Subcellular localization of Heat Shock Factor 1 α and β isoforms

By Jessica Williams

ABSTRACT

Heat shock causes proteins to denature or unfold which alters their function in the cell. Heat shock transcription factor 1 (HSF1) is responsible for the transcriptional response to protect cells from induced stress. Changes occurring in transcript levels of the cell are a measure of how well the HSF1 binds to the promoter regions. Research has been conducted on different isoforms of the HSF1 protein, predominantly α and β isoforms. Isoforms are versions of a protein that are similar, but not identical, and they may serve distinct functions. The goal of this research is to discover the subcellular localization pattern of the HSF1 α and β isoforms as compared to large deletion mutants (αδ and βδ). The experimental approach involved growing NIH 3T3 cells on fibronectin coverslips. Separate dishes of NIH 3T3 cells on fibronectin coverslips were then incubated with a primary (anti-HA tag) and fluorescent secondary antibody (either red or green), and viewed using fluorescent microscopy to visualize tagged cells. We have observed a different pattern of localization for the α and β isoforms. In the absence of heat shock, HSF1 β seems to be mostly localized in the nucleus, while HSF1 α is distributed in both the cytoplasm and the nucleus. Although we expected heat shock to potentially change the subcellular localization of HSF1 α, our preliminary observations suggest there is no striking redistribution of the HSF1 α after heat shock.

INTRODUCTION

Heat shock transcription factor 1 (HSF1) is a cell regulator that helps prevent protein denaturation and cell damage after stress (Pirkkala et al., 2001). Common stressors in the body include significant temperature changes and toxins. Molecular chaperones assist in the physiological responses that maintain homeostasis, such as shivering when your body temperature drops even a few degrees. Different protein isoforms of HSF1 exist and they differ slightly in structure and function. HSF1 α differs from HSF1 β in having an additional peptide segment of 22 amino acids (Goodson and Sarge, 1995). The α and β isoforms have been studied somewhat, but tend to be interchanged without regard to their structural and potentially functional differences. The goal of this study was to compare the subcellular localization of α and β isoforms, to determine if there are consistent differences, both in the presence and absence of heat shock.

METHODS

Cell culture

The experiment was split into three separate days with three overlapping experiments. Day 1 involved cell culture. The NIH 3T3 cells were trypsinized to remove them from the plate, washed, then centrifuged. The trypsin and medium was removed and cells were resuspended in fresh media lacking antibiotics. The NIH 3T3 cells were then combined and incubated at RT for 5 min. Old medium was removed from the NIH 3T3 cells and new medium (lacking antibiotics) was added; the transfections were incubated overnight.

Antibody incubations and fluorescence microscopy

Day 3 involved immunofluorescence, starting with the removal of the old growth medium and the rinsing of the cells. Cells were then fixed with 4% paraformaldehyde/PBS and permeabilized in PBS containing Triton. The primary antibody (mouse anti-HA tag) was diluted 1:100 or 1:200 and each plate was incubated for 1-2 hours after a brief blocking step in BSA/PBS. After the incubation, multiple washes removed the primary antibody and the secondary antibody. Then, the fluorescent anti-mouse antibody was diluted 1:1000 and added to the plates, which were incubated for another hour. After final PBS washes, the coverslips were washed and rinsed with ddH2O. Rinsed coverslips were mounted on slides with a mounting medium (DAPI) and were allowed to set overnight. Slides were analyzed and imaged using an epifluorescence microscope (Olympus model BX51) and digital camera (DP70).

RESULTS

• Localization of C-HA-tagged HSF1 α was seen mainly in the cytoplasm.
• After heat shock, CHA-tagged HSF1 α redistributed to the nucleus, although some cytoplasm staining was seen.
• Localization of C-HA-tagged HSF1 β was seen mainly in the nucleus.
• A large deletion mutant of HSF1 β (C-HA- βδ) was seen mainly in the cytoplasm.

DISCUSSION

Our data supports that HSF1 isoforms vary in cell location and are affected by heat shock. The α isoform was located in the cytoplasm until the cell was put under stress and it moved to the nucleus, suggesting that α mainly functions to reduce stress from heat shock in the nucleus of the cell.

• The β isoform was mainly seen in the nucleus in the absence of heat shock and has yet to be tested in heat shock conditions.
• These results contradicted research that was previously published by Vujanac et al., 2005 in which they generalized that the HSF1 mostly localizes in the cytoplasm and moves to the nucleus under stress.
• In future research, we will be interested in further comparing different isoforms with and without heat stress. We also want to study other forms of stress that could cause HSF1 activation (eg. peroxide treatment) to see the effect on each isoform.

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LITERATURE CITED