Physiological and Transcriptional Responses of *S. cerevisiae* Under Diurnal Temperature Cycles and *ΔSwi4* Mutant Undergoing Cold Shock

Hebly M, de Ridder D, de Hulster EA, de la Torre Cortes P, Pronk JT, & Daran-Lapujade P

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Outline

- **Yeast is an important model organism and regulates gene expression in response to environmental conditions.**
- **Studies on yeast have investigated temperature shock responses, cultures after acclimation, and glycolysis under cyclic conditions.**
- **Hebly et al. (2014) studied yeast under a diurnal temperature cycle to understand physiological and transcriptomal responses.**
- **The yeast developed a circadian rhythm and showed cyclic control of genes related to metabolism, temperature, and the cell cycle.**
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- **Future studies should focus on important cold-shock genes and allow for growth rates independent of temperature cycles.**

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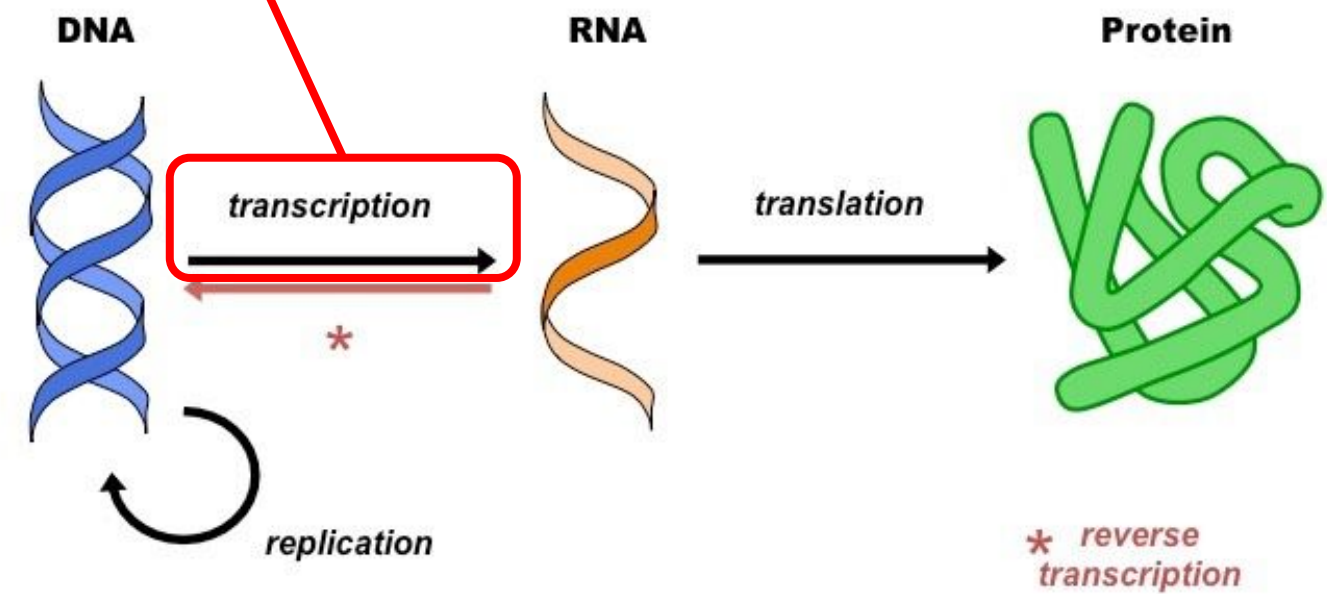
Eukaryotic Gene Regulation Occurs at Multiple Steps Within the Central Dogma of Biology

Transcription is the major step of gene expression regulation.



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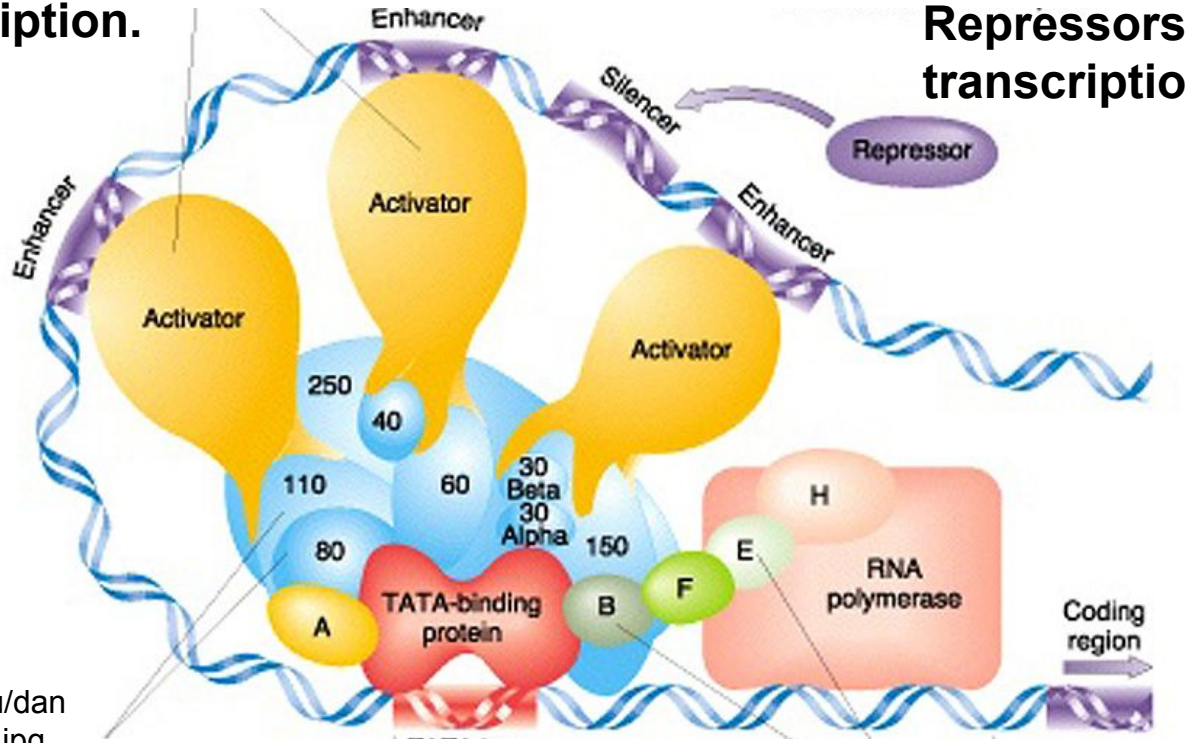
Transcriptional Factors are Proteins that Increase or Decrease Gene Expression

Activators increase transcription.

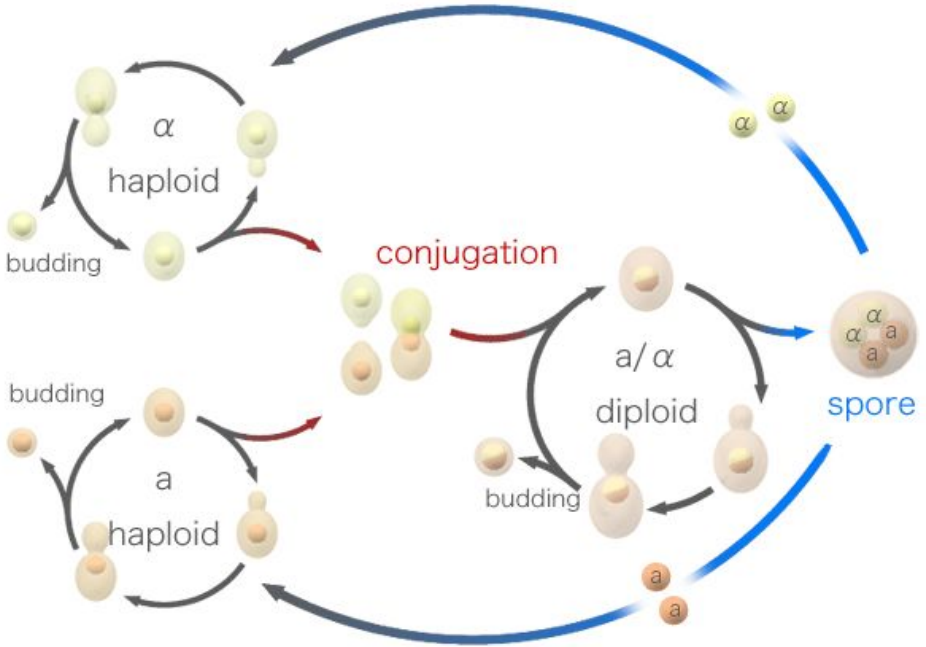
Repressors decrease transcription.



http://www.bio.miami.edu/dana/pix/euktranscripcontrol.jpg

Saccharomyces cerevisiae is an Excellent Model Organism for Eukaryotic Cell Biology

- **Short budding time of ~90 minutes.**
- **Contains only ~6000 genes.**
- **Easy to introduce yeast genes or plasmids.**
- **Enormous data and analysis tools available.**

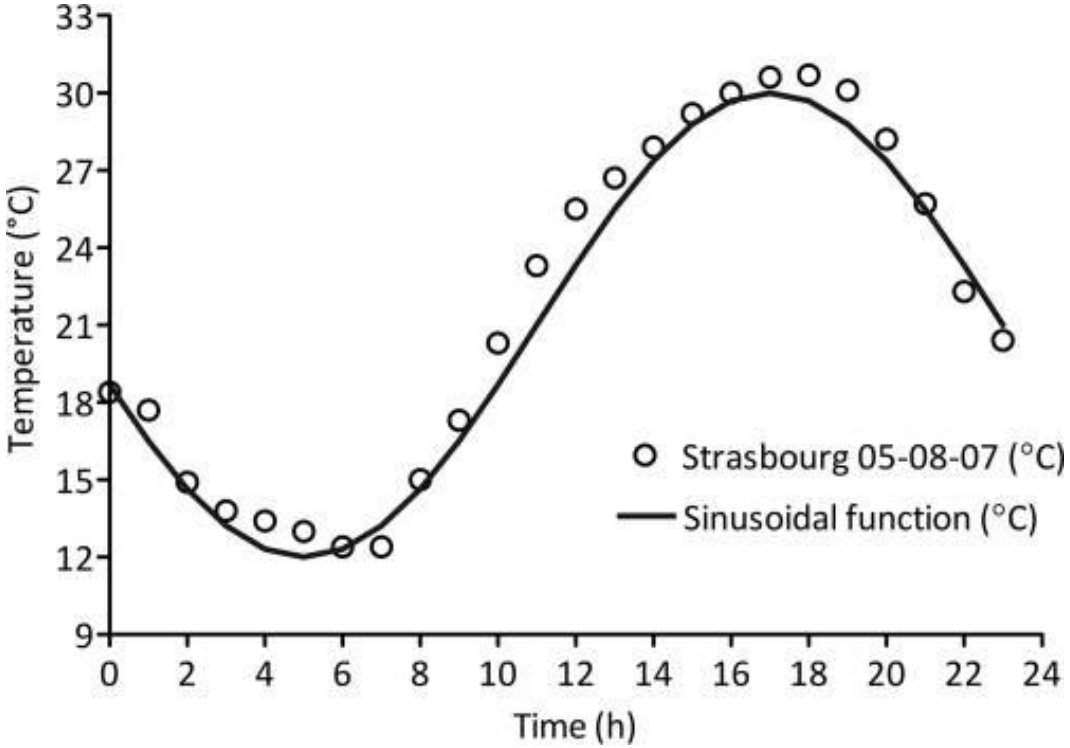


http://wiki.yeastgenome.org/images/e/ea/Yeast_life_cycle.png

Previous Research Has Studied *S. cerevisiae* Gene Expression in Shock and at Various Steady-State Temperatures

- **Past studies focused on acute changes or glycolytic gene expression.**

- **How would a diurnal temperature cycle (DTC) affect:**
  - **a) Physiology?**
  - **b) The entire transcriptome?**



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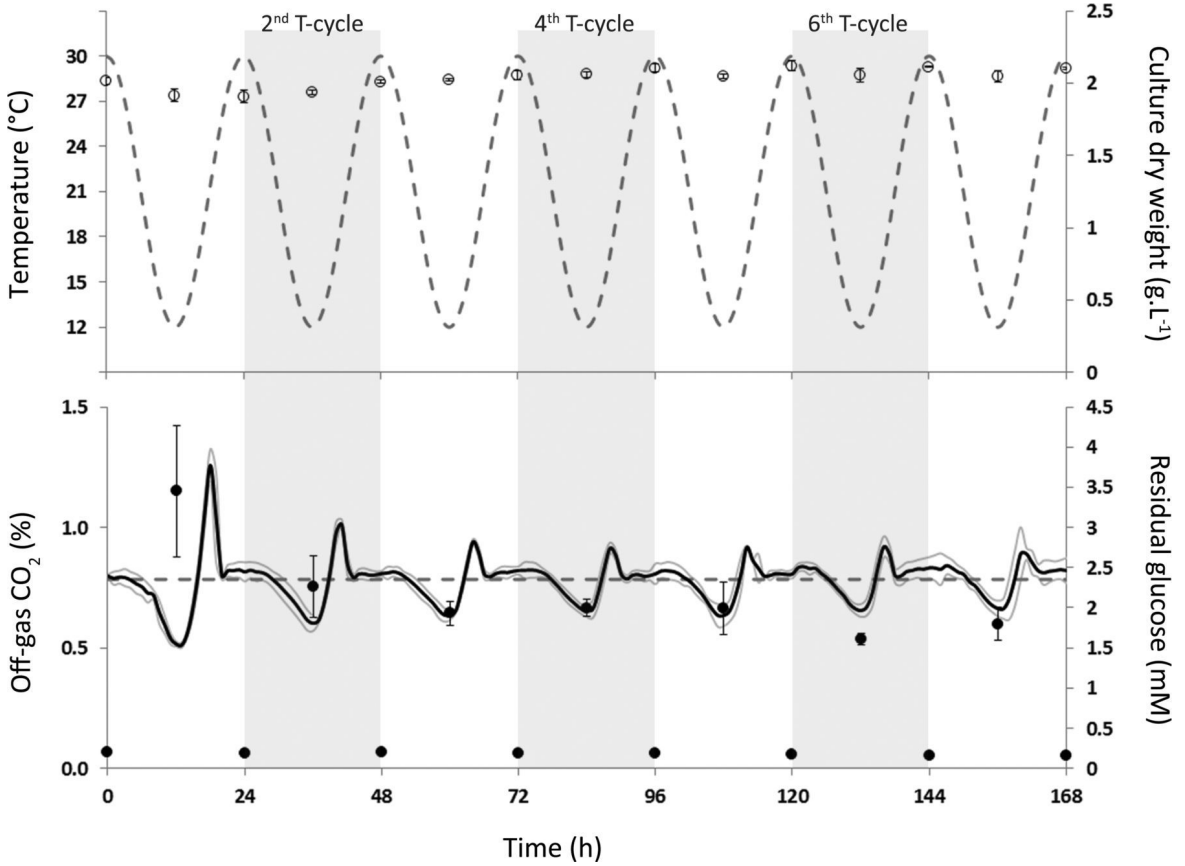
Stabilization of Fluctuation in Residual glucose and CO_2 Levels Suggest Temperature Acclimation

- **Biomass was constant at maximum and minimum temperatures.**
- **CO_2 release and residual glucose levels establish stable cycles inversely related to each other.**



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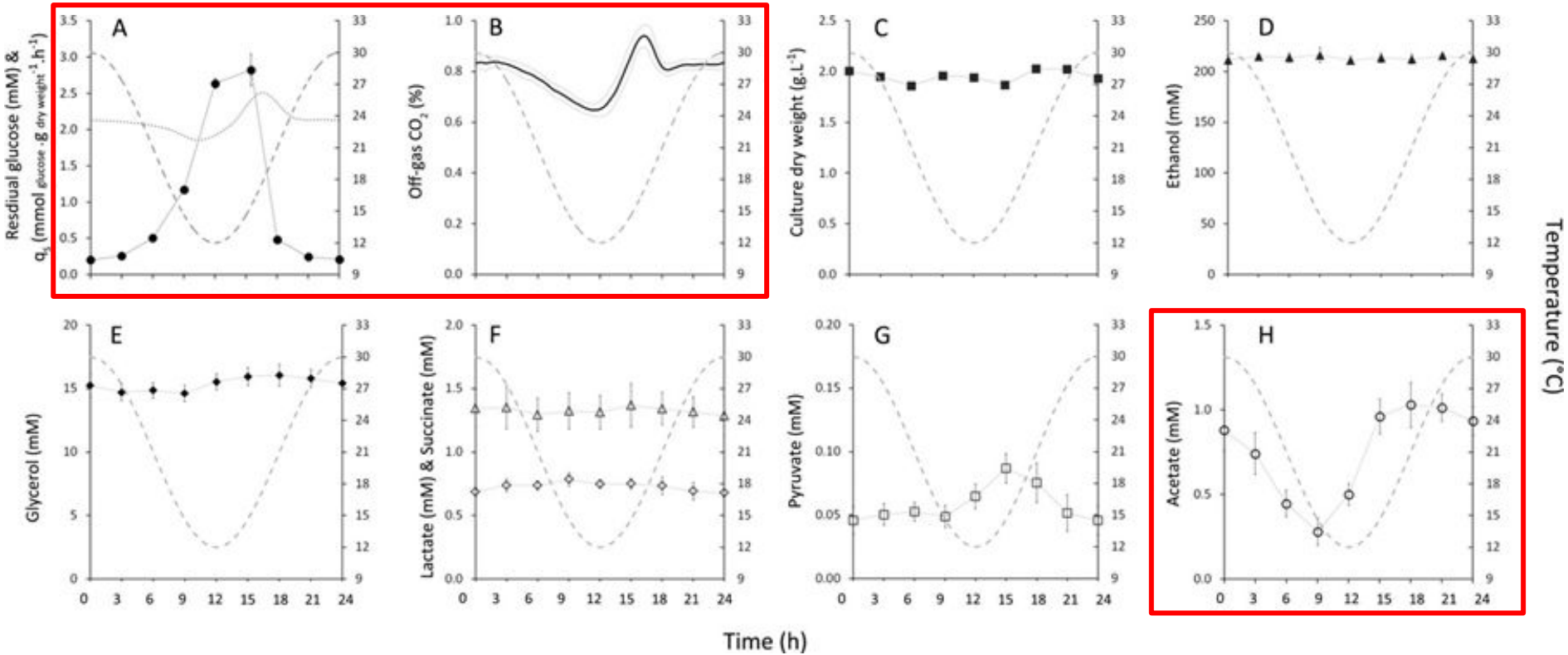
Physiological Analysis Show Related Changes in Some Metabolic Characteristics



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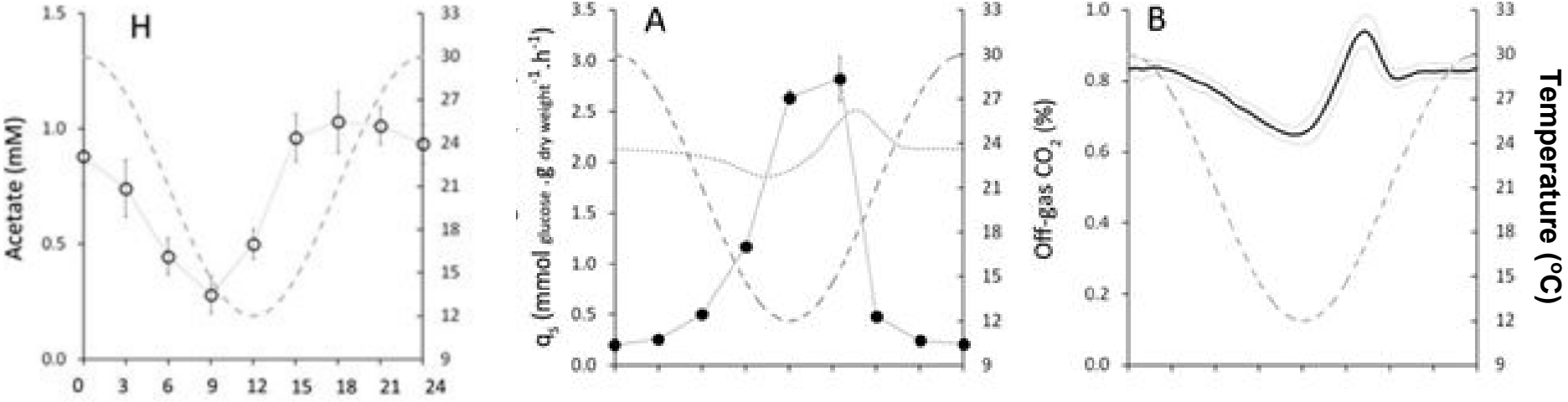
Physiological Analysis Show Related Changes in Some Metabolic Characteristics

Acetate mirrored temperature changes.

Residual glucose responded inversely and asymmetrically to temperature.

CO_2 release fluctuated asymmetrically.



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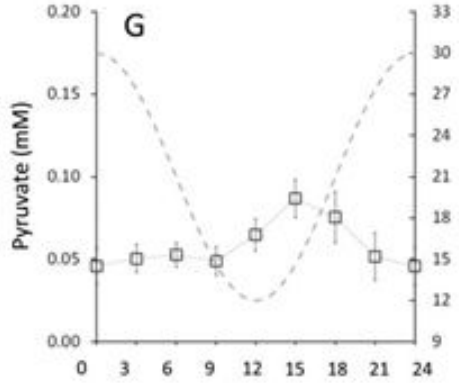
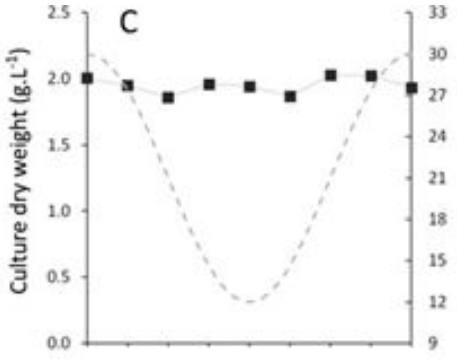
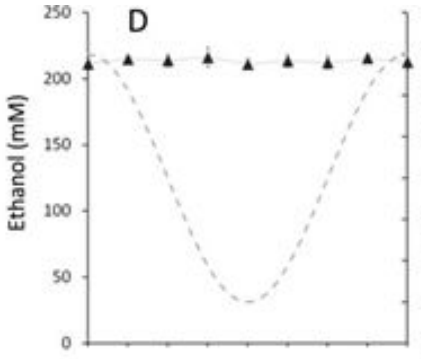
Physiological Changes Show No Change in Some Metabolic Characteristics

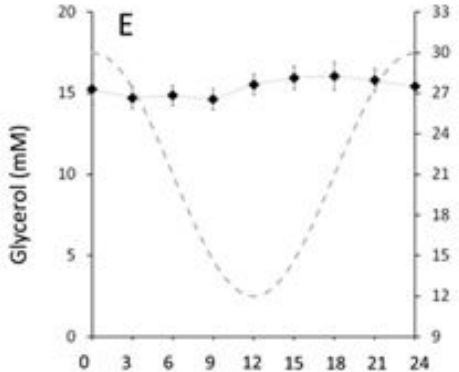
No major changes were seen in:

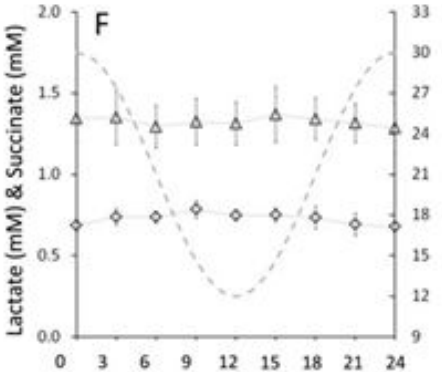
- **(C) Culture dry weight**
- **(D) Ethanol concentration**
- **(E) Glycerol concentration**
- **(F) Lactate and succinate concentrations**
- **(G) Pyruvate concentration**











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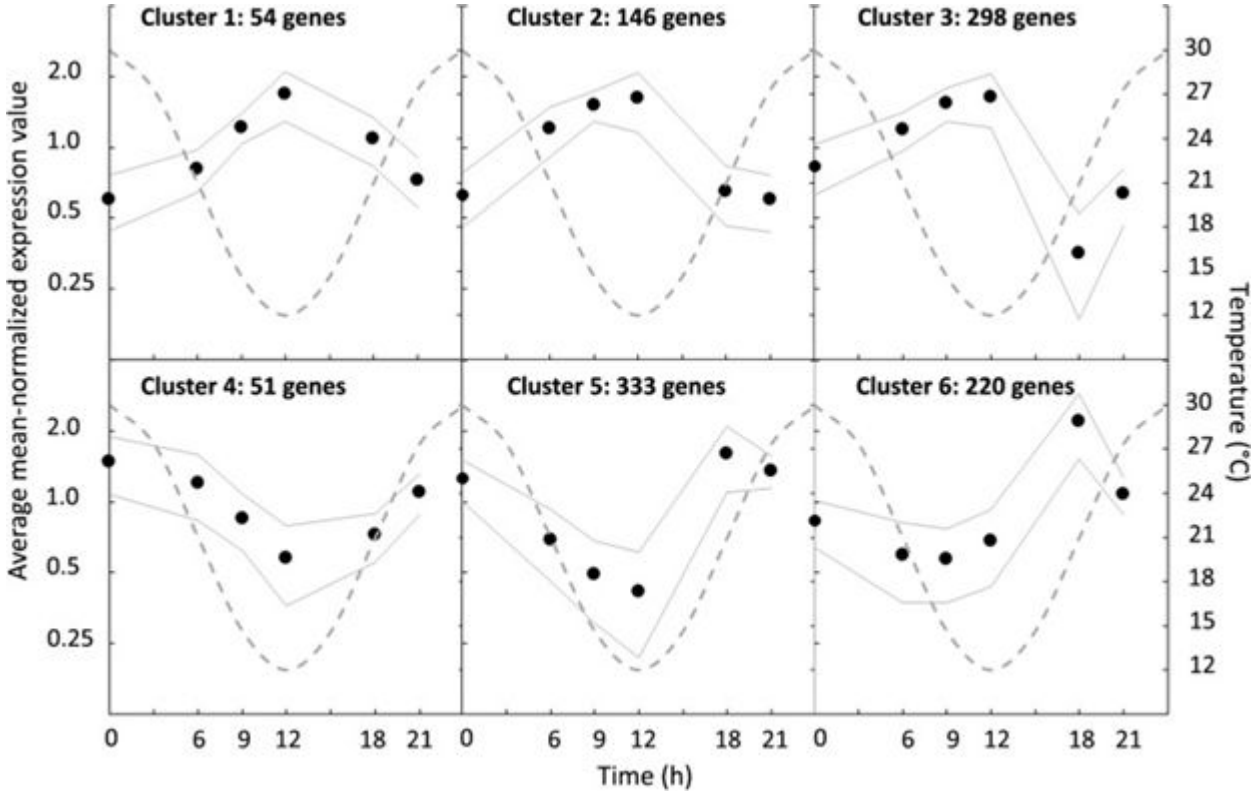
Transcriptome Analysis Showed Major Changes in Gene Expression Dynamics During DTC

- **Clusters included genes with strictly significantly changed expression.**
- **Clusters 1, 2, and 3 had peak expression at 12ºC.**
- **Clusters 4 & 5 had lowest expression at 12ºC.**



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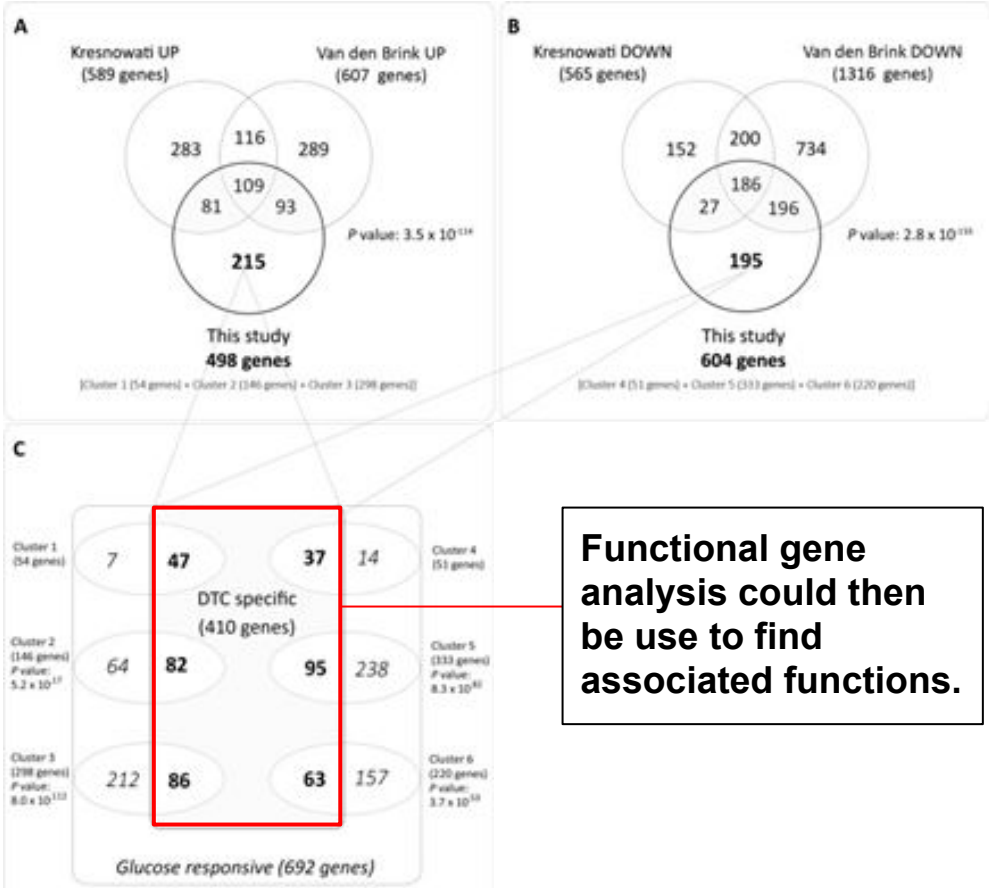
DTC-Specific Genes were Separated from Glucose-Specific Genes

- 215 upregulated genes were glucose-independent.
- 195 downregulated genes were glucose-independent.



Functional gene analysis could then be use to find associated functions.

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Functional Gene Enrichment Showed Associations with Six Major Functions

- 2 categories had no previously assigned function.
- Functions showed relationship to glucose metabolism.

D

Cluster	Enriched functional category (ies) (MIPS functional category or GO category)	No. of significantly changed genes in enriched fun.cat./ total no. of genes in fun.cat.	*P* value
1	none		
2	phospholipid metabolism (01.06.02.01)	6/69	2.3×10^{-4}
	ER to Golgi transport (20.09.07.03)	6/72	3.0×10^{-4}
	Swi6	10/160	3.3×10^{-5}
	Mbp1	10/165	4.4×10^{-5}
	Swi4	9/44	8.5×10^{-5}
	Tec1	6/64	1.5×10^{-4}
	Stb1	4/24	2.2×10^{-4}
	Ino2	4/31	6.1×10^{-4}
3	PROTEIN SYNTHESIS (12)	23/511	1.5×10^{-7}
	Fhl1	16/208	1.2×10^{-8}
	Rap1	9/145	1.3×10^{-4}
4	C-1 compound catabolism (01.05.05.07)	2/5	3.2×10^{-4}
	Bas1	4/39	6.8×10^{-5}
	Gcn4	6/182	5.5×10^{-4}
5	metabolism of arginine (01.01.03.05)	4/20	1.9×10^{-4}
	'de novo' protein folding (GO:0006458)	3/6	6.2×10^{-5}
6	none		

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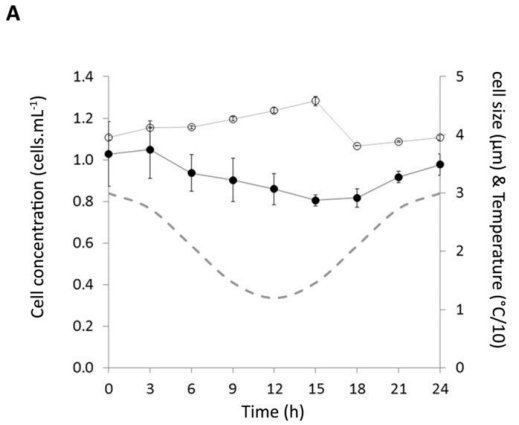
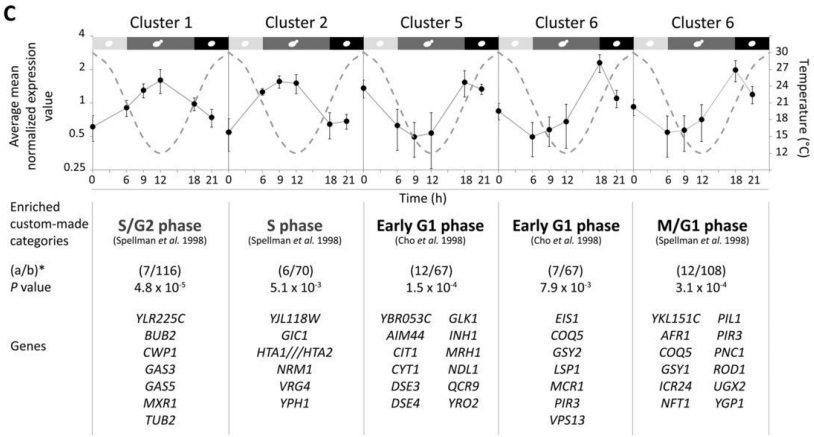
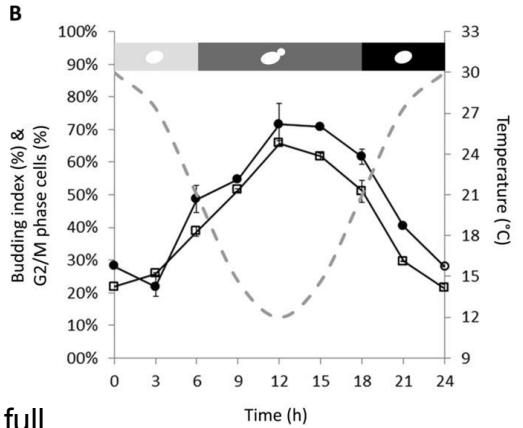
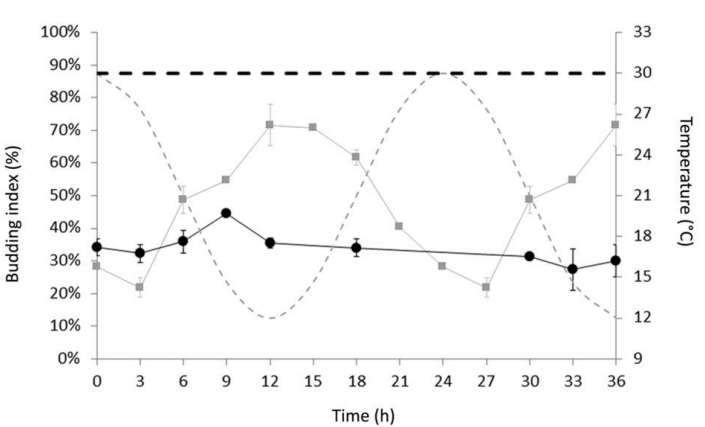
Genes Associated with DTC were Targets of Transcriptional Factors involved in the Cell Cycle

- G2/M-related genes were upregulated at 12ºC.
- Early G1- and M/G1-related genes were upregulated during temperature increase









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Physiological Characteristics of DTC Yeast and Acclimated Yeast were Mostly Similar

- **DTC yeast and yeast acclimated to 12°C or 30°C (Steady State, SS) differed only in glycogen and trehalose content.**

Physiological characteristics of *S. cerevisiae* grown in glucose-limited anaerobic chemostats[a]

Experimental condition	Temp (°C)	Y_{SX} (g glucose · g [dry weight]$^{-1}$)	q_S (mmol · g [dry weight]$^{-1}$ · h^{-1})	q_{EtOH} (mmol · g [dry weight]$^{-1}$ · h^{-1})	q_{CO2} (mmol · g [dry weight]$^{-1}$ · h^{-1})	Carbon recovery (%)	Residual glucose concn (mM)	Glycogen concn (mg glucose equivalent · g [dry weight]$^{-1}$)	Trehalose concn (mg glucose equivalent · g [dry weight]$^{-1}$)	Cell size (μm)	BI (%)
SS	30	0.08 ± 0.004	−2.1 ± 0.15	3.2 ± 0.22	3.7 ± 0.10	95 ± 2.2	0.2 ± 0.03	38 ± 0.2	29 ± 0.2	3.6 ± 0.11	30 ± 3.3
	12	0.09 ± 0.001	−1.8 ± 0.01	2.8 ± 0.01	3.4 ± 0.02	101 ± 0.4	2.1 ± 0.04	121.1 ± 5.7	2.8 ± 0.3	4.4 ± 0.14	65 ± 1.8
DTC	30	0.08[b]	−2.13[c]	ND	3.9 ± 0.12	ND	0.2 ± 0.01	65 ± 0.7	14.5 ± 0.4	4.0 ± 0.04	28 ± 0.2
	12	0.09[b]	−1.96[c]	ND	3.0 ± 0.11	ND	2.6 ± 0.07	50.5 ± 3.6	7.7 ± 0.6	4.4 ± 0.05	72 ± 6.4

[a]Values represent the averages ± standard errors of the mean of at least two independent replicates. SS, steady state; EtOH, ethyl alcohol; ND, not determined.
[b]The biomass yield during DTC was calculated by using the biomass specific glucose consumption rate listed and a specific growth rate of 0.03 h^{-1}.
[c]The profile of the biomass specific glucose consumption rate during DTC is shown in Fig. 3A. The intermediate q_S values of the time intervals of −1.5 h to 1.5 h and 10.5 to 13.5 h, corresponding to the q_S at 30°C and 12°C, respectively, are shown.

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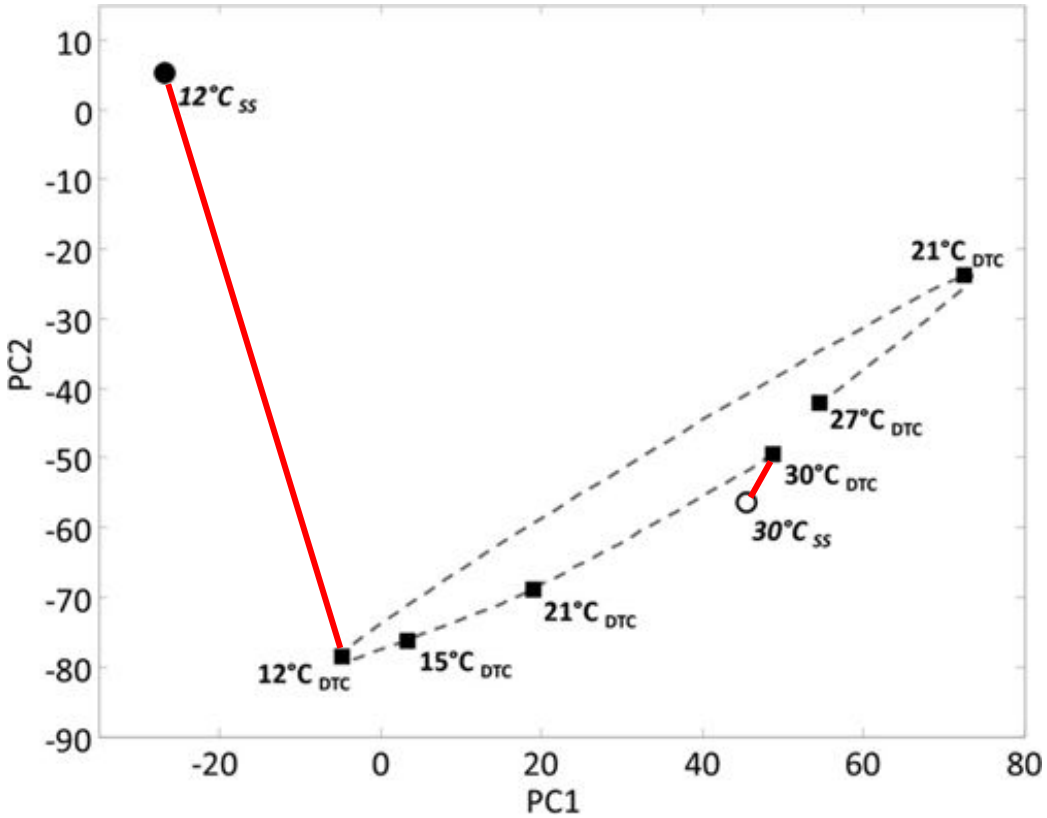
Principle Component Analysis Shows Differences in Overall Gene Expression Between DTC and Steady State

- Overall transcript levels were similar at 30ºC between DTC and SS Yeast.
- Levels differed greatly at 12ºC between DTC and SS Yeast.



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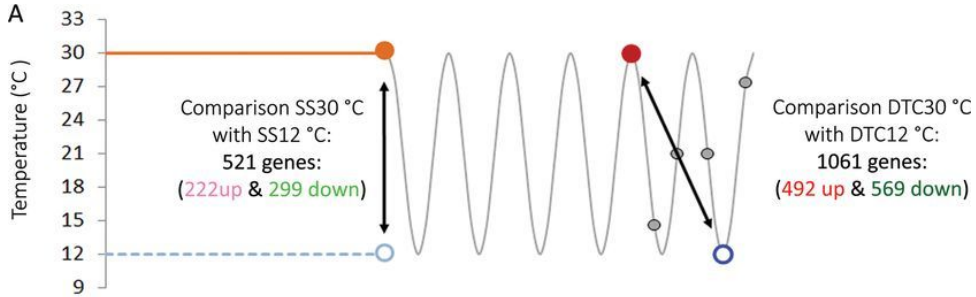
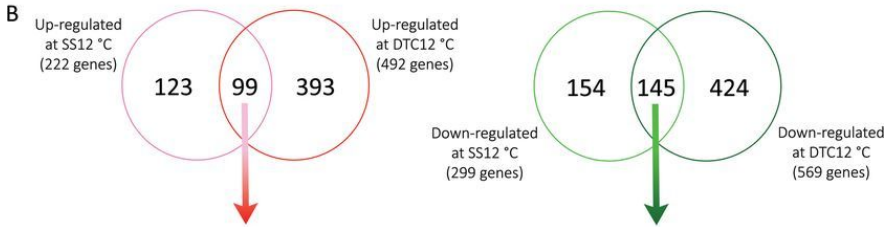
Pairwise Transcriptome Analysis Shows Differences in Gene Expression Between DTC and Steady State

- **(A) Pairwise analysis shows twice as many genes involved in DTC temperature response.**
- **(B) Pooled analysis shows some up- and down-regulated genes changed the same in DTC as SS at 12ºC.**





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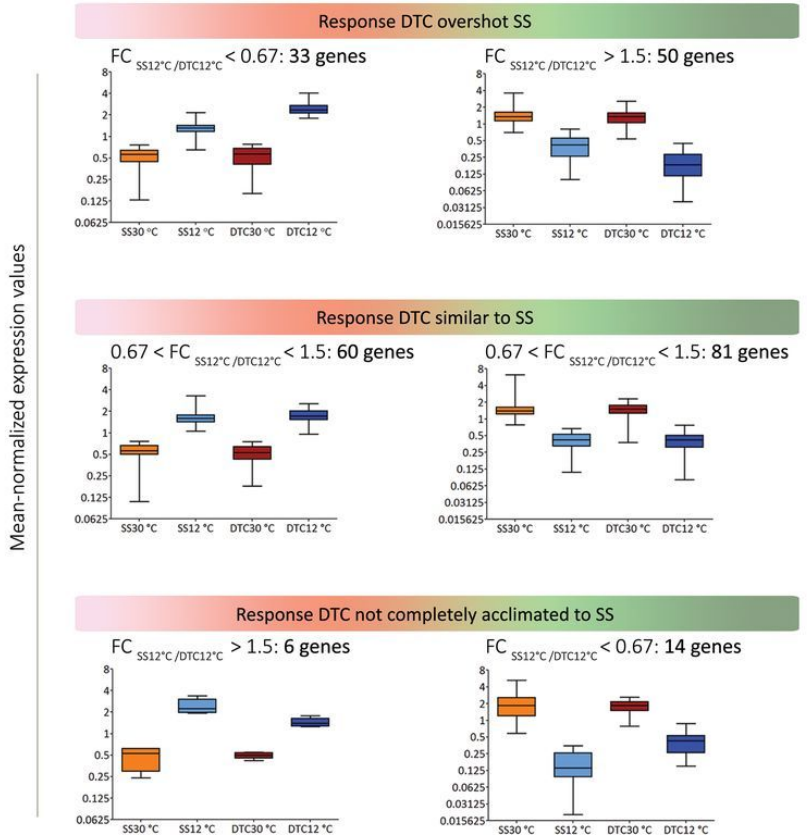
Clustering of DTC and SS Responses at 12ºC Show Differences in Gene Expression Magnitude

83 genes had more pronounced expression in DTC than in SS.

141 genes had similar expression magnitudes.

20 genes in DTC cultures did not reach SS levels.

C



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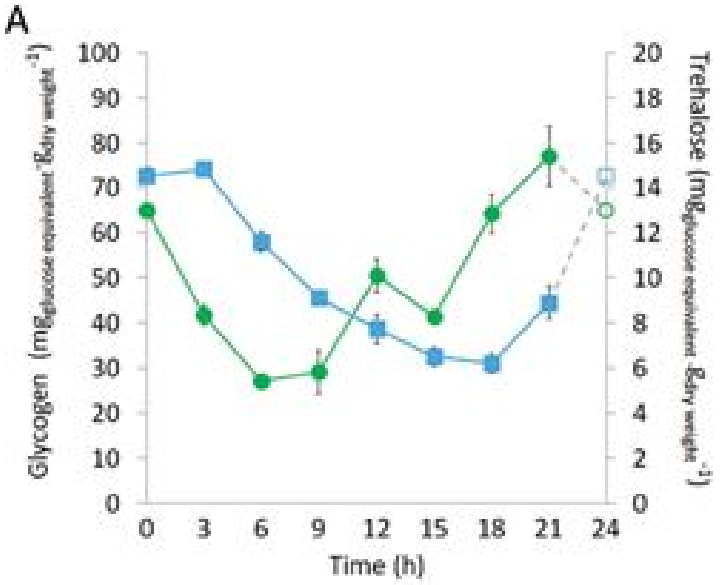
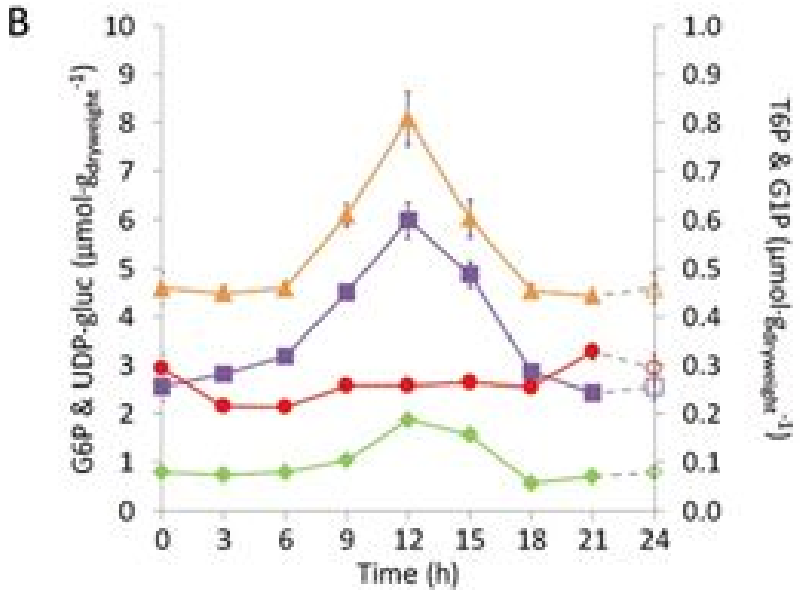
Physiological-Transcriptional Comparison Shows Carbohydrate Reserve is not a Direct Result of Temperature

- Intracellular glycogen and trehalose decrease and diverge.
- But intracellular UDP-glucose and T6P do not change, while G1P and G6P mirror temperature changes.





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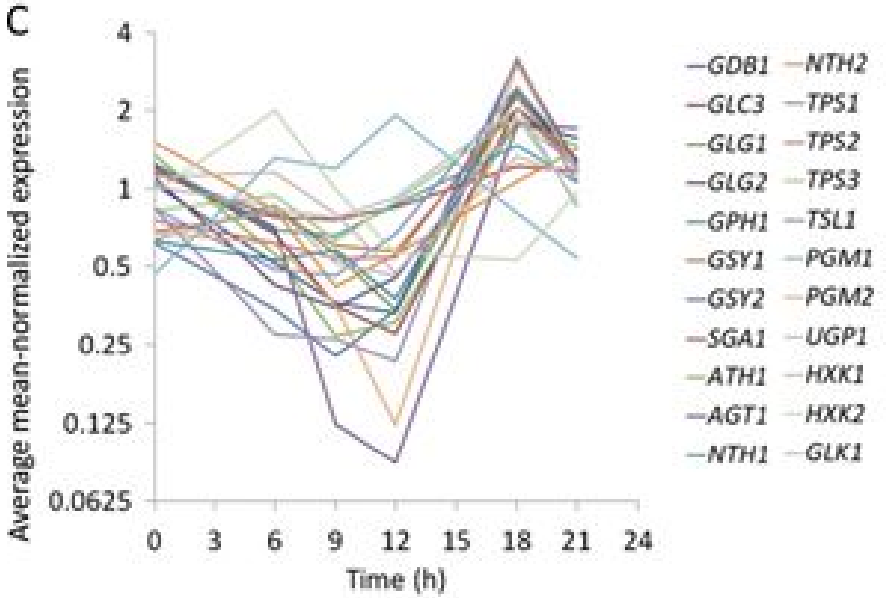
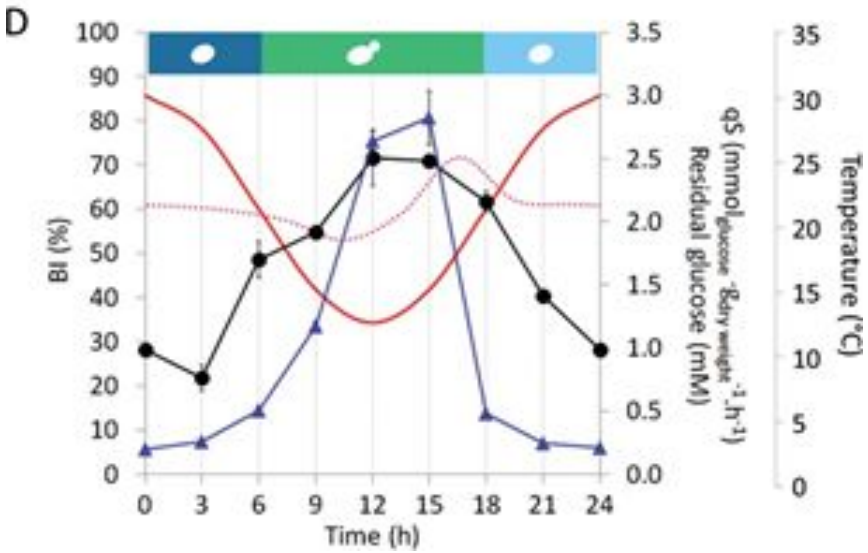
Carbohydrate Profile of *S. cerevisiae* is Related to Imposed Fluctuation in Growth Rate and the Cell Cycle

- **Glycogen and trehalose synthesis/degradation transcription coincides in response to glucose.**
- **Reserve carbohydrate mobilization occurs during late G_1 phase of the cell cycle.**





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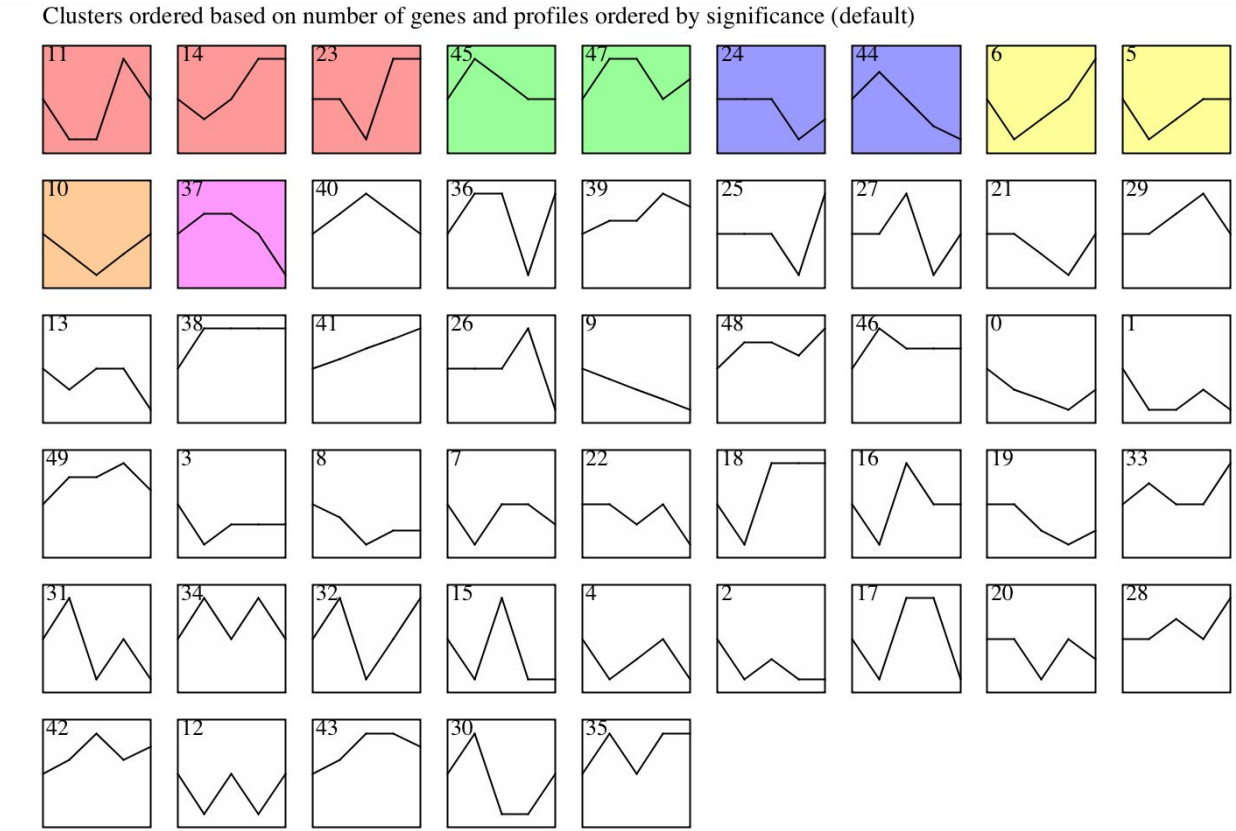
We Monitored Gene Expression of a Yeast Deletion Strain Undergoing Cold-Shock and Recovery

	In-Lab Microarray Experiment	Hebly's Experiment
Strain Type	*Δswi4*	Saccharomyces cerevisiae (CEN.PK113-7D)
Timepoints	30, 60, 90, 120 (minutes)	0, 6, 9, 12, 18, 21… (hours)
Temperature	13℃ with recovery	Diurnal Temp. Cycle of 12-30℃
Media Type	YPD - Rich Media	Silicon Media with anaerobic growth factors

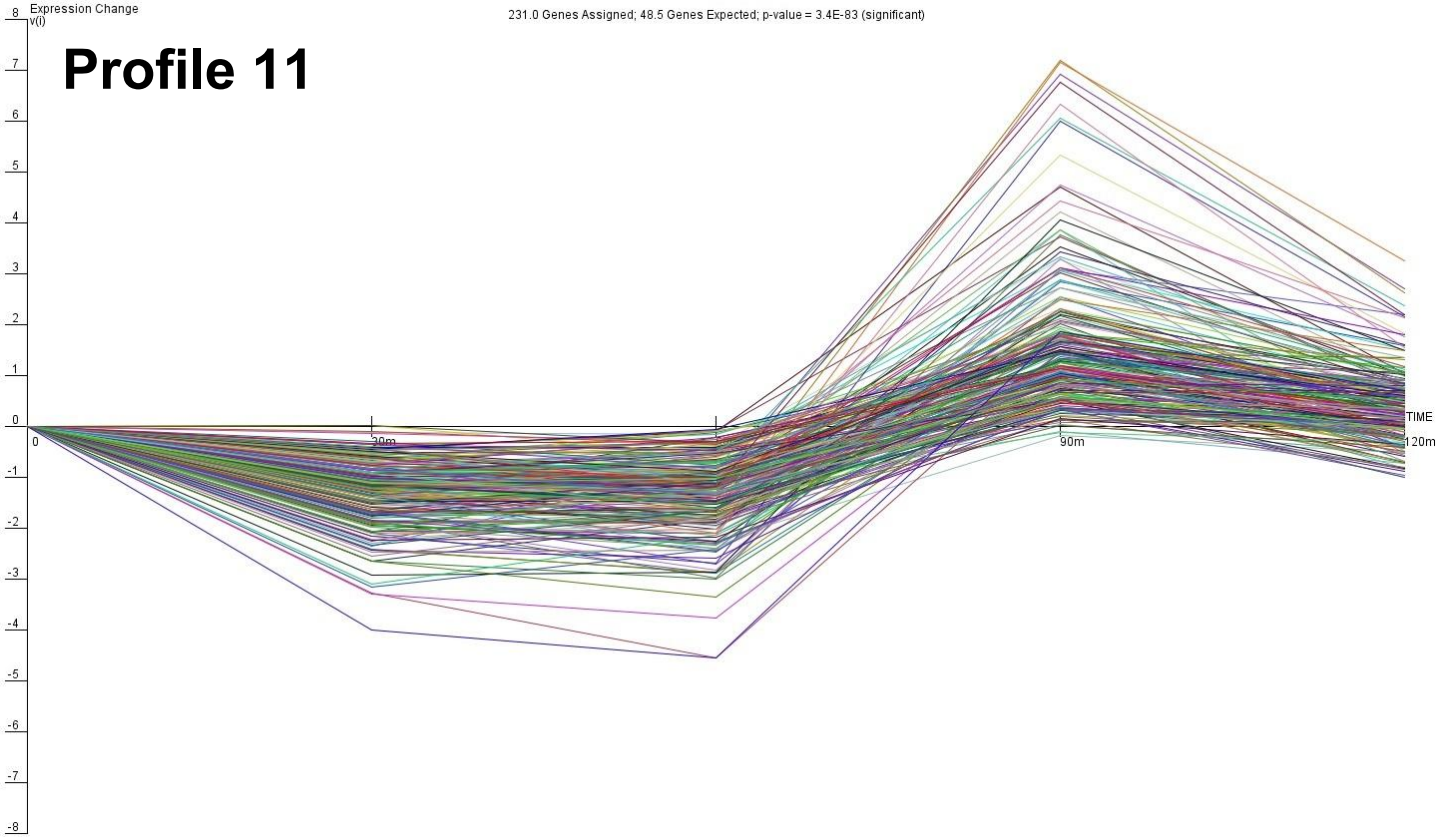
Different Significances Allowed for Analysis of the Deletion Strain's Gene Expression at Varying Levels

ANOVA	*dSWI4*
$p < 0.05$	2782 (45.0%)
$p < 0.01$	1837 (29.7%)
$p < 0.001$	977 (15.8%)
$p < 0.0001$	522 (8.43%)
B & H $p < 0.05$	224 (3.62%)
Bonferroni $p < 0.05$	2080 (33.6%)

Eleven Profiles Showed a Significant Number of Genes Assigned



231 Genes Showed Downregulation During Cold-Shock and Acute Upregulation During Recovery



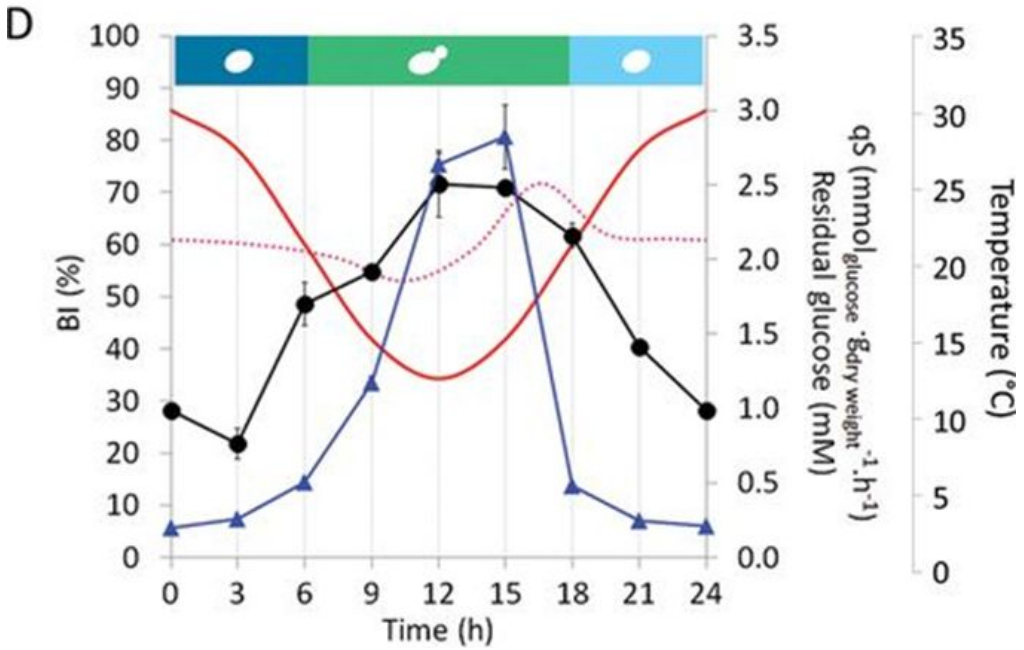
The Largest Gene Cluster Shows a Wide Variety of Functions

GO Term from Profile 11	Gene	Genes Assigned	Genes Expected	Genes Enriched	P-value	Corrected P-value	Fold
cortical actin cytoskeleton	26	13	2.6	10.4	2.60E-07	<0.001	4.9
regulation of cellular component size	29	13	2.9	10.1	1.30E-06	0.004	4.4
carbohydrate metabolic process	109	27	11	16	4.70E-06	0.006	2.5
response to stress	279	46	28.2	17.8	2.70E-04	0.106	1.6
reciprocal meiotic recombination	19	7	1.9	5.1	1.70E-03	0.522	3.6
phosphotransferase activity, alcohol group as acceptor	56	14	5.7	8.3	9.40E-04	0.388	2.5

Some Profile 11 Genes Functions are Related to Important Functions During Cold Shock

- **Chaperonin containing the T-complex polypeptide-1 (CCT) is likely important for cold-shock responses in yeast (Al-Fageeh & Smales 2006).**
  - **CCT binds actin and tubulin, suggesting some cytoskeletal involvement in heat shock (Al-Fageeh & Smales 2006).**
  - **The 4.9 fold decrease in cortical actin cytoskeleton supports the existence of a relationship.**
- **Alcohol phosphotransferase activity reaffirms involvement of decreased metabolism of carbohydrates and increased storage.**
  - **This is one of many of the functions part of a “response to stress.”**

Profile 11 Genes Support Sugar Metabolism Mirroring Cell Cycle Activity



- **Reciprocal meiotic recombination is an important step in meiosis I.**
  - **Carbohydrate metabolic processes decrease and recover in parallel.**
  - **This supports the results of Hebly et al. (2016).**

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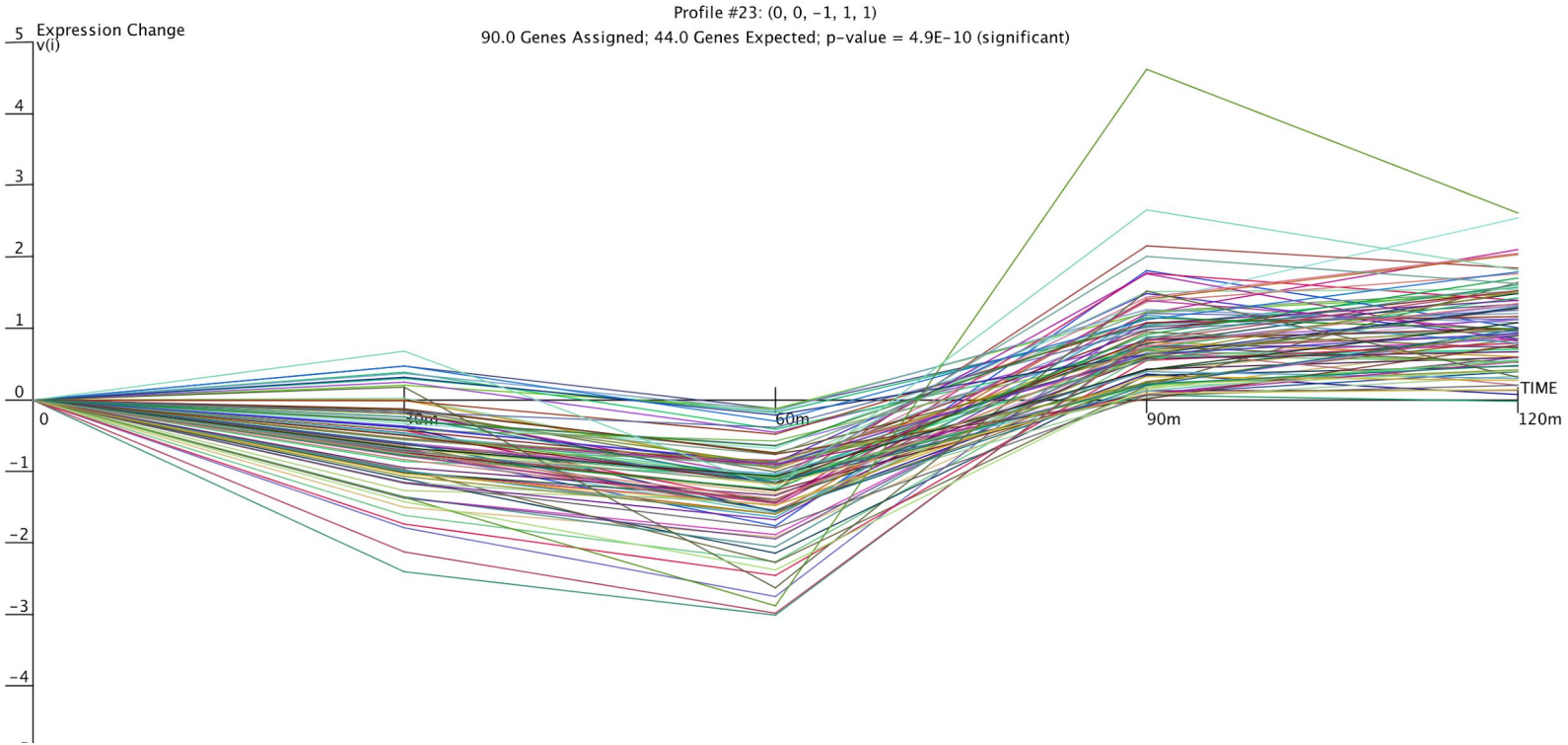
Profile 23 Showed Slight Downregulation During Cold Shock Followed by a More Pronounced Upregulation During Recovery



Profile 23 Shows GO Terms Relating to Energy Metabolism

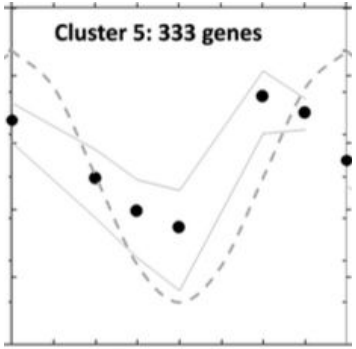
GO Term	Gene	Genes Assigned	Genes Expected	Genes Enriched	P-value	Corrected P-value	Fold
cytoplasmic part	1172	65	46.2	18.8	3.00E-05	0.016	1.4
mitochondrial matrix	99	13	3.9	9.1	8.80E-05	0.034	3.3
oxidoreductase activity	156	12	6.1	5.9	0.02	0.902	2
organelle envelope	209	14	8.2	5.8	0.03	0.974	1.7
protein transport	207	14	8.2	5.8	0.03	0.964	1.7
endosome	64	6	2.5	3.5	0.04	0.986	2.4

Profile 23 Shows Protein Delivery and Cellular Functions Downregulated During Stress to Avoid Excess Energy Use

- **Cytoplasmic Part: Structures in the cytoplasm are not as active during stress resulting in a downregulation during the cold shock.**
- **Mitochondrial Matrix: Contains the enzymes of the tricarboxylic acid cycle (Krebs Cycle), will need to conserve energy during stress and will have a more difficult time creating energy.**
- **Oxidoreductase activity: The catalysis of an oxidation-reduction (redox) reaction is used to create energy which needs to be conserved in a high stress environment.**
- **Organelle envelope and Protein Transport: Both show that protein transportation between organelles is downregulated during low temperatures to conserve energy.**
- **Endosome: Endocytosis is downregulated to conserve energy during the cold shock.**

Profile 23 Showed Similar Results to Cluster 5 of Hebly et al. (2014)

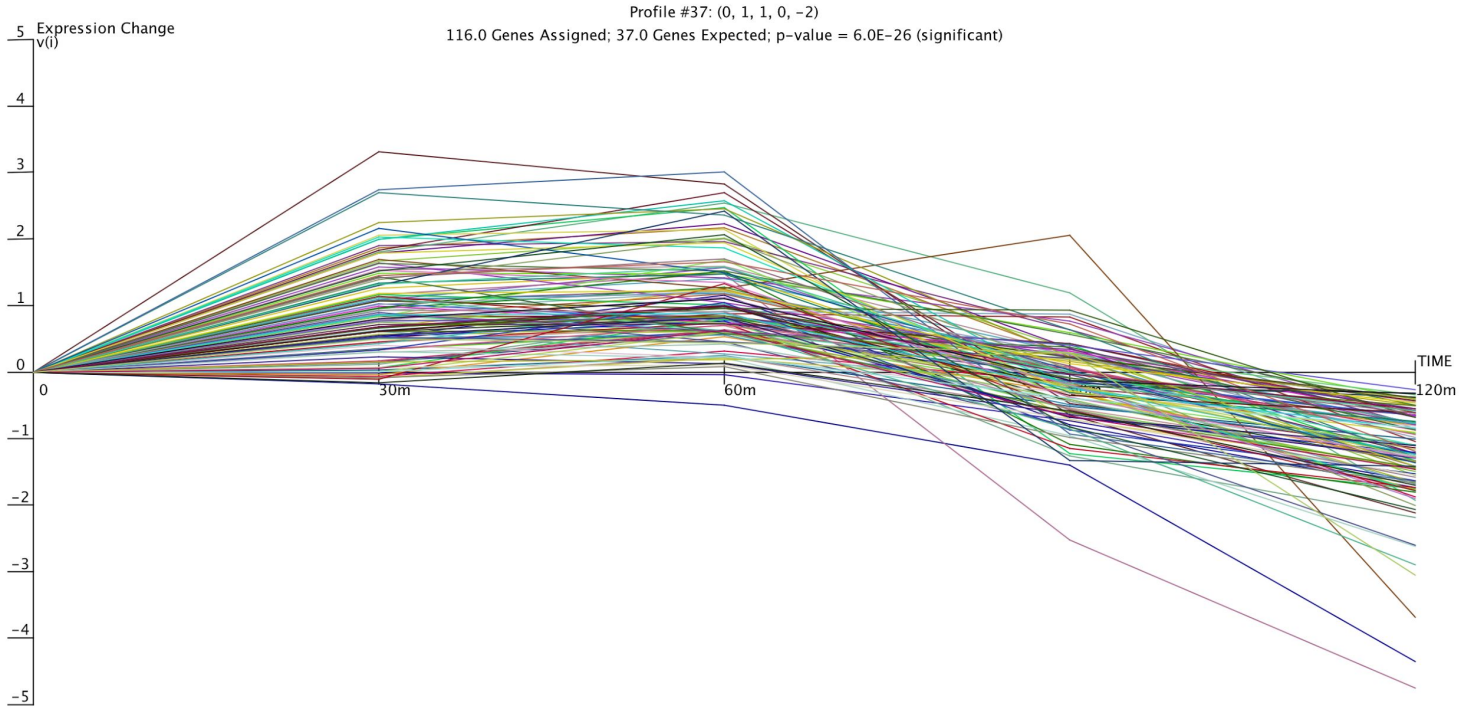
- **Profile 23 and Cluster 5 both were downregulated during cold shock and showed upregulation as the temperature increased.**



- **Both profiles showed a focus on the downregulation of energy metabolism and transport.**

http://aem.asm.org/content/80/14/4433.full

Profile 37 Showed Upregulation During Cold Shock And Mild Downregulation During Recovery



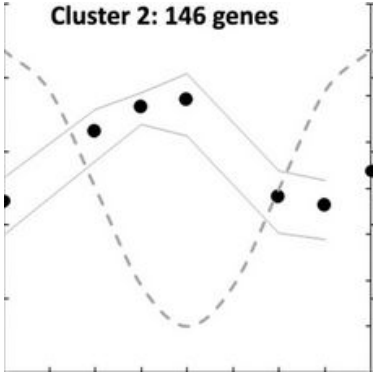
Profile 37 GO Terms Included Processes Related to Conservation of Resources

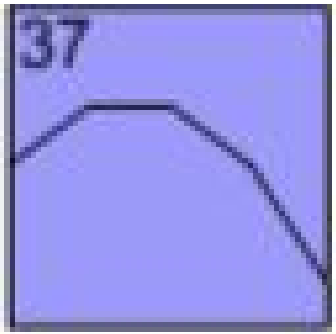
GO Term	Gene	Genes Assigned	Genes Expected	Genes Enriched	P-value	Corrected P-value	Fold
response to nutrient	24	5	1.3	3.7	7.00E-03	.8	4
protein catabolic process	120	13	6.3	6.7	8.7E-03	.843	2.1
regulation of kinase activity	37	6	1.9	4.1	.01	.876	3.1
sulfur compound metabolic process	44	6	2.3	3.7	.03	.978	2.6
ATP binding	245	20	12.9	7.1	.03	.98	1.6
transition metal ion binding	112	11	5.9	5.1	.03	.988	1.9

Profile 37 Resembled Cluster 2 From Hebly et Al. Under Cold Shock Conditions

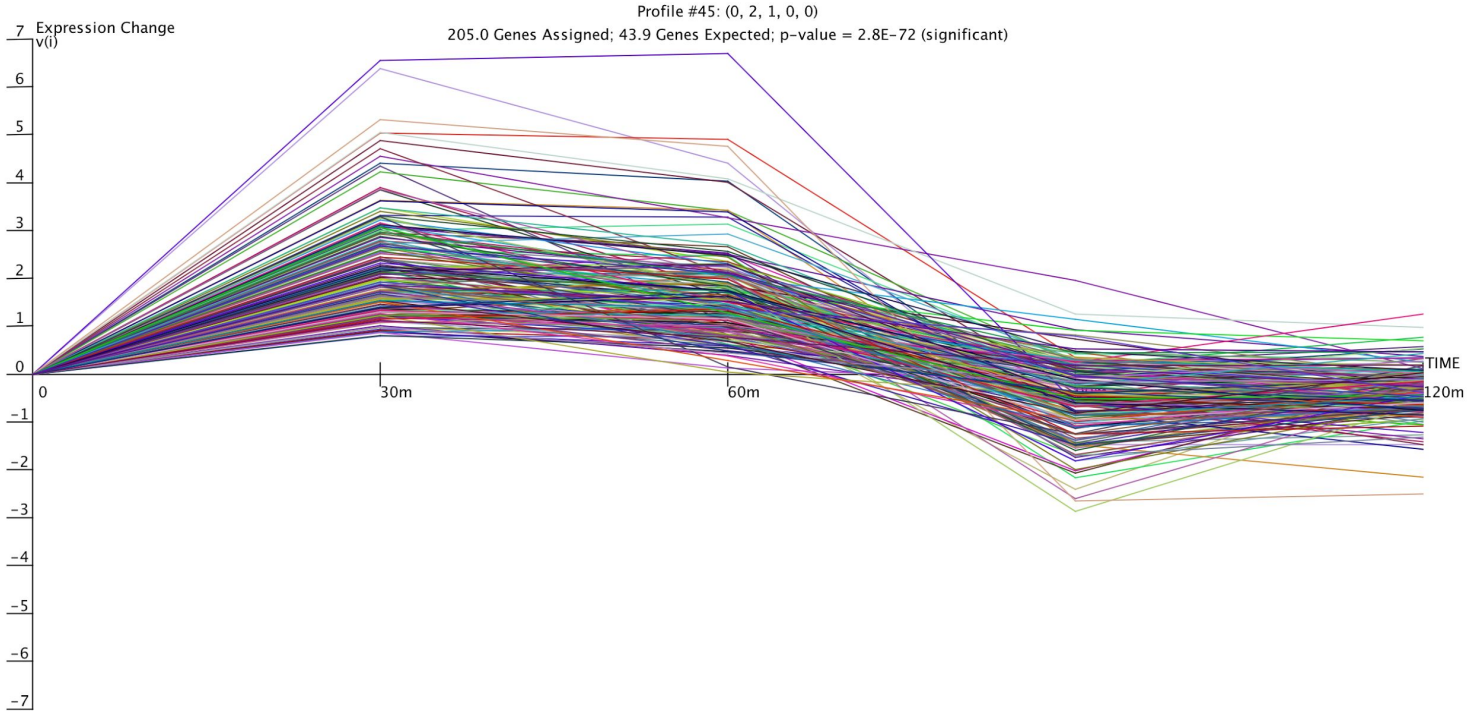
- Profile 37 and cluster 2 moderately upregulate expression and were slightly downregulated after temperatures returned to normal.

- Both are characterized by steady, not sharp increases and decreases in regulation as well as a return to almost baseline values.





Profile 45 Displayed 205 Genes with Drastic Upregulation During Cold-Shock and Downregulation During Recovery

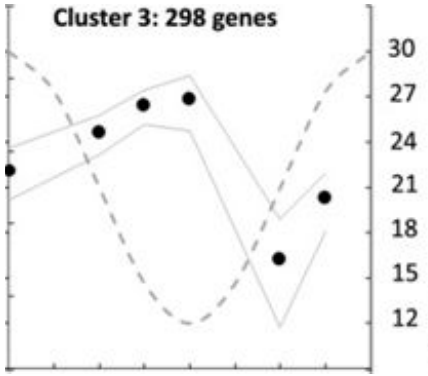


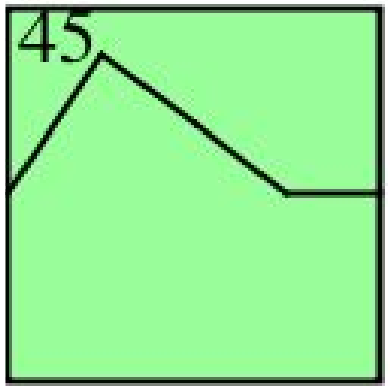
RNA and RNA Processing Genes were Prominent in Profile 45 Genes

GO Term	Gene	Genes Assigned	Genes Expected	Genes Enriched	P-value	Corrected P-value	Fold
RNA metabolic process	504	77	45.2	31.8	8.50E-08	<0.001	1.7
ATP-dependent RNA helicase activity	21	7	1.9	5.1	1.60E-03	0.49	3.7
Nuclear part	438	63	39.3	23.7	2.10E-05	0.014	1.6
Gene expression	628	84	56.3	27.7	8.30E-06	0.002	1.5
U2-type prespliceosome	11	6	1	5	1.50E-04	0.08	6.1
Heterocycle metabolic process	699	85	62.7	22.3	3.50E-04	0.15	1.4

Profile 45 in Cold Shock Showed Similarities to Cluster 3 under Diurnal Temperature Cycling

- Both profile 45 and Cluster 3 were upregulated during cold-shock, but showed downregulation during recovery temperatures.
- Genes enriched in both Cluster 3 and profile 45 are involved in RNA processing.





http://aem.asm.org/content/80/14/4433.full

Protein Synthesis has been Shown in Response to Cold-Shock Conditions

- Cold-shock proteins are synthesized to overcome the deleterious effects of cold shock. (Zhu et al. 2007)
- A majority of the terms found on the GO list of Profile 45 were related to RNA processing, such as:
  - ATP-dependent RNA helicase activity
  - RNA metabolic process
  - U2-type prespliceosome
  - Gene expression

Future Studies Should Account for Natural Conditions and Focus on Important Gene Groups

- **Fluctuations in temperature seen in DTC did not directly induce cyclic responses in gene expression.**
  - **Future studies should use auxostats or fed-batch cultures to mimic natural growth dynamic**
- **Future studies should study other eukaryotes and the use of Monod kinetics under “natural” conditions.**
- **Further work should employ microarray analysis of genes such as those related to the cytoskeleton and RNA processing.**

Summary

- **Yeast is an important eukaryotic model organism and controls gene expression in response to environmental conditions.**
- **Previous studies have investigated temperature shock, acclimated cultures, and glycolytic responses to cyclic conditions.**
- **Hebly et al. (2014) studied yeast under a diurnal temperature cycle to investigate changes in physiology and the transcriptome.**
- **The budding yeast acclimated and developed stable physiological conditions by adjusting metabolic and cell cycle gene expression.**
- **We found similar gene and gene cluster activity in cold shock *Δswi4*.**
- **Future studies should better replicate growth conditions found in nature and include more in-depth microarray analysis of cold-shock genes.**

Acknowledgments

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