Assessing the Cold Shock Response of ΔHAP4 and Wild-type Transcription Factor

Lauren Kelly and Cameron Rehmani Seraji

Department of Biology
Loyola Marymount University

BIOL 398-05/MATH 388-01
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Outline

- Background on the cold shock experimental design
- Significant expression levels in various genes were found by doing statistical analysis of microarray data
- YEASTRACT results yielded 12 significant gene profiles
- Profile 45 gene ontology terms indicate a strong effect on tRNA processes
  - Deletions of HAP4 and SWI5 led to changes in production rates, threshold b parameters, and weight parameters of transcription factors
  - Deletions of HAP4 and SWI5 led to changes in expression of MSN2 and SOK2
- Profile 9 gene ontology terms indicate a strong effect on cytoplasm and its components
  - Deletions of GLN3 and SPT23 led to changes in production rates, weight parameters, and threshold b parameters of transcription factors
  - GLN3 and SPT23 are important in regulating the cold shock response
- Tai et al. (2007) and up/down-regulation of transcription factors
Understanding How Cells Respond to Cold Shock

- Cold Shock is the response to a sudden decrease in temperature and it has not been well studied in the past.
- DNA microarray analysis was performed on data for ΔHAP4 that was generated in the Dahlquist Lab.
  - Red spots represent an increase in expression and green spots represent a decrease in expression.
- The experiment was conducted at 13°C for a total of 60 minutes followed by a recovery at 30°C.
- The experiment was run using ANOVA, STEM, YEASTRACT, GRNsight, and GRNmap
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  o GLN3 and SPT23 are important in regulating the cold shock response
- Tai et al. (2007) and up/down-regulation of transcription factors
According to the ANOVA Results of ΔHAP4, the Amount of Significant Genes Ranges from 75 to 2,479

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>dHAP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>p &lt; 0.05</td>
<td>2479 genes</td>
</tr>
<tr>
<td></td>
<td>40.05%</td>
</tr>
<tr>
<td>p &lt; 0.01</td>
<td>1583 genes</td>
</tr>
<tr>
<td></td>
<td>25.58%</td>
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<tr>
<td>p &lt; 0.001</td>
<td>739 genes</td>
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<tr>
<td></td>
<td>11.94%</td>
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<tr>
<td>p &lt; 0.0001</td>
<td>280 genes</td>
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<tr>
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<td>4.52%</td>
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<tr>
<td>B &amp; H p &lt; 0.05</td>
<td>1735 genes</td>
</tr>
<tr>
<td></td>
<td>28.03%</td>
</tr>
<tr>
<td>Bonferroni p &lt; 0.05</td>
<td>75 genes</td>
</tr>
<tr>
<td></td>
<td>1.21%</td>
</tr>
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</table>
There are More Significant Genes at t30 than Any Other Time Point

<table>
<thead>
<tr>
<th></th>
<th>Cold Shock</th>
<th></th>
<th></th>
<th>Recovery</th>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(t_{15})</td>
<td>(t_{30})</td>
<td>(t_{60})</td>
<td>(t_{90})</td>
<td>(t_{120})</td>
<td></td>
</tr>
<tr>
<td><strong>t test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Log Fold</td>
<td>1182 genes</td>
<td>1242 genes</td>
<td>1207 genes</td>
<td>918 genes</td>
<td>894 genes</td>
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<tr>
<td>Change &gt; 0.25 and</td>
<td>19.10%</td>
<td>20.07%</td>
<td>19.50%</td>
<td>14.83%</td>
<td>14.44%</td>
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<tr>
<td>(p &lt; 0.05)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Log Fold</td>
<td>1048 genes</td>
<td>1067 genes</td>
<td>1057 genes</td>
<td>852 genes</td>
<td>1011 genes</td>
<td></td>
</tr>
<tr>
<td>Change &lt; -0.25 and</td>
<td>16.93%</td>
<td>17.24%</td>
<td>17.08%</td>
<td>13.77%</td>
<td>16.34%</td>
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<tr>
<td>(p &lt; 0.05)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total (p &lt; 0.05)</td>
<td>2479 genes</td>
<td>2479 genes</td>
<td>2479 genes</td>
<td>2479 genes</td>
<td>2479 genes</td>
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<tr>
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<td>40.05%</td>
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<td>40.05%</td>
<td></td>
</tr>
<tr>
<td>Total B &amp; H (p &lt; 0.05)</td>
<td>1735 genes</td>
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<td>1735 genes</td>
<td>1735 genes</td>
<td>1735 genes</td>
<td></td>
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<tr>
<td></td>
<td>28.03%</td>
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<td>28.03%</td>
<td>28.03%</td>
<td></td>
</tr>
<tr>
<td>Total Bonferroni (p &lt; 0.05)</td>
<td>75 genes</td>
<td>75 genes</td>
<td>75 genes</td>
<td>75 genes</td>
<td>75 genes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.21%</td>
<td>1.21%</td>
<td>1.21%</td>
<td>1.21%</td>
<td>1.21%</td>
<td></td>
</tr>
</tbody>
</table>
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Profile #9 and #45 Were Chosen for the Analysis of Significant Transcription Factors
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Gene Ontology Terms of Profile 45 Demonstrate Strong Effect on tRNA Processes

<table>
<thead>
<tr>
<th>GO number</th>
<th>Category Name</th>
<th>#Genes Assigned</th>
<th>Corrected p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0006400</td>
<td>tRNA modification</td>
<td>22</td>
<td>0.002</td>
</tr>
<tr>
<td>GO:0030488</td>
<td>tRNA methylation</td>
<td>11</td>
<td>0.002</td>
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<tr>
<td>GO:0008033</td>
<td>tRNA processing</td>
<td>29</td>
<td>0.004</td>
</tr>
<tr>
<td>GO:0008175</td>
<td>tRNA methyltransferase activity</td>
<td>10</td>
<td>0.004</td>
</tr>
<tr>
<td>GO:0006399</td>
<td>tRNA metabolic process</td>
<td>41</td>
<td>0.001</td>
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</table>
Unweighted Transcription Factor Network of 16 Significant Edges and 32 Nodes
20 Significant Transcription Factors Found in YEASTRACT

- In total, 20 transcription factors were significant.
- Initially deleted all transcription factors that had no regulators or only 1 input.

<table>
<thead>
<tr>
<th>Transcription Factor</th>
<th>p-value from YEASTRACT</th>
<th>Transcription Factor</th>
<th>p-value from YEASTRACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSN2</td>
<td>1.78E-08</td>
<td>ARG80</td>
<td>8.58E-06</td>
</tr>
<tr>
<td>HAP4</td>
<td>0.998</td>
<td>TUP1</td>
<td>9.1E-13</td>
</tr>
<tr>
<td>SFP1</td>
<td>0</td>
<td>PDR1</td>
<td>1.69E-05</td>
</tr>
<tr>
<td>SOK2</td>
<td>1.1E-05</td>
<td>FKH2</td>
<td>7.36E--10</td>
</tr>
<tr>
<td>STB5</td>
<td>1.2E-12</td>
<td>YHP1</td>
<td>0</td>
</tr>
<tr>
<td>MIG2</td>
<td>1.85E-07</td>
<td>INO4</td>
<td>4.86E-05</td>
</tr>
<tr>
<td>ACE2</td>
<td>5E-15</td>
<td>MCM1</td>
<td>1.88E-05</td>
</tr>
<tr>
<td>YLR278C</td>
<td>2.18E-06</td>
<td>SWI5</td>
<td>4.03E-09</td>
</tr>
</tbody>
</table>
Minor Changes in Optimized Production Rate Across the 4 Trials

- MIG2 had the highest production rate in all 4 trials.
- INO4 had the lowest production rate in all 4 trials.
- The initial trial and trial where $b$ was set to 1 have the same value for all genes.
- Deletions of HAP4 and SWI5 caused the production rates to change in all genes.
Deletions of HAP4 and SWI5 Affected the Optimized Threshold b Parameters

- The initial trial and trial where \( b \) was set to 1 have the same value for all genes.
- Large difference between initial trial and HAP4 and SWI5 deletion for 11 different transcription factors.
- INO4 had the lowest optimized threshold b parameters for all trials.
Comparison of Plot Weights Shows Contrasting Values for Multiple Gene Pathways

- The initial trial and trial where b was set to 1 have the same value for all genes.
- Two biggest pathways that stand out on the graph are MSN2→MIG2 and SOK2→MIG2.
- MSN2→MIG2 changed from induction to repression when HAP4 and SWI5 were deleted.
- SOK2→MIG2 changed from repression to induction when HAP4 and SWI5 were deleted.
MSN2→MIG2 Plot Weight Changed from 7.202137 to -6.47552 when HAP4 was Deleted
SOK2→MIG2 Plot Weight Changed from -7.55775 to 5.522197 when HAP4 was Deleted
MSN2→MIG2 Plot Weight Changed from 7.202137 to -5.89503 when SWI5 was Deleted
SOK2→MIG2 Plot Weight Changed from -7.55775 to 7.002023 when SWI5 was Deleted
Line of Best Fit Changes from Repression to Induction Multiple Times for MIG2 in All Trials

Initial

HAP4 Deletion

SWI5 Deletion
The Expression of MSN2 is Very Similar for the Initial and SWI5 Deletion
The Expression of SOK2 is Relatively Similar for the Three Trials

- Initial
- HAP4 Deletion
- SWI5 Deletion
From the Visualized GRN, There are Two Major Regulators in the Network for Profile 45

- The two major regulators are MSN2 and SOK2 and both of these regulators directly regulate MIG2.

- The regulation of MSN2 and SOK2 may be due to the presence of HAP4 and SWI5 in the model.

- Future Studies:
  - Determine why deleting HAP4 and SWI5 causes the regulation of MSN2 and SOK2 to MIG2 to switch to the opposite of its original expression value.
  - Look at the effect of eliminating the transcription factors that have gray arrows from the constructed GRN.
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Gene Ontology Terms from Profile 9 Suggest Strong Effect on the Cytoplasm and its Components

<table>
<thead>
<tr>
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<th>Category Name</th>
<th>#Genes Assigned</th>
<th>Corrected p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0005737</td>
<td>cytoplasm</td>
<td>393</td>
<td>0.001</td>
</tr>
<tr>
<td>GO:0044444</td>
<td>cytoplasmic part</td>
<td>310</td>
<td>0.001</td>
</tr>
<tr>
<td>GO:0022626</td>
<td>cytosolic ribosome</td>
<td>36</td>
<td>0.001</td>
</tr>
<tr>
<td>GO:0002181</td>
<td>cytoplasmic translation</td>
<td>35</td>
<td>0.002</td>
</tr>
<tr>
<td>GO:0044445</td>
<td>cytosolic part</td>
<td>42</td>
<td>0.002</td>
</tr>
<tr>
<td>GO:0005829</td>
<td>cytosol</td>
<td>104</td>
<td>0.006</td>
</tr>
</tbody>
</table>

P-value = 2.8E-158
33 Significant Transcription Factors Found in YEASTRACT

- In total, 33 transcription factors were significant
  - GLN3 and HAP4 showed no significance according to YEASTRACT
- Initially deleted all transcription factors that had no regulators or only one input

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<th>p-value from YEASTRACT</th>
<th>Transcription Factor</th>
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</tr>
</thead>
<tbody>
<tr>
<td>CST6</td>
<td>2.56441E-05</td>
<td>MBP1</td>
<td>1.20671E-06</td>
</tr>
<tr>
<td>CUP9</td>
<td>1.27643E-06</td>
<td>PHO4</td>
<td>1.12068E-05</td>
</tr>
<tr>
<td>FHL1</td>
<td>1.23921E-05</td>
<td>RAP1</td>
<td>2.81392E-05</td>
</tr>
<tr>
<td>GCR1</td>
<td>1.63413E-05</td>
<td>RPN4</td>
<td>1.96342E-05</td>
</tr>
<tr>
<td>GLN3</td>
<td>0.009729</td>
<td>SFP1</td>
<td>4.21512E-05</td>
</tr>
<tr>
<td>HMS1</td>
<td>1.84972E-06</td>
<td>SPT23</td>
<td>1.6982E-05</td>
</tr>
<tr>
<td>HSF1</td>
<td>2.45662E-05</td>
<td>TYE7</td>
<td>1.02978E-05</td>
</tr>
</tbody>
</table>
Initial Network Has 14 Nodes and 33 Edges

- This network was used as the starting point for future experimental deletions
- 3 additional deletions: GLN3, SPT23, and HMS1
Deletion of GLN3 and SPT23 has Effects on Production rates
Deletion of GLN3 and SPT23 has Effects on Optimized Weights
Deletions of GLN3 and SPT23 Affected the Optimized Threshold b Parameters
Deletion of GLN3 Changed the Network Significance

14 nodes, 33 edges
13 nodes, 32 edges
Deletion of SPT23 Led To Various Changes in the Network

14 nodes, 33 edges

13 nodes, 23 edges
Deletion of SPT23 Led To Various Changes in the Network

14 nodes, 33 edges
13 nodes 23 edges
Deletion of SPT23 Led To Various Changes in the Network

14 nodes, 33 edges

13 nodes 23 edges
Deleting HMS1 Did Not Affect Network

14 nodes 33 edges

13 nodes 27 edges
PHO4 Expression and the SPT23 Deletion

Original

PHO4

Deleted HMS1

Deleted GLN3

Deleted SPT23
Production Rate of PHO4 is Reduced

Production Rate of PHO4 is Reduced

Original Rate: 0.676131091
Rate with SPT23 Deletion: 0.08752916

Production rate decreased by ~0.6
Weights Display the Change of Regulation of PHO4
CST6 Affected By GLN3 Deletion

Original

Deleted HMS1

Deleted GLN3

Deleted SPT23
SPT23 and GLN3 Appear to be Important in Regulating Cold Shock Response

- While GLN3 was not significant according to YEASTRACT, the production rates/optimized weights bar charts and the GRNsight visualizations indicate that removing GLN3 changes the network.

- SPT23 consistently regulated many of the transcription factors and was a central part of the GRNsight visualization
  - The deletion of SPT23 changed various things about the network

- Future studies would include why GLN3 appears to have an effect despite the insignificant p-value from YEASTRACT and continuing to manipulate the model to see if other transcription factors play an important role in cold shock response
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Connection to Tai et al. (2007)

- Both Tai et al. (2007) and this project looked at how transcription factors related to specific gene ontology terms react to cold shock.
  - No overlap in transcription factors or GO terms
- Heat maps are another way to visualize the up-regulation and down-regulation that is seen in the GRNsight networks.
- Tai et al. (2007) did not include deletions of transcription factors.
Acknowledgments

Dr. Kam Dahlquist

Dr. Ben Fitzpatrick

LMU Department of Biology

LMU Department of Mathematics
References
