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CHIP interacts with heat shock factor 1 during heat stress

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Abstract **Heat shock factor 1 (HSF1) is a major transactivator of heat shock genes in response to stress and mediates cell protection against various harmful conditions. In this study, we identified the interaction of CHIP (carboxyl terminus of the heat shock cognate protein 70-interacting protein) with the N-terminus of HSF1. Using GST full-down assay, we found that CHIP directly interacts with C-terminal deleted HSF1 (a.a. 1–290) but not with full-length HSF1 under non-stressed conditions. Interestingly, interaction of CHIP with full-length HSF1 was induced by heat shock treatment. The structural change of HSF1 was observed under heat stressed conditions by CD spectra. These observations demonstrate the direct interaction between HSF1 and CHIP and this interaction requires conformational change of HSF1 by heat stress.**
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1. Introduction

Heat shock factors (HSFs) regulate the expression of heat shock proteins (Hsps) against a variety of environmental and developmental stresses [1–4]. Mammals have multiple distinct HSF genes, encoding isoforms denoted HSF1, HSF2 and HSF4. HSF1 is the predominant HSF isoform that responds to thermal and oxidative stress to activate the expression of Hsp genes [2,5].

HSF1 is composed of an amino-terminal DNA-binding domain (DBD), an adjacent coiled-coil trimerization domain (Leucine zipper 1-3, LZ1-3), a central regulatory domain (RD), a second coiled-coil domain (Leucine zipper 4, LZ4), and a carboxyl-terminal transcriptional activation domain (AD) (Fig. 1A). The transcriptional activity of HSF1 is tightly controlled. Under normal physiological conditions, HSF1 largely localizes to the cytoplasm as a monomeric form with low DNA-binding activity [6,7]. Intramolecular interactions between LZ1-3 and LZ4 restrain HSF1 in an inactive state [8–10]. Upon sensing stress, HSF1 undergoes the transition from a monomeric to a homotrimeric complex in which monomers associate through the formation of a three-stranded coiled-coil by the trimerization domain [9,11]. However, precise molecular mechanisms by which HSF1 senses thermal stress to switch from the monomer to the homotrimeric form are poorly understood.

One of the most pronounced consequences of heat stress is the unfolding and the misfolding of proteins. To avoid the cellular damage, aberrant proteins must be either refolded by molecular chaperones or eliminated by the ubiquitin-proteasome protein degradation system. Carboxyl terminus of the Hsc70-interacting protein (CHIP) is a co-chaperone interacts with heat shock cognate protein 70 (Hsc70) and Hsp90 molecular chaperones via a tetratricopeptide repeat motif and inhibits chaperone-dependent protein folding [12–14]. CHIP also stimulates protein degradation by acting as an E3 ubiquitin ligase via a modified ring finger domain called a U-box [13–17]. Recently, Dai and colleagues [18] have shown that CHIP regulates the stress-chaperone response through induced trimerization and transcriptional activation of HSF1. Although they reported the functional relationship between CHIP and HSF1, the molecular mechanism of interaction remains to be elucidated.

In this study, we demonstrate that HSF1 directly interacts with CHIP through its N-terminal region. Interestingly, interaction between CHIP and full-length HSF1 was induced by heat stress suggesting conformational change of HSF1 is required for their interaction. Finally, we observed structural change of HSF1 by circular dichorism (CD) spectra under heat stressed conditions. Our results demonstrate a previously unreported molecular mechanism of HSF1–CHIP interaction.

2. Materials and methods

2.1. Plasmids

Bacterial expression vectors for recombinant glutathione *S*-transferase (GST)-tagged full-length HSF1 (FL) and C-terminal deleted HSF1 (a.a. 1–290) fusion proteins have been described [19]. The open reading frames of Hsc70 (1941 nucleotides) and CHIP (912 nucleotides) corresponding to GenBank™ Accession Nos. BC016179 and AF129085 were amplified by PCR using human skeletal muscle cDNA as a template (CLONTECH). Vectors for the expression of bacterial recombinant His-tagged Hsc70 and CHIP were created using the pET21a vector (Stratagene). The pET-Hsc70 vector was created by inserting a DNA fragment containing the *Hsc70* open reading frame without a stop codon into the 5′-*Bam*HI and 3′-*Xho*I sites of pET21a in-frame with the six-histidine tag. The pET-CHIP construct was created by inserting a DNA fragment containing the CHIP open reading frame without a

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Abbreviations: HSF1, heat shock factor 1; CHIP, carboxyl terminus of the Hsc70-interacting protein; Hsc70, heat shock cognate protein 70; Hsp90, heat shock protein 90; GST, glutathione *S*-transferase

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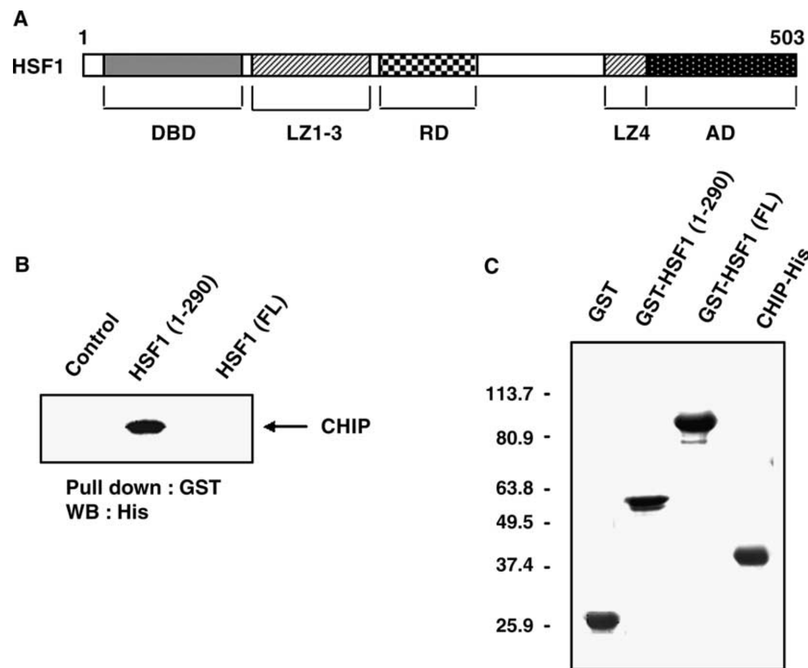

Fig. 1. HSF1 interacts with CHIP through its N-terminal region. (A) Schematic diagram of the HSF1 domain organization. HSF1 is composed of an amino-terminal DBD, an adjacent coiled-coil trimerization domain (LZ1-3), a central RD, a second coiled-coil domain (LZ4), and a carboxyl-terminal transcriptional AD. Domain boundaries were obtained from the SMART and COIL programs. (B) Purified GST-tagged bacterial recombinant HSF1 (1–290) or HSF1 (FL) (1 μg) were incubated equimolar His-tagged CHIP. As a control, equimolar GST was incubated with His-tagged CHIP. GST or GST-HSF1 was pulled down by glutathione-agarose beads. Co-precipitated proteins were resolved on SDS–PAGE and detected by Western blot analysis using anti-His antibody. (C) Purified bacterial recombinant GST-tagged HSF1 (1–290), GST-tagged HSF1 (FL) and His-tagged CHIP were monitored by Coomassie blue staining.

stop codon into the 5′-*Nde*I and 3′-*Not*I sites of pET21a in-frame with the six-histidine tag. Mammalian expression vector for HSF1 have been described previously [19]. Vectors for mammalian expression of N-terminally FLAG-tagged CHIP fusion protein was created by inserting a DNA fragment containing the complete CHIP open reading frame into the 5′-*Kpn*I and 3′-*Not*I sites of pCDNA3.1-NF.

2.2. Protein expression and purification

Bacterial recombinant HSF1 was expressed as an N-terminally GST-tagged fusion protein in *Escherichia coli* BL21 (DE3) Codon Plus cells (Stratagene) and purified by using glutathione-agarose affinity resin as described [19]. Recombinant Hsc70 and CHIP were expressed as C-terminally His-tagged fusion proteins in *E. coli* BL21 (DE3) Codon Plus cells and purified by using Ni^{2+}-agarose affinity resin as described [20].

2.3. GST pull-down assay

GST-tagged HSF1 (1 μg) was added to equimolar His-tagged CHIP in reaction buffer (20 mM Tris–Cl, pH 8.0, 200 mM NaCl, 0.1% Nonidet P-40, 1 mM DTT, 1 mM PMSF, 1% BSA). After 2 h incubation at 4 °C, glutathione-agarose beads were added and incubated for another 1 h at 4 °C. Beads were washed four times with TBST (20 mM Tris–Cl, pH 8.0, 200 mM NaCl, 0.1% Tween-20) and boiled in SDS–PAGE sample buffer. Proteins were resolved by SDS–PAGE and immunoblotted with anti-His antibody (Qiagen).

2.4. Cell culture and immunoprecipitation

HEK 293 cells were maintained at 37 °C with 5% CO_2 in DMEM containing 10% FCS, 50 U/ml penicillin and 50 μg/ml streptomycin. For immunoprecipitation, HEK 293 cells were transiently transfected by using the FuGENE 6 reagent (Roche Molecular Biochemicals) according to the manufacturer's protocol. Thirty hours after transfection, the cells were washed twice with PBS and lysed in RIPA buffer (PBS supplemented with 1% Nonidet P-40, 0.5% sodium deoxycholate, 1 mM PMSF, 1 μg/ml aprotinin, 1 mM sodium orthovanadate). The cell lysates were harvested and incubated at 4 °C for 30 min and cleared by centrifugation at $10\,000 \times g$ for 10 min. The supernatant was incubated with anti-FLAG antibody (Sigma–Aldrich) for 3 h, after which protein G–Sepharose (Amersham Pharmacia) was added and incubated another 1 h. The immunoprecipitates were washed four times with RIPA buffer containing 0.05% SDS and boiled in SDS–PAGE sample buffer. Proteins were resolved by SDS–PAGE and immunoblotted with anti-HSF1 antibody (Abcam), anti-FLAG antibody (Sigma–Aldrich), anti-Hsp70 antibody (Santa Cruz Biotechnology), anti-Hsc70 antibody (Santa Cruz Biotechnology), or anti-Hsp90 antibody (Santa Cruz Biotechnology).

2.5. Characterization of proteins by circular dichroism

For CD experiments, purified recombinant HSF1 was dialyzed in 20 mM HEPES buffer, pH 7.5. The protein was diluted with HEPES buffer to a final concentration of 0.3 mg/ml and treated heat shock at 42 °C for 5 min. CD scans were performed on a Jasco J-810 spectropolarimeter in the far-UV range at 25 °C as previously described [21]. A cell of 0.1 cm optical path was used to obtain spectra at a scan speed of 50 nm/min. Spectra were averaged from four individual scans and results were presented as mean molar ellipticity.

3. Results and discussion

3.1. HSF1 interacts with CHIP through its N-terminal region

To investigate the molecular mechanism of interaction between HSF1 and CHIP, we examined the binding in vitro using GST pull-down assays. GST-tagged full-length HSF1 (FL) and C-terminal truncated HSF1 (a.a. 1–290) were expressed in bacteria and purified. Bacterial recombinant His-tagged CHIP was also purified. Interestingly, CHIP specifically interacts with C-terminal truncated HSF1 (1–290), encompassing the N-terminal DBD and coiled-coil motif (LZ1-3) (Fig. 1B). Under non-stressed conditions, full-length HSF1

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