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Unraveling Complex Interplay between Heat Shock Factor 1 and 2 Splicing Isoforms

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Abstract

Chaperone synthesis in response to proteotoxic stress is dependent on a family of transcription factors named heat shock factors (HSFs). The two main factors in this family, HSF1 and HSF2, are co-expressed in numerous tissues where they can interact and form heterotrimers in response to proteasome inhibition. HSF1 and HSF2 exhibit two alternative splicing isoforms, called α and β, which contribute to additional complexity in HSF transcriptional regulation, but remain poorly examined in the literature. In this work, we studied the transcriptional activity of HSF1 and HSF2 splicing isoforms transfected into immortalized Mouse Embryonic Fibroblasts (iMEFs) deleted for both Hsf1 and Hsf2, under normal conditions and after proteasome inhibition. We found that HSF1α is significantly more active than the β isoform after exposure to the proteasome inhibitor MG132. Furthermore, we clearly established that, while HSF2 had no transcriptional activity by itself, short β isoform of HSF2 exerts a negative role on HSF1β-dependent transactivation. To further assess the impact of HSF2β inhibition on HSF1 activity, we developed a mathematical modelling approach which revealed that the balance between each HSF isoform in the cell regulated the strength of the transcriptional response. Moreover, we found that cellular stress such as proteasome inhibition could regulate the splicing of Hsf2 mRNA. All together, our results suggest that relative amounts of each HSF1 and HSF2 isoforms quantitatively determine the cellular level of the proteotoxic stress response.

Introduction

Proteasome is a major protein complex responsible for regulated degradation of intracellular proteins, and its activity is modified in many disorders. For example, a decrease in proteasome activity is associated with neurodegenerative diseases, whereas an increase catalytic activity is associated with cancers [1]. Thus, proteasome is a prime target in cancer therapy and bortezomib was the first proteasome inhibitor authorized as anti-tumor agent in humans. In a previous work, we have shown that proteasome subunit expression was regulated by heat shock factors [2]. Heat shock factor 1 (HSF1) and heat shock factor 2 (HSF2) belong to the family of transcription factors, which are essential for the expression of heat shock proteins (Hsps) in response to protein insults. HSF1 is the main factor responsible for Hsp induction, which is abolished in HSF1 deficient cells or organism, and cannot be rescued by HSF2 alone [3]. Among proteotoxic stress, proteasome inhibition, but not heat shock, activates HSF2 [4]. Interestingly, it was shown that after treatment with MG132, a classical proteasome inhibitor, HSF1 and HSF2 can form heterotrimers and bind to DNA [5]. The exact role of such heterotrimers is not yet fully understood, but it was proposed that HSF2 could act as a modulator of HSF1 activity [6–7].

Moreover, both HSF1 and HSF2 exist under two different isoforms produced by alternative splicing. This increases the diversity of potential HSF homo and heterotrimers and adds more complexity to HSF regulation. Regarding HSF1, only two publications refer to the existence of two splicing isoforms (Hsf1α and Hsf1β) in mice [8–9]. Hsf1α results from a splicing process maintaining the insertion of the exon 11 (66 bp), and thus producing a longer protein in comparison to the β isoform. This exon is flanked by two introns presenting a consensus-splicing site. The 22 amino acids encoded by exon 11 are located in the C-terminal domain, adjacent to the hydrophobic region C (HR-C) that is important to maintain HSF1 under an inactive form in absence of stress signal. This additional region could form a leucine-zipper pattern possibly involved in the temperature activation of HSF1α [9]. Furthermore, it was shown that the relative quantity of both isoforms was regulated in a tissue specific manner in mice, but surprisingly, their transcriptional activities have never been studied so far. Like HSF1, the long and short HSF2 isoforms are named HSF2α and HSF2β, respectively [10].
HSF2α contains also an alternative exon 11, which is similarly flanked by two introns containing consensus-splicing sites. Hsf2 exon 11 encodes an 18 amino acids region located after HR-C and partially overlapping the activation domain called AD-1. The relative quantity of HSF2 isoforms is tissue specific [8,10]. Activities of HSF2 isoforms have been better documented in studies mainly conducted in K562 erythroleukemia cells, since HSF2 activity is particularly efficient in these cells. Overexpression of HSF2β resulted in a weaker Hsp70 induction compared to HSF2α [11]. This confirmed previous work showing that HSF2α is a more potent transcriptional activator than the HSF2β isoform [10]. However, as K562 erythroleukemia cells contain a mixture of both HSFs in their two existing isoforms, it is difficult to distinguish the actual role of each isoform in the context of heterotrimers.

Since the consequences of proteasome inhibition on HSF1 and HSF2 isoform expression and activity have not yet been fully characterized, we took advantage of existing cells which are deficient for both HSF1 and HSF2. Using defined transfections, we sought to determine the distinct role of each HSF1 and HSF2 isoforms independently of endogenous HSFs. Here, we show that HSF1α and HSF1β exert different transcriptional activity and we provide evidence for a specific repressor role of HSF2β. Finally, our data and analyses establish that the relative quantity of each HSF2 isoforms is regulated in a stress-dependent manner.

Materials and Methods

Animals, Embryos and Cell Culture

Mixed genetic background wild type, Hsf1−/− and Hsf2−−/− mice, previously provided by Dr IJ Benjamın (University of Utah, Salt Lake City) and described elsewhere [12], were used in those experiments. For embryos collection, females were superovulated and mated with males of corresponding genotypes as described in [15]. They were superovulated on the day of coitum (dpc) and embryos were collected at the 2-cell stage with TRIzol reagent (Invitrogen) and 5 µg of RNAs were retro-transcribed using M-MLV RT (Invitrogen). To discriminate Hsf2α and Hsf2β, primers flanking alternative exon (forward: CATGTCATGTTGCTGTCAGC and reverse: 5’-GAGCT- CACTGACTTTTCTATGG-3’) were used in RT-PCR experiment. Primers were designed using Primer3 software (Bio-Rad).

RT-PCR Analysis

One million of WT iMEFs were plated on a 10 cm dish and treated with MG132 at 1 µM during 2 h, 4 h, 6 h, 8 h or 10 h. After 10 h of MG132 treatment, cells were allowed to recover for 1 h, 6 h or 10 h, and then harvested. Total RNAs were extracted with TRIzol reagent (Invitrogen) and 5 µg of RNAs were retro-transcribed using M-MLV RT (Invitrogen). To discriminate Hsf2α and Hsf2β, primers flanking alternative exon (forward: CAGTCTCATGTTGCTGTCAGC and reverse: 5’-GAGCT- CACTGACTTTTCTATGG-3’) were used in RT-PCR experiment. Primers were designed using Primer3 software (Bio-Rad).

RT-real time PCR experiments were performed on blastocysts as described in [15]. Experiments to determine the relative abundance of Hsf2 isoforms used primers to detect the total population of Hsf2 transcripts (Hsf2 forward: 5’-AGGGGGAGTA-
CAACTGCATCG-3' and reverse: 5'- CAGGCCGCAAGCTTACTC-3' [16] and primers designed to amplify only Hsf2α isoform (forward: 5'-AGTTCTGTGCAGATGAACTCC/ACAG-3' and reverse: 5'-GCAGATGCAAGATTTCCATCC-3'). Experiments performed to measure Hsp70.1 transcripts used the following primers: Hsp70.1 forward: 5'-TTGTCCTGATGTAAGTGTGTTAGTATA-3', Hsp70.1 reverse: 5'-GGTTTTTACATTGATGTAAGGCAAA-3'. The experiments were performed at least in duplicate with one or two independent groups of embryos included in each experiment (n = 20 blastocysts). Results were normalized using 18S RNA amplification.

Protein Extracts and Immunoblot Analysis
To evaluate protein expression after transfection, 200 000 Hsf1.2−/− iMEFs per well were seeded in a 6-well plate. Cells were transfected as described above and were harvested after 24 h of transient expression and 8 h of treatment with MG132 at 2.5 μM. To analyze the expression of endogenous HSF2, 1 million of WT cells were plated in a 10 cm dish and were treated the next day with MG132 at 1 μM, or with DMSO for 10 h. Whole cell extracts were prepared with NP-40 lysis buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, 10 mM EDTA, 1% NP-40, 0.2% sarkosyl). Proteins were separated on an 8.5% or a 12% polyacrylamide gel to discriminate HSF2 isoforms and transferred to a nitrocellulose membrane (Amersham Biosciences). Anti-HSF1 (1B4) antibody (ab44819), anti-HSF2 (3E2) antibody (sc-13517) and Anti-GFP antibody (a11122) were purchased from Abcam, Santa Cruz Biotechnology and Molecular Probes, respectively.

Four hours of migration at 40 μA were necessary to separate HSF2α and HSF2β. Ponceau staining was performed to assess equal loading in place of classical actin control, which was excluded from the gel during the long electrophoresis.

Co-immunoprecipitation
Hsf1.2−/− iMEFs were transfected with pHSF1β-EGFP and/or with pCR3.1-HSF2α, or with pCR3.1-HSF2β. As control, cells were co-transfected with pEGFP and pCR3.1-HSF2α, or pCR3.1-HSF2β. Cells were treated with MG132 at 1 μM during 10 h. Anti-GFP antibody (A11122, Molecular Probes) was used for immunoprecipitation, carried out with Nuclear Complex Co-immunoprecipitation kit (Active Motif), according to manufacturer's instructions.

Results
Transcriptional Activities of HSF1 and HSF2 Splicing Isoforms
To characterize the transcriptional activities of HSF1 isoforms, we first co-transfected increasing doses of expression vector coding either HSF1α or HSF1β, in Hsf1.2−/− iMEFs, with pHSE3x-TATA-Firefly luciferase as a reporter gene. Then, cells were treated with MG132 at 2.5 μM for 8 h. As presented in Figure 1A, low quantities of HSF1α expression vector provided high level of transactivation and HSF1β exhibited 1.4 times higher plateau values than HSF1β. These data suggested that the β isoform was more efficient than the α isoform to activate HSF2 gene promoters, were used in similar experiments and gave comparable results since both HSF2 isoforms were unable to significantly trigger Hsp70 or p35 transactivation (data not shown). These data suggest that by itself, HSF2 is a very poor transcription factor, whatever the splicing isoform considered. As previously tested for HSF1, Hsf1.2−/− iMEFs were co-transfected with pEGFP used as a transfection efficiency control, to assess the level of expression of the different vectors (Figure 1E).

Like for HSF1 isoforms, a minimum of 3.125 ng transfected vector is required to observe a visible effect by luciferase assay.
per 1000 cells was necessary to detect HSF2α whereas only 0.78 ng per 1000 cells was required for HSF2β, suggesting that this latter isoform is more stable than HSF2α.

**HSF2β Forms Heterotrimers with HSF1β and Inhibits its Transcriptional Activity**

Previous work from our team had shown that proteasome inhibition was associated with the formation of HSF1/HSF2 heterotrimers [5]. To determine how the different splicing isoforms could impact heterotrimer activity, we co-transfected different combinations of expression vectors with reporter genes in Hsf1.2−/− iMEFs (Figure 2A). HSF1α transcriptional activity was not statistically different in absence or presence of HSF2 isoforms. Likewise, HSF1β transcriptional activity was not altered when co-transfected with HSF2α. However, it was strongly and significantly decreased (3 fold) after co-transfection with HSF2β. The activity of

![Figure 3. Ratio between HSF2α and HSF2β controls HSF1β transcriptional activity.](image-url)

(A) Representative Western-blot showing the expression of HSF2 isoforms after transfection. Hsf1.2−/− iMEFs were co-transfected with pCR3.1-HSF1β and pCR3.1-HSF2α/β to obtain the expression of equivalent amounts of HSF1 and HSF2, and increasing concentration of HSF2β relatively to HSF2α. Cells were treated with 2.5 μM MG132 for 8 h. Protein extracts were loaded on 12% polyacrylamide gel and submitted to a long migration to separate efficiently HSF2 isoforms. HSF2 was revealed by immunobloting. (B) Transcriptional activity induced, with fixed concentrations of HSF1 and total HSF2, but varying combinations of HSF2β/HSF2α.

![Figure 3. Ratio between HSF2α and HSF2β controls HSF1β transcriptional activity.](image-url)

Hsf1.2−/− iMEFs were co-transfected with 12.5 ng of pCR3.1-HSF1β and with the same quantity of pCR3.1-HSF2α/β, as previously described. Diamonds correspond to the experimental data obtained in independent triplicates, and expressed as percentage of maximal activity. Solid line drawn to equation 2 with random multimerization and considering that HSF1 is active only in absence of HSF2β in the trimer. (C) HSE-driven transcriptional activities expected from Eq. 2, with a constant and identical amounts of HSF1 and total HSF2, and when increasing the ratio HSF2β/HSF2α. The transcriptional strength of a trimer is assumed to be proportional to the number of HSF1 monomers present, and the unit of transcriptional strength (k = 1) corresponds to that of an HSF1 monomer. The strength of trimerization is considered as either (i) identical between hetero- and homodimers (plain line), (ii) 10 fold higher for homodimers (dashed line), or (iii) 10 fold higher for heterodimers (dotted line). (D) HSE-driven transcriptional activities drawn to Eq. 2, with constant and equivalent amounts of HSF1 and HSF2, capable to either randomly homo- or heterotrimerize. Plain line: as for panel C, the transcriptional strength of a trimer is proportional to the number of HSF1 monomers included in the trimer and the strength of a HSF1 monomer is set to 1 (k₁ = 3, k₂ = 2, and k₃ = 1). Dashed line: Transcriptional strength independent on whether the trimer contains 1, 2 or 3 HSF1 monomers (k₁ = k₂ = k₃ = 1). Dotted line: same rule with k₁ = k₂ = k₃ = 3.

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Activity of HSF1 and 2 Splicing Isoforms

A

DMSO  MG132  Cont  HS
Hsf 2  a  b
Gapdh

B

Time of MG132 treatment  MG132 10 h + Recovery

Cont  2 h  4 h  6 h  10 h  1 h  6 h  24 h
Hsf 2  a  b
Gapdh

% of isoform

Cont  2 h  4 h  6 h  10 h  1 h  6 h  24 h
% Hsf2 a
% Hsf2 b

C

Time of MG132 treatment  Time of recovery after 10 h of MG132 treatment

Cont  1 h  6 h  10 h  1 h  6 h  10 h  24 h
HSF2  a  b
Ponceau
100 kDa
75 kDa
Protein extracts were loaded on 12% polyacrylamide gel and submitted to a long migration to separate efficiently HSF2 isoforms. HSF2 was revealed by immunoblotting. Ponceau staining was used to verify the equal loading.

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trimer, whereas HSF2β completely inhibits their activity. In addition, HSF trimerization is assumed to occur when bound to DNA, but not in solution. The 3 monomers are supposed to trimerize with microscopic dissociation constants, $K_1$ or $K_1'$, depending on whether they form homo- or hetero-trimer, respectively. For symmetry reasons, one may expect that homotrimers are favored ($K_1 < K_1'$), but considering the close structural relationship between the trimerization domains of the different HSFs, $K_1$ and $K_1'$ could in fact be very similar. All types of HSF trimers are supposed to bind to the DNA with the same intrinsic dissociation constant $K_d$. Global composite constants $K$ or $K'$ are defined such that $K = K_1 + K_2 + K_3$ and $K' = K_1'^2 + K_2' + K_3'$ ($M^5$). There are ten different possible types of trimers, distributing over 27 trimer microstates listed below, so that 11 possible states of HSE can exist, either empty or bound by a HSF trimer. Finally, once bound to DNA, HSF trimers devoid of HSF2β promote transcription from an HSE-driven promoter with transcription rates ($t^{-1}$), depending on the number of HSF1 protomers in the trimer ($k_1$, $k_2$ and $k_3$ for 1, 2 or 3 molecules of HSF1 respectively). Let us define the following variables: $x = [\text{HSF1}^*]$, the concentration of transcriptionally active HSF1, $\alpha = [\text{HSF2}\beta]$ and $\beta = [\text{HSF2}\beta]'$. The probability of the different binding states of HSE can be defined as the ratio of their mass action values over the sum of all of them, written $\Sigma$.

- $P(\text{HSE}_0) = 1/\Sigma$
- $P(\text{HSE}(\text{HSF1})_3) = (x^3/K)/\Sigma$
- $P(\text{HSE}(\text{HSF}2\beta)_3) = (x^3/K)/\Sigma$
- $P(\text{HSE}(\text{HSF}2\beta)_3') = (\beta^3/K)/\Sigma$
- $P(\text{HSE}(\text{HSF}1)_2\text{HSF}2\beta) = (3x^2\beta/K')/\Sigma$
- $P(\text{HSE}(\text{HSF}1)_2\text{HSF}2\beta') = (3x^2\beta/K')/\Sigma$
- $P(\text{HSE}(\text{HSF}2\beta)_3) = (3x^3\beta/K)/\Sigma$
- $P(\text{HSE}(\text{HSF}2\beta)_3') = (3x^3\beta/K)/\Sigma$
- $P(\text{HSE}(\text{HSF}1\text{HSF}2\beta) = (6x\beta/K')/\Sigma$

To go further, a ratio of trimerization affinity is written $R = K/K'$. In the experiment, the total amount of HSF2 is constant but the ratio of the $\alpha$ and $\beta$ isoforms is defined by a variable $z = \beta/\alpha$. The fixed total concentration of HSF2 is $y = [\text{HSF2}] = \alpha + \beta$, so that $\alpha = y/(1+z)$ and $\beta = y/(1+z)$. The transcriptional activity ($a$) of the HSE driven promoter can be defined as $a = \Sigma k_iP_i$, where $P_i$ is the probability of presence of the HSF trimer responsible for the maximal transcription initiation rate $k_i$ [10]. Using the rules and values defined above, the global activity can be written after some algebra

$$k = k_1x^3 + 3R(k_2x^2 + k_3x^2 + k_1x^2) = \frac{K + x^3 + y^3 + 3xy(x + y)}{K + x^3 + y^3}$$

Finally in the experiment described above, the total concentration of transfected HSF is kept constant ($\alpha + \beta = H$). Hence, a variable can be eliminated from Eq. 1, yielding

$$k = \frac{k_3x^3 + 3R(k_2x^2(H-M) + k_1x^2(H-M)^2)}{K + H^3 + 3Hx(H-M)(R-1)}$$

Eq. 2 satisfactorily matches the experimental profile of the global transcriptional activity obtained when increasing the $[\text{HSF2}\beta]/[\text{HSF2}\alpha]$ ratio (Figure 3B). Modifying the relative strength of homo- versus hetero-trimer formation has only marginal influences (Figures 3C, D). Comparison between Figure 3C and Figure 3B suggests that HSF heterotrimers should be stable enough to explain the results, in agreement with the high degree of conservation between the heptad repeat (HR) and the DNA binding domain (DBD) of all HSFs involved in trimerization, regardless of their capacity to initiate transcription.

**Level of Proteasome Activity Regulates the Quantity of Hsf2 Isoforms**

The model described above suggests that the relative concentration of Hsf2 isoforms is involved in modulating the HSF1-dependent transcriptional response. So, to assess the relative quantity of each Hsf2 splicing isoforms in cells, RT-PCRs were performed using MG132 treated, or heat shocked WT IMEs total RNA extracts (Figure 4A). In control and heat shocked cells, Hsf2β was the dominant isoform, whereas in MG132-treated cells, Hsf2α was found to be the major splicing isoform. From these observations, it can be proposed that the way Hsf2 mRNA splicing is regulated depends on the type of stress experienced by the cells. This further implies a Hsf2α to β switch under MG132 treatment. The time course of such switch was analyzed during MG132 exposure (10 h) and after a recovery period (from 1 to 24 h) (Figure 4B). Densitometric analysis showed that Hsf2β represented about 55% of the total Hsf2 transcripts in control cells. After 6 h of treatment, the relative quantity of Hsf2β decreased, whereas the relative quantity of Hsf2α increased to become the dominant isoform (around 55% of the total Hsf2 mRNA). This switch between isoforms is a reversible phenomenon as observed after 24 h of recovery, where the relative quantity of Hsf2α and Hsf2β returned to their initial levels.

The switch in the mRNA isoforms was modest but highly reproducible. Unfortunately, it was more challenging to confirm the isoform switch at the protein level. The WT iMEFs were treated with MG132 at 1 μM for 1 h, 6 h or 10 h and badges of cells were harvested after 1 h, 6 h, 10 h or 24 h of recovery, to detect HSF2 by immunoblot (Figure 4C). In control cells, HSF2α
and HSF2β were barely detectable, because HSF2 is a labile protein, constitutively expressed and degraded. During MG132 treatment, HSF2 was highly stabilized which adds an additional level of regulation, in addition to the translation. Differences between isoforms remained difficult to detect because of the lack of sensitivity of the immunoblot method and the absence of isoform specific antibodies.

**Low Hsf2α/HSF2β Ratio in Blastocyst is Associated with a Weak Response to Proteasome Inhibition**

HSF2 exhibits a developmentally regulated DNA binding activity at the blastocyst stage (E 3.5 d) [19], which remains unexplored so far. This prompted us to examine the relative abundance of HSF2 isoforms, and the blastocyst response to proteasome inhibition. Due to the limited amount of starting material, we designed primers enabling real time RT-PCR to amplify either the total Hsf2 cDNA, or specifically the α isoform. For comparison, the same strategy was applied to testis total extract (Figure 5A). In blastocysts, Hsf2α represented around 4% of total Hsf2 transcripts (implying that the β isoform would count for 96%), while in testis, the proportion of Hsf2α rose up to 46% of Hsf2 total (Figure 5A). When WT blastocysts were treated with MG132 for 4 h, Hsp70 expression assessed by real time RT-PCR (Figure 5B) was only slightly induced (around 1.2 fold), compared to non-treated ones. On the contrary, in Hsf2α−/− blastocysts, Hsp70 was 2.2 fold induced in MG132 treated versus non-treated embryos. In accordance with our previous results, and speculating that in WT blastocysts where HSF2β was the dominant isoform, the response to proteasome inhibition was blunted. Conversely, in Hsf2α−/− blastocyst where there was no HSF2β protein, the Hsp70 response could be elicited.

**Discussion**

Both HSF1 and HSF2 exist as two main splicing isoforms, but very few studies have focused on their respective transcriptional activities. It is uneasy to address this question in cells expressing endogenous HSF1 and 2, because of the coexistence of various HSF complexes, either homo- or hetero-trimers, using potentially both existing isoforms. Therefore, we took advantage of iMEFs genetically depleted for both factors, which provide an invaluable material, we designed primers enabling real time RT-PCR to amplify either the total Hsf2 cDNA, or specifically the α isoform. Our study revealed that the differential splicing of Hsf1 and Hsf2 isoforms is crucial for the modulation of the relative quantity of HSF isoforms. It depends upon the cellular context and the presence of indispensable co-factors, or particular stimuli. The absence of HSF2 transcriptional activity also indicates that its main role is not to directly recruit the transcriptional apparatus, but rather to play a pioneer role in chromatin preparation. This hypothesis is in agreement with the fact that HSF2 can prevent compaction of HSF target genes during mitosis [23]. Our study confirmed the role of HSF2 isoforms in the modulation of HSF1 activity [11,17,24]. While HSF2α does not affect significantly HSF1 transcriptional activity, HSF2β clearly reduces HSF1 activity. Moreover, this repression is specific to the association of HSF1β with HSF2β, under MG132 treatment. Indeed, it was shown that HSF2 is not activated after heat shock treatment, but rather after inhibition of the ubiquitin-proteasome pathway [4]. Therefore, HSF1-HSF2 heterotrimers can be assembled only after proteasome inhibition, and not after heat shock [5], which might explain why HSF2β inhibitory effect cannot be found after heat shock. According to our results, the presence or absence of a short isoform in the heterotrimer appears to be critical for this modulation of HSF activity. We propose that the leucine zipper conserved in the long isoform could create a novel interaction domain. Thus, this domain could stabilize the activation domain in an optimal conformation, while in the short isoforms, the activation domains could be in a more relaxed conformation, resulting in a less efficient co-factors binding, especially in the context of an HSF1β-HSF2β heterotrimer.

The absence of specific antibody for each isoform, and the technical limitations to accurately determine the ratio of isoforms in a trimer, remain a real concern to further decipher HSF2 mechanism in HSF1 inhibition. In an attempt to alternatively address this question, we have developed a mathematical modelling approach. Our model is different from those previously published on the dynamic of the eukaryotic heat shock response [25–27]. In contrast with previous heat shock response models, we did not focus on the various steps involved in the kinetics of Hsp gene activation and its feedback regulation. We rather concentrated on the active HSF trimer that binds to the promoter. We analyzed the impact of the presence of HSF2α and/or HSF2β isoforms on the steady state of HSF1 activity. We postulated that the presence of several HSF isoforms induces the coexistence of different types of heterotrimers. These complexes do not equally transactivate, and they are expected to compete to bind to target promoters. The model we propose satisfies the experimental data obtained by transfection. Based on this model, the strength of the response depends on the proportion of each isoform in the cell. Accordingly, the combination of HSF isoforms synthesized in each cell determines the level of response. This implies that all the cells do not have the same capabilities to respond to proteotoxic stress, and that expression of these HSF isoforms is crucial for the modulation of chaperone expression.

Expression of HSF2 isoforms is tissue-specific. For example HSF2α is the main isoform in testis, while HSF2β is dominant in brain [10]. In addition to this tissue-specific regulation of HSF isoforms, we have shown that the differential splicing of Hsf1 and Hsf2 messenger is also dependent on the type of stress. The switch between the two isoforms is specific to proteasome inhibition, as clearly shown by our RT-PCR experiments.

Taken together our data demonstrate how HSF1 and HSF2 splicing isoforms contribute a new level of complexity to HSF regulation. Our results raise additional questions: how efficient is the proteotoxic response in tissues where HSF1β and HSF2β are dominant? Which evolutionary mechanisms established such complex regulation of the relative quantity of HSF isoforms? It might be critical for stressed cells to possess various ways to...
modulate HSF1 activity, to achieve better adaptation, and thus survival. Heat shock is an acute stress with massive protein alteration, which requires a rapid and high HSP expression. In that case, HSF1 is found in homotrimeric complex, while HSF2 becomes insoluble and is found in the perinuclear fraction [20]. On the other hand, proteasome inhibition induces a proteotoxic stress, which can be considered as a chronic stress with the requirement for longer-term adaptation. This should trigger a more limited response, since it could be deleterious for cells or tissues to express excessive amount of HSPs. Proteasome inhibition activates both HSF1 and HSF2 [29], leading to the formation of a diverse population of HSF heterotrimers, with variable transcriptional efficiency [5]. This mechanism could be the best option to control proteotoxic response in cells exposed to in case of chronic stress.

References


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Author Contributions

Conceived and designed the experiments: YLD SL EC. Performed the experiments: SL LR CLQ FLM PLG. Analyzed the data: YLD SL AA DM EC. Wrote the paper: SL YLD EC.