The Genome-Wide Early Temporal Response of *Saccharomyces cerevisiae* to Oxidative Stress Induced by Cumene Hydroperoxide

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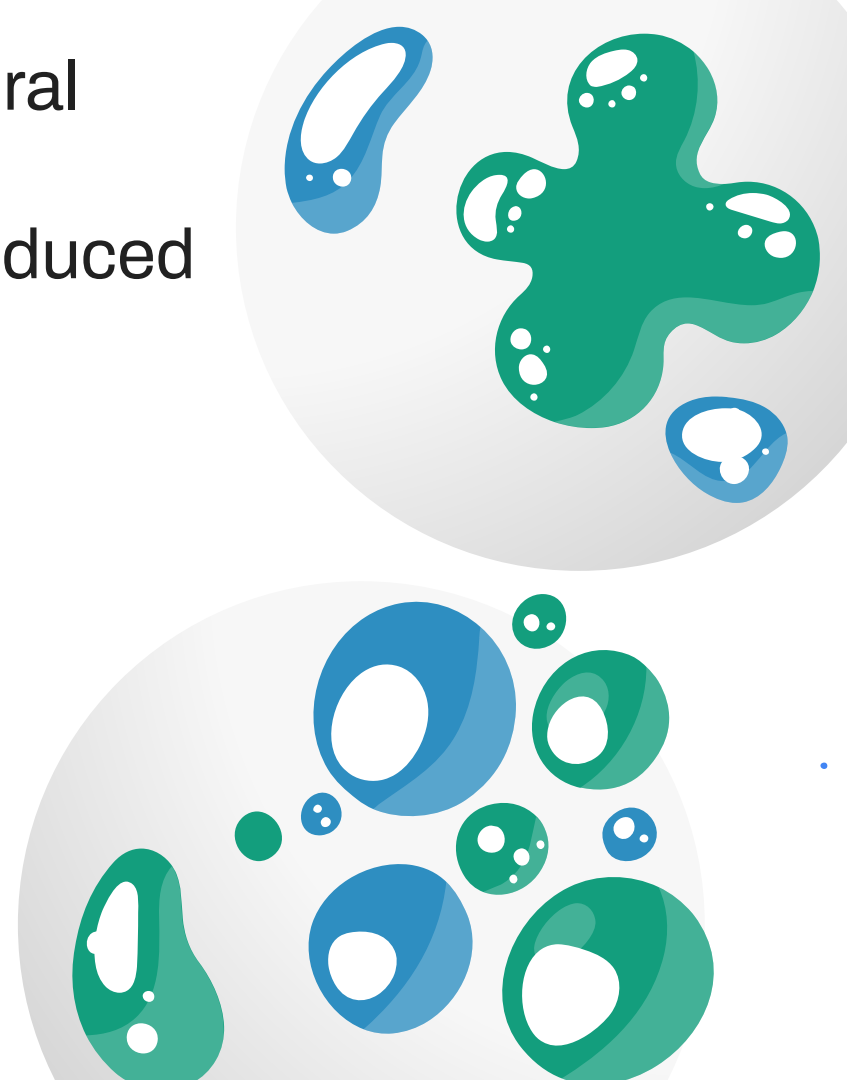


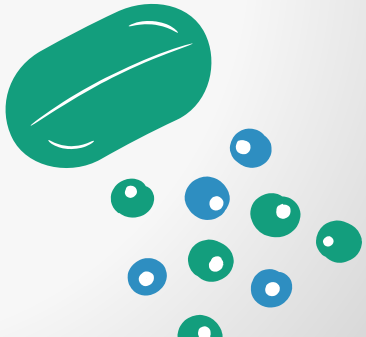
Presented by Hailey Ivanson, Charlotte Kaplan, and Katie Miller, BIOL367

Outline

- Introduction: Cumene hydroperoxide (CHP) triggers the oxidative stress response (OSR) in yeast.
- The OSR can be monitored through yeast's transcriptional response for the first 20 minutes; most notably, the early transcriptional response (0-6 minutes).
- Up- and down-regulation of genes can both explain and be explained by their functions in the yeast OSR.
- CHP and H2O2 as oxidizing agents cause different OSR cascades in yeast, according to their transcriptional responses.

Introduction: Yeast has a physiological response to CHP.

- Cumene hydroperoxide (CHP) is an oxidizing agent that induces an oxidative stress response (OSR) in yeast by creating reactive oxygen species (ROS).
- ROS can cause cellular damage.
- The yeast OSR includes conversion of CHP to cumyl alcohol (COH).



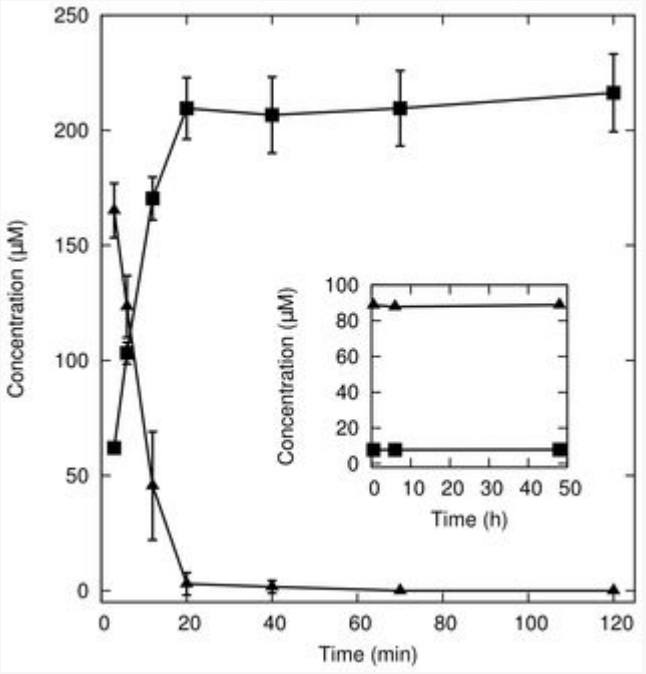
The researchers prepared the yeast for data collection.

- CHP solution was applied to BY4743 strain of *Saccharomyces cerevisiae*.
- Initial culture was grown with 2% (w/v) sucrose and supplemented with uracil L-leucine, and L-histidine, and inoculated into New Brunswick BioFlo fermentors
- Temperature and oxygen were controlled: 30°C and dO2 > 80%
- Transcripts were profiled using the Affymetrix GeneChip system with the Yeast Genome S98 arrays.
- The samples were then amplified with the GeneChip One-Cycle cDNA synthesis kit, and microarray hybridization was performed.
- Raw microarray data was normalized with Robust Multichip Average.
- Then the researchers used 2-way ANOVA gene-by-gene model to determine the significance of difference between 2 time points.

CHP is metabolized rapidly in yeast.

- Most CHP→COH within 20 minutes.
- Yeast is efficient at this process.
- According to Fig. 1, CHP and COH remained stable in the culture medium without yeast.
- This confirms that the yeast actively convert CHP to COH, not just the media itself.

Figure 1. “Time course of oxidant and its product” (Shah et al., 2013).

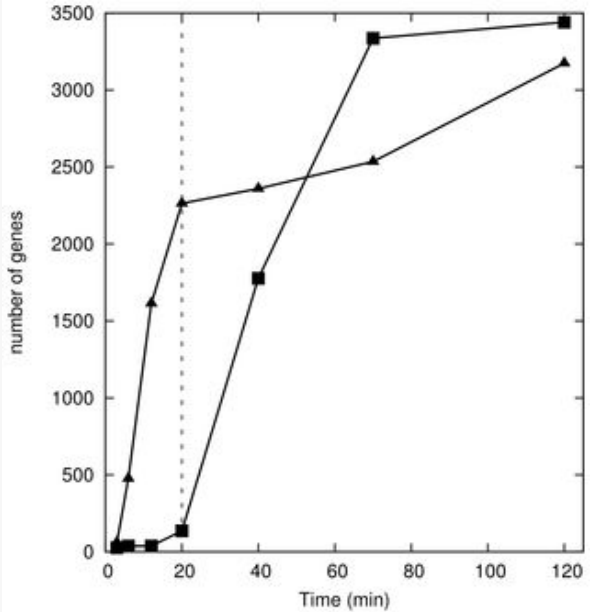


From The Genome-Wide Early Temporal Response of Saccharomyces cerevisiae to Oxidative Stress Induced by Cumene Hydroperoxide (2013). Retrieved from https://doi.org/10.1371/journal.pone.0074939.

Gene expression activity exhibits how yeast genes respond to CHP.

- The researchers study the OSR of yeast to CHP by measuring gene activity over time.
- Control cultures (no CHP) were included to ID any changes unrelated to the oxidant itself.
- Figure 2 reveals major changes to controls at 40 mins.
    - This is unexpected; Minutes 0-20 will be analyzed.
    - All were submitted to Gene Expression Omnibus database.

Figure 2. “Summary of gene expression changes” (Shah et al., 2013).



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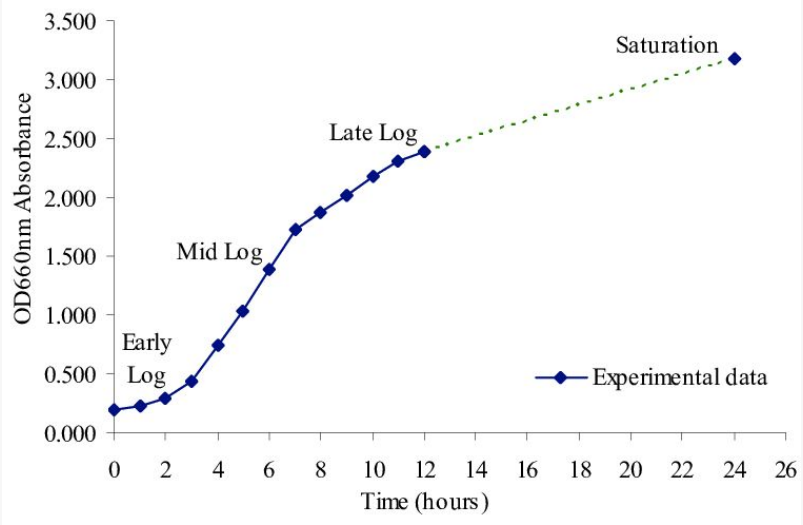
- Figure 2 reveals major changes to controls at 40 mins.
  - This is unexpected; Minutes 0-20 will be analyzed.
  - **Possibly due to letting yeast grow past mid-log phase.**
  - All were submitted to Gene Expression Omnibus database.

Figure 3. Yeast growth chart



From Single Cell Analysis inside Environmental Scanning Electron Microscope (ESEM)-Nanomanipulator System (2010). Retrieved from https://doi.org/10.5772/8858.

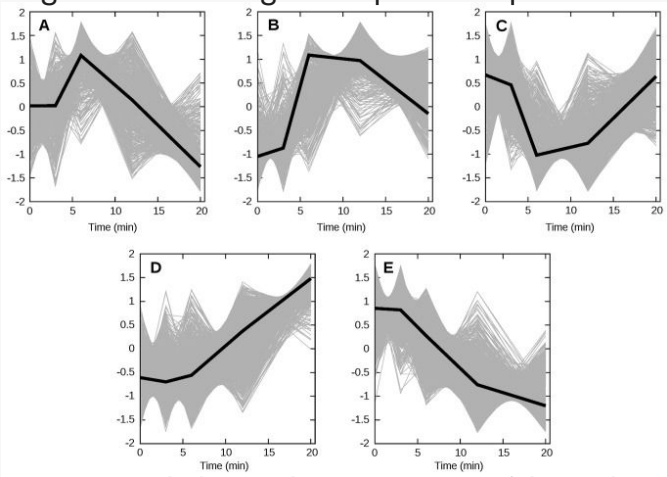
K-means Clustering is utilized to group genes with similar activity patterns over time using TIGR (The Institute for Genomic Research) 3.01.

- Pathways of genes that are overrepresented ($p < 0.01$) are considered significant and grouped into clusters.

Table 1. Pathway analysis of gene expression clusters.

Cluster	Pathway	*P*-value
A	Oxidative phosphorylation	0.0025
B	Galactose metabolism	0.0042
	Starch and sucrose metabolism	0.0062
C	ATP synthesis	9.1 x 10-4
D	Proteasome	1.2 x 10-16
	Ubiquitin-mediated proteolysis	0.0059
	MAPK signaling	0.0081
E	Ribosome	1.8 x 10-14
	Cell cycle	1.3 x 10-5
	RNA polymerase	6.1 x 10-5
	Purine metabolism	1.2 x 10-4
	Pyrimidine metabolism	6.3 x 10-4

Figure 4. Overall gene expression patterns



Both from The Genome-Wide Early Temporal Response of Saccharomyces cerevisiae to Oxidative Stress Induced by Cumene Hydroperoxide (2013). Retrieved from https://doi.org/10.1371/journal.pone.0074939.

The researchers described the clusters by function.

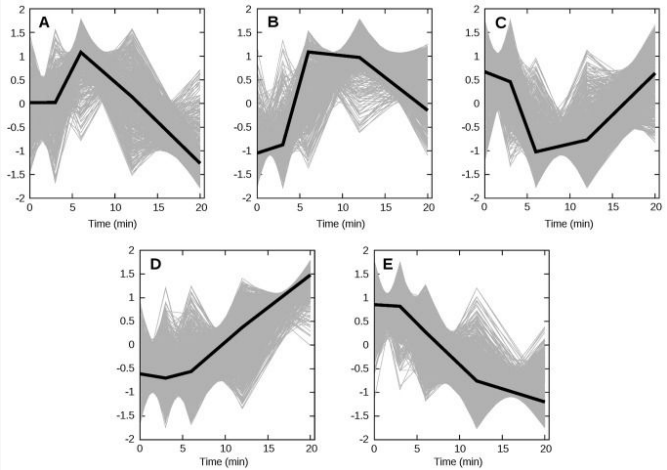
Early Response (Clusters A & B):

- Cluster A showed a quick increase in activity at 6 minutes.
  - Implies immediate OSR defense.
- Cluster B had a slower increase at 12 minutes.
  - Implies a secondary wave of OSR defense.

Adapting to Stress (Clusters C & D):

- Cluster C showed a temporary decrease in activity.
  - Implies redistribution of yeast resources in OSR defense.
- Cluster D showed a gradual increase starting at 20 minutes, potentially related to long-term adjustments for survival.

Figure 4. Overall gene expression patterns



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The researchers described the clusters by function.

Defense Activation (Cluster D):

- Genes involved in proteolysis and managing stress signals were up-regulated.
  - Implies yeast are preparation for cell remodeling in response to stress.

Growth Pause (Cluster E):

- Genes related to growth processes were down-regulated.
  - Implies a shift from multiplication or growth to focus on defense for OSR.

Metabolic Shift (Cluster B):

- Changes in sugar metabolism
  - Imply trehalose production, stress-protective sugar.

☆A and C not described in detail in the paper.

Figure 4. Overall gene expression patterns

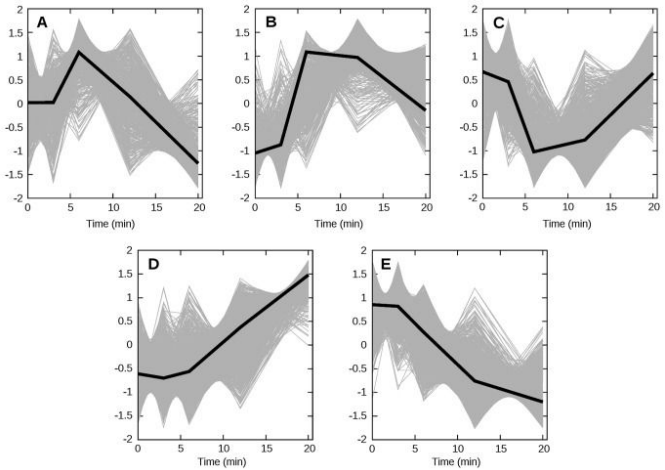

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Figure 5 Shows Patterns of Induction and Repression.

- Graph A:
  - Corresponds to Cluster A, rapidly **induced** genes return to their normal mRNA levels at 12 min, but continue to decrease.
- Graph B:
  - Corresponds to Cluster B, rapidly **repressed** genes return to their normal mRNA levels at 12 min, but continue to increase.
- Researchers wanted to identify induction or repression as early as 3 and/or 6 minutes.

Figure 5. Rapid response genes.

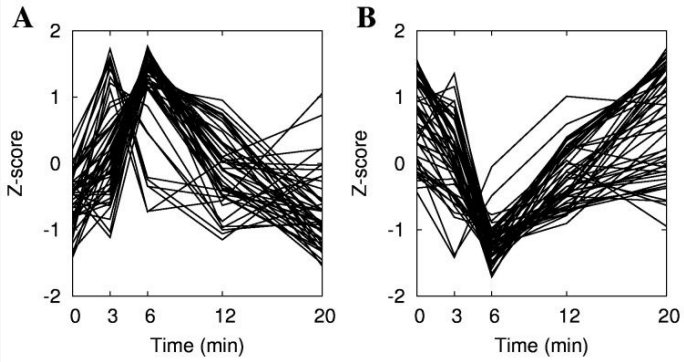

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Figure 5 has some notable mislabeling.

- The y-axis claims to display Z-score, but the figure legend claims that it is a scale of unit standard deviation.
  - Z-scores are only significant indicators of induction or repression if they are above +2 or below −2. Note the values shown.
- The text states there are 44 genes that were significantly induced ($p<0.05$).
- 51 genes were significantly repressed ($p<0.05$.)

Figure 5. Rapid response genes.

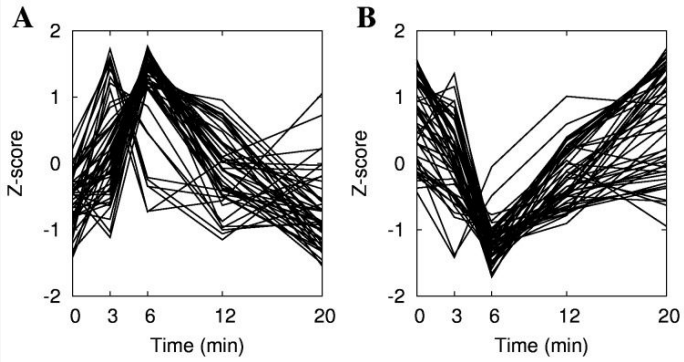

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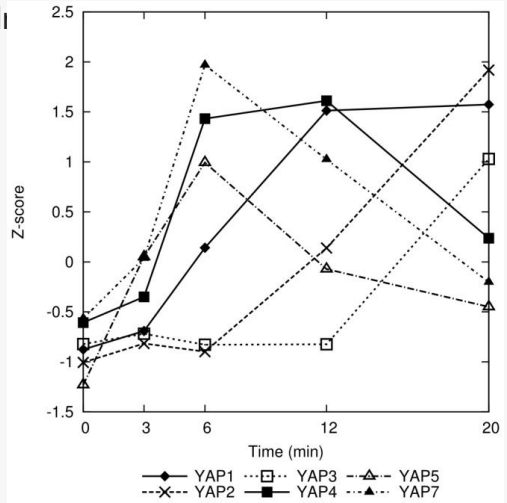
Figure 6 Shows the Expression of YAP Family Genes in Response to Cumene Hydroperoxide.

- Expression of 6 YAP family genes are measured (YAP1, 2, 3, 4, 5, and 7).
- YAP family genes translate to bZIP proteins that act as transcription factors.
- Figure 6 shows the time point the gene expression is induced for each YAP gene.
- The researchers claim this is the first time YAP3, YAP5, and YAP7 have been observed to be induced by oxidative stress.
    - Their functions were previously unknown.

Figure 6. Dynamics of expression of the genes encoding proteins from the yeast YAP family in response to cumene hydr



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YAP Genes Have an Early Induction Response.

- The induction times for transcriptional responses range from 3-12 minutes.
- Other experiments cited could not observe these responses.
    - For example, ChIP-chip needs 15 min of incubation time.
- Figure 6's y-axis is displayed as Z-score, but the legend claims it is in a scale of unit standard deviation.

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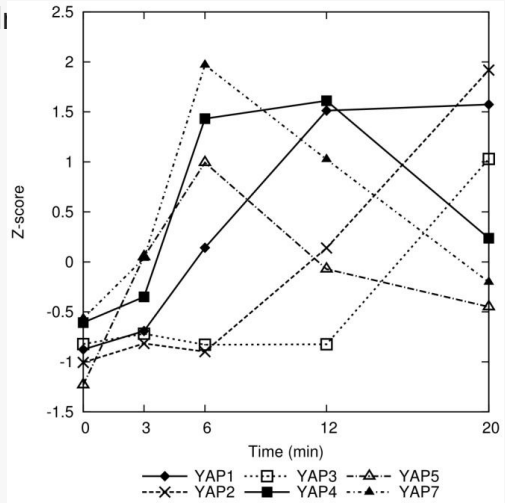

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Figure 7 shows the DNA microarray of transcriptional responses of different regulons.

- Figure 7's DNA microarray has the gene expression response of the genes of three overlapping regulons, YAP1, SKN7, and MSN2/4.
- We can see that there is more overlapping genes between MSN2/4 and YAP1 than any other category.
- Remember: Red is induction and green is repression.
- The log of the ratio of **median** values divided by the median value at time zero is used to quantify intensity.
    - By taking the median value at a time point, they are excluding the data from the other two trials.

Figure 7. Dynamics of the transcriptional response of the YAP1, SKN7, and MSN2/4 regulons.

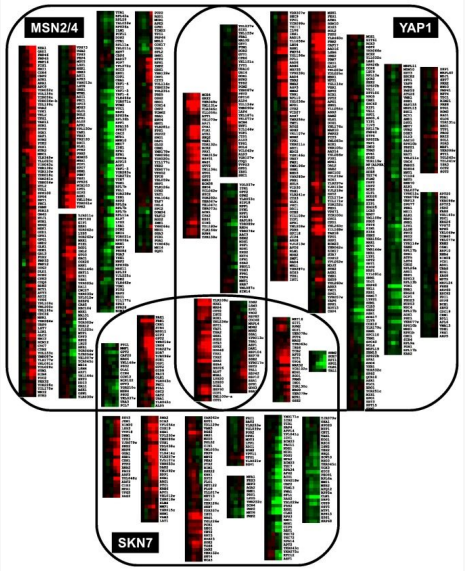

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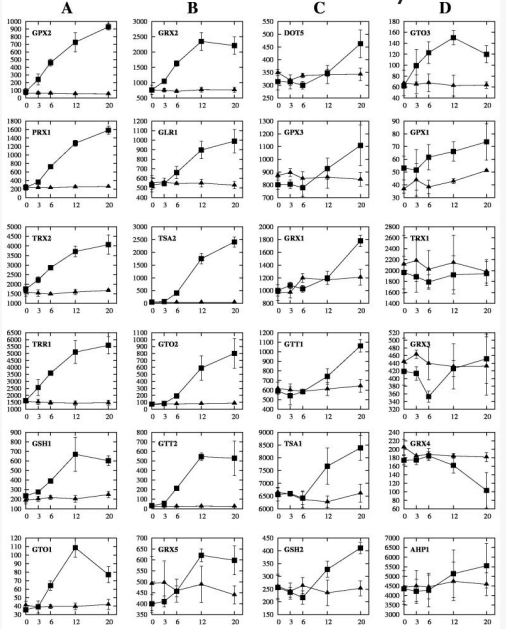
Figure 8 focuses on redox protein-encoding genes grouped into four columns.

- Redox protein-encoding genes are induced with CHP exposure, as seen in Fig. 8.
- Glutathione and thioredoxin systems produce redox proteins that maintain the reduced state of the cytosol.
- Figure 7 is sorted into four different “functions.”
- Increasing L→R in lag time of transcriptional response of genes.
- CHP-treated cultures (squares) compared to controls (triangles).

Figure 8.Dynamics of the transcriptional response of genes involved in the glutathione and thioredoxin systems.



From The Genome-Wide Early Temporal Response of Saccharomyces cerevisiae to Oxidative Stress Induced by Cumene Hydroperoxide (2013). Retrieved from https://doi.org/10.1371/journal.pone.0074939.

Figure 8's columns seem to have many exceptions.

- Column A as well as *GTO3* in column D had no lag in transcriptional response.
  - *Exceptions include GTO1.*
- Column B is delayed by 6 minutes.
  - Exceptions include *GRX2* and *GTT2,* and *GRX5.*
- Column C is delayed by 12 or 20 minutes.
- Column D genes have little to no response other tha the noted *GTO3*.
  - Exceptions include *GPX1.*

Figure 8.Dynamics of the transcriptional response of genes involved in the glutathione and thioredoxin systems.

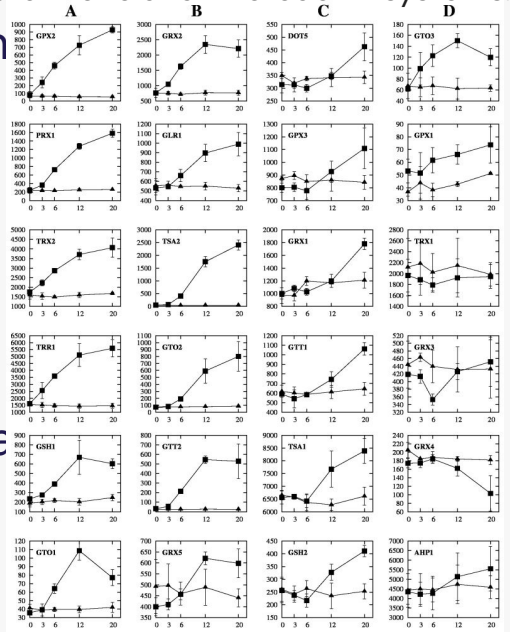


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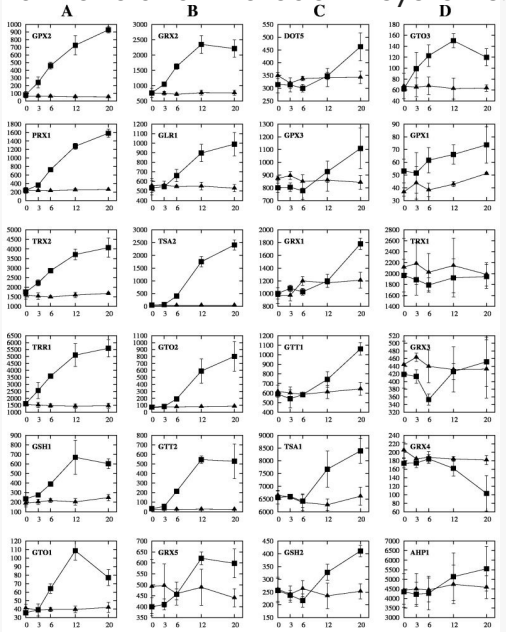
Figure 8's exceptions continue into the genes involved at each transcriptional speed.

- Column sorting is generally ineffective here.
- A gradient effect can be seen, but the number and types of exceptions are too great.
- The researchers described that the "fastest" genes (no associated column) are regulated by Yap1, Msn2/4, and Skn7.
- There are, however, some exceptions to this rule.
    - These do not align with the temporal exceptions noted on the previous slide.

Figure 8.Dynamics of the transcriptional response of genes involved in the glutathione and thioredoxin systems.



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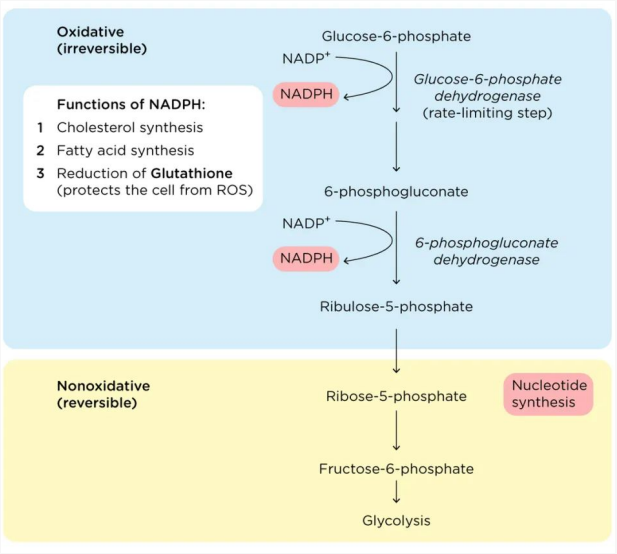
The PPP in yeast is important for its oxidative stress response.

- The pentose phosphate pathway (PPP) produces NADPH, a reducing agent, as well as precursors for important biosynthetic pathways.
  - **Oxidative branch**: Produces NADPH, which helps produce antioxidants by reducing their oxidized forms.
    - Activated when the cell is under oxidative stress.
    - Isoenzyme pairs are either activated or deactivated depending on which branch is active.

Figure 9. The Pentose Phosphate Pathway.

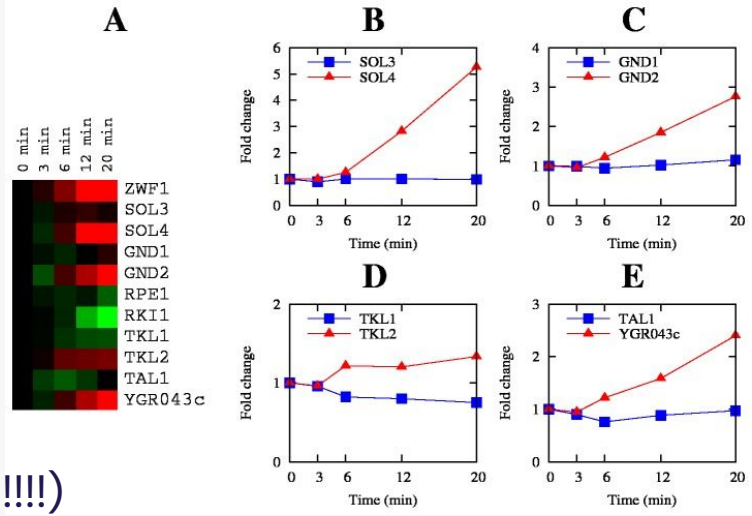


From Jack Westin: The Pentose Phosphate Pathway (n.d.). Retrieved from https://jackwestin.com/resources/mcat-content/glycolysis-gluconeogenesis-and-the-pentose-phosphate-pathway/pentose-phosphate-pathway.

Figure 10 focuses on the regulation of the PPP.

- Figure 10 highlights the isoenzymes involved in the PPP.
- The isoenzyme expressed when exposed to CHP say they are squares.
- The isoenzyme expressed under control conditions say they are triangles.
  - But this is contradictory to the text.
  - SOL4, GND2, TKL2, and NQM1 should be induced under oxidative stress.
    - These are shown as red triangles. (!!!!)
    - Shapes are swapped between Figures 8 and 10, not labeled in Fig. 10.

Figure 10. Dynamics of the transcriptional response of the pentose phosphate pathway.



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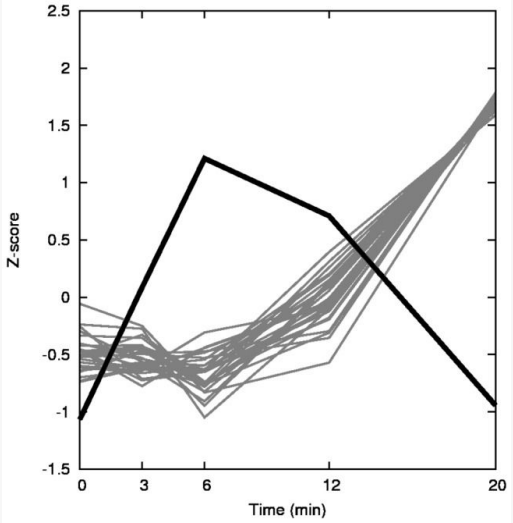
Late-response genes tend to relate to proteasome proteolysis.

- Proteasome proteolysis breaks down partially-oxidized proteins.
  - These are prone to aggregation (bad).
- This occurs late in the OSR (20 mins).
- Proteasome subunit gene transcription is regulated by Rpn4, a transcription factor.
- Figure 9 shows Rpn4 in the cell as a black line.
- The gray curves are the genes of proteasome subunits.
- Their relationship appears to be flipped about the x-axis.
  - Rpn4 is therefore thought to induce the proteasome with CHP exposure.

Figure 11. Dynamics of the transcriptional response of the proteasome genes.



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Exposing yeast to CHP or H_2O_2 yield some differences in their oxidative stress responses.

- CHP and H_2O_2 exposure lead to similar up-regulation of genes involved in stress, glutathione metabolism, and the PPP.
- CHP exposure leads to up-regulation of genes related to cell membrane and cell wall processes while H_2O_2 exposure does not.
    - This is possibly due to CHP's large size; it takes longer to penetrate the cell and therefore damages peripheral cell structures more than H_2O_2.
- CHP exposure leads to down-regulation of genes related to mitochondrial processes while H_2O_2 exposure does not.
    - This is likely because the ETC is not important to CHP tolerance.

Summary

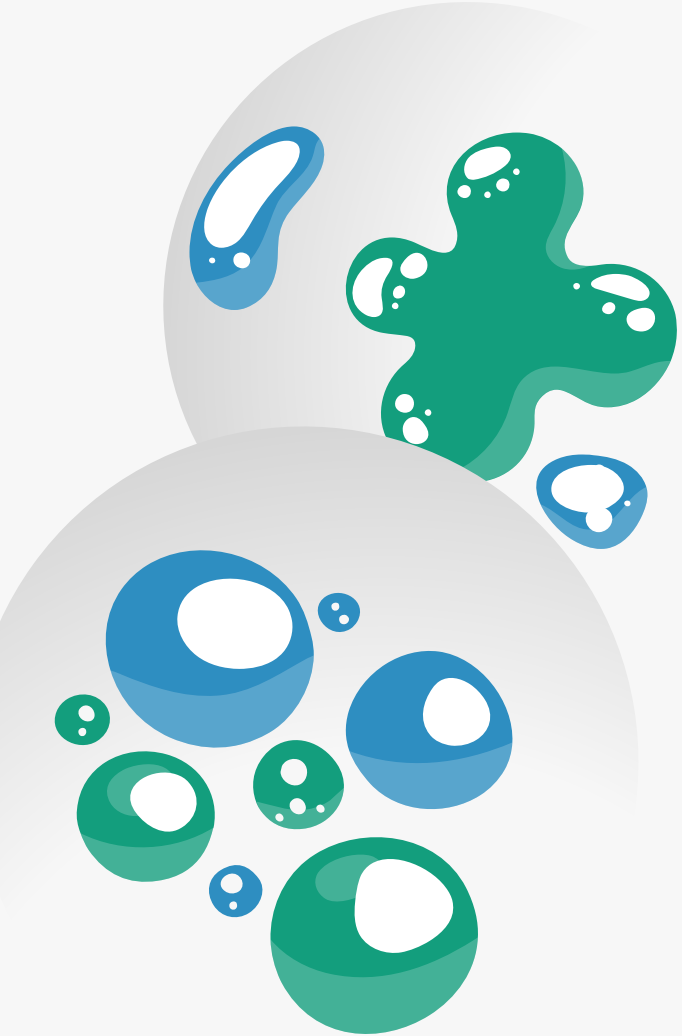
- CHP triggers yeast OSR, changing its transcriptional response.
- The researchers noted that they were the first to publish on the early (0-6 minutes) transcriptional reprogramming of yeast exposed to CHP.
    - Implicated pathways included, most notably:
        - Oxidative phosphorylation and galactose metabolism up-regulation as a first line of defense.
        - ATP synthesis and proteasome proteolysis up-regulation as an adaptive second-line defense.
        - Down-regulation of cell-growth-related genes.
        - Early up-regulation of *RPN4* to trigger Proteasome subunit gene transcription for proteolysis.
        - Redox protein-encoding gene induction in glutathione and thioredoxin systems

Summary (cont.)

- Further, the researchers have found that *YAP3, 5,* and *7* are all up-regulated with CHP exposure, and therefore part of the OSR; their functions were previously unknown.
- CHP and H_2O_2 are managed by yeast OSRs differently.
    - 664 genes are involved in the CHP OSR that are not involved in that of H_2O_2.
        - Genes associated with the cell wall and proteolysis are upregulated.
        - Genes associated with the ETC and mitochondria are downregulated.
- The researchers want to study and the unknown functions of the many genes involved in the yeast OSR to CHP.

Conclusion

- Concerns arise regarding figure caption inconsistencies and poor proofreading, particularly evident in y-axis labeling inconsistencies.
- The y-axis labeling in multiple figures claim to display Z-scores, while the figure legends suggest a scale of unit standard deviation.
- Despite a time frame extending up to 120 minutes, only the data up to 20 minutes was utilized for analysis.
- With this selective use of data, the reliability of recorded trends is thereby brought into question, impacting the quality of this research.



Thanks!

Loyola Marymount University Biology Department
Dr. Dahlquist for creating and teaching Biol 367
Our Biological Databases classmates for listening

References

Biology Major: Mycology and Yeast | Google Slides & PPT. (n.d.). Retrieved April 11, 2024, from https://slidesgo.com/theme/biology-major-for-college-mycology-and-yeast

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