Acclimation of Saccharomyces cerevisiae to Low Temperature: A Chemostat-based Transcriptome Analysis


Lauren Kelly and Cameron Rehmani Seraji

Department of Biology
Loyola Marymount University

BIOL 398-05/MATH 388-01
March 30, 2017
Outline

- Background Information
- Goal of the Present Study
- Methods and Procedures
- In chemostat based cultures, nutrient limitation affected the transcriptional regulation of genes at low temperatures.
- Comparing this chemostat study to other batch culture studies revealed inconsistent gene expression.
- Is the environmental stress response (ESR) an obligatory response to growth at low temperatures?
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Is the environmental stress response (ESR) an obligatory response to growth at low temperatures?
Background Information

- Temperature fluctuations are an evitable environmental factor that affects all organisms.
- Sudden exposure to environmental changes will trigger a dynamic stress-response called adaptation.
- Prolonged exposure to nonlethal stimuli leads to acclimation.
- Chemostat cultures allow for accurate control of specific growth rate, independent of other culture conditions.
- Ms2p/msn4p has been previously suggested to be a transcriptional factor in cold temperature.
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- Comparing this chemostat study to other batch culture studies revealed inconsistent gene expression.
- Is the environmental stress response (ESR) an obligatory response to growth at low temperatures?
Goals of the Present Study

● Tai et al. (2007) aimed to investigate steady-state, acclimatized growth of *S. cerevisiae* at colder temperatures.
  ○ Emphasized transcriptional regulation of the genome.
● They compared their chemostat culture results with those from previous studies in batch cultures and demonstrated the differences between the two.
● They also aimed to further describe the role of ESR genes at low-temperature in chemostat cultures.
In chemostat based cultures, nutrient limitation affected the transcriptional regulation of genes at low temperatures.

Comparing this chemostat study to other batch culture studies revealed inconsistent gene expression.

Is the environmental stress response (ESR) an obligatory response to growth at low temperatures?
Methods and Procedures

- *S. cerevisiae* strain: CEN.PK113-7D (MATα)
- Grown in a chemostat culture (D = 0.03 h⁻¹)
- Four Growth Conditions:
  - 12°C, glucose-limited
  - 12°C, ammonium-limited
  - 30°C, glucose-limited
  - 30°C, ammonium-limited
- Utilized various analytical methods and microarray analysis and compared their data to other *S. cerevisiae* low-temperature transcriptome datasets.
In chemostat based cultures, nutrient limitation affected the transcriptional regulation of genes at low temperatures. Comparing this chemostat study to other batch culture studies revealed inconsistent gene expression. Is the environmental stress response (ESR) an obligatory response to growth at low temperatures?
Growth-Efficiency Was Not Affected by Growth Temperature

Table 1. Physiological characteristics of S. cerevisiae grown in ammonium- and glucose-limited anaerobic chemostat cultures

<table>
<thead>
<tr>
<th>Limiting nutrient</th>
<th>Growth temperature (°C)</th>
<th>$Y_{\text{Glu/X}}$ (gDW · gglucose$^{-1}$)</th>
<th>$q_{\text{Glu}}$</th>
<th>$q_{\text{EtOH}}$</th>
<th>$q_{\text{CO}_2}$</th>
<th>Carbon recovery (%)</th>
<th>Residual glucose (mM)</th>
<th>Residual ammonia (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>12</td>
<td>0.07 ± 0.01</td>
<td>−2.5 ± 0.2</td>
<td>3.8 ± 0.3</td>
<td>4.4 ± 0.3</td>
<td>100 ± 3</td>
<td>2.8 ± 1.1</td>
<td>65.2 ± 2.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>30</td>
<td>0.07 ± 0.00</td>
<td>−2.3 ± 0.0</td>
<td>3.5 ± 0.0</td>
<td>3.8 ± 0.2</td>
<td>95 ± 1</td>
<td>0.3 ± 0.1</td>
<td>61.3 ± 4.5</td>
</tr>
<tr>
<td>Ammonium</td>
<td>12</td>
<td>0.05 ± 0.00</td>
<td>−3.6 ± 0.2</td>
<td>6.1 ± 0.3</td>
<td>6.0 ± 0.6</td>
<td>97 ± 4</td>
<td>90.0 ± 9.8</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Ammonium</td>
<td>30</td>
<td>0.04 ± 0.00</td>
<td>−4.0 ± 0.1</td>
<td>6.8 ± 0.2</td>
<td>7.4 ± 0.2</td>
<td>97 ± 2</td>
<td>85.1 ± 8.2</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

Cultures were grown at 30 and 12°C (D = 0.03 h$^{-1}$). Values represent the mean ± SD of data from three independent steady-state chemostat cultivations. $Y_{\text{Glu/X}}$, biomass yield on glucose; DW, dry weight.

$^a$ Values expressed as mmol · gDW$^{-1}$ · h$^{-1}$.

- Biomass was similar at both temperatures in both nutrient conditions.
- In the 12°C cultures, the residual concentrations of glucose and ammonia were higher than the 30°C cultures.
  - Residual nutrient concentration is temperature dependent.
There was more yeast growth on the ammonium-limited media.

Amount of trehalose was equal in the 12°C media and slightly higher in the 30°C ammonium-limited media.

Overall, yields and fermentation rates were similar in all growth conditions.

- This indicates that the growth temperature did not affect the growth efficiency.
Higher Transcription Levels in Ammonium-limited Cultures

- Of the 1065 genes, 644 genes were down-regulated and 421 up-regulated genes.
- 235 genes showed a consistent down-regulation and up-regulation in the glucose-limited and ammonium-limited conditions.

Figure 1. Global transcriptome responses to anaerobic growth at 12 and 30°C in anaerobic glucose- and ammonium-limited chemostat cultures (D = 0.03 h⁻¹). The Venn diagram shows the number of significant differentially expressed genes between 12 and 30°C in both C and N limitations.
More Genes Were Induced in the Glucose-Limited Cultures

- Temperature-responsive genes were screened for enrichment of specific functional categories.
- C-lim cultures showed the most activity in these categories.
Greater Transcriptional Regulation in N-Lim

- **S. cerevisiae** in N-lim cultures showed stronger transcriptional regulation in these categories.

**Low temperature N-lim specific up (202 genes)**


- Ribosome biogenesis and assembly (GO0042254 p = 2.0E-25)/RNA processing (GO0006396 p = 7.4E-11)/rRNA processing (GO0006364 p = 1.0E-18):
  - ARX1, BRF1, CBF3, CIC1, DBF3, DES1, ENP1, ERG1, GAT1, GAT4, HEM3, IMP1, IMP4, LTM1, MAK5, MRT4, MTH3, NIP7, NUB1, NOC2, NOC4, NOP1, NOP2, NOP58, NOP7, NUG1, POP3, PWP1, RCL1, REX4, RIX1, RIX7, RLP24, RPL12A, RPL3, RPL40B, RRB1, RRP12, RSA3, SSK1, SNU13, TIF5, UTP15, UTP21, UTP22, UTP9, YTM1

**Low temperature N-lim specific down (369 genes)**

- Nitrogen compound metabolism (GO0006807 p = 5.4E-04) and catabolism (GO0044270 p = 2.8E-05)/amine transport (GO015837 p = 1.6E-03)/allantoin metabolism (GO0000256 p = 8.5E-05):
  - ALD2, ALD3, ADY2, DAL1, DAL4, DUL2, CAR2, CHAI, CHAB, SHC1, EKII, GAT1, PUT5, MET4, DAL7, DAL89, ARG1, CAR1, DCO1, ASF3-1, GLT1, CPS1, ARO10, YLR167W, GINS3, PUT1, DAL2, DTR1, GAT1, ATV6, CAN1, PTK2, YMR088C, DUR3, MEP1, MEP2
- Polysaccharide (GO0005976 p = 3.1E-03) and trehalose metabolism (GO0005991 p = 1.1E-03):
  - SHC1, GSY2, GSY1, PCL7, PCL10, PIG2, GSC2, GAC1, TPS2, NTH1, TPS1, TSL1

- **M phase of mitotic cell cycle** (GO000087 p = 5.5E-05) and chromosome segregation (GO0007059 p = 2.8E-03):
  - CDC16, PDS3, ALX1, SIK1, PDS1, TOP1, IRR1, SMC1, SMC3, SUM1, KAR3, RTT101, KEL1, YCG1, TFB1, ASE1, CTF1, GAC1, KIP2, YNL116W

- **Cellular morphogenesis** (GO0000902 p = 2.8E-03):
  - TCO89, ECF4, TOS1, DOP1, TAO3, TOS2, LGE1, SLA1, KEL1, WHI1, BOI2, SCH5, VRP1, RAX1, RNY1, GIM4, WHI5, ZDS1, ABP1

- **Response to stimulus** (GO00050896 p = 3.5E-05):
  - ABF1, ALD3, ASF3-1, BDF1, CAD1, CDC39, CIN5, CTF4, DNA2, FAB1, FAR11, FRT2, FYW6, GAC1, GBE3, HSP104, HSP78, IRE1, ISW2, HCI, MDG1, NMT4, NMT1, MF2, MLH3, MSH6, NCE4, NTH1, PDS1, POL1, POL2, PRB1, PDR3, RAD28, RPN4, RPD1, RRD2, SAN1, SCH9, SHU2, SIS1, SMC1, SLP2, SSA4, TFB1, TPS1, TPS2, TSL1, UBA4, VRP1, WSC4, WWM1, YGR13, YKL075C
### Strong Transcriptional Regulation in Both N-Lim and C-Lim

<table>
<thead>
<tr>
<th>Low temperature C-lim and N-lim up (96 genes)</th>
<th>Low temperature C-lim and N-lim down (139 genes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear export (GO00051168 p = 5.9E-03):</td>
<td>Carbohydrate metabolism (GO0005975 p = 6.4E-04):</td>
</tr>
<tr>
<td>NPL3, HMT1, NMD3, NOG1, ECM1, NOG2</td>
<td>MAL32, SOL4, MAL31, NRG1, MUC1, ATH1, GIP2, MIG1, GUT2, PFK26, PFK27, MAL13, FYV10, NTH2</td>
</tr>
<tr>
<td>Ribosome biogenesis and assembly (GO0042254 p = -2.8E-05) / rRNA processing (GO0006364 p = 1.4E-03):</td>
<td>Response to stimulus (GO00050896 p = 3.1E-03):</td>
</tr>
<tr>
<td>NSR1, CGR1, NOG2, RPF2, ECM1, UTP13, KRR1, NMD3, MAK16, YNL182C, EBP2, EMG1, NOG1, DBP2, IP13, YDR412W</td>
<td>MRK1, NRG1, SSA3, AHA1, HSP26, ATH1, HSF1, PDR5, HSP42, CUP2, HSP30, CRSS, AKR1, FET3, NTH2, MGA2, SSE2, YHR048W, YDR317W, YJL144W</td>
</tr>
<tr>
<td>Transport (GO0006810 p = 8.4E-04):</td>
<td>Transport (GO0006810 p = 8.4E-04):</td>
</tr>
<tr>
<td>COX5B, DAL4, MAL31, AGP1, FCY2, SIT1, HXT5, DIP5, PTR2, PHO84, SUT1, OPT2, CCC2, AAC1, VPS10, BTN2, YHR048W, ARN1, SSA3, GIT1, PDR5, FIT2, SSU1, ATG11, YOR192C, SUL1, MIP6, FUS1, AKR1, MUP1, FTR1, MAL11, FET3, CRS5</td>
<td>COX5B, DAL4, MAL31, AGP1, FCY2, SIT1, HXT5, DIP5, PTR2, PHO84, SUT1, OPT2, CCC2, AAC1, VPS10, BTN2, YHR048W, ARN1, SSA3, GIT1, PDR5, FIT2, SSU1, ATG11, YOR192C, SUL1, MIP6, FUS1, AKR1, MUP1, FTR1, MAL11, FET3, CRS5</td>
</tr>
</tbody>
</table>

- C-lim and N-lim cultures showed similar regulation trends in nuclear export, ribosome biogenesis/assembly, carbohydrate metabolism, and other categories.
There were significantly overrepresented cis-regulatory binding motif in the 5' upstream regions.

There were significantly overrepresented promoter elements that bind known transcription factors or transcription factor pairs according to the ChiP-on-chip analysis.

Overall, More Transcription Factors were Overrepresented

Table 3

(A) 5' upstream cis-regulatory motif

<table>
<thead>
<tr>
<th>Regulatory cluster</th>
<th>Motif name</th>
<th>Putative-binding protein</th>
<th>Promoter element</th>
<th>occ</th>
<th>Expected occ</th>
<th>occ F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Temperature C-lim Up</td>
<td>—</td>
<td>—</td>
<td>TGA</td>
<td>AAA</td>
<td>206</td>
<td>113.04</td>
</tr>
<tr>
<td>Low Temperature C-lim Up</td>
<td>Low Temperature N-lim Up</td>
<td>PAC</td>
<td>—</td>
<td>CGA</td>
<td>TGAG</td>
<td>57</td>
</tr>
<tr>
<td>Low Temperature N-lim Down</td>
<td>GATAA</td>
<td>Gln3/Gat1/Dna10/Gat3</td>
<td>—</td>
<td>AGA</td>
<td>TAAG</td>
<td>203</td>
</tr>
<tr>
<td>Low Temperature C- and N-lim Up</td>
<td>PAC</td>
<td>—</td>
<td>CGA</td>
<td>TGAG</td>
<td>30</td>
<td>8.39</td>
</tr>
<tr>
<td>Low Temperature C- and N-lim Down</td>
<td>—</td>
<td>—</td>
<td>CGT</td>
<td>CCAC</td>
<td>13</td>
<td>2.85</td>
</tr>
</tbody>
</table>

(B) Overrepresentation of transcription factors (TF) binding targets

<table>
<thead>
<tr>
<th>Regulatory cluster</th>
<th>Factor</th>
<th>p value</th>
<th>K1</th>
<th>P0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Temperature C-lim Up</td>
<td>Mbp1p</td>
<td>1.6E-03</td>
<td>10</td>
<td>65</td>
</tr>
<tr>
<td>Low Temperature C-lim Down</td>
<td>Hap2/Hap1</td>
<td>3.9E-05</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Low Temperature N-lim Up</td>
<td>Phd1p</td>
<td>9.9E-06</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Low Temperature N-lim Down</td>
<td>Glc3p</td>
<td>3.6E-05</td>
<td>19</td>
<td>203</td>
</tr>
<tr>
<td>Low Temperature N-lim Down</td>
<td>Hap2-Hap1</td>
<td>1.3E-03</td>
<td>7</td>
<td>51</td>
</tr>
<tr>
<td>Low Temperature C- and N-lim Up</td>
<td>Glc3p</td>
<td>2.1E-07</td>
<td>20</td>
<td>92</td>
</tr>
<tr>
<td>Low Temperature C- and N-lim Down</td>
<td>Glc3-Dna82</td>
<td>5.8E-07</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Low Temperature C- and N-lim Down</td>
<td>Hap2-Dna82</td>
<td>6.8E-05</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>

(A) Significantly overrepresented cis-regulatory binding motifs in 5' upstream regions. (B) Significantly overrepresented promoter elements that bind known transcription factors (TF) or TF pairs according to ChiP-on-chip analysis (Harbison et al., 2004). C-lim, glucose-limited; N-lim, ammonium-limited.

1 The number of occurrences of the promoter element in the regulatory cluster.
2 The expected number of occurrences of the promoter element in a randomly chosen cluster of genes of the same cluster size.
3 The probability of finding the number of patterns with the same level of overrepresentation, which would be expected by chance alone.
4 Number of genes in category in cluster.
5 Number of genes in category in genome.
In chemostat based cultures, nutrient limitation affected the transcriptional regulation of genes at low temperatures.

Comparing this chemostat study to other batch culture studies revealed inconsistent gene expression.

Is the environmental stress response (ESR) an obligatory response to growth at low temperatures?
259 Genes Found in Common in Three Batch-Culture Studies

- Venn diagram compares the genes from the three different batch culture studies.
- Responses of genes was not consistent.
- The heat map shows the transcript ratio of the 259 genes found in common.
11 Genes Show Consistent Regulation

- Three genes that were consistently up-regulated are involved in lipid metabolism.
  - SFK1, YPC1, and YEL073C
- Three genes that were consistently down-regulated encode transporters.
More Genes Regulated in Chemostat-Cultures

- Genes were taken from Castrillo et al (2007) and Regenberg et al (2006).
- 25% of low-temperature down-regulated genes had altered transcript levels.
- 10% of the low-temperature up-regulated genes had altered transcript levels.
In chemostat based cultures, nutrient limitation affected the transcriptional regulation of genes at low temperatures.

Comparing this chemostat study to other batch culture studies revealed inconsistent gene expression.

Is the environmental stress response (ESR) an obligatory response to growth at low temperatures?
Extensive Overlap between the ESR Genes and the Genes in the Batch-Cultures

- 50% of consistently low-temperature up-regulated genes and 13% of low-temperature down-regulated genes found in the batch-culture studies were found in the ESR genes from Gasch et al. (2000)
- Proposed that the ESR mechanism becomes induced with the growth temperature is decreased.
Comparison of Gene Regulation in the Gasch et al. (2000) and the Chemostat Cultures

- 233 genes up-regulated and down-regulated in the study by Gasch et al. (2000) had an opposite transcriptional response in the low-temperature chemostat cultures.
Chemostat Cultures Offer a New Perspective on Transcriptional Regulation at Low Temperatures

- Genes that were consistently up- or down-regulated in both the batch cultures and the chemostat cultures coded for crucial cell functions.
- Prior studies had different results because they used batch-cultures and Tai et al. (2007) used chemostat-cultures.
- ESR is not the main response to low temperatures.
- Transcriptional responses to low-temperature and low specific growth rate can be dissected by the use of chemostat cultures.
Works Cited

Acknowledgments

Dr. Kam Dahlquist
Dr. Ben Fitzpatrick
LMU Department of Biology
LMU Department of Mathematics