Induction of the DNA-binding and transcriptional activities of heat shock factor 1 is uncoupled in *Xenopus* oocytes

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Abstract

The DNA-binding and transcriptional activities of the heat shock transcription factor 1 (HSF1) are repressed under normal conditions and rapidly upregulated by heat stress. Here, we tested for the ability of various stress agents to activate HSF1 in the *Xenopus* oocyte model system. The HSE-binding activity of HSF1 was induced by a number of chemical stresses including cadmium, aluminium, iron, mercury, arsenite, ethanol, methanol, and salicylate. HSE-binding was not induced by several stresses known to induce the synthesis of hsps in other cell types in different organisms including zinc, copper, cobalt, manganese, recovery from anoxia, UV-irradiation, and increased pH. The inability of several known inducers of the stress response to activate the HSE-binding ability of HSF1 suggests that certain stress activation pathways may be absent or inactive in oocytes. The transcriptional activity of oocyte HSF1 was induced by heat, cadmium, and arsenite, but many of the agents that induced HSE-binding failed to stimulate HSF1-mediated transcription. The apparent uncoupling of inducible HSE-binding and transcriptional activities of HSF1 under a variety of stress regimes indicates that these events are regulated by independent mechanisms in the oocyte. © 1998 Elsevier Science B.V.

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1. Introduction

Cells respond to elevated temperature by transiently increasing the synthesis of a family of highly conserved heat shock proteins (hsp) which function under both normal and stressful conditions as molecular chaperones mediating the folding, assembly, translocation, and degradation of proteins (reviewed in Refs. [1–5]). In addition to heat shock, this response is also induced by a number of different agents (heavy metals, alcohols, oxidants, amino acid analogs, and metabolic inhibitors) and adverse physiological conditions (fever, ischemia, tissue trauma, and bacterial and viral infections). Hsps are also differentially expressed under normal conditions in cell type specific patterns during growth and differentiation. The expression of hsps during stress in eukaryotes is regulated primarily at the level of transcription by the action of heat shock transcription factors (HSFs). HSF genes have been isolated in a number of species, and higher eukaryotes have been found to encode multiple HSF family members (reviewed in Ref. [6]). The HSF family member that is universally responsive to heat and other stresses has been termed HSF1 [7–10]. HSF1 acts through the
heat shock regulatory element (HSE) that is found in the promoters of all hsp genes [11,12].

Hsp expression is proportional to the severity of stress and switched off upon resumption of normal physiological conditions. It therefore appears that HSF1 is subject to complex regulatory mechanisms under both normal and stress conditions (reviewed in Refs. [6,13,14]). In unstressed metazoan cells, HSF1 is present as an inert non-DNA-binding monomer [15,16] which must become activated by stress. Numerous studies show that there are several key points in the HSF1 regulatory pathway, the first of which is conversion of the oligomeric state from monomers to homotrimers and acquisition of DNA-binding to the HSE [7,8,17–20]. The second step in the activation pathway of HSF1 involves changes to the transcriptional activation domain. Some stresses, such as indomethacin and salicylate have been shown to activate HSF1 DNA-binding ability but fail to induce the transcription of hsp genes [21,22]. Thus, activation of DNA binding and transcriptional competence appear to be regulated independently and it has been postulated that to activate transcription HSF1 must undergo a second conformational change once it has trimerized [23–26]. The final step of HSF1 regulation is deactivation or attenuation. Upon removal of stress HSF1 dissociates into monomers and ceases to activate transcription [15,27,28]. If cells are heated for an extended period, HSF1 loses its DNA-binding and transcriptional activities in a process called attenuation.

One of the central questions of the stress response is the mechanism by which cells detect various unrelated stress stimuli and signal the activation of HSF1. There are a number of inherent features of HSF1 that appear to regulate its activity. Detailed mutagenic analyses suggest that the monomeric form is stabilized by intramolecular interactions between leucine zipper motifs at the amino- and carboxy-terminal regions [8,17,29]. Activation of HSF1 involves the disruption of these intramolecular interactions and the formation of intermolecular coiled coils with other HSF1 monomers [17,29]. Thus, the suppression of DNA-binding activity under normal conditions is regulated at least in part by hydrophobic sequences within HSF1 itself, although other regions of the molecule have recently been implicated in this regulation [30]. It is unlikely that HSF1 oligomerization is regulated by the absolute environmental temperature because such a simple model does not account for the activation of HSF1 by multiple unrelated stresses. Also, it appears that activation of HSF1 molecules expressed in heterologous systems is reprogrammed according to the appropriate physiological temperatures of the host cells suggesting that HSF1 is under negative regulation by cellular factors [15,17,28,31–33].

Observations that HSF1 is constitutively phosphorylated on serine and threonine residues before stress and inducibly hyperphosphorylated after stress have led to speculation that HSF1 could be regulated in some fashion by cellular kinases and/or phosphatases [7,8]. It was recently reported that repression of HSF1 could be modulated by constitutive phosphorylation [34]. However, the functional relevance of hyperphosphorylation remains to be elucidated, as the current body of evidence does not allow for a definitive correlation of with DNA-binding or transcriptional activities [18,20,22,35–37].

It is known that induction of HSF1 by stress is associated with several independently regulated steps leading to the acquisition of HSE-binding and transcriptional activities, however, there are several unanswered questions regarding how these steps are regulated in vivo, the potential role of hyperphosphorylation, and whether common or multiple distinct signaling pathways are involved. Most studies to date have concentrated on the regulation of HSF1 in response to heat shock, so a detailed evaluation of how various chemical inducers compare to heat in a given model system is lacking. In the present study, we examine how different classes of stresses affect key regulatory steps in the HSF1 activation pathway in Xenopus oocytes. The oocyte has emerged as a convenient model system in which to study induction of HSF1. This is illustrated by expression of cloned Drosophila HSF in oocytes showing partial suppression of HSE-binding at the normal growth temperature of Xenopus [15], and the employment of oocytes by Baler et al. [7] and Zou et al. [24,29] for mutagenic analyses of human HSF1. We recently reported the existence of distinct inducible and developmentally regulated HSE-binding activities of endogenous HSF molecules in Xenopus oocytes [38]. The DNA-binding activity of HSF1 in oocytes is induced by heat, but remains active throughout prolonged stress treatments sug-
suggesting that factor(s) regulating attenuation of HSE-binding ability are limiting or modified in the oocyte.

Here, we performed a detailed examination of the stress response in *Xenopus* oocytes at the level of induction of HSE-binding activity and transcriptional activation potential. The ability of a wide variety of known stressors to induce HSS-binding activity of *Xenopus* HSF1 was tested, and this activity was compared to transcriptional activation as measured by induced expression from microinjected hsp 70 promoters. The results of these experiments suggest that multiple cellular pathways are required for full activation of oocyte HSF1 in response to stress, and that induction of DNA-binding and transcriptional activities are regulated independently in oocytes.

### 2. Experimental procedures

#### 2.1. Oocytes

*X. laevis* frogs were purchased from Xenopus I (Ann Arbour, MI). Ovary portions were surgically obtained from adult female frogs and follicle cells were removed from oocytes by treatment in calcium free OR2 buffer (82.5 mM NaCl, 2.5 mM KCl, 1 mM MgCl$_2$, 1 mM NaH$_2$PO$_4$, 5 mM Heps, pH 7.8, 10 mg/l streptomycin sulfate, 10 mg/l benzyl penicillin) containing 2 mg/ml collagenase type II, Sigma for 2–3 h at 18°C. Oocytes were washed extensively and allowed to recover overnight in OR2 as above (as above +1 mM CaCl$_2$ [39]), at 18°C. Oocytes were staged according to the criteria described by Dumont [40]. Control oocytes were maintained at 18°C in OR2, chemically stressed oocytes were incubated in OR2 supplemented with chemical stressors at indicated concentrations, and heat shocked oocytes included as positive controls for HSF1 activation were incubated in OR2. The duration and severity of stress exposures for each experiment are indicated in the figures. In all experiments, a minimum of 20 oocytes were used for each sample. Following stress treatments, oocytes were quickly washed in OR2, and collected for protein extracts or expression analysis (see Section 2.2).

#### 2.2. Protein extracts

For protein extracts, oocytes were homogenized in Buffer C (50 mM Tris–Cl, pH 7.9, 20% glycerol, 50 mM KCl, 0.1 mM EDTA, 2 mM dithiothreitol, 10 μg/ml aprotinin and 10 μg/ml leupeptin [41]) in a Dounce homogenizer with a tight fitting pestle. Homogenates were transferred to eppendorf tubes and spun for 5 min at 15,000 × g (4°C). The resultant supernatants were removed to a fresh tube, immediately frozen in liquid nitrogen, and stored at −80°C. Oocytes (stage VI) were homogenized in 10 μl buffer C per oocyte. Under the conditions described, a single oocyte yields approximately 20 μg soluble protein.

#### 2.3. Gel mobility shift assays

DNA mobility shift assays were performed essentially as described by Ovsenek and Heikkila [42]. DNA-binding reactions with stage VI oocyte samples contained 10 μl extract (one embryo equivalent is approximately 20 μg soluble protein). The relative amounts of protein in all samples was determined by Coomassie staining of SDS–polyacrylamide gels, and extract volumes were adjusted so that equal protein concentrations were added to each binding reaction. HSE oligonucleotide probes used in these assays were as described in Ovsenek and Heikkila [42]. Binding reactions were performed in the presence of 1 μg poly (dl–dc), 10 mM Tris (pH 7.8), 50 mM NaCl, 1 mM EDTA, 0.5 mM dithiothreitol, and 5% glycerol, in a final volume of 20 μl. Reactions were incubated on ice for 20 min, and immediately loaded onto 5% non-denaturing polyacrylamide gels containing 6.7 mM Tris–Cl (pH 7.5), 1 mM EDTA, 3.3 mM sodium acetate. Gels were electrophoresed for 2.5 h at 150 V, dried, and exposed overnight to X-ray film.

#### 2.4. Oocyte injections and CAT assays

Plasmid constructs used for microinjection experiments were the human CMV-CAT and the *Xenopus* Hsp70-CAT clones (kindly provided by Dr. Alan Wolfe, NICHD, National Institutes of Health, Bethesda, MD) previously described in Landsberger et al. [43]. Following defolliculation, oocytes were incubated for several hours at 18°C, after which healthy oocytes were selected and injected into nuclei with 20 nl of a solution containing 2 ng/μl (40 pg) of either CMV-CAT or Hsp70-CAT plasmid using a
Narashige IM 300 microinjector. After incubation overnight at 18°C, healthy oocytes were selected and stressed for either 1 or 2 h by heat shock at 33°C, or treated with indicated stress agents at 18°C. Following these treatments, oocytes were incubated for an additional 12 h at 18°C, washed in OR2 and assayed for CAT activity. As a control for oocyte injections, DNA was recovered from at least five individuals out of the pool of injected oocytes, and the equivalency of injected plasmid DNA was confirmed by Southern blotting. CAT assays were performed using 1 oocyte equivalent of whole cell extract from uninjected or injected oocytes as previously described [42]. A pool of at least 20 oocytes were used for each analysis. The acetylated products were separated by thin layer chromatography and visualized by autoradiography.

3. Results and discussion

Since most studies examining the regulation of HSF1 have focused mainly on the response of cells to thermal stress, an evaluation of how various chemical inducers and sub-optimal physiological conditions compare to induction of HSF1 by heat shock is lacking. We recently demonstrated that the HSE-binding activity of endogenous HSF1 is induced upon exposure of Xenopus oocytes to heat shock and to two different chemical stresses, cadmium and arsenite [38]. In the present work, we aimed to gain a more comprehensive understanding of the stress conditions that induce HSF1 in oocytes. The HSE-binding activity of HSF1 was assayed after treatment of stage VI oocytes with a wide range of stresses previously shown to induce the synthesis of hsps in various model systems. Gel mobility shift assays were performed with equal amounts of protein extracts and a radiolabeled HSE oligonucleotide probe (Fig. 1). The specific HSE–HSF1 complex was not present in unstressed controls, but was induced to high levels in oocytes treated for 1 h with cadmium, iron, mercury, heat shock, aluminum, methanol, ethanol, or salicylate (Fig. 1A). Maximal levels of HSF1 activation for each of these stress treatments was determined by assaying protein extracts made from oocytes exposed to a range concentrations for 1 h. The data presented in Fig. 1B shows maximal induction of HSF1 after 1 h treatments with 70 mM salicylate, 10% ethanol, and 14% methanol. Similar experiments were performed to determine optimal concentrations for stress treatments with iron, mercury, and aluminum (data not shown), as well as cadmium and arsenite [38]. The results of these experiments show that exposure of oocytes to various unrelated stress conditions can lead to activation of the DNA-binding activity of HSF1. Relatively high concentrations of these compounds, compared to those reported for other cell types [44] were required to elicit a response in Xenopus oocytes.

We observed that the HSE-binding activity of HSF1 was not induced upon exposure of oocytes to a number of different chemical stresses and environmental conditions previously shown to induce hsp
Fig. 2. The HSE-binding activity of HSF1 is not induced by exposure of oocytes to azetidine or zinc. (A) Gel mobility shift assay of protein extracts from stage VI oocytes that were incubated for 2 h at 18°C in the indicated concentrations of L-azetidine 2-carboxylic acid or zinc chloride, or exposed to a heat shock temperature of 33°C for 2 h (HS). (B) Time course of HSE-binding activity in oocytes incubated in 1, 10 or 50 mM azetidine. Extracts from control (C) and heat shocked (HS) oocytes were included. The heat-inducible HSE-binding complex is indicated beside each panel.

synthesis in different cell types. As expected, the inducible HSF-bandshift indicative of HSF1 trimerization was detected after heat stress (Fig. 2A), but was not detected after 2 h exposures of oocytes to various concentrations of zinc chloride or after incubation with the metabolic inhibitor azetidine. Similar results were obtained after exposure of oocytes to sodium azide, copper, cobalt, manganese, recovery from anoxia, UV-irradiation, and increased pH, and the transcriptional activity of HSF1, as measured by transcription assays, was not upregulated by these treatments (data not shown). Activation of HSF1 was not observed even after increasing the severity of each of these stress treatments to levels resulting in oocyte death within 1 h (data not shown). We also exposed oocytes to longer incubations with each of these apparent non-activators in order to determine if activation of HSF1 could be accomplished under a more extended stress regime. The HSE-binding activity of HSF1 was not induced in oocytes even after prolonged exposure to azetidine (Fig. 2B), and similar results were observed when oocytes were treated with each of the stresses mentioned above (data not shown). It is interesting that the HSE-binding activity of oocyte HSF1 was not induced by several stresses previously shown to induce the stress response in other model systems. We speculate that cells require multiple signal transduction mechanisms to activate HSF1 in response to different stresses, and that some of these may be absent or inactive in the oocyte.

It has been postulated that inducible HSE-binding and transcriptional competence are regulated independently and that full activation of HSF1 involves further conformational changes in addition to those leading to trimerization [23–26]. Some agents, such as indomethacin and salicylate have been shown to activate the HSE-binding ability of HSF1 but fail to induce HSF1-mediated transcription [21,22]. In order to determine the relationship between activation of HSE-binding and transcription in the oocyte model system, we tested for upregulation of HSF1-mediated transcription under each of the stress conditions shown in Fig. 1 to induce of HSE-binding. In these experiments, oocytes were microinjected with a CAT reporter construct under the control of the *Xenopus* Hsp70B promoter (Hsp70-CAT), and then stressed under conditions that give rise to maximal HSE-binding activity. CAT activity was low in un.injected controls, and in unstressed Hsp70-CAT-injected oocytes (Fig. 3). CAT activity was induced to high levels in heat, cadmium, and arsenite treated oocytes, an indication that the transcriptional activity of HSF1 is induced in oocytes by these stress conditions. Interestingly, many of the agents and stress conditions that activated HSE-binding, including ethanol,

Fig. 3. Comparison of the transcriptional activation of oocyte HSF1 by stresses known to induce HSE-binding. CAT assays were performed on stage VI oocytes injected with Hsp70-CAT or CMV-CAT plasmid DNAs and subjected to the stresses indicated above the panel. Stress conditions used were identical to those used in Fig. 1, determined to be maximal for induction of HSE-binding activity. The positions of chloramphenicol and acetylated chloramphenicol are shown on the right of each panel.
methanol, mercury and salicylate, failed to stimulate HSF1-mediated transcriptional activity as measured by expression from the Hsp70 promoter. It was important to rule out the possibility that some of the stress treatments used in these experiments caused a general inhibition of CAT expression. In these experiments, oocytes were microinjected with a CAT reporter under the control of the cytomegalovirus promoter (CMV-CAT) and stressed in parallel with Hsp70-CAT-injected oocytes under identical conditions. Equal levels of expression from CMV-CAT was observed after each treatment, indicating that CAT expression was not negatively affected by any of the stress conditions used in this experiment. Therefore, the lack of HSF1 mediated expression after mercury, ethanol, methanol and salicylate treatments was due to the inability of these stress agents to bring about the modifications required to activate the transcriptional activation domain. Due to the apparent uncoupling of inducible HSE-binding and transcriptional activities of HSF1, we conclude that these events are regulated independently in the Xenopus oocyte.

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References

[22] N.A. Winegarder, K.S. Wong, M. Sopta, J.T. Westwood, Sodium salicylate decreases intracellular ATP, induces both heat shock factor binding and chromosomal puffing, but


42. N. Ovsenek, J.J. Heikkila, DNA sequence specific binding activity of the Xenopus heat shock transcription factor is heat inducible before the midblastula transition, Development 110 (1990) 427–433.
