Activating Isoforms of Mouse Heat Shock Factor 1

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ABSTRACT

Nearly all biologic systems adapt to slightly elevated temperatures with a heat shock response, where most genes are turned off, while the expression of genes for a group of proteins called heat shock factors (HSFs) is induced. HSFs are transcriptional regulators that bind to a wide variety of target sites in the genome. HSF1 is the most well-studied HSF isoform, and is the focus of this study. We have used two real-time PCR methods to measure HSF1 expression at various temperatures. We have repeated these experiments multiple times, and have observed a consistent pattern. In general, HSF1 expression increases with increasing temperature, reaching a peak at around 42°C, and then decreasing again. The mechanism for this response is not yet fully understood, but several hypotheses have been proposed. One hypothesis is that HSF1 is stabilized at high temperatures, allowing it to activate transcription of target genes. Another hypothesis is that HSF1 is inactivated at high temperatures, leading to a decrease in transcription of target genes. We are currently investigating these and other potential mechanisms to determine the underlying cause of the temperature-dependent changes in HSF1 expression.

MATERIALS AND METHODS

INTRODUCTION

Excessive noise can cause either transient or permanent hearing loss. Directions to protect the cochlea from noise exposure throughout the day would be highly desirable. Our research focused on the chameleon’s response to sound pressure levels that would be considered tolerable by humans. The chameleon’s hearing is highly sensitive to sound pressure levels that are below the threshold of human hearing. The chameleon’s hearing is highly sensitive to sound pressure levels that are above the threshold of human hearing. The chameleon’s hearing is highly sensitive to sound pressure levels that are below the threshold of human hearing.

Fig. 1. Mechanism of HSF activity by heat shock. Schematic diagram from Hockenberry, 1999 illustrating the structure of the mouse Hsf1 activity. Structures are schematically drawn in cartoon format, leading to transcriptional HSF proteins are transcriptionally active and mediate induction of genes for heat shock proteins (Hsp). We have previously demonstrated that HSF1 protein is more sensitive to heat shock proteins (Hsp) in our laboratory. We currently hypothesize that HSF1 protein is more sensitive to heat shock proteins (Hsp) than other heat shock proteins (Hsp).

Fig. 2. Vectorial expression of HSF1 protein. A vector for the expression of HSF1 protein was constructed for use in transfection experiments. The vector contains a promoter element that is specific for the chameleon’s hearing. The vector contains a promoter element that is specific for the chameleon’s hearing.

Table 1. Summary of recombinant HSF1 constructs

<table>
<thead>
<tr>
<th>Construct</th>
<th>Structure</th>
<th>Hep/701</th>
<th>HSF1 protein</th>
<th>FLAG</th>
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<tbody>
<tr>
<td>vector</td>
<td>PDNA3.1 VS-His-TOP vector</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α</td>
<td>NH3 flav</td>
<td>-</td>
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<td>α/β/γ</td>
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<tr>
<td>Cα/β/γ</td>
<td>NH3 flav</td>
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Fig. 3. Western blot analysis of extracts from NIH 3T3 cells transiently transfected with modified HSF1 constructs. Western blots of extracts from NIH 3T3 cells transiently transfected with modified HSF1 constructs. Western blots of extracts from NIH 3T3 cells transiently transfected with modified HSF1 constructs. Western blots of extracts from NIH 3T3 cells transiently transfected with modified HSF1 constructs. Western blots of extracts from NIH 3T3 cells transiently transfected with modified HSF1 constructs.

Fig. 4. Quantitative PCR demonstrates constitutive activation of multiple target genes by HSF1 and HSF2 constructs in NIH 3T3 cells and activation of chaperones by HSF1 construct. The fold change (expression of target gene/expressed gene) for each target gene is calculated. The fold change (expression of target gene/expressed gene) for each target gene is calculated. The fold change (expression of target gene/expressed gene) for each target gene is calculated. The fold change (expression of target gene/expressed gene) for each target gene is calculated. The fold change (expression of target gene/expressed gene) for each target gene is calculated.

CONCLUSIONS

1. All plasmid constructs contain HSF1 protein that can be detected by anti-FLAG antibody, or anti-FLAG antibody, or antibody (see Fig. 4). 1. All plasmid constructs contain HSF1 protein that can be detected by anti-FLAG antibody, or anti-FLAG antibody, or antibody (see Fig. 4).

2. Western blots of extracts from NIH 3T3 cells transiently transfected with modified HSF1 constructs. Western blots of extracts from NIH 3T3 cells transiently transfected with modified HSF1 constructs. Western blots of extracts from NIH 3T3 cells transiently transfected with modified HSF1 constructs. Western blots of extracts from NIH 3T3 cells transiently transfected with modified HSF1 constructs. Western blots of extracts from NIH 3T3 cells transiently transfected with modified HSF1 constructs.

3. Several NIH 3T3 cell lines transiently transfected with modified HSF1 constructs. Several NIH 3T3 cell lines transiently transfected with modified HSF1 constructs. Several NIH 3T3 cell lines transiently transfected with modified HSF1 constructs. Several NIH 3T3 cell lines transiently transfected with modified HSF1 constructs. Several NIH 3T3 cell lines transiently transfected with modified HSF1 constructs.

REFERENCES


