The Influence of Promoter Architecture on Targeted Gene Activation by Complex Transcription Factor Signaling

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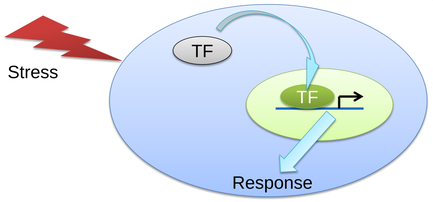
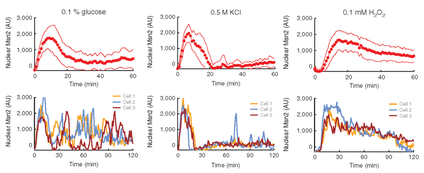
Abstract

Our studies focused on Msn2, a transcription factor in budding yeast responsible for mediating the response to a number of stresses, such as oxidative stress and starvation. Based on the identity of the stressor, Msn2 enters the nucleus with different patterns, such as entering and leaving the nucleus rapidly or periodically. However, how the promoter is able to understand these different signals is not well understood. Our project's goal was to elucidate how the positioning of histones within promoter sequences allowed genes to be differentially activated by these various signals. We selected the DDR2 gene promoter, which possesses Msn2 binding sites both in and out of its well-defined nucleosome binding positions. Then, we systematically modified the position of its binding sites relative to the nucleosomes and utilized a fluorescent reporter that allowed us to quantify relative expression levels. We focused on how the promoter processes three aspects of the incoming Msn2 signal: the duration (how long Msn2 is present in the nucleus), the intensity (the maximum amount of Msn2 within the nucleus), and the frequency of oscillations (how quickly we move Msn2 in and out of the nucleus). We then fit these results to a set of differential equations describing how transcription factor binding can lead to protein production. With these results, we hope to identify how all of these binding sites cooperate to analyze complex Msn2 signaling.

Background



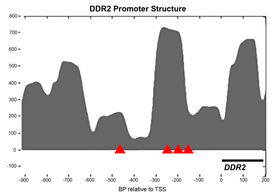
- Upon stress, the cell will often activate transcription factors, which subsequently translocate to the nucleus to induce expression of appropriate stress response genes



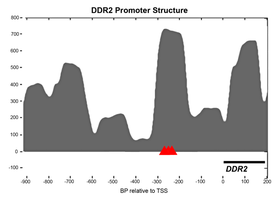
- Msn2 is a general stress transcription factor found in *Saccharomyces cerevisiae* [1]. It has been shown to translocate into the nucleus with different patterns based on different types of stresses [2]
- **Purpose**: How is the same transcription factor able to activate a presumably different gene expression profile using these different signals? Quantitatively characterizing how the promoter region is able to process these complex signals would be illuminating not only for the general stress response, but also gene regulation in higher organisms
- Chromatin might be part of the answer, since it has been shown to influence the threshold and dynamic range of promoters [3]
- **Specific Goal**: To elucidate how the positioning of Msn2 binding sites relative to nucleosomes influences the promoter's ability to process these complicated signals

Experimental Approach

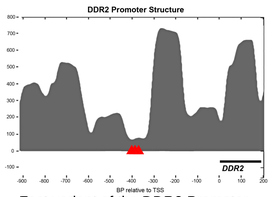
Generation of Promoter Variants



Promoter Architecture for DDR2: Nucleosome positioning was determined with MNase-seq and depicted as gray peaks. Red triangles depict locations of Msn2 Binding Motifs

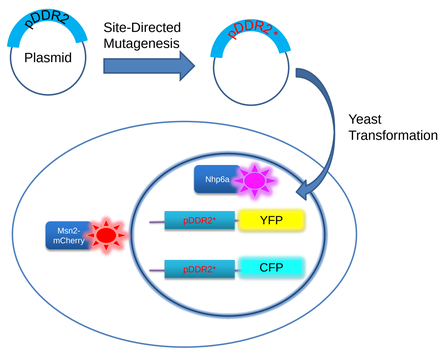


Slow variant of the DDR2 Promoter



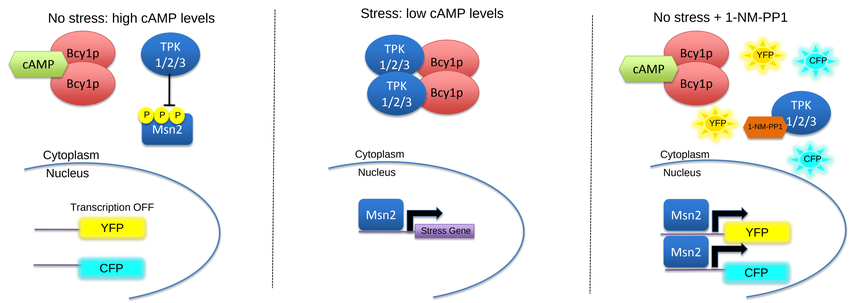
Fast variant of the DDR2 Promoter

- We selected the DDR2 promoter for its well defined nucleosomes and multiple Msn2 binding sites both in and out of the nucleus
- We then formed a dozen mutants manipulating the positioning of Msn2
  - Four of the mutants consisted of a single binding site knockout
  - Two of the mutants were a "slow" and "fast" variant consisting of three binding sites within and outside of a nucleosome, respectively
  - The remaining represented a spectrum of promoter speeds from the slow and fast promoters

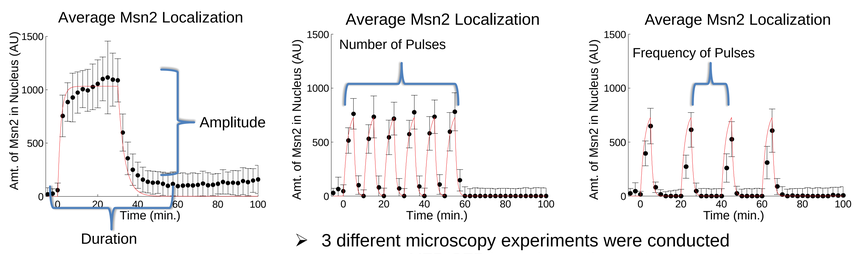


- Promoter variants were first formed using site-directed mutagenesis
- Mutants were then transformed upstream of a YFP and CFP dual reporter in a preexisting yeast strain with Msn2 and Nhp6a (a nuclear protein) fluorescently tagged to track Msn2 and identify the nucleus

Controlling Msn2 Signals



- Msn2 is normally prevented from entering the nucleus by phosphorylation by PKA
- By adding an inhibitor for PKA, it is possible to force Msn2 in and out of the nucleus on the order of a few minutes



- 3 different microscopy experiments were conducted measuring YFP/CFP intensity, while manipulating key features of the incoming Msn2 signal

Analyzing Gene Expression

$$P_0 \underset{d_1}{\overset{\frac{k_1 \cdot [TF]^n}{K_d + [TF]^n}}{\rightleftharpoons}} P_1 \xrightarrow{k_2} \text{mRNA} \xrightarrow{k_3} \text{pre-YFP} \xrightarrow{k_4} \text{mature-YFP}$$

$$\frac{d[P_0]}{dt} = d_1 \cdot [P_1] - \frac{k_1 [TF]^n}{K_d + [TF]^n} \quad (1)$$

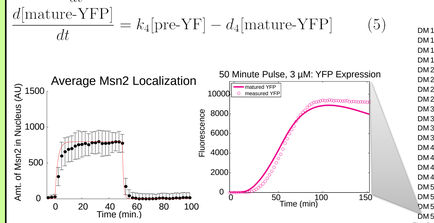
$$\frac{d[P_1]}{dt} = \frac{k_1 [TF]^n}{K_d + [TF]^n} - d_1 [P_1] \quad (2)$$

$$\frac{d[\text{mRNA}]}{dt} = k_2 [P_1] - d_2 [\text{mRNA}] \quad (3)$$

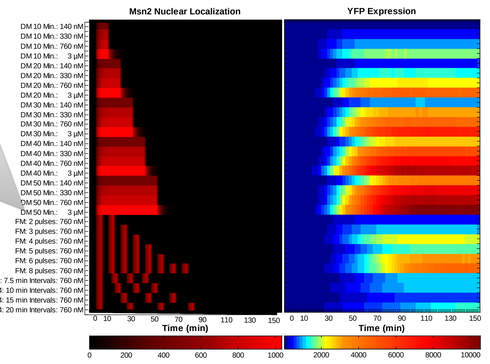
$$\frac{d[\text{pre-YFP}]}{dt} = k_3 [\text{mRNA}] - (d_3 + k_4)[\text{pre-YFP}] \quad (4)$$

$$\frac{d[\text{mature-YFP}]}{dt} = k_4 [\text{pre-YF}] - d_4 [\text{mature-YFP}] \quad (5)$$

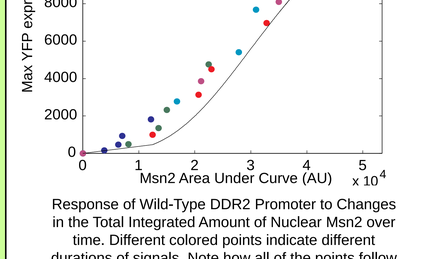
- For each cell over a time period of 2.5 hours, Msn2 localization and CFP/YFP expression were measured
- The results were fit to a set of ordinary differential equations (ODEs) describing activation of the promoter region followed by transcription of mRNA and translation of fluorescent protein [3]



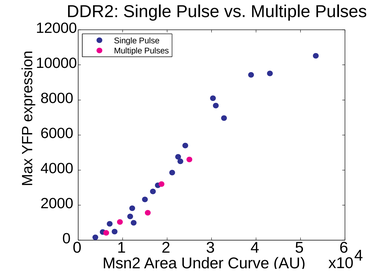
Typical Results of Microscopy Experiment



Gene Expression Data from all 30 Conditions



Response of Wild-Type DDR2 Promoter to Changes in the Total Integrated Amount of Nuclear Msn2 over time. Different colored points indicate different durations of signals. Note how all of the points follow the same general pattern regardless of duration.



Response of Wild-Type DDR2 Promoter to single vs. multiple pulses. As can be seen, DDR2 responds similarly with both the single pulse and multiple pulses

Conclusions

- DDR2 appears to respond strongly to Amplitude Modulation, but not Duration or Frequency Modulation. It effectively integrates the total amount of Msn2 in the nucleus over time
- Other properties of the DDR2 promoter, such as the threshold and dynamic range, can also be inferred from the data
- The next immediate step is to characterize all of the mutants and compare the behavior with the wild-type
- Futures studies could:
  - Characterize using ChIP-Seq how Msn2 binding changes over time after induction with the inhibitor
  - Try to create a completely synthetic promoter and predict its behavior given predicted promoter architecture and positioning of Msn2 binding sites

Acknowledgments

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2. Lam, F.H., Steger, D.J., and O'Shea, E.K., "Chromatin decouples promoter threshold from dynamic range". *Nature*, 2008. **453**(7192): p. 246-50.
3. Kim, H.D. and O'Shea, E.K., "A quantitative model of transcription factor-activated gene expression". *Nature Structural & Molecular Biology*, 2008. **15**(11): p. 1192-8.