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Short Communication

Analysis of *in vivo* binding of yeast heat shock factor to promoter DNA

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The *in vivo* binding of yeast heat shock factor (HSF) to promoter domains of stress-inducible genes was examined using chromatin immunoprecipitation (ChIP) combined with PCR. The promoter-specfic PCR amplification of DNA immunoprecipitated with antibodies against HSF showed that yeast HSF was bound constitutively to the promoters of *GTT2*, *YLL058*W, *COQ6*, *GND2* and *YGR066C* genes, inducibley to the *MRS2* gene. HSF-binding consensus sites prediction indicated the promoter sequences of the stress-inducible genes contain heat shock elements (HSEs). These results suggest the genes are potential HSF-regulated targets.

Key words: Yeast, heat shock factor, heat shock elements, chromatin immunoprecipitation.

INTRODUCTION

In response to elevated temperatures and physiological stresses, eukaryotic cells rapidly activate a preexisting heat shock factor (HSF) with its high-affinity binding to heat shock promoter domains called heat shock element (HSE) to regulate expression of stress-inducible genes, therefore playing a central role in the control of many cellular processes (Morimoto et al., 1994).

The baker's yeast, *Saccharomyces cerevisiae*, harbors only a single HSF which is indispensable for cell survival (Sorger and Pelham, 1988; Wiederrecht et al., 1988). Previous work showed, under heat-induced conditions, the yeast HSF trimers constitutively bind to HSEs comprised of multiple inverted nGAAn tandem repeats (Jakobsen et al., 1988) to activate transcription of heat-induced genes functioning as molecular chaperones (Craven et al., 1996; Treger et al., 1998). Recent reports (Hahn et al., 2004; Yamamoto et al., 2005) have shown HSF trimers bind to promoter sequences of some stress-inducible genes to encode proteins that involve in growth, differentiation, disease, aging, and reprogramming metobolism path-ways.

To gain further insight into the regulation of yeast HSF, the paper reports the *in vivo* binding of yeast HSF to the promoters of the stress-induced genes.

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MATERIALS AND METHODS

Cell culture and heat shock

Yeast cells (*S. cerevisiae*, strain Y190) were grown in 100 ml SD/-leu media to an OD_{600} of 0.6 at 25℃. Cell cultures were heat-shocked at 39℃ for 15 min. The non-heat shocked controls were treated at 25℃ for 15 min.

Formaldehyde crosslinking

Formaldehyde was added directly to aliquots of yeast cell cultures at a final concentration of 1% for 5 min to freeze DNA-proteins interactions. Crosslinking was quenched by the addition of glycine to a final concentration of 0.125 M. The cells were harvested in 1.5 ml eppendorf tubes by centrifugation and washed once in cold TBS.

Chromatin immunoprecipitation

Yeast cells were votexed with glass beads, and chromatin was sheared to 0.1-0.6 kp average length at 4℃ using a BRANSON Digital Sonifier. The extracts were centrifuged at 12,000 g at 4℃ for 10 min, and the supernatants were filtered through a 0.22 µm filter.

Around 20 µg of chromatin DNA were incubated with 30 µl (1.0 µg) of antibody for more than 4 h at 4℃. Control samples were prepared by adding 30 µl control serum, which were taken through all sebsequent steps in parallel with the experimental groups. 30 µl protein-A agarose (Santa Cruz) preinculbated with 1% BSA were added to immunocomplexes, and incubated for additional 30 min. Subsequently, the resin was washed and immunoprecipitated material was eluted according to the paper (Hecht et al., 1996). The

Table 1. Primers used for promoter-specific PCR amplification.

Primers (5' - 3', sense / antisense)	Correspondent downstream genes
CACAGATCCTGGTACGTT, GCTGACGTTACAGACCTT	*PHO5*
TCAACTAAAATCTGGAGAAAA, CGGAACGTTTAGAAGCTGTCATT	*SSA1*
AGGTTCTTTCTGCTCGTGA, CTTTTCCTTGGTTTCCTCG	*MRS2*
GCCTACTTTCCTTCTTG, TGCAATTGGGAGTACGT	*GTT2 / YLL058W*
GTAGAGCGTTGCACTTGT, TGCCACCTGTCAGAATTG	*COQ6 / GND2*
ATGGAGCACAGGTAAAC, GAGGTGCTCAGTAAAAG	*YGR066C*
CTGAAGACCTGTTACAG, CGTAATGAGAGGCAAGT	*RAD14 / TOM40*

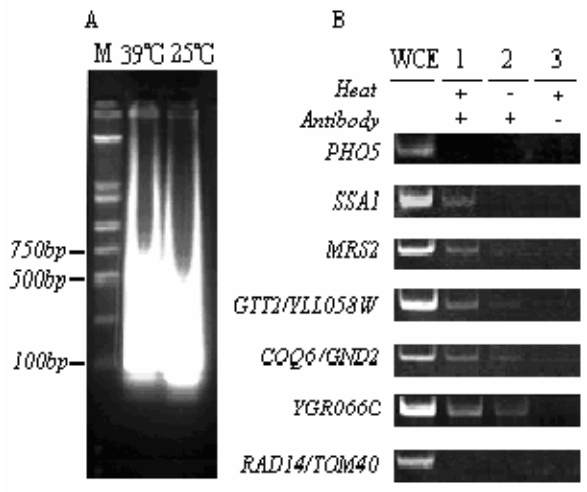

Figure 1. Analysis of the in vivo binding of yeast HSF response to heat shock. **A.** Migration analysis of the nuclear DNA complexes of the heat shocked and non-heat shock cells on a agarose gel. **B.** Determination of HSF-binding to potential targets by PCR. WCE, Whole cell extracts used as template for control amplification. Heat +, Yeast cells were heat-shocked at 39℃ for 15 min; Heat-, Control samples were treated at 25℃ for 15 min. Antibody +, DNA immunoprecipitated with antibodies against HSF; Antibody -, DNA immunoprecipitated with control serum. The *SSA1* promoter was used as a positive control, while the *PHO5* promoter as a negative control.

immunoselected DNA was released by digestion with 1% proteinase K, extracted by phenol/choroform (1:1), precipitated with ethanol. The resulting DNA was dissolved in 20 µl TE.

PCR

3% of each immunoprecipitated DNA was used to amplify promoter regions of the stress-inducible and non-stress inducible genes with primers (Table 1). PCR reactions were performed for 25 cycles of 20 s at 94℃, 20 s at 50-55℃, and 20 s at 72℃. 6 µl of PCR products were loaded onto a 10% polyacrylamide gel and analysed by staining with Ethidium bromide.

Computational analysis

The upstream promoter sequences of the stress-induced genes were derived from NCBI, and yeast HSF-binding consensus sites were screened. Stress inducible expression assays were performed according to the published datum (http:// web.wi.mit.edu/young/environment) (Gasch et al., 2000).

RESULTS AND DISCUSSION

The formaldehyde-crosslinking nuclear extracts of the heat-shocked as well as non-heat shocked yeast cells were electrophoresised on a 0.8% agarose gel and stained with Ethidium bromide. Figure 1A indicated the nuclear DNA complexes of the heat shocked cells were migrated more slowly than that of the cells treated at 25℃ on a gel, suggesting additional heat-shocked regulatory proteins bound to genetic DNA.

The *in vivo* binding of HSF to the promoter regions of stress inducible expression genes (Table 2) were screened by promoter-specific PCR amplification of immunoprecipitated DNA. PCR results (Figure 1B) showed, upon heat stress, the *SSA1* promoter using as a positive control gave a PCR signal, however the *PHO5* gene promoter reported as a non-HSF binding target site did not. Under heat shock and non-heat shocked conditions, the promoters of *GTT2*, *YLL058*W, *COQ6*, *GND2* and *YGR066C* genes exhibited signals at the same positions as the controls using total DNA as template, but the heat-shocked samples gave more strong signals, indicateing the binding of yeast HSF was constitutive. The *MRS2* demonstrated a signal only in the present of heat shock, which implied yeast HSF bound inducibley to the promoter. Some non-stress inducible genes including *RAD14* and *TOM40* did not give signals, and they were not regulated by HSF *in vivo*. In this experiments, all negtive controls immunoprecipitated by control serum did not give PCR signals.

HSEs consist of multiple inverted 5'-nGAAn-3' tandem repeats, which is functionally conserved from yeasts to humans (Xiao et al., 1991; Wu 1995; Liu et al., 1997). To detect yeast HSEs, we analysed the promotor squences within 1000 bp of the 5'-untranslated regions of yeast genes (*MRS2*, *GTT2*, *YLL058*W, *COQ6*, *GND2* and *YGR066C*). There are HSE-like sequences such as 5'-a**GAA**ag**GAA**gctgaa**GAA**a-3' motifs, 5'-t**GAA**tt**GAA**c-3' and 5'-a**GAA**at**GAA**a-3' motifs in the promotor regions of

Table 2. The heat-inducible binding of HSF and mRNA expression of genes response to stresses.

Gene	Stress-inducible expression[a]	Biochemical function or gene product[b]	I*n vivo* binding analysis[c]
PHO5	-	acid phosphatase	-
SSA1	+	heat shock protein 70	+
MRS2	+	magnesium ion transporter	+
GTT2	+	tolerance oxidant stress	+
YLL058W	+	molecular function unknown	+
COQ6	+	monooxygenase	+
GND2	+	6-phosphogluconate dehydrogenase	+
YGR066C	+	o-succinylhomoserine (thiol)-lyase	+
RAD14	-	repair of nucleotide excision	-
TOM40	-	translocase of the outer membrane	-

[a]Data derived from the http:// web.wi.mit.edu/young.environment (Gasch et al., 2000). + = Enhanced mRNA expression; - = inhibition of mRNA expression.
[b]From http:// www.yeastgenome.org.
[c] + = *In vivo* binding of HSF; - = *in vivo* non-binding of HSF.

MRS2; gap-type HSEs (Yamamoto et al., 2005): 5'-c**GAA**agatttc**TTC**at**TTC**t-3' motifs distributed in the promotor domains shared by *GTT2* and *YLL058W*, and 5'-t**TTC**ttaagtg**TTC**tg**TTC**a-3' for *YGR066C*; perfect-type HSE (Jakobsen et al., 1988): 5'-t**TTC**tt**GAA**ac**TTC**a-3' motifs for *COQ6* and *GND2*. It may show that a upstream genetic sequence harboring regulartory elements exerts effects on one to multigenes (Shostak and Yamamoto, 2005). The transcriptions of the genes were dramatically induced in stress conditions such as heat shock, acid shock, alkaline shock and osmotic shifts as well (Gasch et al., 2000). The genes coding proteins function as tolerance oxidant stress, and involve in other biochemical pathways.

Conclusion

Response to heat shock, yeast HSF constitutively bound to the promoters of the stress inducible expression genes including *GTT2*, *YLL058W*, *COQ6*, *GND2* and *YGR066C*, however, inducibly to the *MRS2*, not to the *RAD14* and *TOM40* that are not induced by stresses.

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