

Coactivator ASC-2 mediates heat shock factor 1-mediated transactivation dependent on heat shock

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Abstract Upon exposure to elevated temperatures, mammalian cells increase the expression of the heat shock proteins (HSP) through activation of the heat shock factor 1 (HSF1). Since most transcription factors require coactivators for efficient transcriptional activity, we tried to identify the coactivator(s) that interacts with and modulates the activities of HSF1. In vitro glutathione S-transferase (GST) pull-down assay revealed that HSF1 strongly interacts with activating signal cointegrator (ASC)-2 and weakly with cyclic adenosine monophosphate responsive element binding protein (CBP). We also show that cotransfection of ASC-2, but not CBP, potentiates HSF1-mediated transactivation based on its cognate element (heat shock element, HSE) linked to luciferase reporter. The molecular interaction of HSF1 and ASC-2 was stimulated by heat shock in cells and the overexpression of HSF1-interacting domain of ASC-2 inhibited the specific induced protein association and HSF1-mediated transactivation. Taking these results together, we suggest that ASC-2 is a novel coactivator for HSF1 and heat shock stress may contribute the strong active transcription complex through sequential recruitment of HSF1 and ASC-2. © 2004 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.

Key words: HSF-1; ASC-2; Heat shock; Transactivation

1. Introduction

When cells are exposed to stressful conditions such as heat shock and heavy metals, they increase the expression of a specific set of proteins referred to as the heat shock proteins (HSPs) that serve as chaperone by facilitating protein folding and assembly and intracellular transport [1–4]. Increased expression of the HSPs in response to stresses is transcription-

ally mediated by the heat shock factor (HSF) [5,6]. HSF1, one of the HSF families (HSF1–4), is known as a major mediator for the heat shock response. HSF1 consists of the N-terminal DNA binding domain (DBD), regulatory domain, and C-terminal transactivation domain (TAD). In normal cells, HSF1 exists in complex with regulatory proteins such as HSP70 and HSP90, which repress the DNA binding activity of HSF1 [7–9]. Exposure of cells to proteotoxic stresses results in the appearance of non-native polypeptides and the requirement for molecular chaperones. As a consequence of the release of interacting chaperones, HSF1 monomer homotrimerizes, translocates to nucleus, binds to the heat shock element (HSE), composed of inverted repeats of the 5-bp sequence 5'-nGAAn-3', and is hyperphosphorylated, and becomes transcriptionally competent [10–14].

Most transcription factors require coactivators to exhibit their full transcriptional activity. Cyclic adenosine monophosphate responsive element binding protein (CBP)/p300, one of the major coactivators whose roles in the transcription are widely studied, potentiates the transactivational activity of a variety of transcription factors including nuclear receptors and myoD [26,27]. Activating signal cointegrator (ASC)-2 is first known to identify as a transcriptional coactivator of nuclear receptors [28] and is introduced under different names by others with variously termed TRBP, PRIP, and RAP250 [29–31]. ASC-2, a typical ligand and AF2-dependent interactor of nuclear receptors, enhances receptor transactivation, either alone or in conjunction with SRC-1 and CBP/p300. ASC-2 can also mediate transactivation of a variety of transcription factors including SRF, AP-1, and NF- κ B in addition to nuclear receptors [32,33]. Certain functions of coactivators could be modulated by a number of different signal transduction pathways and external stimuli. For instance, CBP can be stimulated to exert its histone acetyltransferase (HAT) enzymatic activity through phosphorylation by p44 extracellular regulated kinase (ERK) and is calcium-sensitive such that it may act as a regulatory switch for CaM kinase II, CaM kinase IV, and protein kinase A-induced transcription [34]. SRC-1 is also phosphorylated by ERK1/2 in response to progesterone and EGF [35].

In this study, we tried to find out the coactivators that interact with and modulate the activities of HSF1. Here we show that HSF1 strongly interacts with coactivator ASC-2 and that expression of ASC-2 potentiates HSF1-mediated transactivation based on HSE linked to luciferase reporter. We further show that heat shock enhances the protein–protein interaction of ASC-2 and HSF1 in cells.

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Abbreviations: HSP, heat shock protein; HSF, heat shock factor; CBP, cyclic adenosine monophosphate responsive element binding protein; ERK, extracellular regulated kinase; ASC, activating signal cointegrator; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; GST, glutathione S-transferase

2. Materials and methods

2.1. Cell culture and heat shock treatment

HepG2 cells were grown in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 1% penicillin–streptomycin (Life Technologies, Inc.) and 10% (v/v) fetal bovine serum (Life Technologies, Inc.) at 37°C in a humidified incubator with 5% CO₂. For heat shock treatment, cells were plated and stabilized for 24 h at 37°C and then exposed to heat shock of 43°C for 30 min and recovered at 37°C for several hours if that was required.

2.2. Plasmid constructs of HSF1

The GAL4N-HSF1 fusion proteins were constructed by amplification of HSF1 (amino acid residues 1–529, 1–399, and 395–529) using specific primers, and subcloned into plasmid pCMX-1, which encodes GAL4N DBD (residues 1–147). The HSE-Luc was constructed by ligating the –122 to –90 fragment of the Hsp70 promoter (–122GATCCGGCGAAACCCCTGGAATATTCCTCCGACCT–90) into the heterologous reporter construct pGL2basic. pcDNA3/p38β expression vector was kindly provided by Dr. Jiahui Han (The Scripps Research Institute, La Jolla, CA, USA).

2.3. Transient transfection and promoter analyses

The cells were plated at about 8×10^4 cells per 35 mm dish and cultured for 24 h before transfection. After incubation, the cells were transfected with 200 ng of β-galactosidase expression vector pSV110 and 200 ng of reporter gene, along with HSF1, ASC-2, or CBP expression vector using SuperFect or PolyFect as instructed by the manufacturer (Qiagen). Total amounts of expression vectors were kept constant by adding decreasing amounts of the pcDNA3 to transfections. 2–3 h after transfection, the cells were washed and refed with Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cells were harvested 44–48 h later, and luciferase activity was assayed, and the results were normalized to the β-galactosidase expression. All the results represent the mean of three independent experiments. The basal activity of the luciferase reporter in the presence of empty expression vector was normalized to 1.0, and the activities of the remaining transfection reaction were expressed relative to this, as fold activation of the basal promoter.

2.4. Glutathione S-transferase (GST) pull-down assay

The GST-HSF1 fusion constructs or GST alone were expressed in *Escherichia coli*, bound to glutathione-Sepharose-4B beads (Amersham Pharmacia Biotech), and incubated with labeled proteins expressed by in vitro translation by using the TNT-coupled transcription–translation system, under conditions as described by the manufacturer (Promega) in GST pull-down reaction buffer (25 mM HEPES, pH 7.6, 20% glycerol, 120 mM NaCl, 0.2 mM ethylenediamine tetraacetic acid (EDTA), 1 mM dithiothreitol (DTT), 0.1% Triton X-100, 0.1% bovine serum albumin) for 8 h at 4°C. After washing three times with PBST, the bound proteins were eluted with 10 mM reduced glutathione in 50 mM Tris–Cl, pH 8.0, 120 mM NaCl and boiled with an equal volume of 2× LSB at 100°C for 3 min prior to electrophoresis. After electrophoresis, the gel was dried and analyzed with the Molecular Imager Fx (Bio-Rad).

2.5. Coimmunoprecipitation assay between ASC-2 and HSF1

Nuclear extracts (500 μg) were incubated with 20 μg of anti-HSF1 IgG at 4°C for 8 h with gentle agitation. Immune complexes were washed three times with lysis buffer, the precipitates were boiled with an equal volume of 2× LSB at 100°C for 3 min and analyzed by 4 and 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

2.6. Gel electrophoresis and Western blotting

Cells were washed in ice-cold phosphate-buffered saline (PBS) and lysed in ice-cold lysis buffer (150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 50 mM Tris–Cl, pH 7.5, 10% glycerol) containing 1 mM DTT, 1% protease inhibitor cocktail (Sigma), and 1 mM phenylmethylsulfonyl fluoride (PMSF), 20 mM NaF and phosphatase inhibitor, 5 mM Na₃VO₃ at 4°C for 15 min. The protein concentration of cell lysates was determined with Bradford reagent (Bio-Rad) using bovine serum albumin (BSA) as a standard. After heating at 100°C for 3 min, equal amounts of proteins was separated by SDS–PAGE. The resulting gels were either stained with Coomassie blue or transferred

to polyvinylidene difluoride (PVDF) (Immobilon-P) membrane (Schleicher and Schuell). For Western blotting, the membrane was blocked with 1% skim milk in TBS-T (0.05% Tween-20 in TBS) for 1 h at room temperature on a shaker. After washing the membrane with TBS-T, it was probed with antibodies to ASC-2 and TBP antibody (Santa Cruz Biotechnology) for 1 h at room temperature. After washing the membrane with TBS-T three times, it was incubated with 1:2500 diluted horseradish peroxidase (HRP)-conjugated secondary antibody and the proteins were visualized by the enhanced chemiluminescence detection system according to the recommended procedure (Amersham Pharmacia Biotech).

2.7. Mammalian two-hybrid assay

HepG2 cells were seeded with growth medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics, and cotransfected with expression vectors encoding Gal4-DBD fusions (pCMX/Gal4N-, pCMX/Gal4N-HSF1) and VP16 activation domain fusions (pCMX/VP16-, pCMX/VP16-ASC-2) as well as the previously described Gal4-tk-luc reporter plasmid. After 48 h, cells were harvested and the luciferase activity was normalized to the β-galactosidase expression. All the results represent the average of at least three independent experiments.

2.8. Chromatin immunoprecipitation analysis

Cells were lysed for 5 min in L1 buffer (50 mM Tris pH 8.0, 2 mM EDTA, 0.1% NP-40 and 10% glycerol) supplemented with protease inhibitors. Nuclei were pelleted at 3000 rpm and resuspended in L2 buffer (50 mM Tris pH 8.0, 0.1% SDS and 5 mM EDTA). Chromatin was sheared by sonication, centrifuged and diluted 10 times in dilution buffer (50 mM Tris pH 8.0, 0.5% NP-40, 0.2 M NaCl and 0.5 mM EDTA). Extracts were pre-cleared for 3 h with 60 μl of a 50% suspension of salmon sperm-saturated protein A agarose. Immunoprecipitations were carried out overnight at 4°C. Immunocomplexes were collected with salmon sperm-saturated protein A for 30 min and washed three times (5 min each) with high-salt buffer (20 mM Tris pH 8.0, 0.1% SDS, 1% NP-40, 2 mM EDTA and 0.5 M NaCl) followed by three washes in no salt buffer (1× TE). Immunocomplexes were extracted in 1× TE containing 2% SDS, and protein–DNA cross-links were reverted by heating at 65°C overnight. After proteinase K digestion, DNA was extracted with phenol–chloroform and precipitated in ethanol. About one-twentieth of the immunoprecipitated DNA was used in each polymerase chain reaction (PCR). The PCR conditions were as follows: 1.25 U of *Taq* DNA polymerase (Amersham Biosciences), 100 ng of each primer, 200 μM deoxyribonucleoside triphosphate (dNTP), 2.5 μl of 10× *Taq* buffer and double-distilled water to a final volume of 25 μl: 94°C for 180 s; 34 cycles at 94°C for 45 s, 60°C for 60 s and 72°C for 60 s; final elongation at 72°C for 10 min.

3. Results

3.1. Interaction between HSF1 and coactivators, ASC-2 and CBP

As transcription factors require coactivators for their efficient transcriptional activity, we tried to identify the coactivator(s) that interacts with and modulates the activities of HSF1. From our preliminary experiments, ASC-2 coactivator was phosphorylated by heat shock treatment (data not shown). This finding led us to examine a possibility that a certain coactivator including ASC-2 be involved in HSF1-involved transcriptional regulation mediated by heat shock. To find out coactivator(s) that interacts with HSF1, we performed GST pull-down assay in vitro. GST alone and GST fusion to HSF1 were expressed in *E. coli*, purified, and incubated with coactivators including CBP, SRC-1, ASC-1 and ASC-2, which were transcribed and translated in vitro using rabbit reticulocyte lysates. In vitro GST pull-down assay revealed that GST-HSF1 fusion protein, but not GST alone, strongly interacts with coactivator ASC-2 and moderately with CBP (Fig. 1). In comparison to the input (first lane in

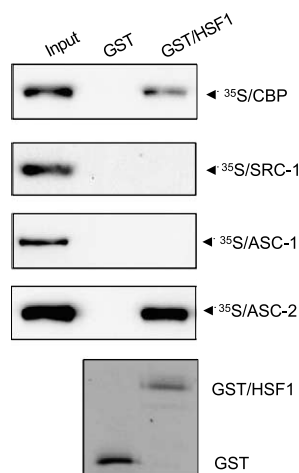


Fig. 1. Interaction between HSF1 and coactivators, CBP and ASC-2. Equal amounts (1 μ g) of glutathione agarose-immobilized GST and GST-HSF1 proteins were incubated with 35 S-radiolabeled full-length CBP, SRC-1, ASC-1 or ASC-2. After being washed three times, the bound proteins were eluted with reduced glutathione, resolved by SDS-PAGE, and analyzed with autoradiography. The input lanes represent \sim 25% of the total protein.

Fig. 1), ASC-2 and CBP were retained by the hybrid GST-HSF1 protein. In contrast, ASC-1 and SRC-1 did not associate with HSF1 under experimental condition employed in this study (Fig. 1).

3.2. Effects of coactivator overexpression on HSF1-mediated transactivation

To study the involvement of the coactivators, ASC-2 and CBP, on transactivation of gene expression by HSF1, it was employed transfection experiments employing the Gal4 full-length (amino acid residues 1–529), Gal4 N-terminal DBD (1–399 amino acids), or Gal4 C-terminal TAD (395–529 amino acids) of HSF1. Briefly, this system employs an expression plasmid encoding each domain and full length of HSF1 fused to the DBD of Gal4 and a Gal4 responsive reporter plasmid, Gal-luciferase. The advantage of this assay is that Gal4-HSF1/TAD^(395–529) is exclusively nuclear and is regulated independently with phosphorylation on DBD of HSF1, allowing the effects of genes of interest on transactivation by HSF1 to be assessed. The TAD alone of HSF1 showed a full transactivation of Gal4 element-linked luciferase reporter, whereas the DBD and full length of HSF1 did not activate transcription relative to that of the TAD (data not shown). For subsequent transfection experiments, we used the Gal4 fused to HSF1-TAD construct as an activator. HepG2 cells were transfected with mammalian expression plasmids encoding Gal4N-HSF1 and coactivators, ASC-2 and CBP, along with Gal4 binding element-conjugated luciferase reporter. As shown in Fig. 2A, cotransfection of ASC-2 with HSF1-TAD showed an increased transcriptional activity synergistically but CBP overexpression did not enhance HSF1-dependent transactivation.

We confirmed the enhanced HSF1-mediated transactivation by ASC-2 through a transient transfection experiment using full-length HSF1 expression plasmid and HRE-Luc reporter gene with the plasmids encoding ASC-2 and CBP. ASC-2 enhanced the HSF1-mediated activation of transcription with a two-fold difference, but CBP alone did not show the

synergistic effect on the transactivation of HSF1 (Fig. 2B). Taken together the results of Figs. 1 and 2, it is suggested that ASC-2 directly associates with HSF1 and activates HSF1-driven transcription as a coactivator.

3.3. Delineation of HSF1-interacting region of ASC-2

Since ASC-2 coactivator cooperatively increased the HSF1-mediated transactivation (Fig. 2), we focused the physical interaction of HSF1 and ASC-2 protein for the synergistic transactivation. To identify the interaction region of two proteins, GST pull-down experiment was employed using in vitro translated ASC-2 constructs and glutathione resin-conjugated GST-HSF1 proteins. The full-length ASC-2 protein (amino acids 1–2063) specifically interacted with HSF1 protein (Fig. 3A). Subsequently, it was carried out to further identify the HSF1-interacting region of ASC-2 through the same experiments using ASC-2-deleted mutants. As shown in Fig. 3, HSF1 protein interacted with the C-terminal domain (co4.5) of ASC-2 specifically and also weakly with the N-terminal one (co1). The co4.5 domain of ASC-2 was also identified with an interaction region for TBP, TFIIA, CBP, and SRC-1 [28,33]. The reciprocal strategy was used to delineate the region of

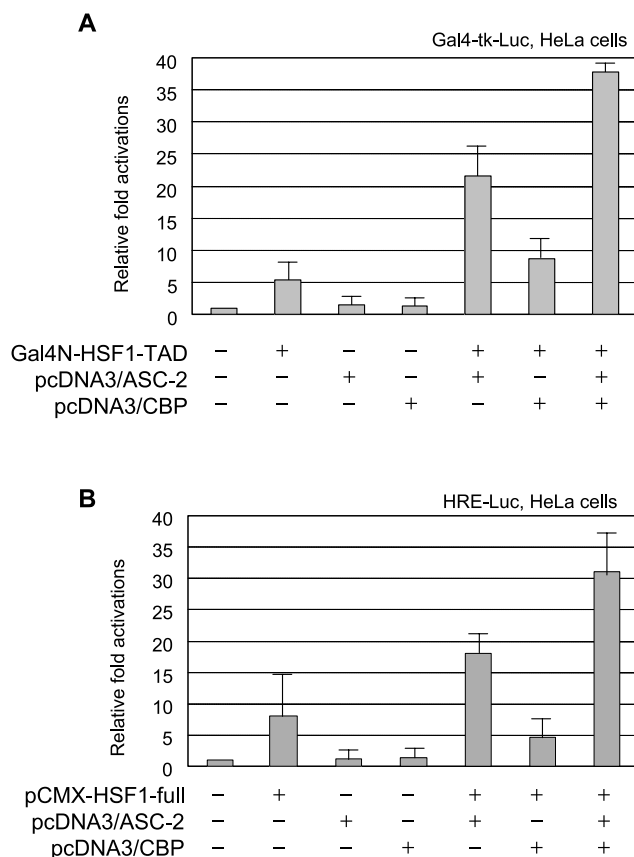


Fig. 2. ASC-2 synergistically increases HSF1-mediated transactivation. A: Coactivator ASC-2 effectively increases HSF1-mediated transactivation. HepG2 cells were cotransfected with plasmids encoding Gal4N-HSF1/TAD, CBP or ASC-2, along with a Gal4-tk based reporter construct. After 48 h post-transfection, cells were harvested for measurement of luciferase activity. B: HepG2 cells were cotransfected with plasmids encoding full-length HSF1, CBP or ASC-2, along with HRE reporter construct. Luciferase activity was determined in cell lysates 48 h later, and the values (\pm S.E.M.) from at least two independent experiments performed in triplicate are shown in the form of a bar graph.

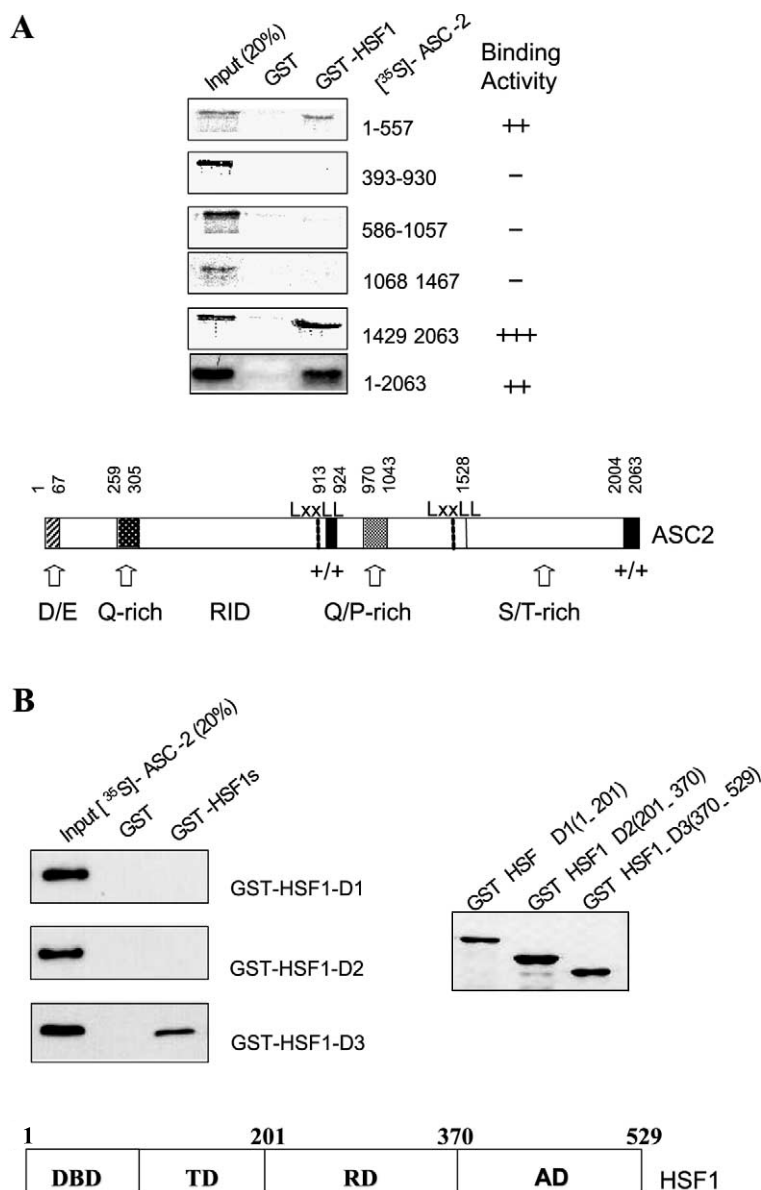
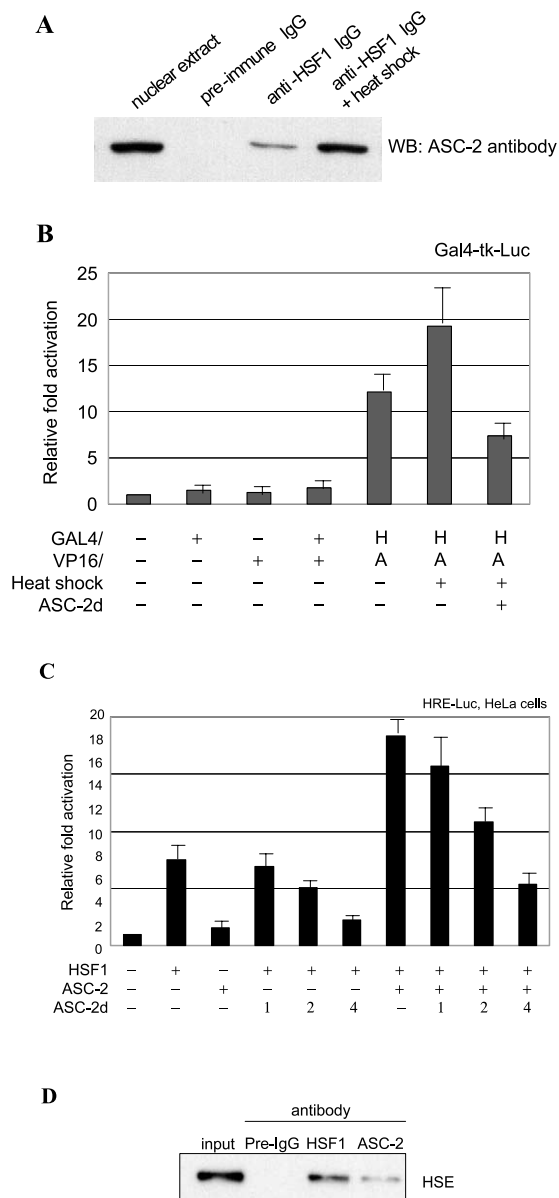


Fig. 3. Delineation of each interacting region of HSF1 and ASC-2 proteins. A: Delineation of HSF1-interacting region of ASC-2. GST-HSF1 protein was purified from *E. coli* and conjugated with glutathione resin. ^{35}S -labeled in vitro translated ASC-2-deleted mutants were produced using rabbit reticulocyte lysates. After being washed three times, the bound proteins were eluted with reduced glutathione, resolved by SDS-PAGE, and analyzed with autoradiography. The input lanes represent 20% of the total protein. The diagram at bottom part shows the schematic representation of coactivator ASC-2, in which the N-terminal acidic (D/E), glutamine-rich (Q-rich), receptor-interacting (RID), glutamine/proline-rich (Q/P-rich) and serine/threonine-rich (S/T-rich) sequences are indicated with arrows. B: Delineation of ASC-2-interacting region of HSF1. GST-HSF1-deleted proteins (designated with D1, D2, and D3) were purified from *E. coli* and conjugated with glutathione resin. ^{35}S -labeled in vitro translated ASC-2 protein was produced using rabbit reticulocyte lysates. After being washed three times, the bound proteins were eluted with reduced glutathione, resolved by SDS-PAGE, and analyzed with autoradiography. The input lanes represent 20% of the total protein. The diagram at bottom part shows the schematic representation of HSF1, in which the DBD, trimerization domain (TD), regulatory domain (RD), and activation domain (AD) are indicated.

HSF1 required for interaction with ASC-2. After preparing GST-HSF1-deleted proteins (HSF1-D1, 1–201; HSF1-D2, 201–370; HSF1-D3, 370–529) from *E. coli*, the proteins were immobilized on glutathione-Sepharose beads and incubated with in vitro translated ^{35}S -labeled full-length ASC-2. The labeled ASC-2 strongly associated with the C-terminal activation domain of HSF1, but not with the N-terminal DBD and central regulatory domain (Fig. 3B). These results indicate that the C-terminal region of ASC-2 specifically interacts with the activation domain of HSF1.

3.4. Heat shock treatment increases the protein interaction of HSF1 and ASC-2 in vivo

The association between HSF1 and ASC-2 was further characterized by coimmunoprecipitation analysis. HSF1 was immunoprecipitated from freshly prepared HepG2 nuclear extracts, gel-fractionated, and analyzed for ASC-2 coprecipitation by Western blotting with anti-ASC-2 antibody. As shown in Fig. 4A, endogenous ASC-2 was detected as a coprecipitant in HSF1 immunoprecipitates. A parallel immunoprecipitate formed with preimmune serum failed to show ASC-2 immu-



noreactivity (Fig. 4A, second lane). There is a possibility that HSF1 efficiently associates with ASC-2 dependent on extracellular and endogenous signals. Since heat shock stress is a representative extracellular signal for HSF1-mediated gene activation, we examined the protein association of HSF1 and ASC-2 using *in vivo* protein interaction assays after treating heat shock. The heat shock treatment potentially increased the association of HSF1 and ASC-2 in cells (Fig. 4A, fourth lane).

We confirmed the *in vivo* protein interaction of HSF1 and ASC-2 in cells via mammalian two-hybrid assay using GAL4-HSF1 and VP16-ASC-2 expression plasmids in the presence or absence of heat shock treatment. As a result of direct interaction *in vivo*, heat shock treatment increased efficiently the molecular interaction of GAL4-HSF1 and VP16-ASC-2 approximately 19-fold (Fig. 4B). The transient overexpression of HSF1-interacting region (ASC-2d, amino acids 1429–2063) of ASC-2 inhibited the increased protein interaction of HSF1 and ASC-2 by heat shock. These results are consistent with the interpretation that HSF1 is capable of associating with ASC-2 *in vitro* and *in vivo* and that heat shock enhances

Fig. 4. Heat shock treatment increases the protein interaction of HSF1 and ASC-2 *in vivo*. **A**: Coimmunoprecipitation of endogenous HSF1 and ASC-2. HepG2 cells were exposed to heat shock of 43°C for 30 min or not. Nuclear extracts (500 µg) were incubated with 20 µg of anti-HSF1 IgG or preimmune IgG and immune complex was subjected to electrophoresis and Western blot analysis with anti-ASC-2 antibody. **B**: The mammalian expression plasmids encoding GAL-HSF1 and VP16-ASC-2 or the empty vector of GAL and VP16 were transfected into HepG2 cells with or without the transient expression of HSF1-interacting ASC-2 construct (ASC-2d). 48 h after transfection in the absence or presence of heat shock, cells were harvested for luciferase activities. All the transfection results were normalized to β-galactosidase activity, and the presented results represented the average of three independent experiments, with fold induction over the level observed with the reporter alone. H, HSF1; A, ASC-2. **C**: The HSF1-interacting region of ASC-2 plays a role as a dominant negative protein in HSF1-mediated transactivation. HepG2 cells were transfected with expression plasmids (each 1 µg) HSF1, ASC-2, and/or increasing amounts of ASC-2d (amino acids 1429–2063) (1, 2, and 4 µg) as indicated and the HSE promoter-luciferase reporter, as indicated. 48 h after transfection, cells were harvested and luciferase activity was determined. **D**: ChIP analysis of factor occupancy on HSE promoters. Following formaldehyde cross-linking, soluble chromatin was prepared. After IP with antibodies against the indicated proteins (HSF1 and ASC-2), precipitated DNAs were used in PCR analysis. Input shows the starting chromatin extracts.

the protein interaction between HSF1 and ASC-2 in cells. In addition, the HSF1-interacting region (ASC-2d) of ASC-2 acted as a dominant negative mutant in HSF1-mediated transactivation in the presence or absence of ectopic ASC-2 overexpression, as shown in Fig. 4C. This evidence provides a more convincing result that explains the ASC-2 enhancement of HSF1-mediated transactivation by the protein–protein interaction in cells.

In order to determine whether ASC-2 is directly recruited to HSF1-dependent promoter in the heat shock-treated cells, ChIP analysis was performed. After heat shock treatment, cells were lysed and solubilized chromatin was immunoprecipitated, initially with antibodies against HSF1 or ASC-2, and recovered DNAs were amplified by PCR using promoter-specific primers. It is clear from the data in Fig. 4D that ASC-2 recruitment to the HSF1-recognized promoter was confirmed in cells. Collectively, these findings support the notion that heat shock controls the recruitment of essential components of the transcriptional activation machinery and consequently the efficiency of HSF1-dependent transcriptional activation.

4. Discussion

Most transcriptional factors require coactivators for their full activities. In this study, we investigated the coactivators that interact with and modulate the transcriptional activity of HSF1. *In vitro* GST pull-down assay and coimmunoprecipitation revealed an intact interaction between HSF1 and coactivators, CBP and ASC-2 (Figs. 1 and 4A). In addition, co-transfection of ASC-2, but not CBP, with HSF1 showed an increased transcriptional activity dependently of HSF1 (Fig. 2), indicating that ASC-2 acts as a coactivator of HSF1. ASC-2 has been demonstrated to control other transcriptional factors such as mitogenic transcription factors, including AP-1, NF-κB, and SRF as well as the nuclear hormone receptor superfamily [33]. This is the first report that ASC-2 may enhance HSF1-driven transcriptional activity.

In unstressed cells, HSF1 is constitutively phosphorylated on serine residues 303, 307, and 363 in the regulatory domain by glycogen synthase kinase-3 β , ERK, c-Jun N-terminal kinase/protein kinase C, respectively [15–21], which repress the transcriptional activity of HSF1. HSF1 can be hyperphosphorylated in response to proteotoxic stresses such as heat shock and heavy metals. Hyperphosphorylation of HSF1 involves in the acquisition of transactivation function and in the activation of the factor without affecting HSF1 trimerization and DNA binding activity [22–24]. Recently, CaM kinase II has been demonstrated to be responsible for the HSF1 hyperphosphorylation and activation [25]. In addition, the HSP expression in response to stresses such as heavy metals and hypoxia has been suggested to be regulated by p38 kinase that is one of mitogen-activated protein kinase family proteins, and has been implicated in a variety of cellular functions such as cell proliferation, differentiation, and apoptosis, although its precise role in the stress response has been not well elucidated. In contrast, the roles of p38 kinase in HSF1 activation have been not well investigated. Since profile of p38 β kinase activation in response to heat shock profile is very similar to that of HSF1 hyperphosphorylation (data not shown) and both HSF1 and ASC-2 proteins possess the tentative phosphorylation sites for p38 kinase, we speculated that p38 β may be linked to HSF1 transcriptional activation.

For active transcription process upon stress signaling, chromatin modification by several coactivators including CBP/p300, P/CAF, and SRC-1 may be necessary [36,37]. Although ASC-2 has a homology with the activation domain of CBP/p300, it lacks an inherent HAT activity. As CBP/p300 was found to interact with ASC-2, the recruitment of ASC-2 into HSF1 transcription complex may drive active chromatin structure for transcription by HAT proteins, such as CBP/p300. Finally, it is interesting to note that the HSP promoter may be a typical enhancosome, comprised of a series of *cis* elements, including binding sites for HSF family proteins and several different classes of transcription factors. Our finding that ASC-2 interacts with HSF1 protein may shed some light into how the HSP enhancosome is regulated upon stresses, since ASC-2 functionally associates with other classes of transcription factors including AP-1, SRF, NF- κ B, and nuclear receptors [33]. ASC-2 can stabilize the assembly of HSP enhancosome through association with HSF1 and other transcription factors. In addition, ASC-2 may enhance the function of HSP enhancosome by juxtaposing components of the transcriptional machinery in a more favorable orientation, and may also play a role in recruiting transcriptional coactivators. Perhaps one mechanism by which p38 kinase increases the transactivation potential of ASC-2 is to promote the interaction of ASC-2 with these members of the transcriptional basal machinery. It should be interesting in the future to test directly these possibilities.

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