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Stress Responses through Heat Shock Transcription Factor in *S. cerevisiae*

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Abstract

Heat Shock Transcription Factor (HSF), and the promoter Heat Shock Element (HSE), are among the most highly conserved transcriptional regulatory elements in nature. HSF mediates the transcriptional response of eukaryotic cells to heat, infection and inflammation, pharmacological agents, and other stresses. While HSF is essential for cell viability in yeast, oogenesis and early development in *Drosophila*, extended life-span in *C. elegans*, and extra-embryonic development and stress resistance in mammals, little is known about its full range of biological target genes. We used whole genome analyses to identify virtually all of the direct transcriptional targets of yeast HSF, representing nearly three percent of the genomic loci. The majority of the identified loci are heat-inducibly bound by yeast HSF, and the target genes encode proteins that have a broad range of biological functions including protein folding and degradation, energy generation, protein secretion, maintenance of cell integrity, small molecule transport, cell signaling, and transcription. Approximately 30% of the HSF direct target genes are also induced by the diauxic shift, in which glucose levels begin to be depleted. We demonstrate that phosphorylation of HSF by Snf1 kinase is responsible for expression of a subset of HSF targets upon glucose starvation.

Results and Discussion

Identifying the genome-wide targets of S. cerevisiae HSF

To begin to understand the precise molecular responses to stress, and the central role played by HSF, we have used chromatin immunoprecipitation (ChIP) combined with DNA microarray approaches to identify virtually all of the direct target genes bound by *S. cerevisiae* HSF *in vivo*. We determined the *in vivo* association of *S. cerevisiae* HSF with each genomic locus based on 14 independent crosslinking experiments under unstressed conditions (30°C) and 20 independent crosslinking

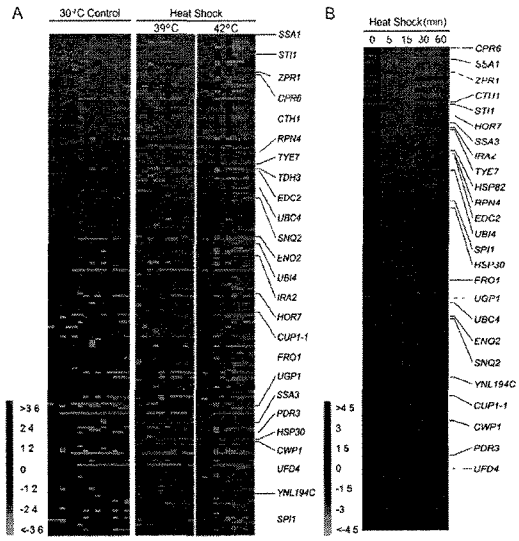

Fig. 1. Genome-wide binding distribution of HSF. Cy5/Cy3 ratios of genomic fragments with enrichment values above the threshold are displayed using a red and green color scale. Grey cells indicate missing data that was filtered out. (A) Each column represents an IP performed after independent HSF crosslinking carried out under 30°C unstressed conditions or after a 20minute heat shock at the indicated temperature. Genes downstream of selected target loci are shown on the right. (B) Binding of HSF to all targets during the heat shock time course. HSF IP samples prepared at the indicated times during the 39°C heat shock were analyzed by DNA microarrays.

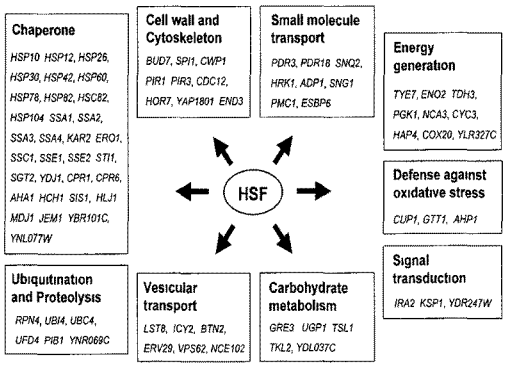

Fig. 2. The constellation of HSF target genes in the yeast genome. Representative HSF targets are categorized according to their known or hypothetical functions based upon sequence homology.

experiments under heat shock conditions (20 minutes at 39°C or 42°C). To set an appropriately stringent threshold for defining HSF targets, we used the fact that the physiological targets of yeast HSF are expected to be transcriptionally induced by heat shock. Based on the relationship between enrichment (E) of a genomic locus and the expression level of the gene downstream of it as measured by a moving-window average analysis (Fig. 1), we set our operational threshold for defining targets at 97.7. Loci with (E) values greater than this threshold under heat shock conditions tend to be strongly induced by heat shock and are thus defined as the putative physiological binding targets of HSF *in vivo*.

Of all loci evaluated, 210 loci representing sequences upstream of 165 distinct open reading frames had enrichment values above this threshold (Fig. 1A). Although HSF appears to bind many of its strongest target promoters equally well at 30°C and after heat shock, other prominent target loci such as the promoters of the *SSA3* and *HSP30* genes showed clear heat-inducible binding. We further confirmed heat-inducible binding of HSF to the targets by CHIP during heat shock time course (Fig. 1B). This is the first evidence demonstrating that, similar to higher eukaryotic cells, *S. cerevisiae* HSF binds to many of its targets in a heat regulated manner in a much broader way than previously appreciated.

The constellation of yeast HSF target genes

The products of many of these HSF target genes are known or predicted to function in a wide range of cellular processes (Fig.2). These

include protein chaperone functions, ubiquitination and proteolysis, vesicular transport, maintenance of the cell wall and cytoskeleton, small molecule transport, carbohydrate metabolism, energy generation, signal transduction, and oxidative stress defense mechanisms, suggesting a much broader and central role for HSF in the physiological response to heat shock and other stresses.

Regulation of HSF by Snf1 kinase under glucose starvation condition

It has been known that yeast HSF can be activated by various environmental stresses such as oxidative stress and glucose starvation, but the mechanisms by which HSF is activated are poorly understood. According to the expression data base, about 30% of HSF targets are induced by diauxic shift as well as heat shock. When cells grown in the presence of 2% glucose were limited for glucose by shifting to a medium containing 0.05% glucose, induction of several HSF target genes was observed after 1.5 or 3 h, which was dependent on Snf1 kinase, a key regulator involved in gene expression under glucose starvation conditions, and C-terminal activation domain of HSF (Fig. 3). However Snf1 did not affect heat-induced expression of HSF targets (data not shown). We demonstrated that Snf1 phosphorylates HSF in vitro (Fig. 4). In addition, Snf1 phosphorylated HSF in vivo in response to glucose limitation, without any effect on heat-induced phosphorylation of HSF (Fig. 5). Furthermore, enhanced chromosomal HSF DNA binding to low affinity target promoters such as *SSA3* and *HSP30* , occurred in a Snf1-dependent manner (data not shown).The fact that Snf1-dependent phosphorylation of HSF is only required for activation of HSF by glucose starvation, but not by heat shock, suggests that HSF activity might be regulated by differential phosphorylation via different kinases in response to a variety of stress conditions.

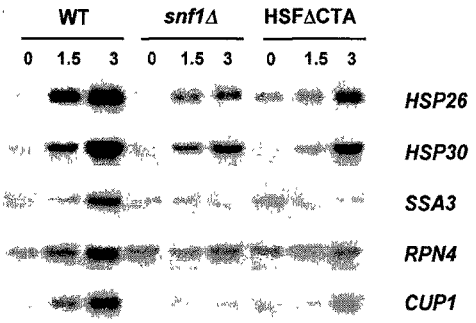

Fig.3. Snf1-dependent induction of HSF targets in response to glucose limitation. MCY1093 and its *snf1Δ* or HSFΔCTA derivative were grown in SC medium containing 2% glucose and then shifted to medium with 0.05% glucose for the indicated times in hours (h) Expression levels of the indicated direct HSF target genes were detected by RNA blot analysis

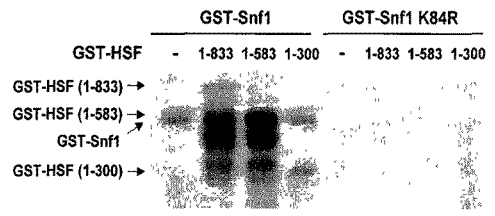

Fig. 4. Phosphorylation of HSF by Snf1 *in vitro*. GST-Snf1 or GST-Snf1 K84R proteins were purified from yeast strain RSY620 and used in *in vitro* kinase assays in the presence of GST fused to full-length HSF (GST-HSF), or truncated derivatives of HSF [GST-HSF (1-583)], or [GST-HSF (1-300)] purified from *E coli*. Reaction components with fractionated by SDS-PAGE and detected by autoradiography.

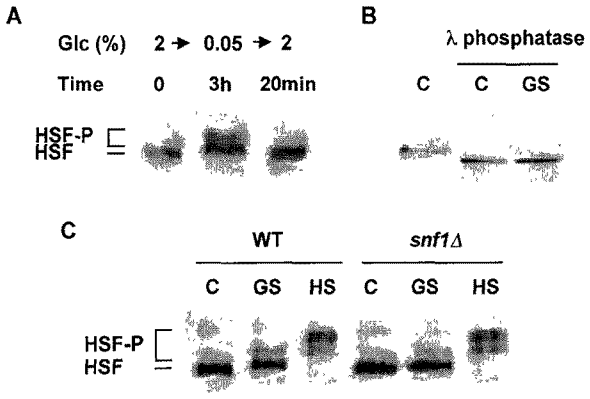

Fig.5. Snf1-dependent phosphorylation of HSF in response to glucose deprivation. (A) Phosphorylation of HSF under glucose starvation conditions. Strain MCY1093 which had been grown in the presence of 2% glucose was transferred to medium with 0.05% glucose for 3 h, cells sampled, and glucose added to a final concentration of 2% to the medium for 20 min. HSF was detected from total cell extracts by SDS-PAGE followed by immunoblotting with antibody against HSF. The phosphorylated forms of HSF are indicated as HSF-P. (B) Confirmation of HSF phosphorylation in response to glucose starvation Cell extracts from cells grown in 2% glucose (C) or from cells grown in 0.05% glucose glucose for 3 h (GS) were treated with λ phosphatase for 30 min and HSF proteins were detected as described in (A) (C) Glucose deprivation-induced HSF phosphorylation is Snf1-dependent. MCY1093 (WT) and the isogenic *snf1Δ* strain were grown in the presence of either 2% glucose (C), 0.05% glucose for 3 h (GS) or heat shocked at 39 °C for 30 min (HS), and HSF was detected and identified as described in (A).

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