

Combinatorial Control of MIG1 & MIG2 are Likely Regulators of the Early Cold Response in Yeast (*S. cerevisiae*)

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Biomathematical Modeling (BIOL 388)

Outline

- Background on cold shock response in yeast
- Objectives
- Methods
- STEM Analysis reveals 7 significant gene clusters
- Profile 45 associated with RNA and ribosomal regulation & Profile 22 associated with cell recovery
- GRNmap and GRNsight models illustrate a need for increased data collection
- Proposed transcription factors involved in regulating cold shock response
- Future directions

Background: Cold Response in Yeast (*S. cerevisiae*)

- Environmental stress response (ESR) regulates gene expression when exposed to environmental stress
- Cold response in yeast includes an early and late cold response
 - Late cold response is consistent with ESR transcriptional changes
 - Early cold response shows opposite expression profiles compared to ESR genes from previous findings (Schade et al., 2004)
 - Little known about which transcription factors regulating early cold response
- DNA microarray experiments have been demonstrated to be effective in visualizing and quantifying transcriptional changes

Experimental Purpose

Generate a gene regulatory model for the early cold response in yeast using microarray data

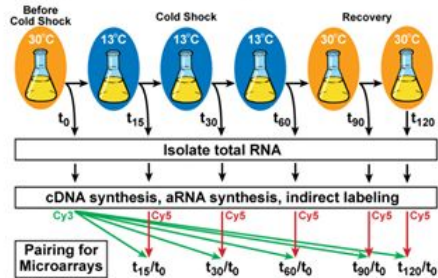
Questions

1. What is the relative contribution of each transcription factor to the regulation of gene expression?
2. How do in silico alterations affect the accuracy of the model?

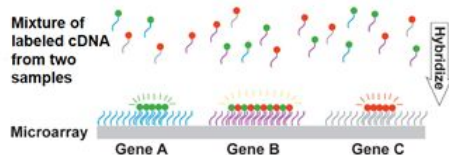
Methods

DNA Microarray

Experimental Design and Methods



DNA Microarrays Measure the Expression of Thousands of Genes Simultaneously



Data Analysis

ANOVA

STEM Clustering

YEASTRACT

Gene Regulatory Matrix (GRNsight)

$$\frac{dx_i}{dt} = \frac{P_i}{1 + e^{-(\sum \omega_{ij} x_j - b_i)}} - d_i x_i$$

GRNmap Modeling

Model Visualization (GRNsight)

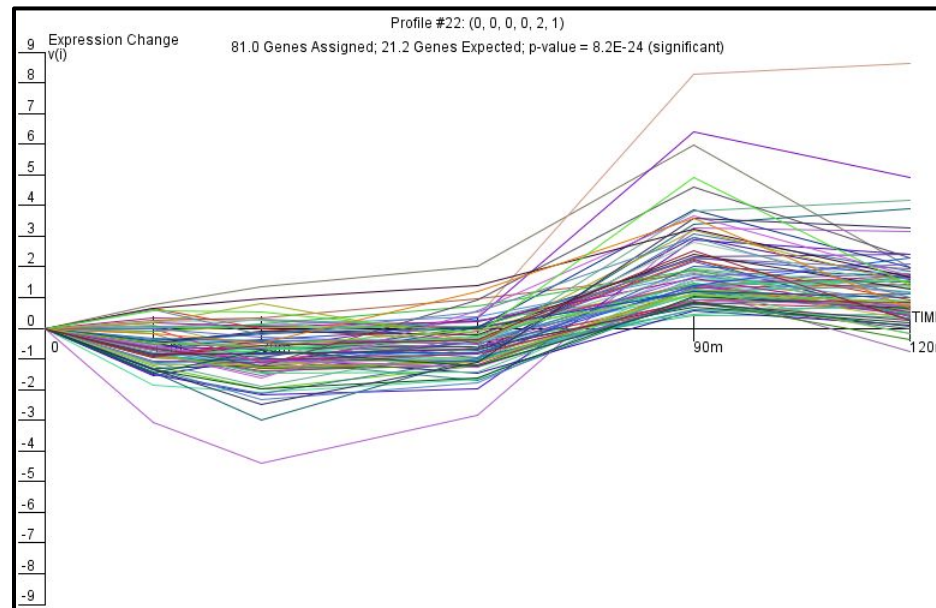
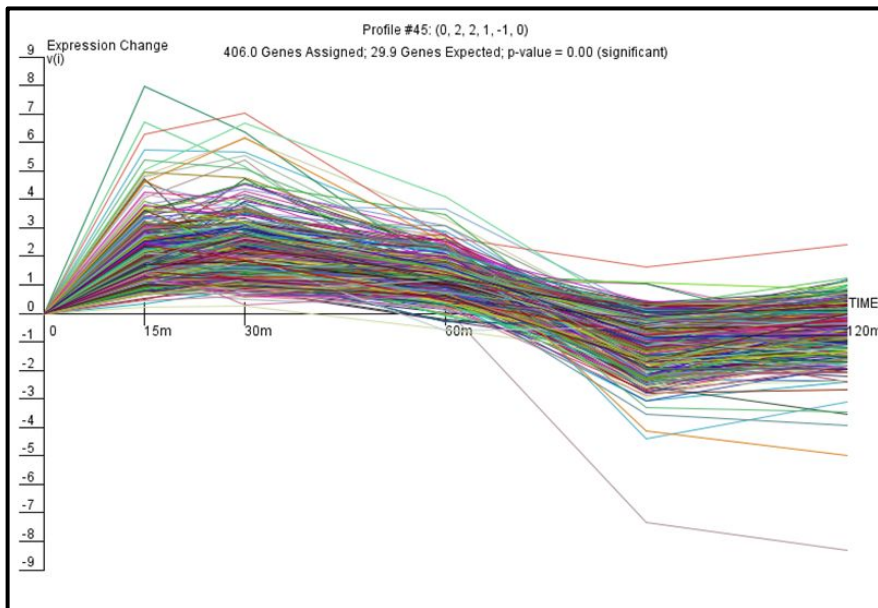
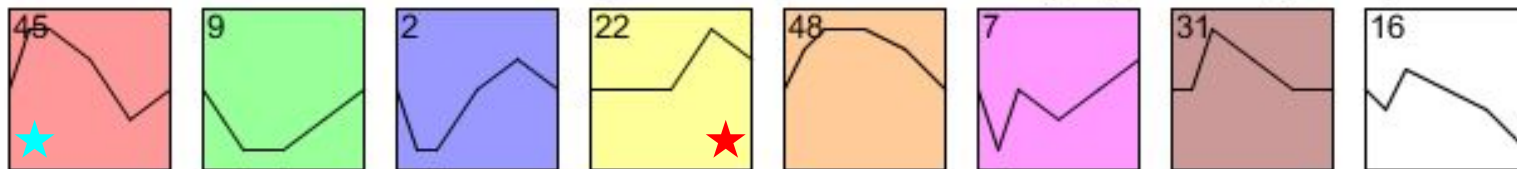
In silico
experiments

The Bonferroni p-value Resulted in the Least Amount of Genes with a Significant Change in Gene Expression at Any Time-point

ANOVA	<i>ΔGLN3</i>
p < 0.05	2135 (34.5%)
p < 0.01	1204 (19.5%)
p < 0.001	514 (8.31%)
p < 0.0001	180 (2.91%)
B & H p < 0.05	1185 (19.1%)
<u>Bonferroni</u> p < 0.05	45 (0.727%)

Significant Gene Clusters for dGLN3

Clusters ordered based on number of genes and profiles ordered by significance (default)



Significant gene ontology terms for profiles 45 and 22

Profile 45 GO Terms: Ribosome and RNA Regulation

Ribosome assembly

Ribonucleoprotein complex export from nucleus

snRNA metabolic process

Nucleolar metabolic process

Nitrogen compound metabolic process

snoRNA binding

Profile 22 GO Terms: Metabolism and Transport

Amide transport

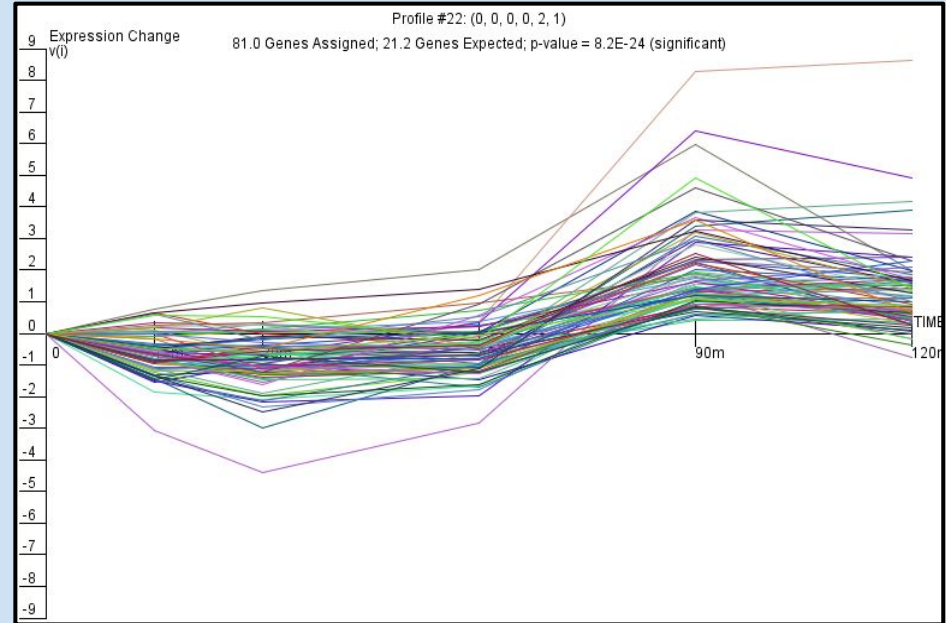
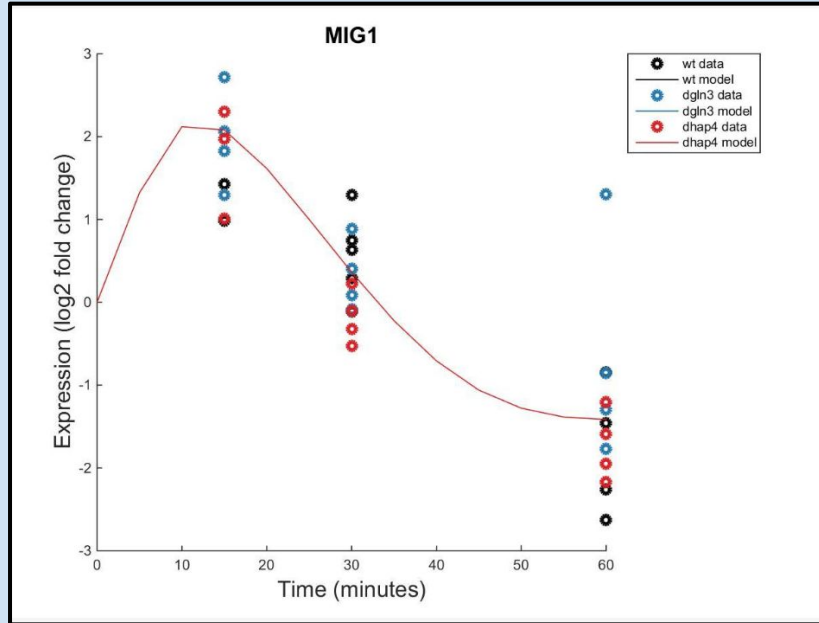
Cytoplasmic Vesicle

Membrane protein complex

Organonitrogen compound metabolic process

Phosphorus Metabolic Process

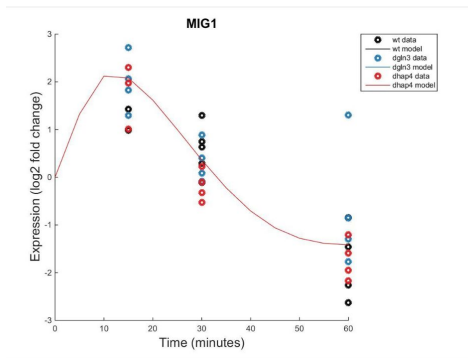
MIG1 as Most Dynamic Gene in Profile 22 Cluster



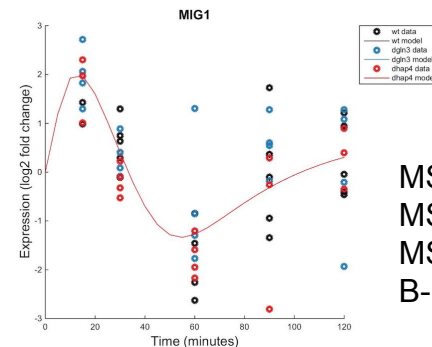
MIG1 involved in glucose repression (Kayikci & Nielsen, 2015).

Extending Data Points Reveals Gene Behavior During Recovery

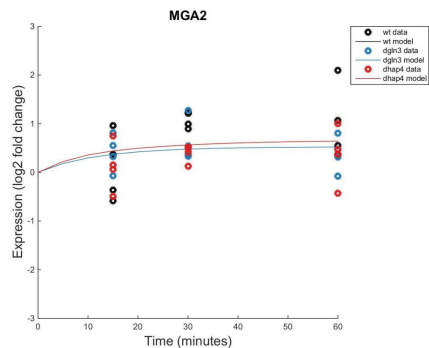
MSE WT: 0.606
MSE dGLN3: 0.791
MSE dHAP4: 0.686
B-H p-value: 0.077



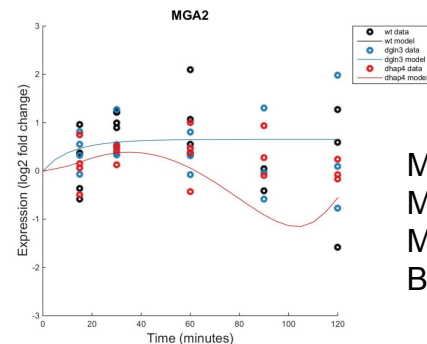
MSE WT: 0.741
MSE dGLN3: 0.982
MSE dHAP4: 0.918
B-H p-value: 0.077



MSE WT: 0.432
MSE dGLN3: 0.132
MSE dHAP4: 0.233
B-H p-value: 0.416

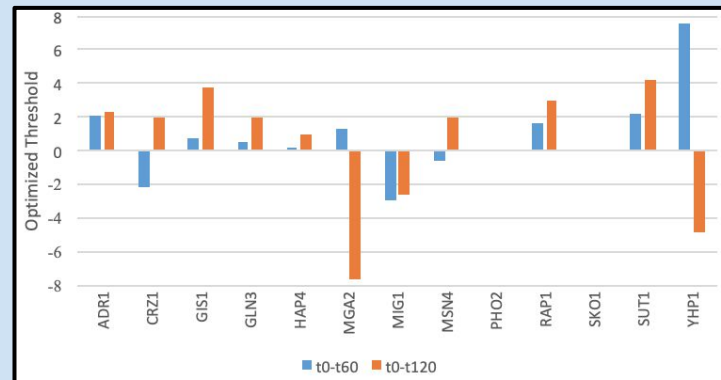
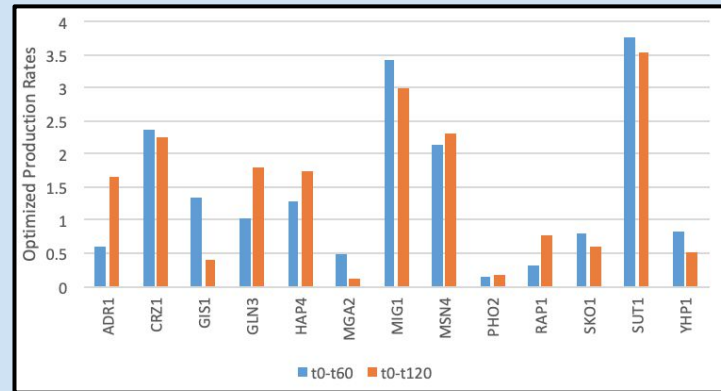
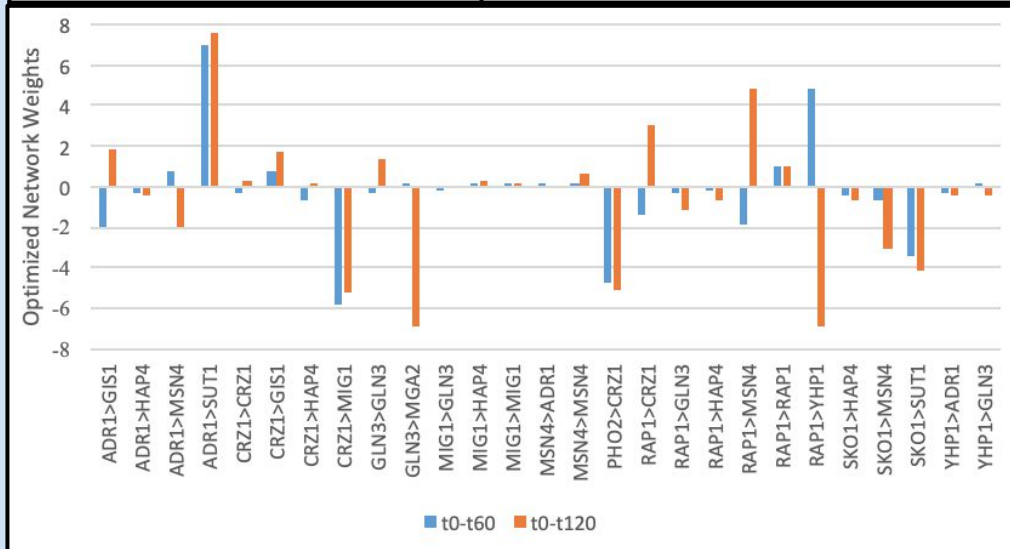


MSE WT: 2.235
MSE dGLN3: 0.428
MSE dHAP4: 0.489
B-H p-value: 0.416



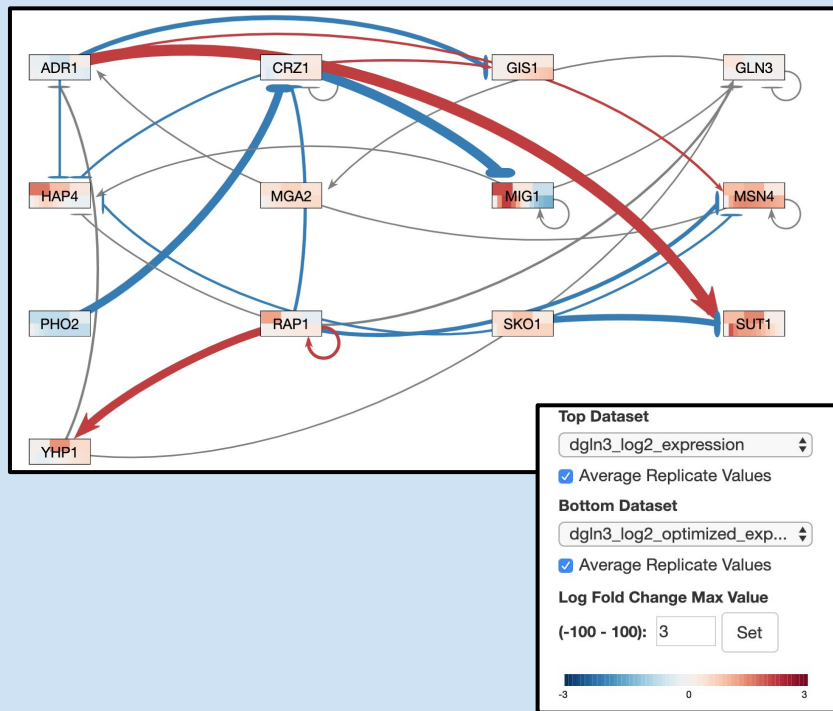
Including t90 and t120 Timepoints had Considerable Effect on Optimized Thresholds and Network Weights

Run	LSE:minLSE
t0-t60	1.331
t0-t120	1.436

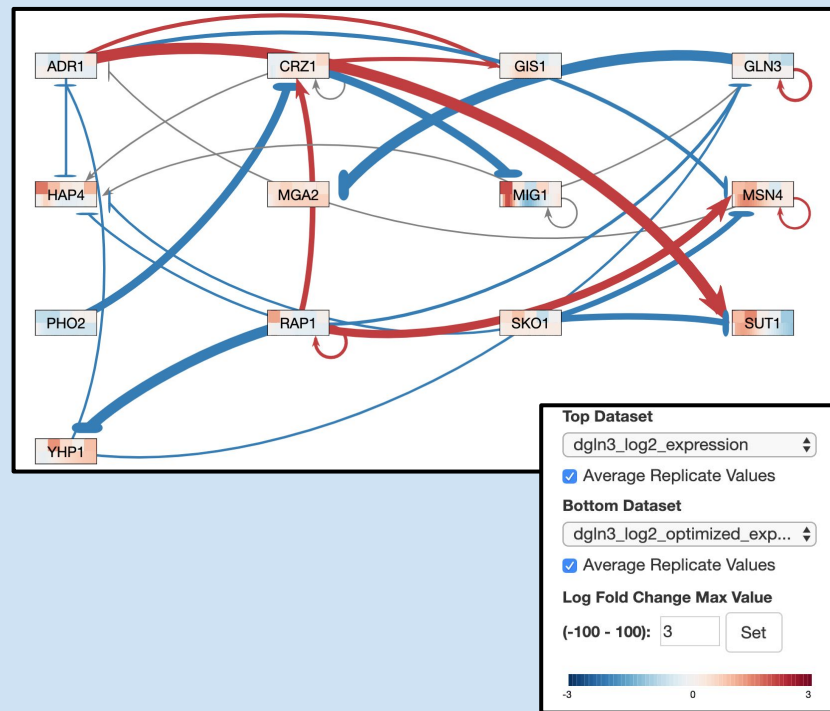


Extending Data Points Caused Change in Network Relationships

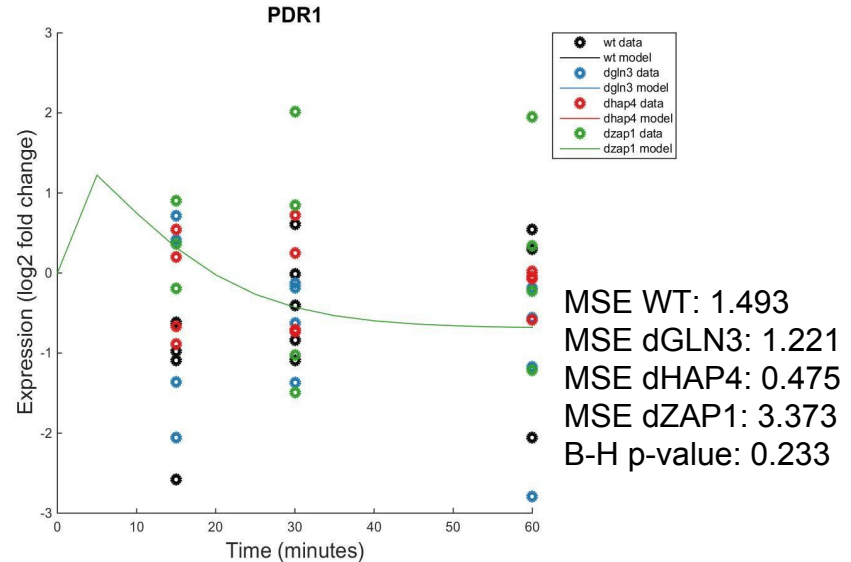
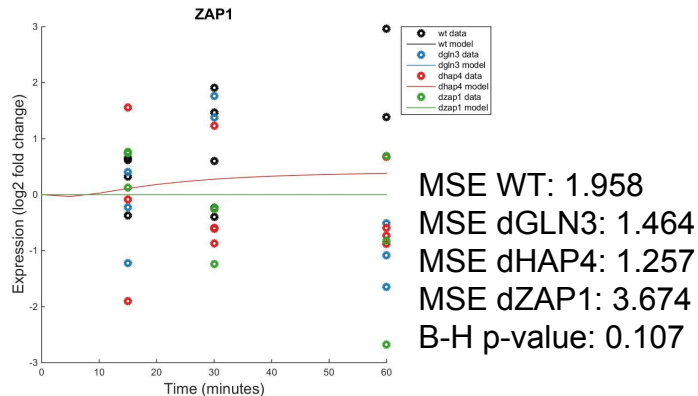
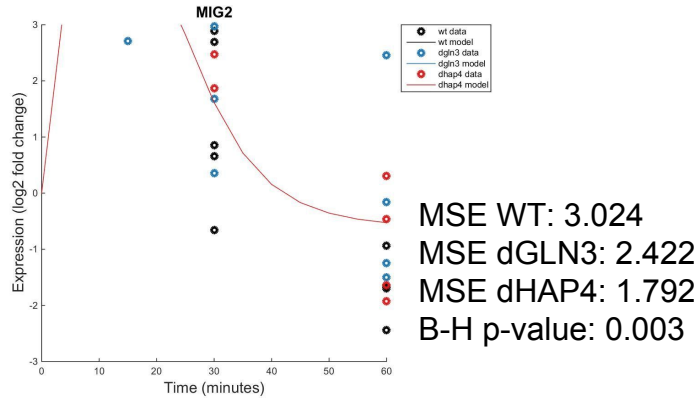
t0-t60 dGLN3



t0-t120 dGLN3

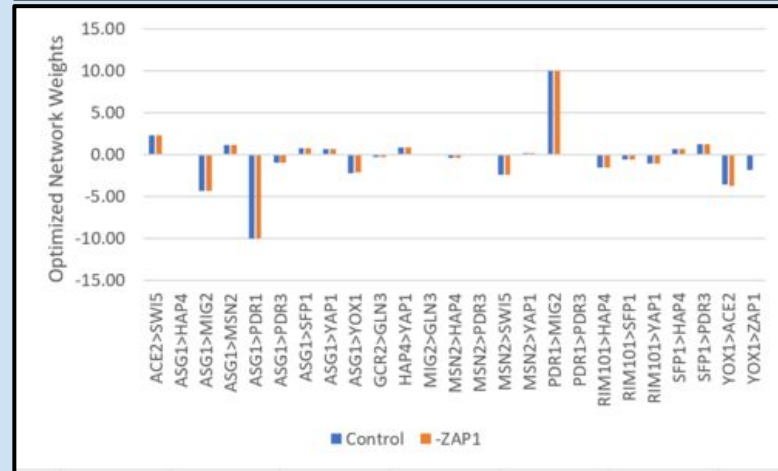
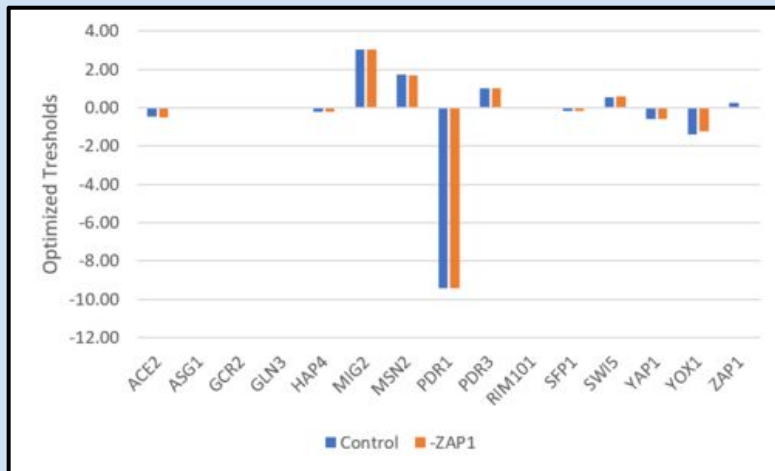
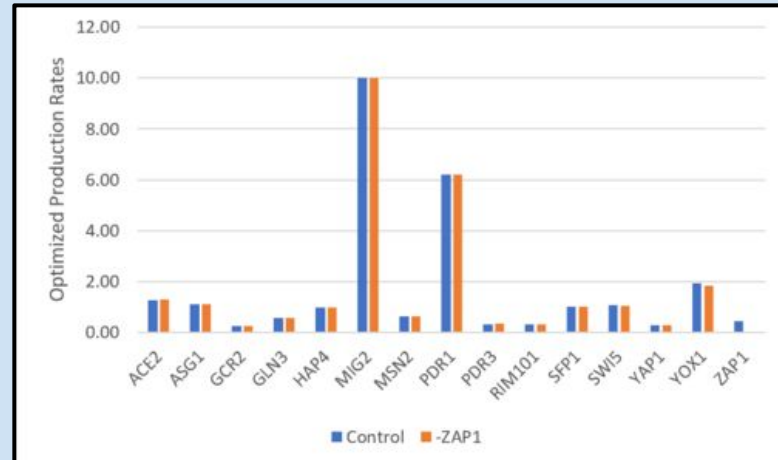


Data May Need to be Collected Prior to 15 minutes for “Full Picture”



Removal of ZAP1 Had Minimal Effect on Model

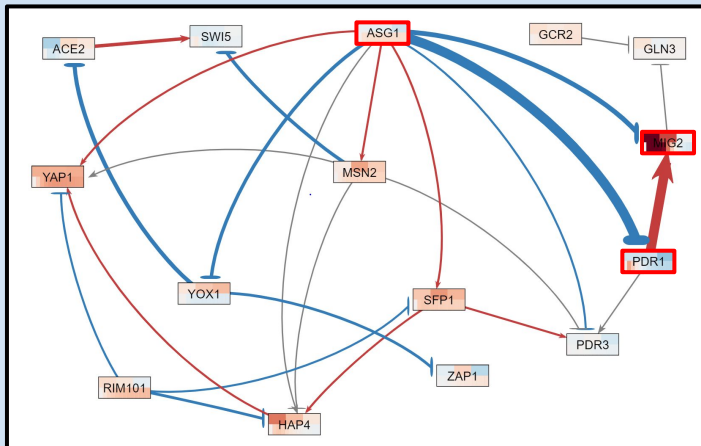
Run	LSE:minLSE
Initial	1.551
Without ZAP1 Control	1.505
Without ZAP1	1.450



Transcription Factors Likely to Regulate Cold Response

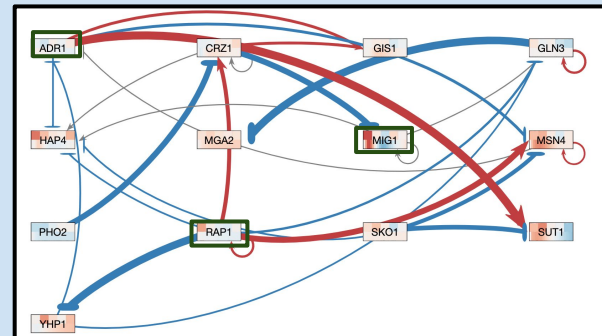
Profile 45

ASG1	Most edges
★MIG2	High b and P
PDR1	Most repressed, strongest activator



Profile 22

ADR1	Numerous edges, strongest activator
★MIG1	Most dynamic
RAP1	Controls two genes that reverse regulation behavior



Summary & Conclusions

- Genes of profile 22 are largely associated with cell recovery after experiencing cold shock.
 - GO terms suggest that genes of this profile help restore normal metabolic and homeostatic activity after cold shock disruption.
- Including t90 and t120 timepoints did not improve the fit of the model, but helped give a better understanding of how the cell is able to recover.
- Genes of profile 45 are associated with RNA and ribosomal regulation.
- Removing ZAP1 had minimal effects on the model.
- MIG1 & MIG2 are likely regulators of the transcriptional response to cold shock.
 - Effectors in glucose repression--repress transcription of genes involved in metabolism of poor-carbon sources in presence of glucose (Karunanithi & Cullen, 2012)

Future Directions

- Examine other significant profiles
- Collect data for time-points before 15 minutes
- Run models separately
 - t0-t60 versus t90-t120 to compare cold shock and recovery period distinctly

Acknowledgments

We would like to thank:

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Dr. Fitzpatrick

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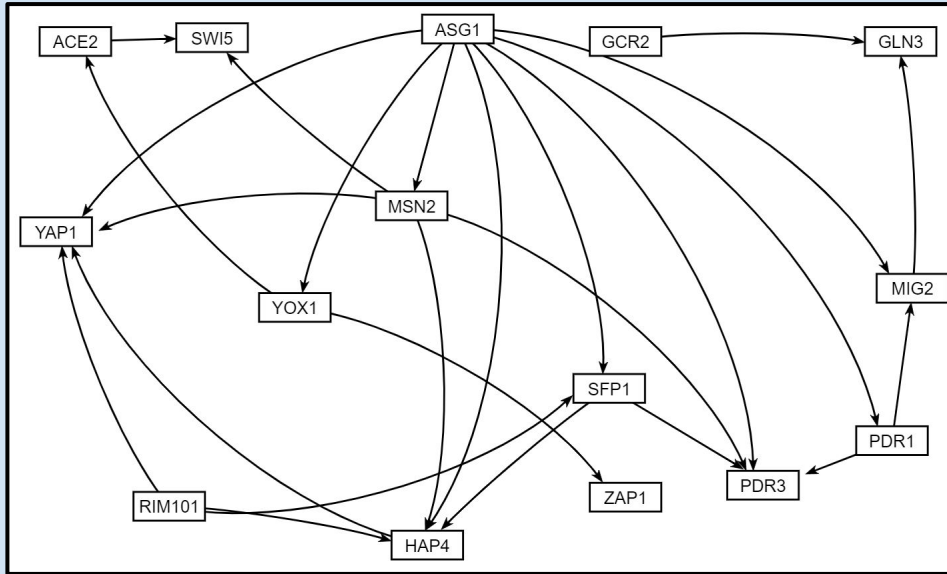
References

Karunanithi, S., & Cullen, P. J. (2012). The filamentous growth MAPK pathway responds to glucose starvation through the Mig1/2 transcriptional repressors in *Saccharomyces cerevisiae*. *Genetics*, 192(3), 869-887.

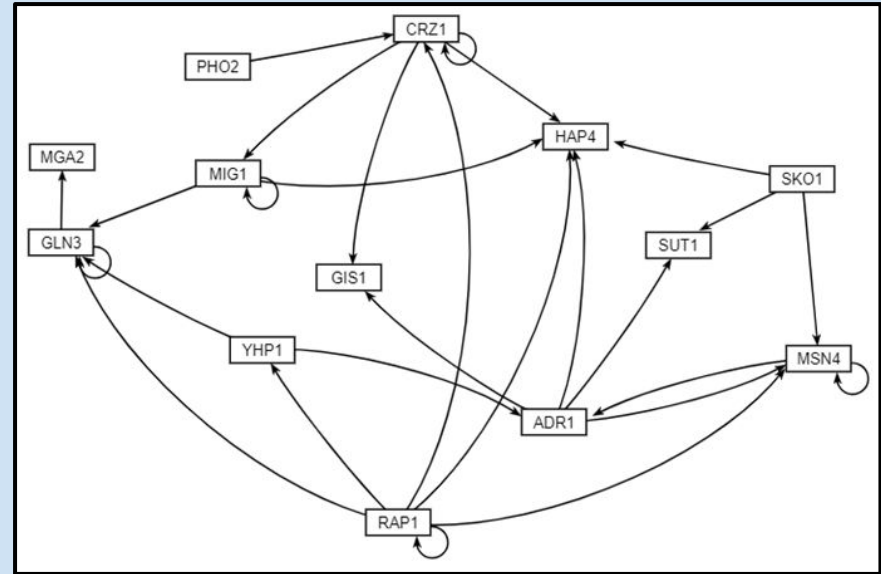
Kayikci, Ö., & Nielsen, J. (2015). Glucose repression in *Saccharomyces cerevisiae*. *FEMS yeast research*, 15(6), fov068.

Schade, B., Jansen, G., Whiteway, M., Entian, K. D., & Thomas, D. Y. (2004). Cold adaptation in budding yeast. *Molecular biology of the cell*, 15(12), 5492-5502. DOI: 10.1091/mbc.e04-03-0167

Unweighted regulatory gene networks



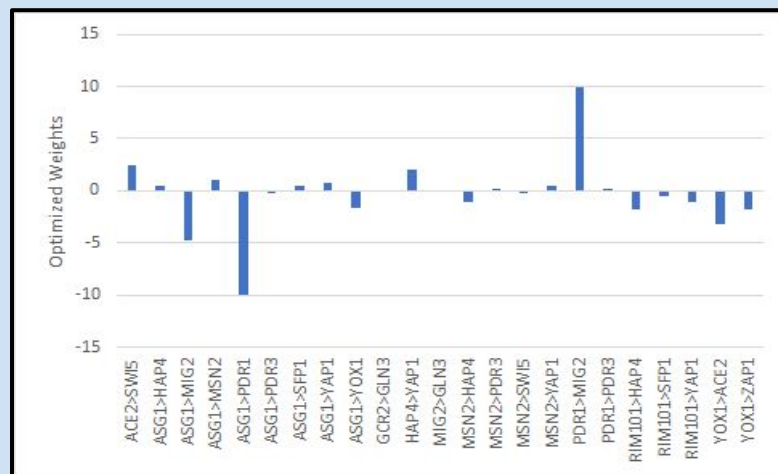
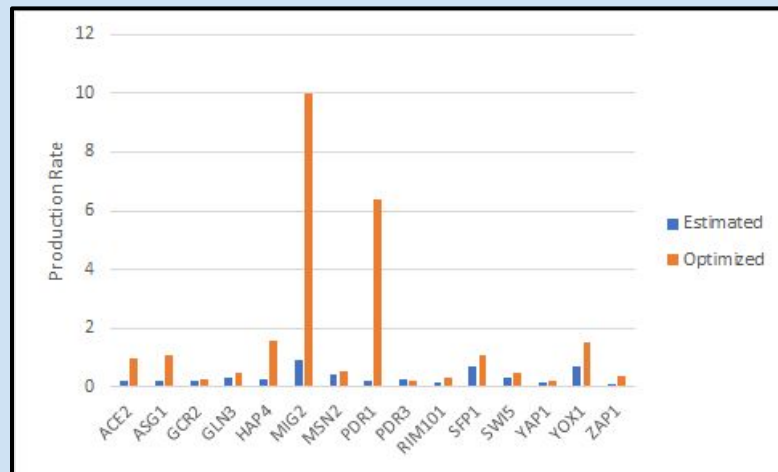
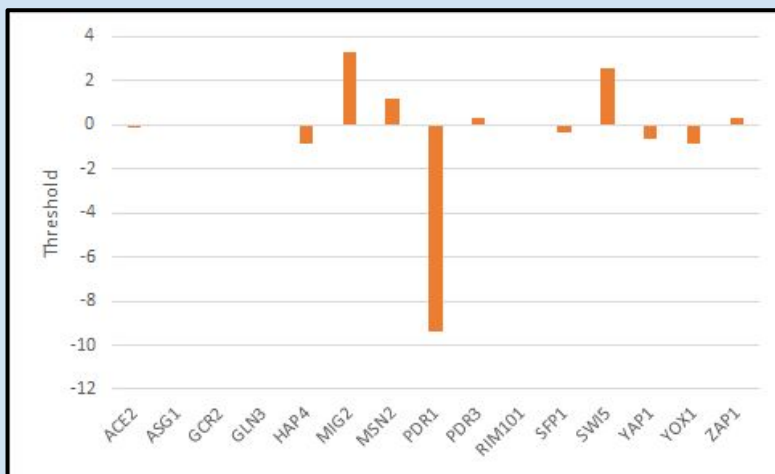
Profile 45



Profile 22

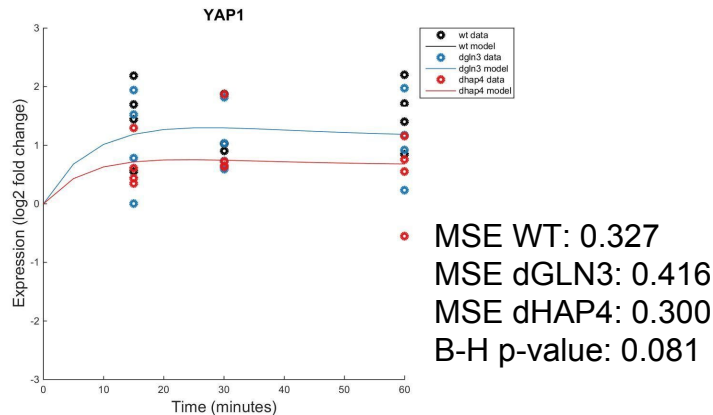
Optimized Parameters Differ from Initial Estimations

Run	LSE:minLSE
Initial	1.551

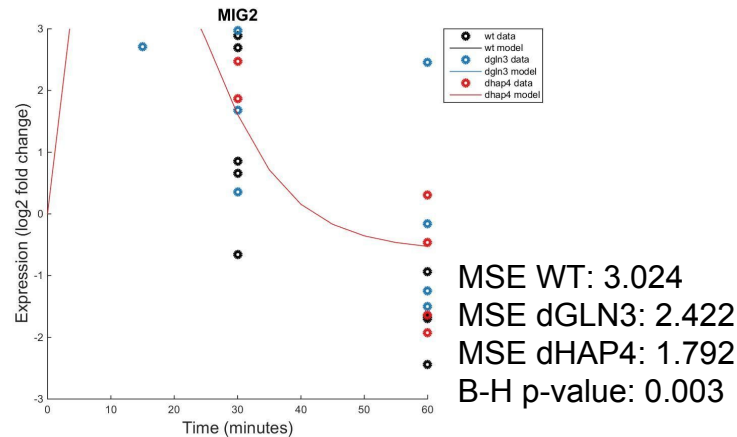
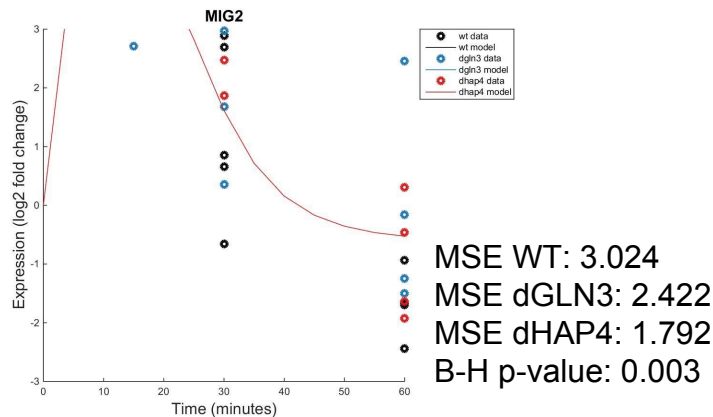
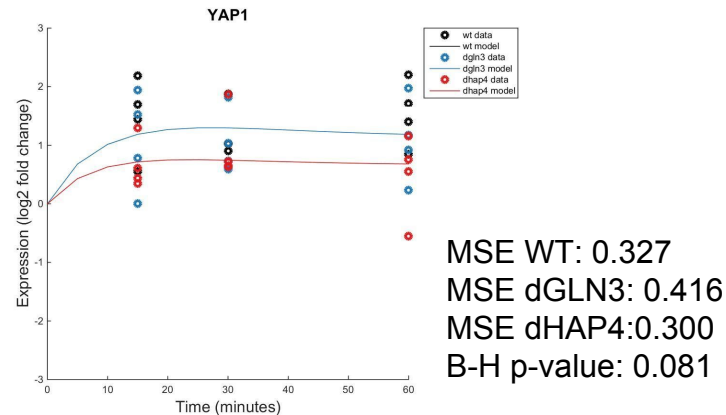


Removal of ZAP1 from the model did not alter the fit

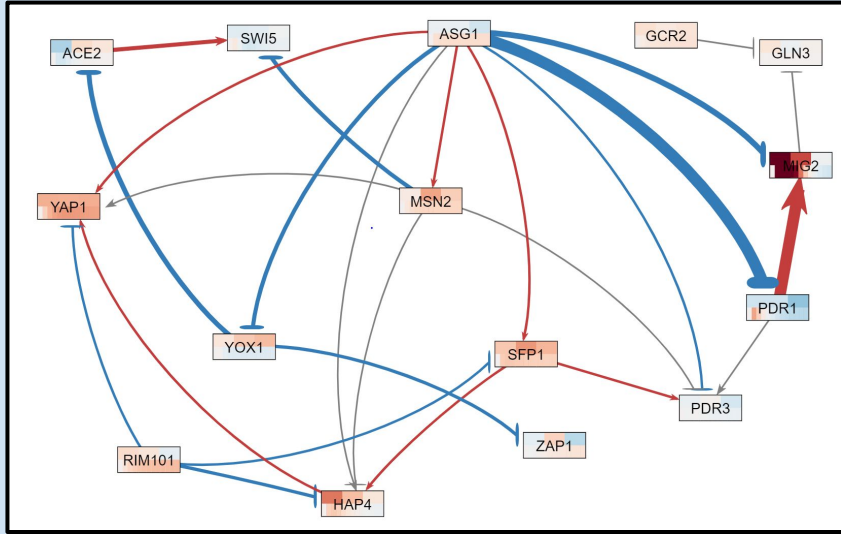
Control



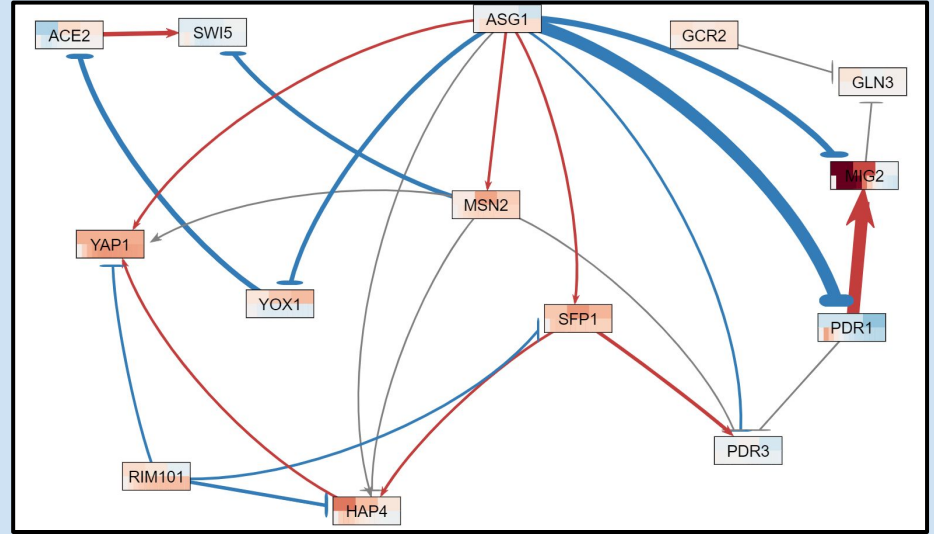
-ZAP1



Removal of ZAP1 did not alter the regulatory matrix



Control



-ZAP1