GPH1*, *GYS2*, *HNT1*, *HSP12*, *HSP26*, *HSP42*, *HSP78*, *HSP104*, *HXK1*, *INH1*, *KRE1*, *LEE1*, *LRE1*, *MCR1*, *MDH1*, *MDJ1*, *MEF2*, *MEK1*, *MEP1*, *MIG2*, *MIH1*, *MRPL15*, *NCE2*, *NHP6B*, *PAI3*, *PBN1*, *PGM1*, *PGM2*, *PIG2*, *POR2*, *PPH21*, *PRC1*, *PTP2*, *PUT4*, *RAD16*, *RAS2*, *RET1*, *RPA14*, *RPB4*, *RPLA2*, *RTS1*, *SGA1*, *SIP18*, *SMC2*, *SNF1*, *SRA5*, *STE14*, *STE23*, *STF2*, *SUA7*, *TDH3*, *TFS1*, *TPS1*, *TPS2*, *TPS3*, *TSL1*, *UTR4*, *SBP1*, *XBP1*, *YAT1*, *YRB1*, *ZWF1*, *YAL053W*, *YAR029W*, *YBL077W*, *YBL078C*, *YBR044C*, *YBR138C*, *YBR139W*, *YBR149W*, *YBR183W*, *YBR230C*, *YBR254C*, *YBR255W*, *YBR285W*, *YCR087W*, *YDL025C*, *YDL085W*, *YDL124W*, *YDL223C*, *YDR233C*, *YDR276C*, *YDR399W*, *YDR453C*, *YER035W*, *YER036C*, *YER037W*, *YFR017C*, *YGL036W*, *YGL037C*, *YGL096W*, *YGL157W*, *YCR043C*, *YGR067C*, *YGR071C*,

YGR086C, *YGR122W*, *YGR130C*, *YGR223C*, *YGR224W*, *YGR235C*, *YGR280C*, *YHL021C*, *YHR009C*, *YHR161C*, *YHR172W*, *YIL113W*, *YIL124W*, *YIR016W*, *YIR038C*, *YJL070C*, *YJL103C*, *YJL193W*, *YKL026C*, *YKL090W*, *YKL091C*, *YKL107W*, *YKL151C*, *YKR075C*, *YKR076W*, *YLL055W*, *YLR080W*, *YLR257W*, *YLR345W*, *YLR390W*, *YLR421C*, *YLR422W*, *YLR425W*, *YML066C*, *YML101C*, *YMR031C*, *YMR084W*, *YMR172C-A*, *YMR181C*, *YMR206W*, *YMR262W*, *YMR277W*, *YMR322C*, *YMR323W*, *YNL077W*, *YNL083W*, *YNL134C*, *YNL144C*, *YNL179C*, *YNL321W*, *YNR007C*, *YNR013C*, *YNR014W*, *YOL026C*, *YOL087C*, *YOL153C*, *YOL163W*, *YOR060C*, *YOR066W*, *YOR134W*, *YOR171C*, *YOR173W*, *YOR352W*, *YOR391C*, *YPL017C*, *YPL230W*, *YPL247C*, *YPL280W*, *YPL281C*, *YPR003C*, *YPR061C*. Further information about the genes and the proteins that they encode can be obtained by accessing the Stanford Saccharomyces Genome Data base at <http://genome-www.stanford.edu/Saccharomyces> or Proteome's Yeast Protein Data base at <http://www.proteome.com/YPDhome.html>.

REFERENCES

- Martinez-Pastor, M. T., Marchler, G., Schuller, C., Marchler-Bauer, A., Ruis, H., and Estruch, F. (1996) *EMBO J.* **15**, 2227–2235
- Schmitt, A. P., and McEntee, K. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 5777–5782
- Kobayashi, N., and McEntee, K. (1993) *Mol. Cell. Biol.* **13**, 248–256
- Treger, J. M., Magee, T. R., and McEntee, K. (1998) *Biochem. Biophys. Res. Commun.* **243**, 13–19
- Marchler, G., Schuller, C., Adam, G., and Ruis, H. (1993) *EMBO J.* **12**, 1997–2003
- Schuller, C., Brewster, J. L., Alexander, M. R., Gustin, M. C., and Ruis, H. (1994) *EMBO J.* **13**, 4382–4389
- Varela, J. C., Praekelt, U. M., Meacock, P. A., Planta, R. J., and Mager, W. H. (1995) *Mol. Cell. Biol.* **15**, 6232–6245
- Ni, H. T., and LaPorte, D. C. (1995) *Mol. Microbiol.* **16**, 1197–1205
- Gounalaki, N., and Thireos, G. (1994) *EMBO J.* **13**, 4036–4041
- Rose, M. D., Winston, F., and Hieter, P. (1990) *Methods in Yeast Genetics: A Laboratory Course Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Treger, J. M., and McEntee, K. (1990) *Mol. Cell. Biol.* **10**, 3174–3184
- McClanahan, T., and McEntee, K. (1984) *Mol. Cell. Biol.* **4**, 2356–2363
- Sanchez, Y., and Lindquist, S. L. (1990) *Science* **248**, 1112–1115
- Boy-Marcotte, E., Perrot, M., Bussereau, F., Boucherie, H., and Jacquet, M. (1998) *J. Bacteriol.* **180**, 1044–1052
- Farrelly, F. W., and Finkelstein, D. B. (1984) *J. Biol. Chem.* **259**, 5745–5751
- Leonhardt, S. A., Fearson, K., Danese, P. N., and Mason, T. L. (1993) *Mol. Cell. Biol.* **13**, 6304–6313
- Wotton, D., Freeman, K., and Shore, D. (1996) *J. Biol. Chem.* **271**, 2717–2723
- Amin, J., Ananthan, J., and Voellmy, R. (1988) *Mol. Cell. Biol.* **8**, 3761–3769
- Boorstein, W. R., and Craig, E. A. (1990) *EMBO J.* **9**, 2543–2553
- Boorstein, W. R., and Craig, E. A. (1990) *Mol. Cell. Biol.* **10**, 3262–3267
- Gross, D. S., Adams, C. C., English, K. E., Collins, K. W., and Lee, S. (1990) *Antonie Leeuwenhoek* **58**, 175–186
- Smith, B. J., and Yaffe, M. P. (1991) *Mol. Cell. Biol.* **11**, 2647–2655
- Sorger, P. K. (1990) *Cell* **62**, 793–805
- Tamai, K. T., Liu, X., Silar, P., Sosinowski, T., and Thiele, D. J. (1994) *Mol. Cell. Biol.* **14**, 8155–8165
- Inoue, Y., Tsujimoto, Y., and Kimura, A. (1998) *J. Biol. Chem.* **273**, 2977–2983
- Sass, P., Field, J., Nikawa, J., Toda, T., and Wigler, M. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 9303–9307
- Oh, D., and Hopper, J. E. (1990) *Mol. Cell. Biol.* **10**, 1415–1422
- Powers, S., Kataoka, T., Fasano, O., Goldfarb, M., Strathern, J., Broach, J., and Wigler, M. (1984) *Cell* **36**, 607–612
- Smid, A., Riva, M., Bouet, F., Sentenac, A., and Carles, C. (1995) *J. Biol. Chem.* **270**, 13534–13540
- Woychik, N. A., and Young, R. A. (1989) *Mol. Cell. Biol.* **9**, 2854–2859
- Strunnikov, A. V., Hogan, E., and Koshland, D. (1995) *Genes Dev.* **9**, 587–599

**Transcriptional Factor Mutations Reveal Regulatory Complexities of Heat Shock
and Newly Identified Stress Genes in *Saccharomyces cerevisiae***

Janet M. Treger, Anthony P. Schmitt, John R. Simon and Kevin McEntee

J. Biol. Chem. 1998, 273:26875-26879.

doi: 10.1074/jbc.273.41.26875

Access the most updated version of this article at <http://www.jbc.org/content/273/41/26875>

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 30 references, 20 of which can be accessed free at
<http://www.jbc.org/content/273/41/26875.full.html#ref-list-1>