Genome-wide expression analysis of yeast response during exposure to 4 degrees C

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Murata, Y., Homma, T., Kitagawa, E., Momose, Y., Sato, M. S., Odani, M., Shimizu, H., Hasegawa-Mizusawa, M., Matsumoto, R., Mizukami, S., Fujita, K., Parveen, M., Komatsu, Y., Iwahashi, H. (2006). Genome-wide expression analysis of yeast response during exposure to 4 C. *Extremophiles*, *10*(2), 117-128.

Outline

- Stress response systems in organisms are well studied--except for near freezing response of yeast.
- Common methods were used whenever possible, resulting in more easily reproducible work.
- Cold-shock induces up-regulation of genes and down-regulation of genes that affect cell survival.
- The paper does a poor job of comparing itself to previous work and of suggesting future work.

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Organisms have mechanisms which allow them to withstand environmental stresses

- Under heat stress, increased number of heat shock proteins
- Under cold stress, cell membrane permeability and enzyme activity decrease, expression of cold shock proteins increases
- Efficient translation of mRNA at low temperatures in *E. coli*

Data is lacking for cold responses in yeast at temperatures below 10C

- Exposure to these conditions occurs in natural environments
- Information would be useful in understanding cellular response and adaptation during organism preservation
- This paper examines yeast gene expression 6-48 hours after 4C exposure
    - Differences between cultures *maintained* at 35C or 4C

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Samples were carefully prepared during all phases of the experiment

- Strain S288c was grown in the following conditions:
  - Grown at 25°C until mid-log phase
  - Cooled to 4°C and harvested after 6, 12, 24 & 48 h
  - 2 Controls grown continuously in 4°C & 35°C environments.
- RNA was extracted by the hot-phenol method.
- mRNA was purified with an mRNA purification kit.
- The probes were prepared by mixing 1-2µg of mRNA with two primers and a dNTP nucleotide mix.
  - Reverse transcribed cDNA
  - Probes were purified using a G-50 column

Microarray Hybridization and scanning

- 5,952 targets on microarray
- Mixture of labeled cDNA, DPEC-treated water and 2× hybridization solution
- Hybridized overnight at 65°C
- Scanarray 4000 scanner and GenePix 4000 software used to locate spots on chip
- At least 5 different cultures used
  - Temperature regulated if 3/5 tests are significant
  - ratio >2.0 and <0.5 considered significant
- Hierarchical cluster algorithm by GeneSpring
  - Clusters data as tool for analysis

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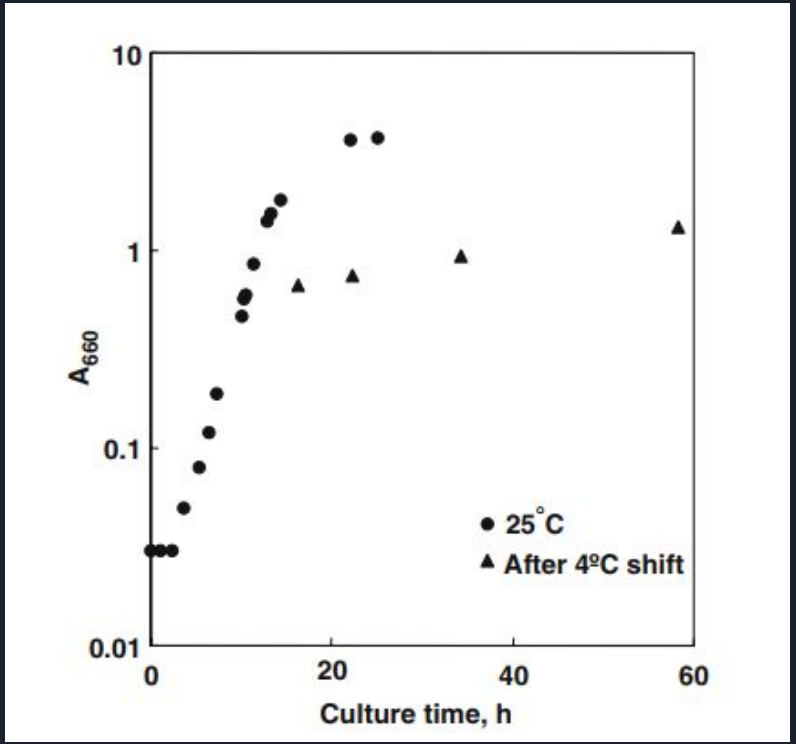
Growth rate of yeast cells decreased after exposure to 4°C

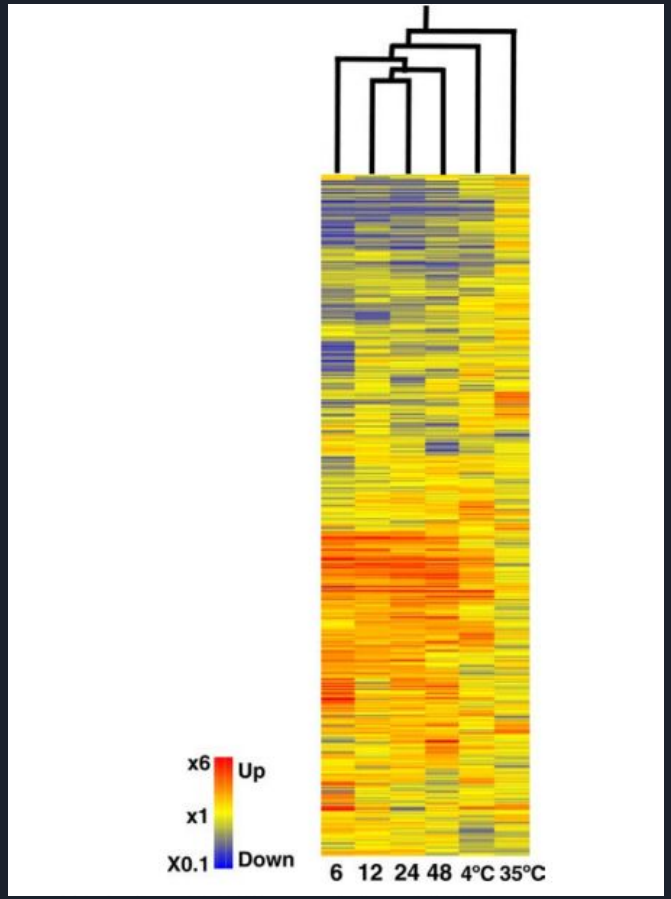
- Growth occurred with a doubling time of 50 h after cells were transferred to 4°C
- Similar to growth when cells are cultured at 4°C for months



Up and down-regulation of genes occurred according to what functions were needed for cell viability

- Genes involved in cell rescue, defense and virulence, and energy and metabolism were upregulated
- Genes involved in protein synthesis, binding functions, activity regulation and fate were downregulated

Hierarchical cluster analysis reveals that gene expression in cold shock yeast is more similar to gene expression in yeast grown at 4°C than 35°C



Genes involved with energy were induced by cold shock

- Trehalose and glycogen synthesis and break down
- Glycolysis and gluconeogenesis
- Aryl-alcohol dehydrogenase
  - Function in yeast is unclear

Genes involved in metabolism were induced

- Phospholipid synthesis (membrane synthesis)
- Methionine biosynthesis pathway
  - Important in the cold acclimation process

Genes involved in cell defense, rescue, and virulence were induced

- Cold shock-inducible protein (cell wall)
  - Function unknown
- Seripauperin protein (stress proteins)
- Heat shock proteins
  - Protein folding
- Detoxification of active oxygen species

Many genes involved in protein synthesis were repressed

- Ribosomal protein large subunit
- Ribosomal protein small subunit
- Others

Transcription of some genes go into overdrive after cold shock, others don’t

- Cold-shock causes up-regulation of genes that lead to
  - Energy preservation and cold tolerance
  - Membrane maintenance and permeability increases
  - Detoxification of active oxygen species
  - Revitalization of enzyme activity
- Causes down-regulation of genes that lead to growth
  - Allows yeast to adapt to new environment

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Many references to previous papers, but little comparison of data

- Similar observations to previous cold-shock studies
  - HSPs were induced by exposure to extreme cold
  - Trehalose synthesis was shown to be important at low temperatures
- Some differences
  - TPS1 and TPS2 genes found to have smaller fold induction than previous study
  - May be explained by difference in carbon source
- No suggestions for further research

We would like to suggest the following ideas for future research

- How different types of stresses lead to similar or different changes in gene expression (e.g. salinity, pressure, chemicals, etc)
- If there is significant change in gene expression depending on the temperature used for cold shock (ie cold shock at 2°C, 4°C and 6°C and 8°C).

Summary

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References

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