# Using a Sequence-Specific Chromatin Remodeling Protein to Alter Heat Shock Response in S. Cerevisiae

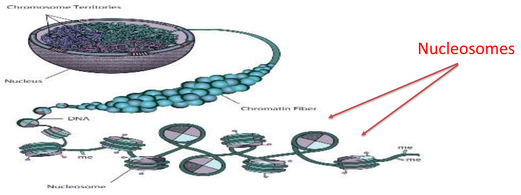
**Uriel Jean-Baptiste[1], Zena Jensvold[2], Laura McKnight[2], Jeffrey McKnight[2]**
**[1]Florida State University, 600 W College Ave, Tallahassee, FL, 32306 ; [2] University of Oregon, Institute of Molecular Biology, 1318 Franklin Blvd, Eugene, OR 97403**

## Abstract

Eukaryotic DNA is packaged into chromatin to fit in the nucleus. One of the organizational subunits of chromatin are nucleosomes, which are composed of DNA wrapped around histone proteins. These nucleosomes can be rearranged by proteins called chromatin remodelers. For this project, we looked at the nucleosome remodeling protein Chd1. It has been shown that the Chd1 protein fused with a foreign DNA binding domain (DBD) can remodel nucleosomes toward the specific site of that foreign DBD (McKnight et al. 2011). We are using this knowledge to design fusion proteins where the DBD of Chd1 has been replaced with a variety of different domains, targeting Chd1 to different sites in the genome. The specific fusion for this project used the DBD of Hsf1, a protein that recognizes a conserved heat shock element (HSE) motif in the yeast S. cerevisiae. When induced, Hsf1 turns on stress genes that allow the cells to survive in stressful environments. With this foreign DBD fused to the Chd1 remodeler, we aim to target nucleosomes to genomic HSE sites, blocking access for Hsf1 and thereby modulating the heat shock response. This project will demonstrate the feasibility of using engineered nucleosome positioning to control a physiological response in vivo.

## Introduction

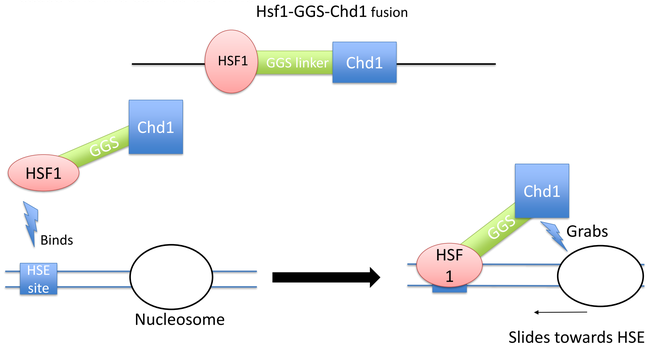
- The chromatin is a macromolecule consisting of DNA, RNA, and proteins. The nucleosome is one of the organizational subunits of the chromatin and consists of 8 histone proteins. Between each nucleosome is linker DNA that can be accessed and transcribed, but wrapped around the nucleosome is ≈147 base pairs of DNA that cannot be accessed for transcription, so that section is essentially silenced.




- In the McKnight lab we are interested in finding the location of the nucleosomes in the genome and move them to specific sites to turn off certain genes. By using the catalytic core of the chromatin remodeler Chd1 with the DNA binding domain of numerous transcription factors (TF), such as; gal4, xbp1, as well as the RNA guided endonuclease dcas9 and many more, we are able to move the nucleosome to the recognized site of that transcription factor. With the chd1 catalytic core fused to so many TF, we are building an arsenal of nucleosome remodelers that can block the transcription of any desired gene.

- The TF I am working with is Hsf1, and it is responsible for inducing a heat shock response when the cell is in a highly stressful environment. This Hsf1 protein binds both constitutively and inducibly to the HSE site. In an unstressed cell, the Hsf1 protein is constitutively phosphorylated, but when the cell is stressed, the protein becomes hyper-phosphorylated and adopts an activated conformation.

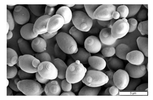
- If our Chd1-Hsf1 protein should move the nucleosome to the HSE site and keeps the Hsf1 protein off of the chromatin, then we expect the heat shock response to cease and the cells to die when stressed.




## Methods

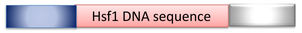

Flag Cassettes with Hsf1 homology were amplified by PCR and transformed into yeasts cells This flag tag will help us keep track of HSF1 binding.

Transformation

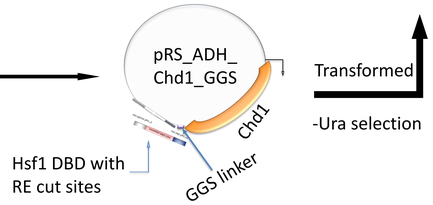


End product is a yeast strain with our flag tag and the plasmid with our Chd1-Hsf1 fusion protein!

Using yeastgenome.org, we determined the DNA binding Domain of the Hsf1 protein and added HindIII and AfeI sites at the ends of the DNA sequence.




2nd half AfeI site  1st half HindIII site




Transformed

-Ura selection

We ligated the Hsf1 DNA binding domain to a plasmid containing the Chd1 catalytic core along with the same restriction enzyme cut sites.

Questions:
**1)** Will our fusion protein prevent the heat shock response in the yeast cells.
-We exposed two yeast strains with only our Flag tag along with two others with our fusion protein to three different temperatures: 30°C, 42°C, and 50°C. We expect the yeast strains with just our flag tag to be able to tolerate higher temperatures.
**2)** Does our protein displace Hsf1 at their target sites?

## Results

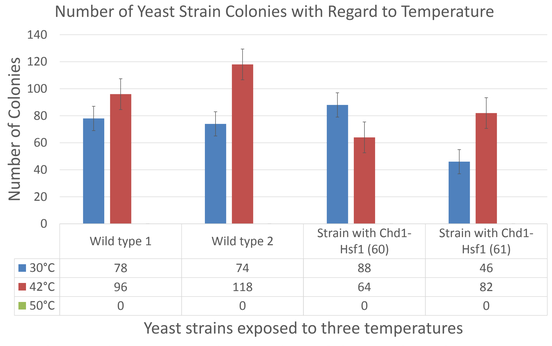



Figure 1: This graph represents the raw data of the number of yeast strains with regards to the three different temperatures. On this graph, the number of colonies is plotted against the four yeast strains under the three different temperatures. As shown, more colonies survived at 42°C , except for yeast strain 60, and no colonies survived at 50°C.

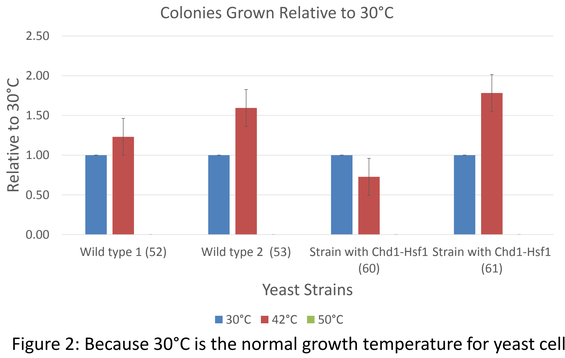



Figure 2: Because 30°C is the normal growth temperature for yeast cells, this graph is the representation of the number of colonies that grew when exposed to 42°C and 50°C relative to the number of colonies that grew when exposed to 30°C.

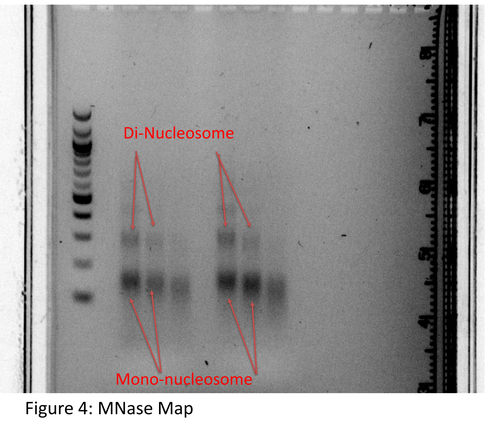



Figure 4: MNase Map
This figure represents an MNase map of the yeast chromatin. The arrows are pointing at mono and di-nucleosomes. We can map the positions of nucleosomes by sequencing.

## Discussion

**Question 1:**
- We were not expecting the samples that were exposed to high temperatures to have more colonies grown than the cells that were exposed to the 30°C.
- After replica-platting the cells on a different plate that selects for cells that lack our plasmid, we discovered that the colonies grew on those other plates.
- This suggests that there was a selection pressure against our fusion protein, thus, the yeast threw our plasmids out.
- This selection pressure and pipetting error could explain why so many colonies Grew from samples exposed to higher temperatures.

**Question 2:**
- The MNase seq. results are important for in vitro sliding assays with our fusion protein.
- We can use the MNase seq. digested chromatin to see if our fusion protein was functional.

## Future Work

- To better explain our results, we plan on performing more heat shock tests with our yeast strains.
- Our fusion protein is ligated into an expression vector, so the next step is to express and purify the protein for in vitro experiments.
- We will map genomic positions of nucleosomes with and without our fusion proteins and track Hsf1-flag binding.

## Acknowledgements

I would like to thank Jeffrey McKnight for allowing me to be a member of the McKnight lab for these past ten weeks, it was a wonderful time and I had a lot of fun. Furthermore, I would like to thank Laura McKnight and Zena Jensvold for being my mentors and giving me this wealth of knowledge. I have gained a lot from being in this lab and I will never forget this experience.

**Reference**: McKnight, J. N.; Jenkins, K. R.; Nodelman, I. M.; Escobar, T.; Bowman, G. D. Molecular and Cellular Biology 2011, 31 (23), 4746–4759.

This material is based upon work supported by the National Science Foundation award #1460735. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.