# Heat shock transcription factors regulate heat induced cell death in a rat histiocytoma

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Heat shock response is associated with the synthesis of heat shock proteins (Hsps) which is strictly regulated by different members of heat shock transcription factors (HSFs). We previously reported that a rat histiocytoma, BC-8 failed to synthesize Hsps when subjected to typical heat shock conditions (42°C, 60 min). The lack of Hsp synthesis in these cells was due to a failure in HSF1 DNA binding activity. In the present study we report that BC-8 tumor cells when subjected to heat shock at higher temperature (43°C, 60 min) or incubation for longer time at 42°C, exhibited necrosis characteristics; however, under mild heat shock (42°C, 30 min) conditions cells showed activation of autophagy. Mild heat shock treatment induced proteolysis of HSF1, and under similar conditions we observed an increase in HSF2 expression followed by its enhanced DNA binding activity. Inhibiting HSF1 proteolysis by reversible proteasome inhibition failed to inhibit heat shock induced autophagy. Compromising HSF2 expression but not HSF1 resulted in the inhibition of autophagy, suggesting HSF2 dependent activation of autophagy. We are reporting for the first time that HSF2 is heat inducible and functions in heat shock induced autophagic cell death in BC-8 tumor cells.

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## 1. Introduction

Stress response or heat shock response is a universal phenomenon and heat shock proteins (Hsps) form the most ancient defense system in all living systems at cellular level (Feder and Hofmann 1999; Sreedhar and Csermely 2004; Katschinski 2004). Hsp synthesis is tightly regulated by heat shock transcription factors (HSFs) at transcriptional level (Morimoto 1998). The cytoprotective effects of Hsps were attributed primarily to their chaperone activities, which minimize the proteotoxicity induced by the accumulation of unfolded or denatured proteins upon stress (Katschinski 2004). HSFs alone can function in the maintenance of cellular homeostasis that include regulation of cell cycle, cell proliferation, redox homeostasis, cell death mechanisms etc. (Pirkkala *et al* 2001; Katschinski 2004; Sreedhar and Csermely 2004; Sreedhar *et al* 2006).

Between the two major heat shock factors, HSF1 regulates the induced transcription of heat shock genes in response to stress, whereas HSF2 play a major role during differentiation and development (Morimoto 1998). HSFs are highly conserved and upon activation bind to the specific DNA elements called heat shock elements (HSEs) consisting of tandem inverted repeats of the short consensus sequence, 5′-*nGAAn*-3′ present upstream in the heat shock gene promoters (Wu 1995). HSF1 is constitutively expressed and localizes

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Abbreviations used: [($Ca^{2+}$)i], intracellular calcium; DioC6(3), 3′,3′-dihexyloxacarbocyanine iodide; Hsc70, heat shock cognate protein 70; Hsp, heat shock protein; Hsp70, heat shock protein 70; H2-DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; HSE, heat shock element; HSF, heat shock transcription factor; lamp2a, lysosomal-associated membrane protein; MDC, monodansylcadaverine; Rhod-2, 1-[2-Amino-5- (3-dimethylamino-6-dimethylammonio-9-xanthenyl) phenoxy]-2-(2-amino-5-methylphenoxy)ethane-N,N,N′,N′- tetracetic acid chloride; ROS, reactive oxygen species.

to the cytoplasm as a non-DNA binding inactive monomer under normal physiological conditions. Upon stress, the HSF1 trimerizes, hyperphopshorylates, translocates to the nucleus and binds to HSEs, and thereby activates the heat shock gene transcription. HSF2 possesses a constitutive DNA binding activity, which, however, is abolished by heat stress (Sarge *et al* 1991). Thus HSF2 is refractory to heat shock and cannot be substituted for HSF1. Hsp70, the major cytoprotective protein can be synthesized by both HSF1 and HSF2; however, these two factors have differential specificity for the synthesis of Hsps (Mathew *et al* 2001).

Though heat shock response is a universal phenomenon, we previously reported that BC-8, a rat macrophage cell line, failed to synthesize Hsps when exposed to typical heat shock condition (42°C for 60 min), and in the absence of Hsp synthesis these cells undergo CD95 mediated apoptosis (Sreedhar *et al* 1999, 2000). Exogenous expression of inducible Hsp70 only transiently protected BC-8 cells from heat-induced apoptosis (Sreedhar *et al* 2000), suggesting that an *in vivo* stress response is required for thermotolerance. Since regulated expression and DNA-binding activity of HSF1 or HSF2 may decide the fate of cells during stress, we studied the effect of heat shock on these molecules and on cell survival and cell death. In the present study we show that mild heat shock induced HSF2 and its DNA binding activity, which correlated with the activation of heat shock induced chaperone-mediated autophagy. We discuss the possible role of HSF2 in cell survival in the absence of functional HSF1 in BC-8 cancer cells.

## 2. Materials and methods

### 2.1 *Cell culture and heat shock conditions*

Rat histiocytic tumor cells, BC-8 (a single clone of AK5 tumor) was grown in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (50 $\mu$g/ml). Cells (1 x $10^6$/ml) were given heat shock at 42, 42.5 or 43°C in a water bath (Julabo) by immersing the culture flask in the water for 30 or 60 min followed by recovery at 37°C.

### 2.2 *Propidium iodide staining for microscopy and FACS analysis*

Control and heat shocked BC-8 cells were collected after 12 h of recovery at 37°C, washed with PBS, stained with 50 $\mu$g/ml propidium iodide (PI, containing 0.1% triton X-100 and 22 $\mu$g/ml RNase in 0.1% sodium citrate). Cells were either visualized under a fluorescence microscope (Nikon) or analyzed by fluorescence activated cell sorting (FACS-Calibur, BD).

### 2.3 *Comet assay*

Control and heat shocked BC-8 cells were washed with phosphate buffered saline, pH 7.6 (PBS) and subjected to comet assay analysis as described earlier (Sreedhar *et al* 1999). Comet analysis was done under neutral (pH 7.6) as well as alkaline (pH 12.0) conditions, stained with ethidium bromide (5 ng/ml) (Sreedhar *et al* 1999), and appearance of comets was visualized using a fluorescence microscope (ApoTome, Carl Zeiss).

### 2.4 *Analysis of autophagic vacuoles by microscopy*

BC-8 cells after heat shock and 12 h of recovery at 37°C were washed with serum free DMEM and incubated with 20 nM/ml monodansylcadaverine (MDC, Molecular Probes) for 15 min at 37°C with 5% constant $CO_2$ supply. Cells were washed twice with PBS and immediately analyzed using ApoTome (Carl Zeiss).

### 2.5 *Western blot analysis*

Twenty micrograms of total cell lysate from BC-8 control and heat shocked cells was collected in HEPES lysis buffer (pH 7.6) and run on 10% SDS-PAGE, transferred onto nitro-cellulose membrane (Amersham Biosciences). The membrane was first blocked with 3% BSA, 0.1% Tween-20 followed by incubation with appropriate antibodies at room temperature. The antigen-antibody complexes were visualized using a BM-chemiluminescence kit (Roche, Switzerland). Antibodies for HSF1, HSF2, and cytochrome c were obtained from Santa Cruz Biosciences (Santa Cruz, USA). Antibodies for Hsp90, gp96, Hsc70, Hsp70, caspases, and ERK1/2 were obtained from StressGen (USA). Anti glyceraldehyde-3-phosphate dehydrogenase antibodies were purchased from Abcam (USA) while anti-tubulin, anti-actin antibodies and proteasome inhibitor, MG-132, were purchased from Sigma (USA).

### 2.6 *Electrophoretic gel mobility shift assay*

Total cell extract (25 $\mu$g) from control and heat shocked BC-8 cells was incubated with γ-p32 dATP labeled heat shock elements ("*n-GAA-n*" repeats) to examine the HSF-HSE interaction as a mobility shift of the labelled oligo as described earlier (Swamynathan *et al* 1996).

### 2.7 *RNA isolation and RT-PCR*

Total RNA from control and heat shocked BC-8 cells was isolated using trizol. 5 micro grams of total RNA was reverse

transcribed using AMV RT-PCR kit from Promega (USA). HSF2 transcript specific primers used for polymerase chain reaction were: forward primer, 5′-ATT CAT ATG CTA AAC ACA AAT GGA GCC C-3′, and reverse primer, 5′- ATT GAA TTC CTT TCC TAA CAG GTT AAT GTT-3′ (AC No. X61754). The autophagy specific gene lamp2a transcript were amplified using forward primer 5′- GTC TCA AGC GCC ATC ATA CT-3′ and reverse primer 5′-AAG GAC TCC TAT AGT GGG TGA CGA-3′. For normalization of PCR reactions, actin transcript specific forward primer 5′-ATC TGG CAC CAC ACC TTC TAC AAT GAG CTG CG-3′ and reverse primer 5′-CGT CAT CCT GCT TGC TGA TCC ACA TCT GC-3′ (Kiffin *et al* 2004) were used for the RT-PCR reactions.

### 2.8 *Immunofluorescence and confocal microscopy*

BC-8 cells, fixed in 4% formaldehyde for 10 min, washed with PBS and permeabilised by 0.5% Triton X-100 and 0.05% Tween-20 for 2 min, were incubated with either anti-HSF1 or anti-HSF2 antibody followed by secondary antibody conjugated with fluorescene isothiocyanate (FITC, Sigma); the HSF staining was visualized using a confocal imaging microscope (Carl Zeiss).

### 2.9 *Measurement of mitochondrial membrane potential*

Mitochondrial membrane potential ($\Delta\psi$ m) was determined by the retention of mitochondria specific dye DiOC6(3) (Molecular Probes). Cells were loaded with 20 nM of DiOC6(3) after heat shock and during recovery at 37°C. After removal of the medium, cells were washed twice with PBS, and the concentration of retained DiOC6(3) was measured using flow cytometer (FACS Calibur, BD, USA).

### 2.10 *Isolation of cytosol and mitochondria fractions*

BC-8 cells were harvested in PBS and the mitochondria and cytosolic fractions were separated as explained earlier (Sreedhar *et al* 2002). Twenty micrograms of total protein was separated on 10% SDS-PAGE, immunoblotted with anti-cytochrome c antibody as described earlier.

### 2.11 *Studies with proteasome inhibitor*

BC-8 cells (1 x $10^6$/ml) treated with reversible proteasome inhibitor MG-132 (200 nM/ml) before and during heat shock were analyzed for cell death by fluorescence activated cell sorting (FACS Calibur, BD, USA), and the cell lysates were immunoblotted for assaying levels of HSF1, HSF2 and Hsc70 proteins as above.

### 2.12 *Antisense oligonucleotides for HSF1 and HSF2*

Antisense nucleotides for the coding region of mouse *HSF1* (AC No. X61753), 5′- CGC TGC ACC GGG GCC CAC GGC CAG ATC CAT-3′, and for the coding region of mouse *HSF2* (AC No. X61754), 5′- GAA AGC CGG CAC GTT GGA ACT CTG CTT CAT-3′, were synthesized. Cells (1 x $10^6$/ml) were pre-incubated with 2 $\mu$g of antisense oligo for 6 h and then subjected to heat shock followed by further addition of 4 $\mu$g of the given oligo during recovery. Cell viability and induction of apoptosis was monitored after 12 h of recovery at 37 °C.

## 3. Results

### 3.1 *BC-8 cells exhibited differential heat sensitivity at different heat shock conditions*

BC-8 cells responded differently to heat shock in incubation time and temperature dependent manner. Exposure of BC-8 cells to typical heat shock condition (42°C for 60 min, a condition where most of the mammalian cells respond to stress) resulted in cell shrinkage, condensation of chromatin and membrane blebbing, followed by the formation of apoptotic bodies (figure 1A), and double strand DNA damage on neutral comet assay (figure 1B), characteristics of apoptotic cell death (Sreedhar *et al* 1999). Increasing the heat shock temperature to 43°C or longer incubation of cells at 42°C resulted in cell swelling (figure 1A) and DNA damage as observed by alkaline comet assay (figure 1B) confirming necrosis. A decrease in the heat shock incubation time by 30 min decreased accumulation of cells in sub-G1; however, in contrast to cells undergoing apoptosis or necrosis, these cells appeared morphologically normal with no detectable DNA damage (figure 1C, results not shown). Hence we have monitored the activation of autophagy under these heat shock conditions .The RT-PCR analysis of autophagy marker, *lamp2a*, showed a temperature dependent increase, which was not detected either in the control cells or at higher heat shock temperatures (figure 1D). Cell death at these conditions was further correlated with increased vacuolization and fusion of these vacuoles, which was detected by staining of cells with monodansylcadaverine, a dye that specifically stains acidic lysosomes or autophagosomes (figure 1E, F).

### 3.2 *Mild heat shock resulted in induced HSF2 expression and its DNA-binding activity*

We previously showed that BC-8 cells failed to synthesize inducible Hsps at sub-lethal heat shock condition (Sreedhar *et al* 1999), which was due to lack of HSF-HSE interaction

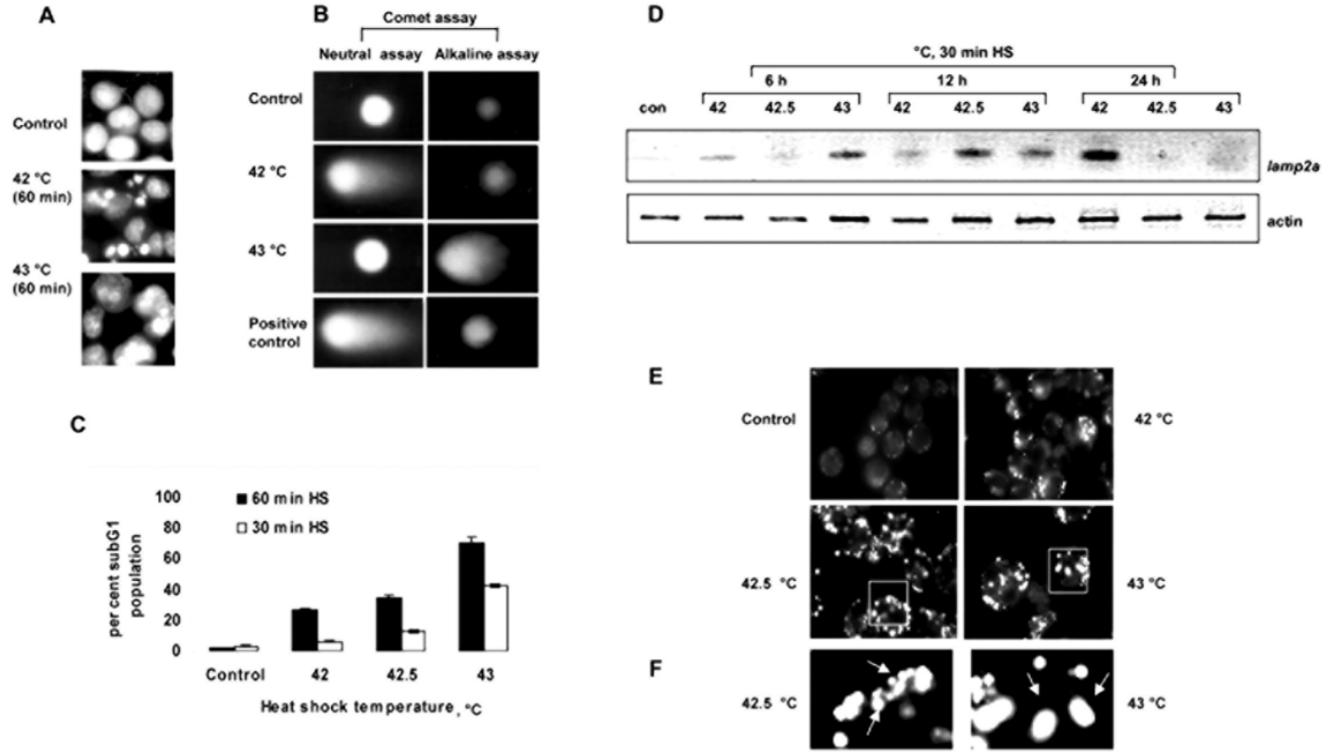


**Figure 1.** Heat shock induced activation of apoptosis and necrosis in BC-8 tumor cells. **(A)** appearance of apoptotic bodies and swelling of cells under sub-lethal and lethal heat shock temperatures respectively. Cells were fixed in methanol and stained with propidium iodide. **(B)** Neutral (for apoptosis) or alkaline (for necrosis) comet assays to assess the extent of DNA damage following sub-lethal or lethal heat shock. Anti AK-5 serum was used as a positive control for the induction of apoptosis. **(C)** FACS for sub-G1 populations of BC-8 cells after 12 h at 37°C recovery following exposure to 42°C either for 60 min or 30 min. The values represent are means of three independent experiments (± standard deviation). **(D)** RT-PCR analysis of *lamp2a* expression in heat shocked BC-8 cells. **(E)** Increase in acidic lysosomes and enhanced vacuolization following the induction of autophagy seen in monodansylcadaverine stained cells following different temperature exposures. **(F)** magnified images of boxed areas in **(E)** showing fusion of lysosomes.

(Sreedhar *et al* 2000, 2002). In agreement with our earlier observations, in the present study also, mild heat shock also failed to induce Hsp70, Hsp90 and gp96 (figure 2A6, A7 and A8, respectively), but we observed induction of the constitutively expressed protein, Hsc70 (figure 2A1). In our earlier study (Sreedhar *et al* 2000) we reported an increase in HSF1 mRNA under sub-lethal heat shock condition. In the present, we observed proteolysis of HSF1 upon mild heat shock treatment (figure 2A3) with a concomitant decrease in its mRNA expression (figure 2B1). In contrast, another heat shock factor, HSF2 was induced both at mRNA and at protein levels, however only under mild heat shock conditions (figure 2A4 and 2B3). The HSF1 and HSF2 expression in these cells was further confirmed by HSF2-immunofluorescence studies. HSF1 cellular localization (cytoplasmic/nuclear ratio) was decreased with an increase in heat shock temperature, whereas HSF2 levels were significantly increased both in the cytoplasm and nucleus (figure 2C). Subsequently the DNA-binding ability of HSF2 was monitored by modified EMSA analysis. EMSA data suggested that heat shock indeed induced HSF-HSE interaction which was observed as a shift in the gel (compare figure 2D1 with 2D5). However to confirm HSF2 specific DNA binding, protein samples (cell lysates) were pre- incubated with either HSF1 or HSF2 polyclonal antibodies and were subjected to EMSA analysis. Results show that cell lysates incubated with HSF1 antibody failed to inhibit HSF-HSE interaction, whereas lysates incubated with HSF2 antibody inhibited this interaction (compare figure 2D7 with 2D8).

### 3.3 *Heat shock induced a change in mitochondrial integrity but not the release of cytochrome c*

A change in the redox homeostasis alters the fate of cells. Earlier we have proposed a cross talk between cellular signaling and redox homeostasis in BC-8 cells upon sub-lethal heat shock (Sreedhar *et al* 2002). Therefore in the present study we measured the release of superoxide radicals in BC-8 cells using a fluorophore, H2-DCFDA

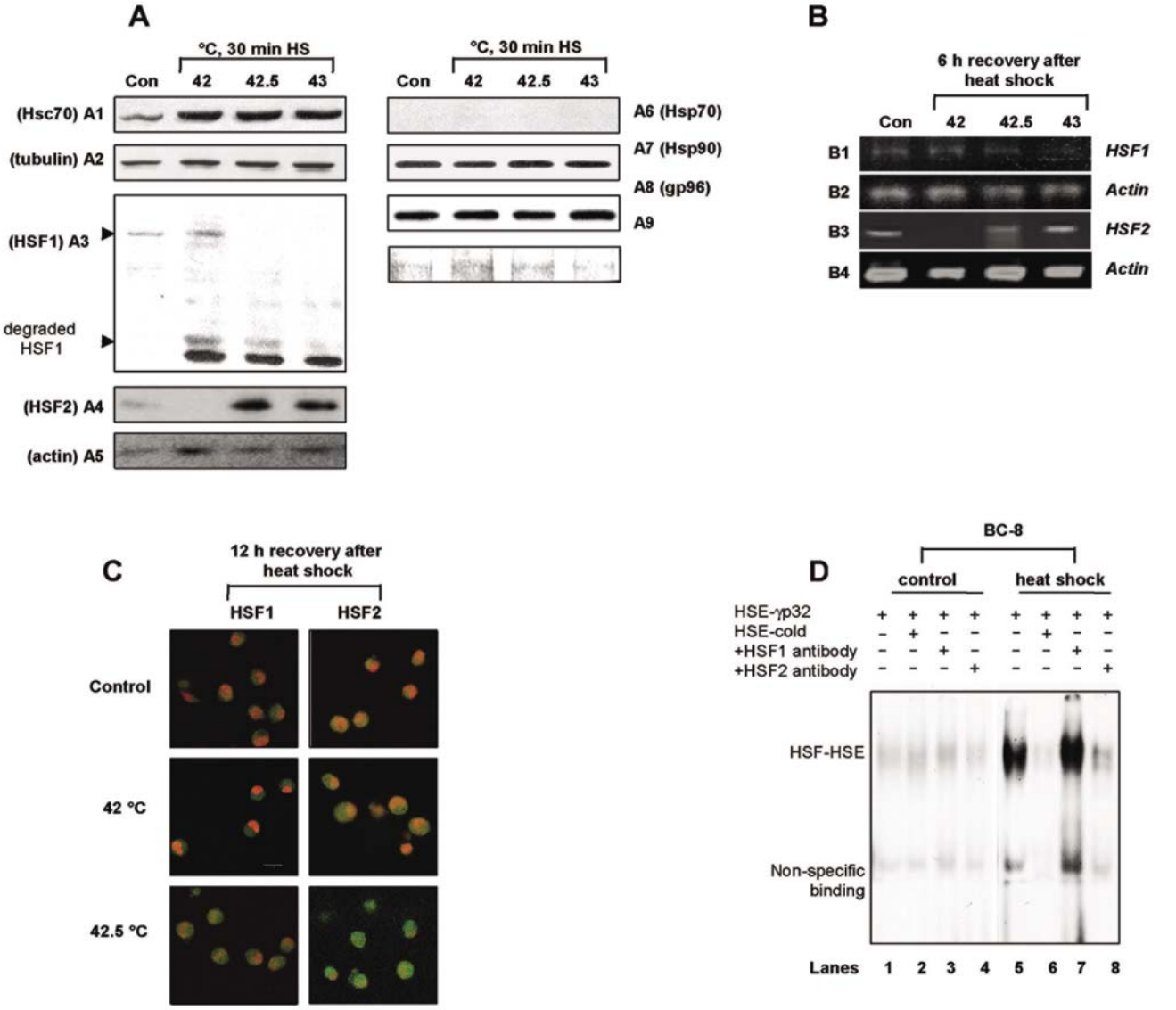


**Figure 2.** **(A)** Western analysis of stress response in BC-8 tumor cells upon mild heat shock with antibodies for Hsc70 (A1), control tubulin (A2), HSF1 (A3),. HSF2 (A4) and actin (A5) blot for loading control, Hsp70 (A6). Hsp90 and gp96 (A7 and A8, respectively) and Poncue-s stain for loading control (A9). **(B)** RT-PCR analysis of HSF1 (B1) and HSF2 HSF2 mRNAs;. Actin (B2 and B4) was used for normalization of amplification in each case. **(C)** immunofluorescence analysis of HSF1 and HSF2. Green color indicates HSF conjugated to FITC and the red color is nuclear staining with propidium iodide. The picture represented is a merging of these two. Please note an increase in HSF2 cytoplasm/nucleus localization compared to HSF1. **(D)** electrophoretic mobility shift assay for measuring HSF DNA-binding activity. 25 micrograms of total cell lysate was used for binding assay either with cold or γp32 labeled HSE (40000 cpm) consensus sequence and analyzed on 4% native PAGE. +HSF1Ab and +HSF2Ab represent cell lysates pre-incubated with HSF1 or HSF2 antibodies respectively. Cold HSE represents HSE not labeled with γp32 which is used for competition assay.

and measured the intracellular calcium [$(Ca^{2+})$i] using Rhod-2 fluorescence. There was no significant increase in either intracellular ROS levels or in the intracellular calcium [$(Ca^{2+})$i] levels in BC-8 cells under these heat shock conditions (data not presented). Further to understand the effect of heat shock on mitochondria in autophagic cell death, we monitored the changes in mitochondrial transmembrane potential ($\Delta\Psi$ m) using DioC6(3) fluorophore. Compared to control cells, heat shocked cells showed significantly altered membrane potential, which was once again, heat shock temperature dependent (figure 3A). It is believed that $\Delta\Psi$m dissipation compromises mitochondrial integrity and releases pro-apoptotic factors such as cytochrome c to the cytosol which subsequently activate cell death

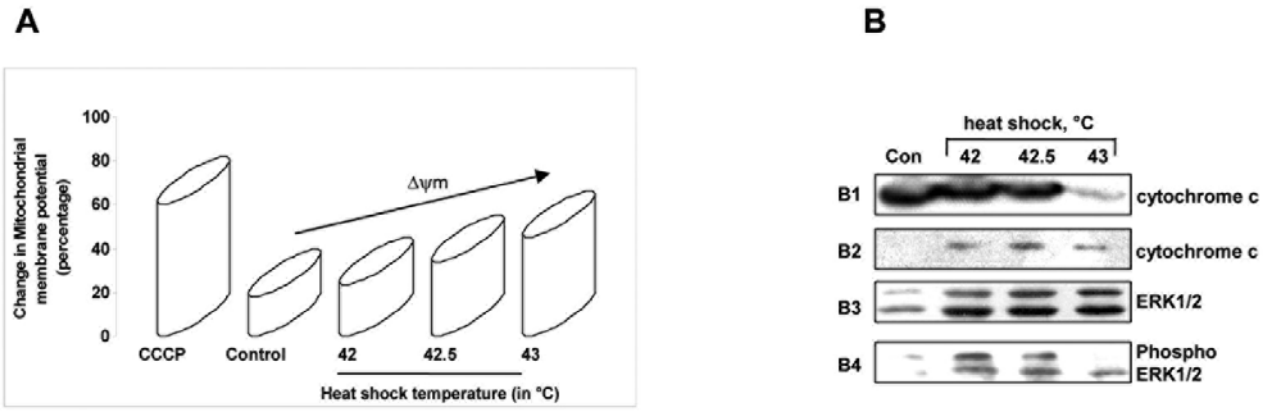


**Figure 3.** Effect of heat shock on mitochondrial transmembrane potential ($\Delta\psi$ m) and survival signaling. **(A)** BC-8 cells after heat shock was incubated with DiOC6(3) and fluorophore treated cells were subjected to FACS analysis . Note a gradual $\Delta\psi$m dissipation in BC-8 cells which is once again temperature dependent. CCCP was used as a positive control to induce $\Delta\psi$ m. **(B)** Analysis of cytochrome c release from mitochondria. The cytosol and mitochondria fractions were separated as explained in materials and methods and immunoblotted with anti cytochrome c antibody, showing no significant release of cytochrome c from mitochondria. BC-8 cell lysates after heat shock were immnoblotted with non-phosphorylated and phosphorylated ERK1/2 antibody showing activation of survival signaling.

pathways. Hence, we monitored cytochrome c release to asses whether $\Delta\Psi$m dissipation is activating the cell death. Our immunoblot analysis showed that cytochrome c was not released from mitochondria [compare figure 3B1 (mitochondrial fraction) with 3B2 (cytosolic fraction)]. The $\Delta\Psi$ m dissipation in these cells was assumed to be associated with macroautophagy, a selective process but not lethal to cells (Kiffin *et al* 2004; Gonzalez-Polo *et al* 2005). If autophagy is a survival mechanism upon heat shock, it may be expected to activate survival signalling. Hence we tested for the activation of mitogen kinases ERK1/2. Activation of mitogenic signalling molecules such as ERK1/2 (figure 3B3 and 3B4) further confirmed that mild heat shock in BC-8 cells is activating survival pathways, and autophagy thus seems to be a cytoprotective mechanism.

### 3.4 *HSF2 expression but not HSF1 is required for the activation of heat shock induced autophagy*

Since HSF1 was degraded upon heat stress (figure 2A3), we wanted to inhibit proteasome function and monitor whether inhibition of HSF1 proteolysis has any role in cell death mechanism. BC-8 cells were treated with reversible proteasome inhibitor, MG-132 before and after heat shock and HSF1 protein levels were monitored. Inhibition of the proteasome inhibited HSF1 proteolysis (figure 4A1); however, it did not inhibit activation of heat induced autophagy (figure 4A4). Further, proteasome inhibition increased basal levels of HSF2 expression, which was further enhanced by heat shock (figure 4A2). To understand the importance of HSFs in the activation of heat induced autophagy, siRNA for both HSF1 and HSF2 were made and BC-8 cells were treated with these oligos. HSF1-siRNA treatment compromised HSF1 expression in BC-8 cells (figure 4B1); however, compromising HSF1 in these cells did not inhibit heat shock induced autophagy or Hsc70 expression (figure 4B1 and 4B2). HSF2-siRNA compromised HSF2 expression, gradually decreased Hsc70 expression (figure 4C1, 4C2 and 4C3), and inhibited heat shock induced autophagy (figure 4C5). Subsequent studies using HSF2 compromised cells suggested that these cells are highly sensitive to multiple stresses (results not shown).

## 4. Discussion

We report a novel observation that heat shock transcription factor HSF2 is heat inducible while HSF1 is proteolytically degraded upon mild heat shock conditions in a rat histiocytoma cell line. We propose a cross talk between the major heat shock transcription factors, HSF1 and HSF2 such that in the absence of HSF1, HSF2 functions in cytoprotection.

Stress response plays a major role in acquired thermotolerance and resistance to heat induced cell death (Sreedhar and Csermely 2004; Sreedhar 2006). From our earlier studies using BC-8 cells and heat shock treatments we demonstrated that absence of inducible stress response sensitizes these cells to heat induced apoptosis by both extrinsic and intrinsic pathways (Sreedhar *et al* 1999*;* Sreedhar *et al* 2000, 2002). In contrast to sub-lethal heat shock which resulted in apoptosis, mild heat shock treatment

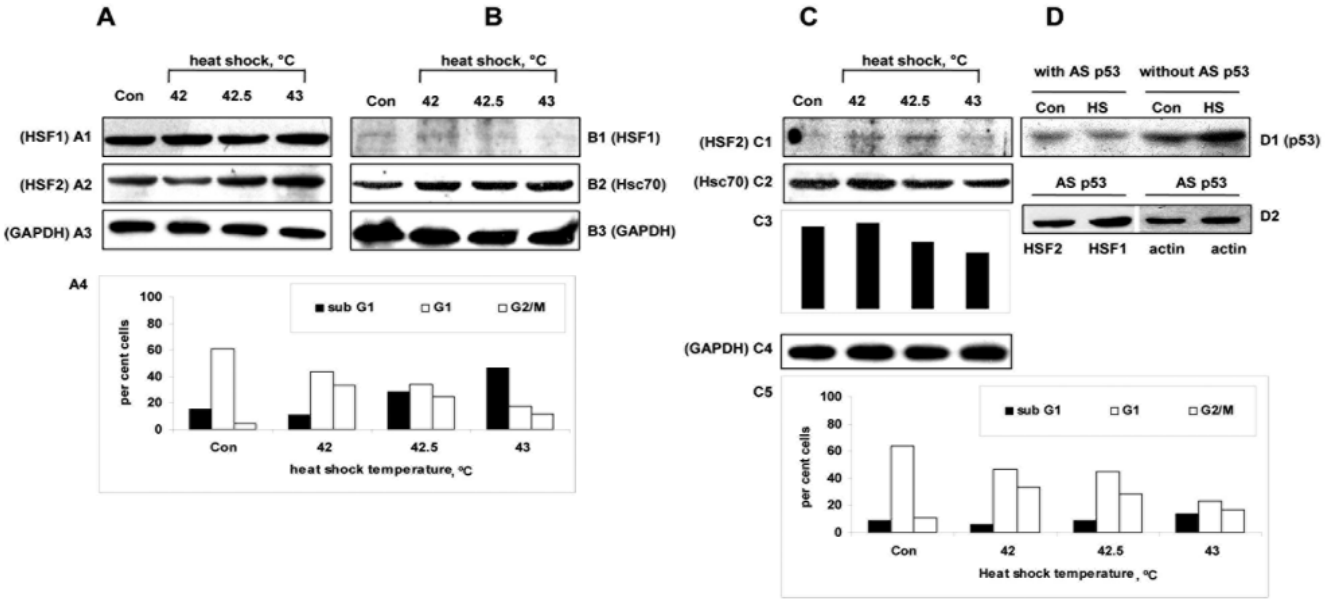


**Figure 4.** **(A)** Effect of proteasome inhibition on expression and stability of HSFs in BC-8 cells. Western blot analysis with anibodies for HSF1 (A1), HSF2 (A2), and GAPDH (A3). FACS analysis of MG-132 treated cells (A4). **(B)** Effect of HSF1 antisense oligonucleotides on Hsc70 induction. Western blot analysis with antibodies for HSF1 (B1), Hsc70 (B2), and GAPDH (B3). **(C)** Effect of HSF2 antisense oligonucleotides on Hsc70 induction and cell survival was determined by western blotting with antibodies for HSF2 (C1), Hsc70 (C2), and bar diagram represents the densitometric scan of Hsc70 blot (C3). GAPDH blot for loading control (C4). FACS analysis of BC-8 cells treated with HSF2 antsisense oligonucleotides (C5). **(D)** Effect of antisense p53 nucleotides on BC-8 cells. Western blot analysis of cells subjected to heat shock (HS) at 42ºC, 60 min) both in the presence (with AS p53) and and in the absence (without AS p53) of p53 antisence nucleotides with antibodies for p53 (D1), HSF1 and HSF2 (D2). Note p53 antisense showing no effect on HSF expression.

significantly reduced number of sub-G1 population, which was not associated with the activation of caspases, caspase-3 or -2 (data not presented). Hence cell death induced at these heat shock conditions was assumed to be different from apoptosis or necrosis. Interestingly mild heat shock activated type II programmed cell death, which was thought to be a cytoprotective mechanism towards cell survival (Kiffin *et al* 2004). Contrary to the expected inducible Hsp synthesis, an enhanced expression of only Hsc70, a heat shock cognate protein, was observed. Surprisingly, we also observed proteolysis of HSF1 and induced expression of HSF2 in response to mild heat shock treatment in BC-8 tumor cells. The induced expression of HSF2 and its enhanced DNA binding activity correlated with the activation of chaperone mediated autophagy. It is reported that HSF2 functions only during development and differentiation (Morimoto 1998), but conditions like exposure of cells to hemin, which mimics the *in vitro* cell differentiation in erythroleukemia cells (Sistonen *et al* 1992) and proteasome inhibition (Mathew *et al* 1998) can also induce HSF2 DNA binding activity. However, its role in cell death pathways is not known. Unlike HSF2, HSF1 has a role in CD95 mediated cell death pathway where CD95 expression and its binding to the receptor inhibits DNA binding activity of HSF1 (Schett *et al* 1999; Sreedhar *et al* 2000). Although non-conventional roles of heat shock factors are emerging, almost all of them have been attributed to HSF1 protein (Pirkkala *et al* 2001).

In the present study we showed that under mild heat shock conditions HSF1 is selectively degraded whereas HSF2 attained enhanced DNA binding activity. Proteasome inhibitor, MG-132, inhibited HSF1 proteolysis, did not affect HSF1 protein levels and it failed to inhibit activation of autophagy. Our finding that HSF2 shows enhanced DNA binding at mild heat shock, but not at higher temperature, is in agreement with earlier studies that HSF2 has a threshold for its DNA binding activity which is abolished at elevated temperatures (Sarge *et al* 1991). We are demonstrating for the first time that HSF2 is heat inducible at lower heat shock conditions. Unlike HSF1, HSF2 is a short lived protein and hence, its activation is always accompanied by increased synthesis and decreased degradation, similar to the $\sigma$32 transcription factor in *E. coli* (Tilly *et al* 1989). In accordance with this, we also found enhanced levels of HSF2 transcripts. To further elucidate the physiological significance of induced HSF2-DNA binding in the absence of HSF1 in BC-8 tumor cells, we generated *in vitro* knockouts for HSF1 and HSF2 using siRNA. HSF1 knockout cells when subjected

to mild heat shock were still sensitive to heat shock induced autophagy, whereas siRNA for HSF2 inhibited autophagy induced by heat shock.

When cells are not equipped with protective mechanisms such as synthesis of Hsps, they may activate alternate survival pathways at the expense of certain cellular components on exposure to less damaging cellular insults. Autophagy is one such mechanism associated with cannibalization of short lived/abnormal/non-functional proteins or organelles by proteasome/ubiquitin system and is thought to be a house keeping mechanism involved in the maintenance of cytoplasmic homeostasis (Oshumi and Mizhushima 2004; Bergamini *et al* 2003). In the absence of oxidative damage or protease activation, the cell death experienced by BC-8 cells was associated with activation of survival signaling. In support of this, $\Delta\psi$ m dissipation also did not result in the release of pro-apoptotic factors such as cytochrome c.

Involvement of HSF2 in autophagy activation suggests that in the absence of HSF1, this transcription factor may function in cell survival. To validate our observations with other cellular stress, we treated BC-8 tumour cells with the anticancer drug such as geldanamycin and monitored for HSF-DNA binding. Similar to mild heat shock, geldanamycin also enhanced HSF2-DNA binding, which is correlated with the synthesis of Hsc70 (Aftab *et al* 2006, and other unpublished observations). From these studies we propose that in the absence of HSF1-DNA binding, HSF2 functions in deciding the fate of cells. The differential regulation of cell death pathways correlating with differential expression of HSFs in BC-8 tumor cells suggests that these transcription factors probably exhibit additional cellular functions than the known conventional ones. Further studies are required to understand the molecular mechanism of HSF involvement in stress management and developing resistance to classical cell death pathways. Since HSFs are already in the pharmacological perspective towards treatment (Soti *et al* 2005; Sreedhar *et al* 2006), our study on differential regulation of HSFs may have some clinical importance.

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