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

Austin Dias & Leanne Kuwahara
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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**

Before Cold Shock → 15°C → Cold Shock → 15°C → 15°C → Recovery

- Isolate total RNA
- cDNA synthesis, aRNA synthesis, indirect labelling
- Pairing for Microarrays

DNA Microarrays Measure the Expression of Thousands of Genes Simultaneously

- Mixture of labeled cDNA from two samples

**Data Analysis**

- ANOVA
- STEM Clustering
- YEASTRACT
- Gene Regulatory Matrix (GRNsight)
- \( \frac{dx_i}{dt} = \frac{p_i}{1 + e^{-(\sum_{j}x_j - b_i)}} - d_i x_i \)
- 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

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>ΔGLN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>p &lt; 0.05</td>
<td>2135 (34.5%)</td>
</tr>
<tr>
<td>p &lt; 0.01</td>
<td>1204 (19.5%)</td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td>514 (8.31%)</td>
</tr>
<tr>
<td>p &lt; 0.0001</td>
<td>180 (2.91%)</td>
</tr>
<tr>
<td>B &amp; H p &lt; 0.05</td>
<td>1185 (19.1%)</td>
</tr>
<tr>
<td>Bonferroni p &lt; 0.05</td>
<td>45 (0.727%)</td>
</tr>
</tbody>
</table>
Significant Gene Clusters for dGLN3

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

Profile #45: (0, 2, 2, 1, -1, 0)
406.0 Genes Assigned; 29.9 Genes Expected; p-value = 0.00 (significant)

Profile #22: (0, 0, 0, 6, 2, 1)
94.0 Genes Assigned; 21.2 Genes Expected; p-value = 9.2e-24 (significant)
**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: 0.432
MSE dGLN3: 0.132
MSE dHAP4: 0.233
B-H p-value: 0.416

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

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

<table>
<thead>
<tr>
<th>Run</th>
<th>LSE:minLSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>t0-t60</td>
<td>1.331</td>
</tr>
<tr>
<td>t0-t120</td>
<td>1.436</td>
</tr>
</tbody>
</table>

![Graph showing optimized network weights and production rates for t0-t60 and t0-t120](image)
Extending Data Points Caused Change in Network Relationships

t0-t60 dGLN3

Top Dataset
- dgl3_log2_expression
- Average Replicate Values

Bottom Dataset
- dgl3_log2_optimized_expression
- Average Replicate Values

Log Fold Change Max Value
(-100 - 100): 3

Set

-3 0 3

-3 0 3

t0-t120 dGLN3
Data May Need to be Collected Prior to 15 minutes for “Full Picture”

MSE WT: 3.024
MSE dGLN3: 2.422
MSE dHAP4: 1.792
B-H p-value: 0.003

MSE WT: 1.958
MSE dGLN3: 1.464
MSE dHAP4: 1.257
MSE dZAP1: 3.674
B-H p-value: 0.107

MSE WT: 1.493
MSE dGLN3: 1.221
MSE dHAP4: 0.475
MSE dZAP1: 3.373
B-H p-value: 0.233
Removal of ZAP1 Had Minimal Effect on Model

<table>
<thead>
<tr>
<th>Run</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>1.551</td>
</tr>
<tr>
<td>Without ZAP1 Control</td>
<td>1.505</td>
</tr>
<tr>
<td>Without ZAP1</td>
<td>1.450</td>
</tr>
</tbody>
</table>
### Transcription Factors Likely to Regulate Cold Response

#### Profile 45

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASG1</td>
<td>Most edges</td>
</tr>
<tr>
<td>⭐️ MIG2</td>
<td>High b and P</td>
</tr>
<tr>
<td>PDR1</td>
<td>Most repressed, strongest activator</td>
</tr>
</tbody>
</table>

#### Profile 22

<table>
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<th>Factor</th>
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</tr>
</thead>
<tbody>
<tr>
<td>ADR1</td>
<td>Numerous edges, strongest activator</td>
</tr>
<tr>
<td>⭐️ MIG1</td>
<td>Most dynamic</td>
</tr>
<tr>
<td>RAP1</td>
<td>Controls two genes that reverse regulation behavior</td>
</tr>
</tbody>
</table>
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:

Dr. Dahlquist and her laboratory
Dr. Fitzpatrick
LMU Department of Biology and Department of Mathematics


Unweighted regulatory gene networks

Profile 45

Profile 22
Optimized Parameters Differ from Initial Estimations

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Removal of ZAP1 from the model did not alter the fit

Control

- ZAP1

\[
\text{MSE WT: 0.327} \\
\text{MSE dGLN3: 0.416} \\
\text{MSE dHAP4: 0.300} \\
\text{B-H p-value: 0.081}
\]

\[
\text{MSE WT: 3.024} \\
\text{MSE dGLN3: 2.422} \\
\text{MSE dHAP4: 1.792} \\
\text{B-H p-value: 0.003}
\]
Removal of ZAP1 did not alter the regulatory matrix