# Epitope Tagged Plasmids for HSF Isoform Expression in Mouse Cells

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## Abstract

Organisms must survive a variety of stressful conditions, including sudden temperature increases that damage important cellular structures and interfere with essential functions. Proteins activated in response to elevated temperatures are called heat shock proteins (hsps). Heat shock proteins bind to misfolded proteins to repair them, promoting survival. The main regulator of genes for hsps is called Heat Shock Transcription Factor 1 (HSF1). Humans, mice, and other vertebrates express four different types of HSF1, called isoforms, which differ structurally in the presence or absence of either of two short regions of 28 and 22 amino acids. We have launched this study in order to investigate (1) what the separate roles of the individual isoforms are and (2) to learn whether they functionally interact with each other. We have constructed four sets of plasmids, which enable the individual mouse HSF1 isoforms to be expressed in tissue culture cells, with or without an activating deletion. Each set contains specific protein tags [either an octapeptide known as FLAG or a section of the flu virus "spikey" protein hemagglutinen (HA)] with one set tagged at the beginning (N-FLAG or N-HA) and another set tagged at the end (C-FLAG or C-HA) for each tag. Plasmid constructs were made by overlap extension PCR or subcloning of restriction fragments into the expression vector pcDNA3.1/V5-His-TOPO. The DNA sequences of the plasmid constructs were confirmed by dideoxy sequencing. The production of the tagged proteins was detected after transient transfection of NIH 3T3 cells via Western analysis. Three of the four sets are complete or mostly complete as confirmed by binding of HA or FLAG primary antibodies and later reincubation with HSF1 primary antibodies. The fourth set (N-HA tagged HSF1 isoforms) do not appear to be expressed in mouse cells following transient transfection. The three confirmed sets of plasmid constructs comprise highly specific tools for investigating HSF1 function in mouse cells.

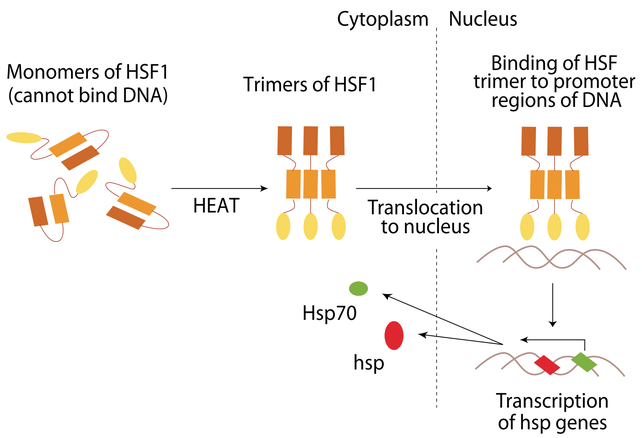


**Figure 1. Regulation of heat shock protein (hsp) transcription by HSF1.** Figure adapted from Pockley 2001.

## Introduction

- The heat shock response is critical for organisms' tolerance to stress. Components of the heat shock pathway promote homeostasis; while their imbalance contributes to cancer, heart disease, and other aging-related disorders.
- The main stress sensor in organisms is the regulatory transcription factor, heat shock factor 1. We recently demonstrated that four different protein isoforms (HSF1 α, β, γ, δ) are expressed in mouse cells and contribute to the heat shock response.
- In this study, we prepared and validated "tagged" genetic constructs to enable us to test specific hypotheses regarding the function, interactions, and subcellular locations of HSF1 isoforms.

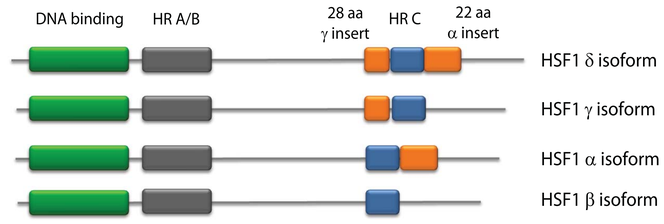


**Figure 2. Diagram of mouse HSF1 isoforms showing conserved domains.** green = DNA binding; gray = hydrophobic oligomerization; blue = hydrophobic repeat and isoform specific inserts of 28 aa (δ and γ) and 22 aa (δ and α).

## Experimental Procedures

### 1) Preparation and confirmation of expression constructs

- Primers were designed to incorporate FLAG or HA epitope sequences at the C-terminus or N-terminus and used in PCR on HSF1 cDNAs for the different isoforms.
- The purified PCR products were subcloned into pcDNA3.1/V5-His-TOPO, a mammalian expression vector.
- The constructs were confirmed by dideoxy sequencing on a Beckman CEQ8000 system.

### 2) Confirmation of protein tags using Western blots

- Epitope tagged constructs were introduced into NIH 3T3 cells growing in 60 mm dishes by transient transfection using Lipofectamine LTX (Life Technologies).
- Cell extracts were separated by polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes using a semi-dry blotter (Bio-Rad).
- Western blots were carried out using primary antibodies to the epitopes (FLAG or HA), followed by secondary antibodies coupled to HRP enabling chemiluminescent detection. Subsequent blots were stripped and reprobed with primary antibodies to HSF1 or b-tubulin (control).

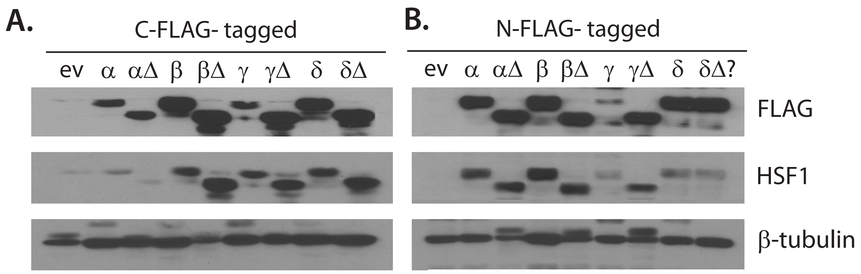


**Figure 3. Western blot analysis detecting FLAG.** (A) Cells expressing C-FLAG and (B) N-FLAG-tagged HSF1 isoforms. b-tubulin is expressed in all extracts, including vector

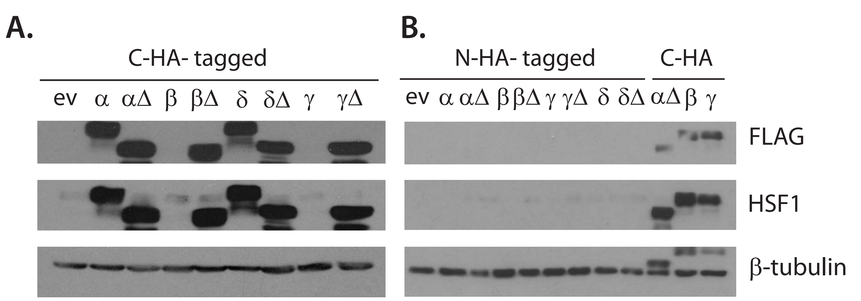


**Figure 4. Western blot analysis detecting HA.** (A) Cells expressing C-HA and (B) N-HA-tagged HSF1 isoforms. β-tubulin is expressed in all extracts, including empty vector (ev). C-HA-β and CHA-γ proteins were not expressed in this experiment, but were detected when the experiment was repeated with new plasmid DNA (Fig. 4B)

## Results

- Epitope tagged constructs were made using PCR, subcloned into pcDNA3.1/V5-His-TOPO vectors, and confirmed by DNA sequencing.
- Sets tested together for protein expression consisted of each of the HSF1 isoforms (α, β, γ, δ) and corresponding constitutive deletion mutants (αΔ, βΔ, γΔ, δΔ) tagged at either the amino terminus (N-FLAG or N-HA) or the carboxy terminus (C-FLAG or C-HA).
- N-FLAG and C-FLAG constructs of HSF1 isoforms are expressed in mouse NIH 3T3 cells and can be identified using anti-FLAG antibodies (Sigma Chemical) or antibodies to HSF1 (Cell Signalling), Figures 3A and 3B.
- C-HA constructs of HSF1 isoforms are expressed in NIH 3T3 cells as confirmed by binding to anti-HA antibodies (Cell Signalling) or to antibodies to HSF1; Figure 4As and 4B.
- N-HA constructs of HSF1 isoforms are either not expressed or encoded proteins are rapidly degraded in NIH 3T3 cells, as only endogenous HSF1 or control protein (β-tubulin) were detected in Western blots; Figure 4B.

## Future Work

**The N-FLAG, C-FLAG, and C-HA constructs will be used in future projects aimed at:**

- Examining HSF1 isoform function (expression studies in mouse NIH 3T3 cells or hsf1-/- mouse embryo fibroblasts).
- Studying subcellular localization of HSF1 isoforms under native vs. stress conditions (immunofluorescence microscopy, as in preliminary experiments shown in Figure 5A and 5B).
- Examining potential interactions of HSF1 isoforms to form heterodimers or trimers (co-immunoprecipitation experiments and analysis on native gels).

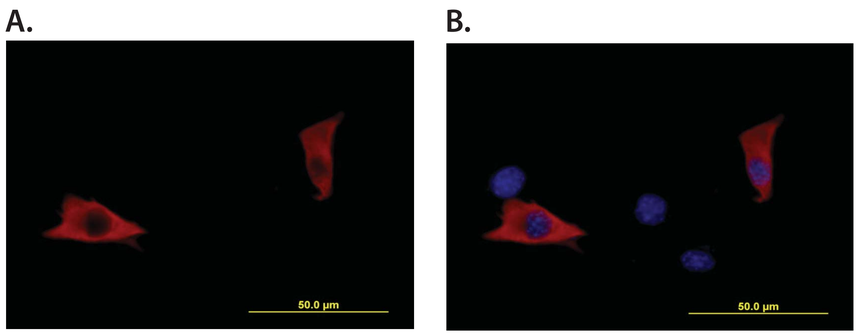


**Figure 5. C-HA-Tagged-HSF1α in NIH 3T3 cells.** The HSF1α isoform was stained with Alexa Red 594 (Red) and the nuclei of the cells were stained blue with DAPI. (A) Alexa Red 594 staining of HA-HSF1α in the cytoplasm. (B) Shows a composite of (A) and DAPI stained nuclei. Images taken by Blake Moses 2010.

## References and Acknowledgements

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