Physiological and transcriptional responses of anaerobic chemostat cultures of *Saccharomyces cerevisiae* subjected to diurnal temperature cycles.

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

(2014) *App. and env. microbiology*, 80(14), 4433-4449.

Colin Wikholm, Matthew Allegretti, Matthew Oki, and Mia Huddleston
BIOL 368: Bioinformatics Laboratory
December 6, 2016

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.
- Future studies should allow for growth rates independent of temperature cycles.

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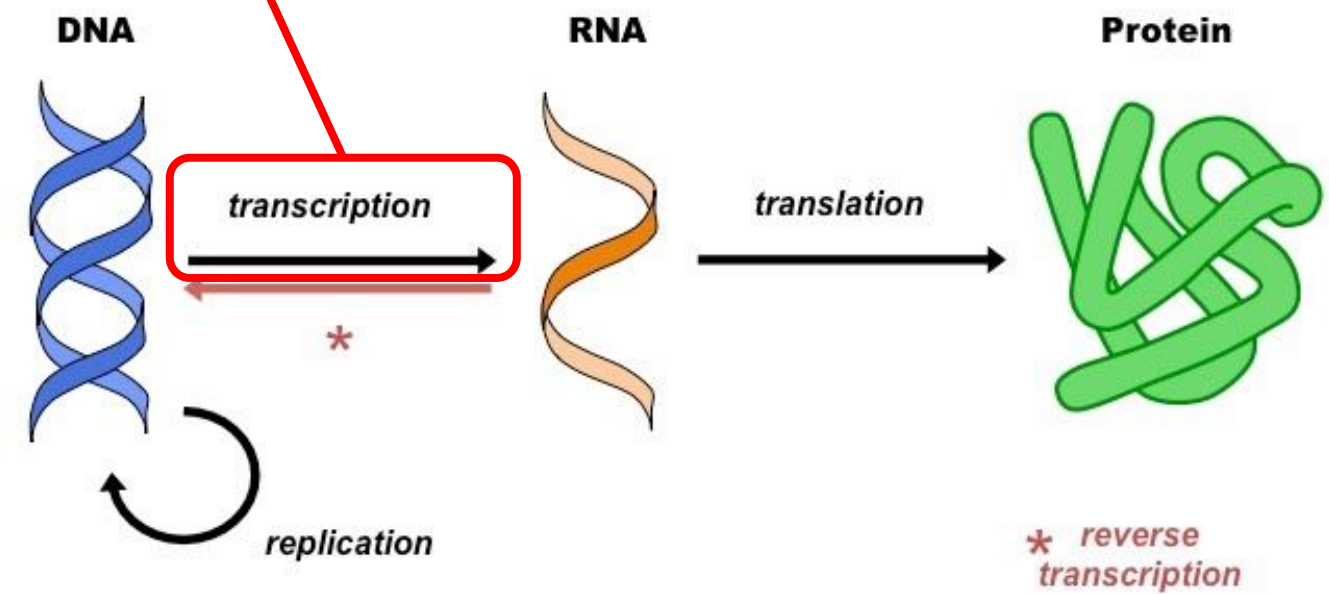
Eukaryotic Gene Regulation Occurs at Multiple Steps Within the Central Dogma of Biology

Transcription is the major step of gene expression regulation.

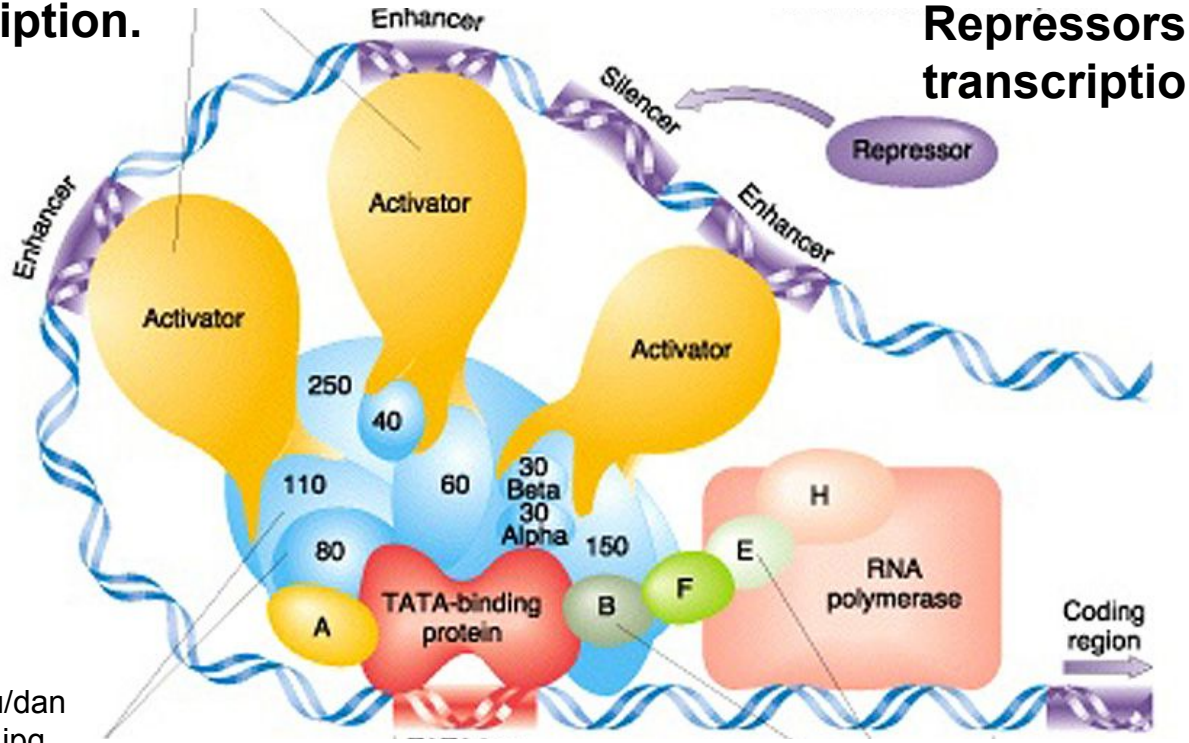


http://ib.bioninja.com.au/_Media/central-dogma_med.jpeg

Transcriptional Factors are Proteins that Increase or Decrease Gene Expression

Activators increase transcription.

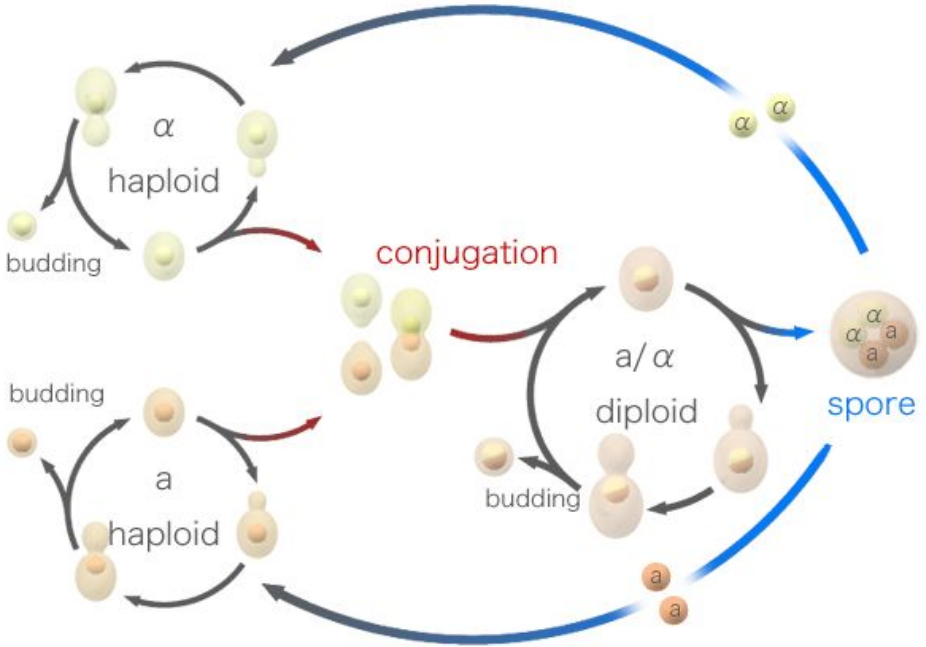
Repressors decrease transcription.



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.**

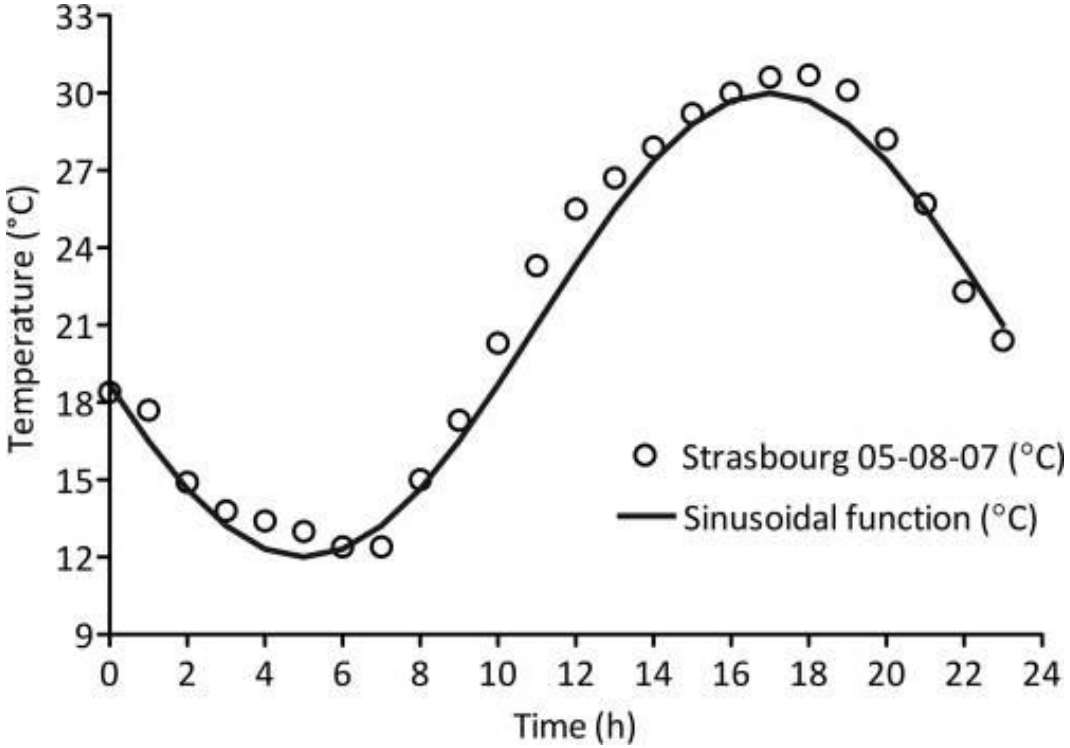


http://wiki.yeastgenome.org/images/e/ea/Yeast_life_cycle.png

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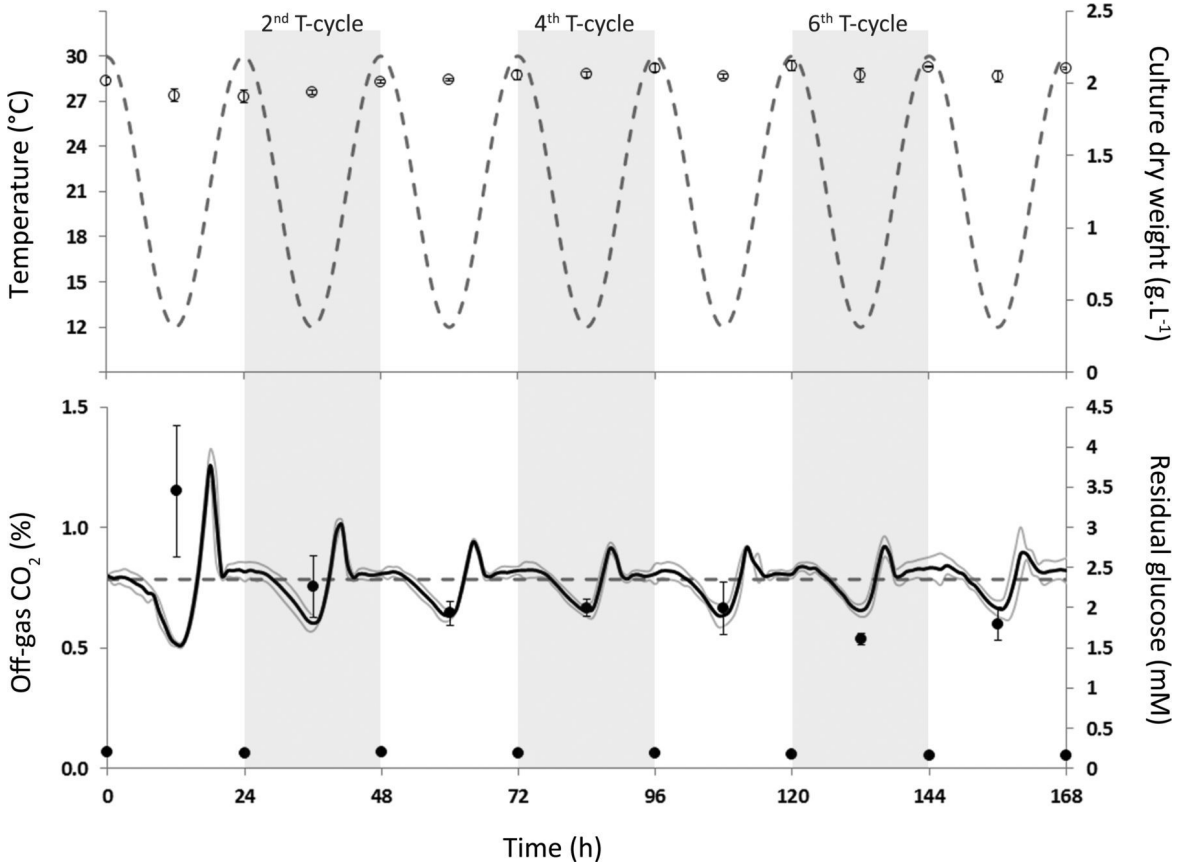
Stabilization of Fluctuation in Residual glucose and CO_2 Levels Suggest Temperature Acclimation

- **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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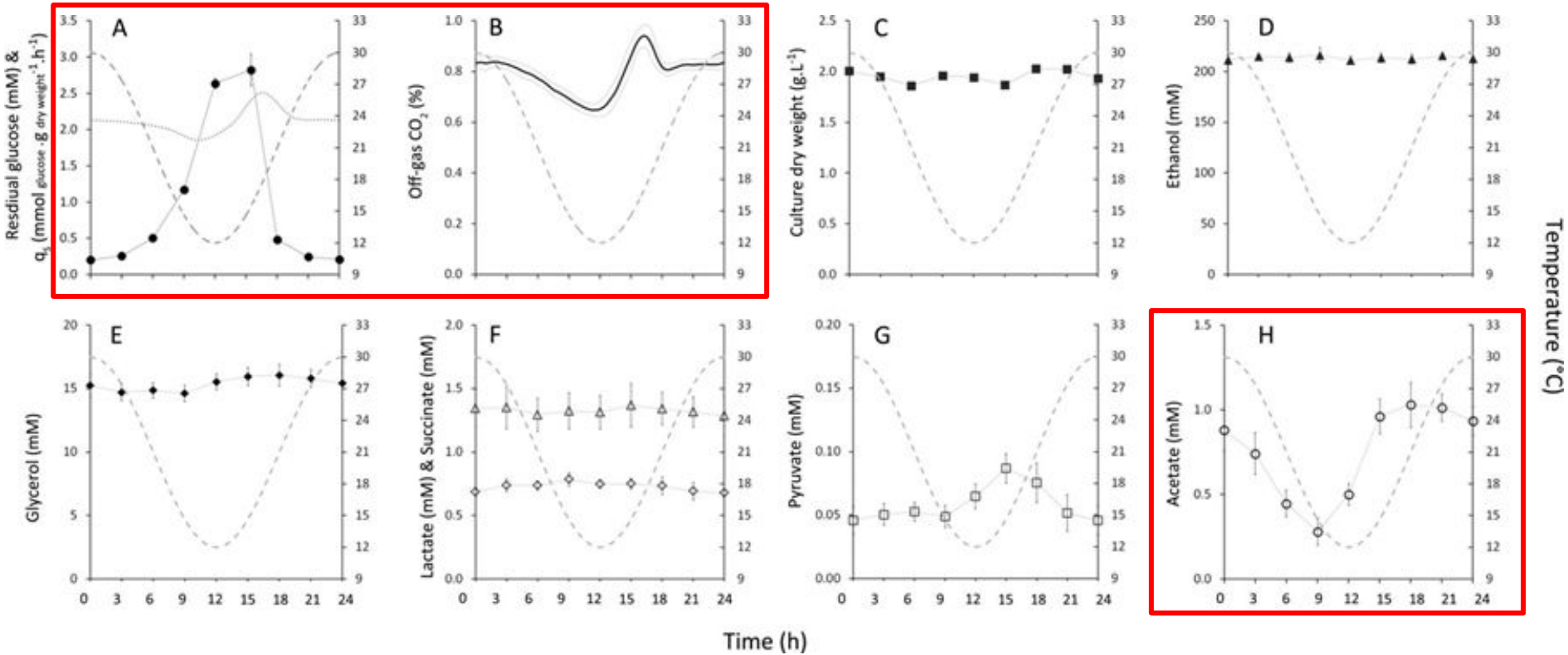
Physiological Analysis Show Related Changes in Some Metabolic Characteristics



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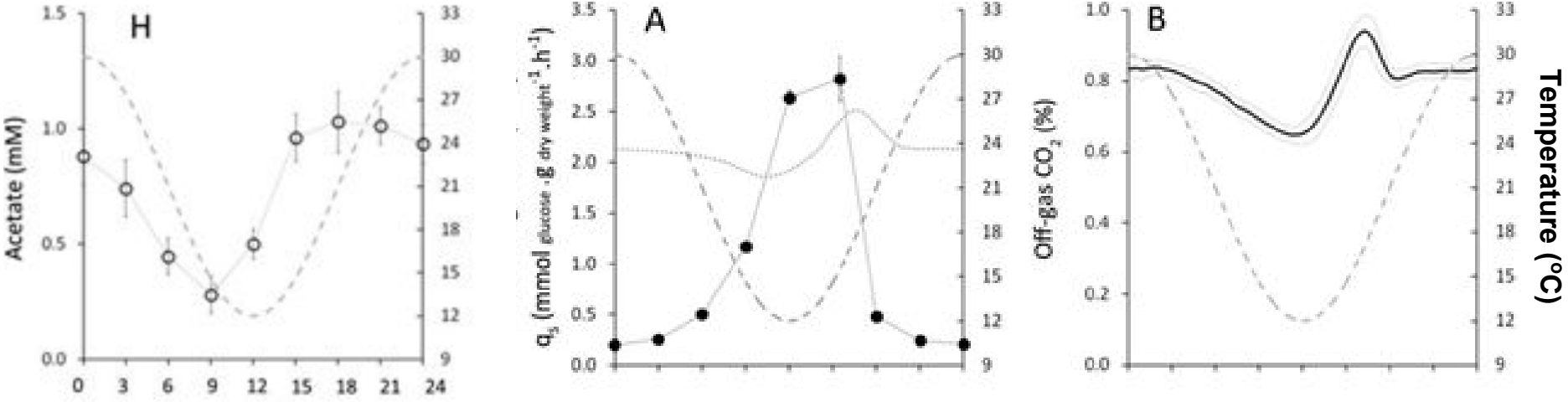
Physiological Analysis Show Related Changes in Some Metabolic Characteristics

Acetate mirrored temperature changes.

Residual glucose responded inversely and asymmetrically to temperature.

CO_2 release fluctuated asymmetrically.



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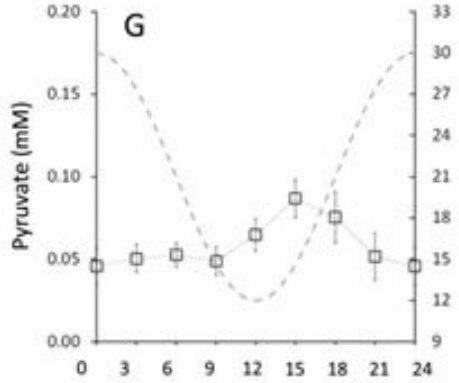
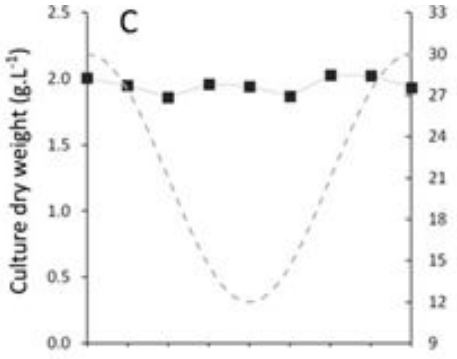
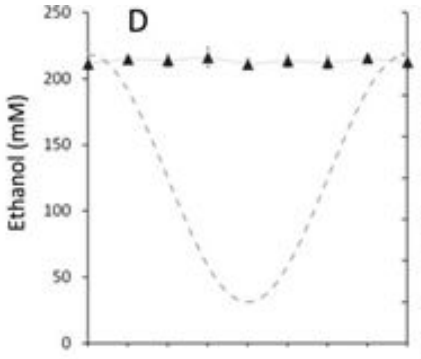
Physiological Changes Show No Change in Some Metabolic Characteristics

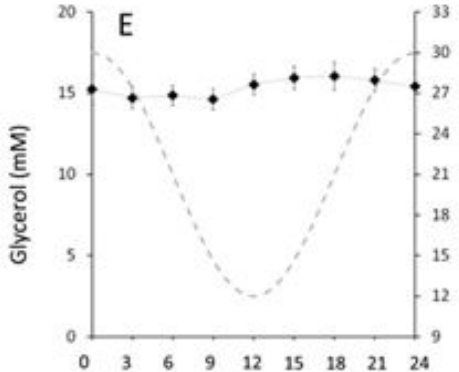
No major changes were seen in:

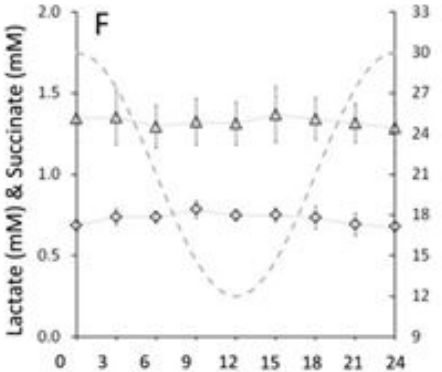
- **(C) Culture dry weight**
- **(D) Ethanol concentration**
- **(E) Glycerol concentration**
- **(F) Lactate and succinate concentrations**
- **(G) Pyruvate concentration**











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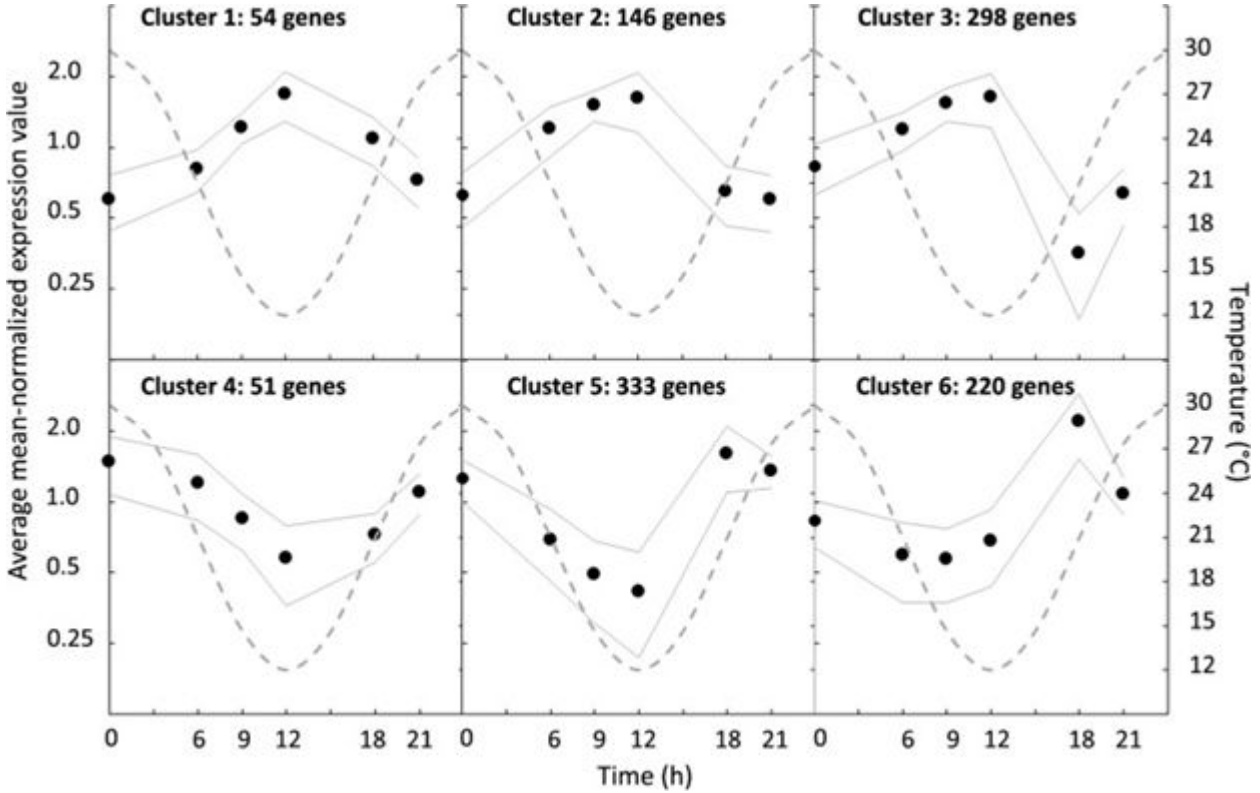
Transcriptome Analysis Showed Major Changes in Gene Expression Dynamics During DTC

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



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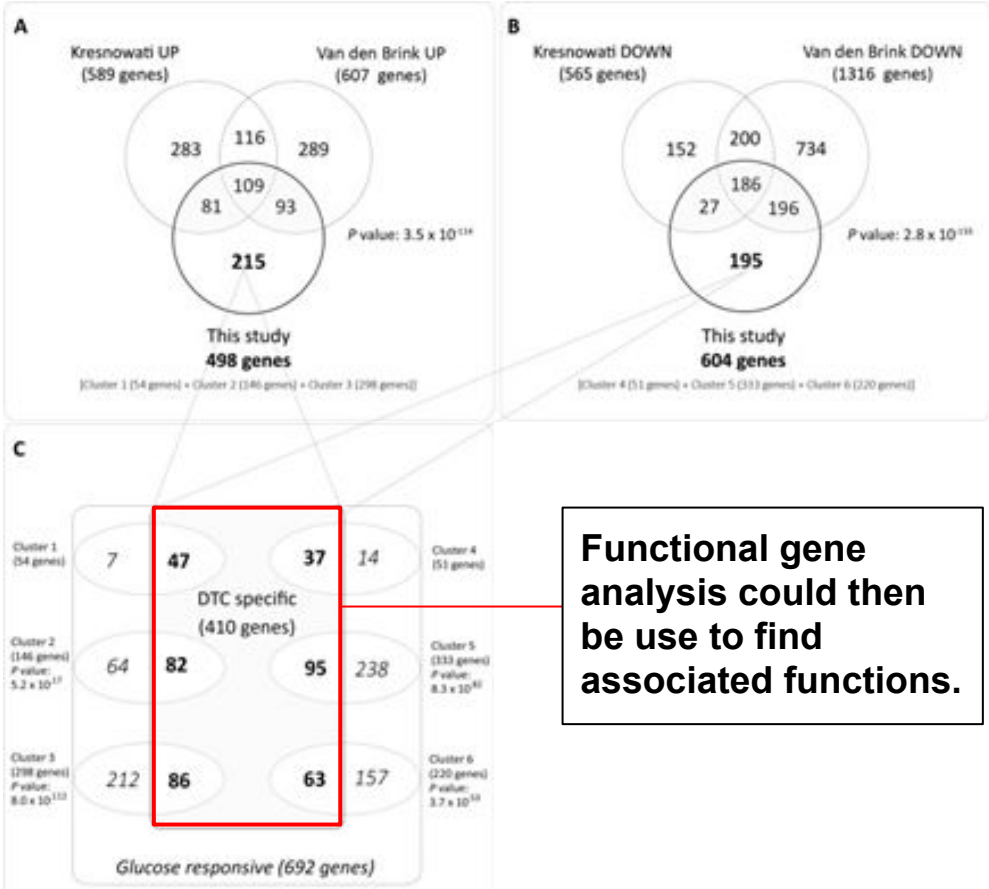
DTC-specific genes vs glucose-specific genes (comparing DTC results to glucose dataset)

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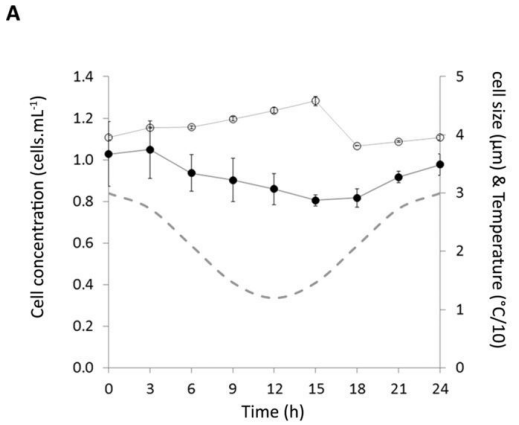
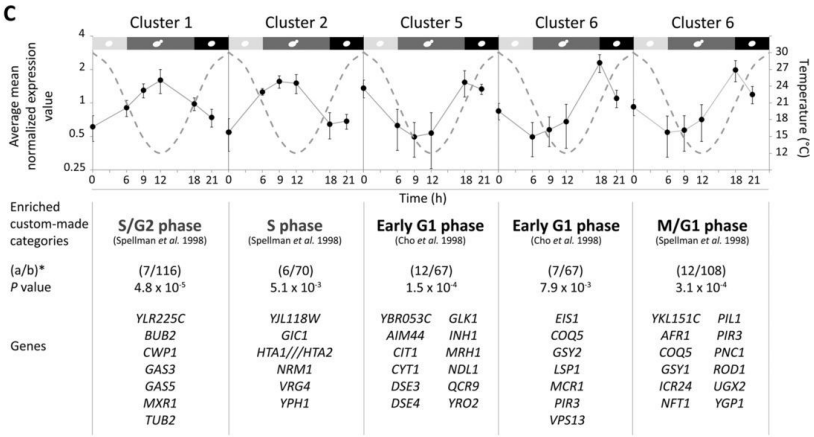
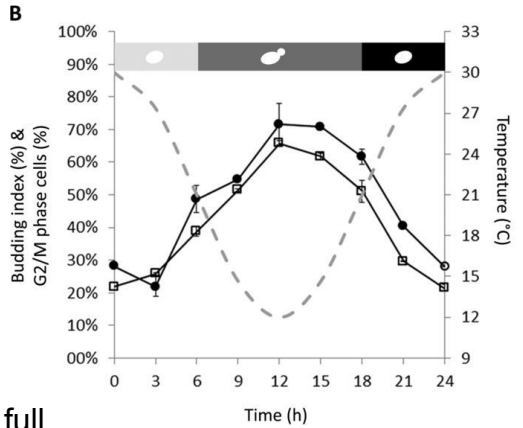
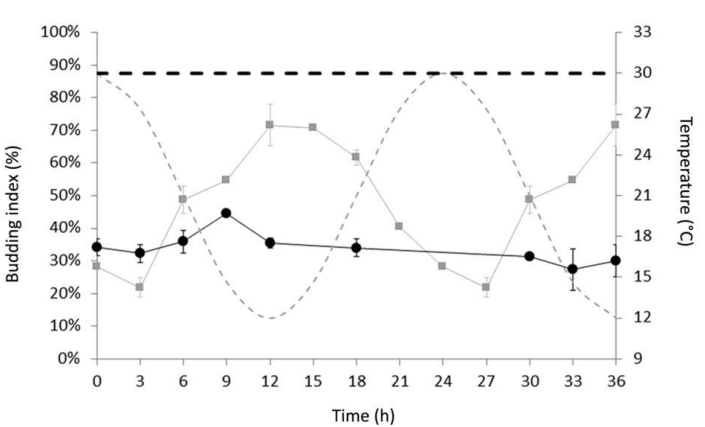
Genes Associated with DTC were Targets of Transcriptional Factors involved in the Cell Cycle

- 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]

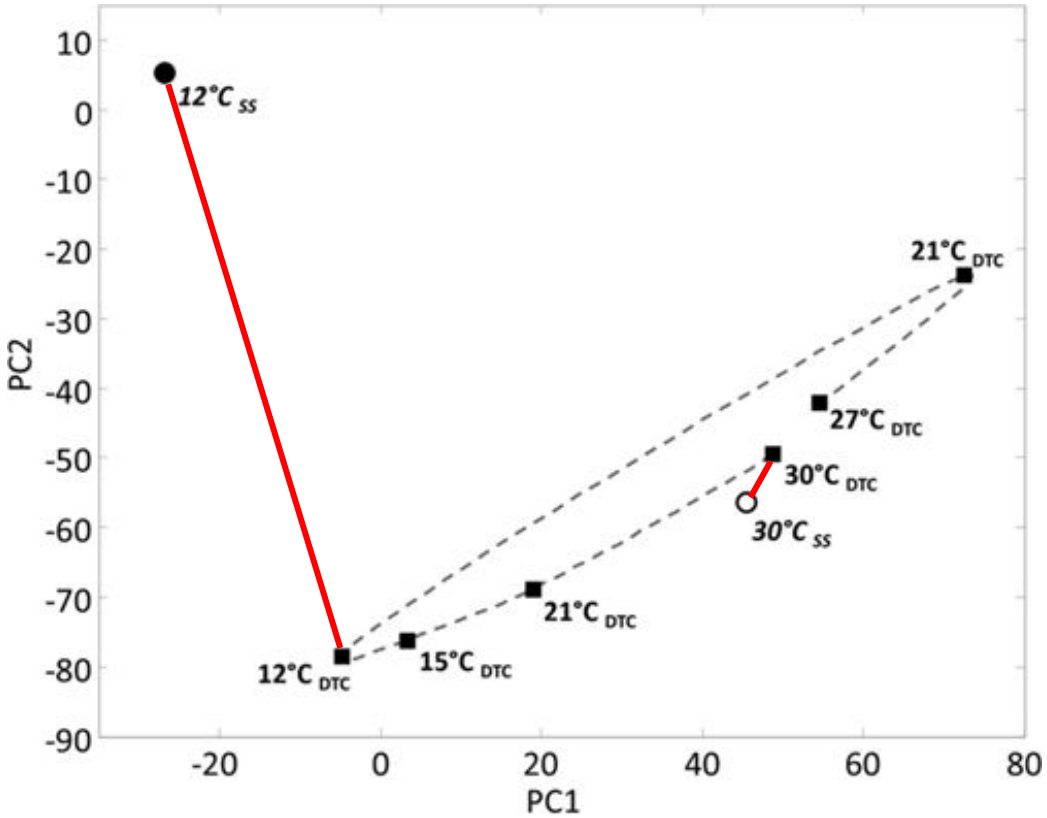
Experimental condition	Temp (°C)	Y_{SX} (g glucose · g [dry weight]$^{-1}$)	q_S (mmol · g [dry weight]$^{-1}$ · h^{-1})	q_{EtOH} (mmol · g [dry weight]$^{-1}$ · h^{-1})	q_{CO2} (mmol · g [dry weight]$^{-1}$ · h^{-1})	Carbon recovery (%)	Residual glucose concn (mM)	Glycogen concn (mg glucose equivalent · 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.

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

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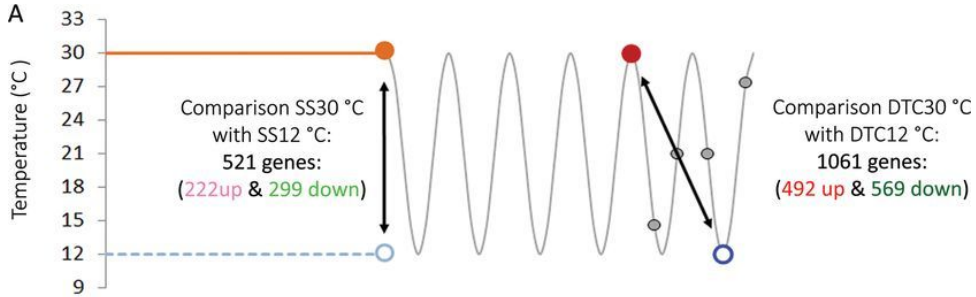
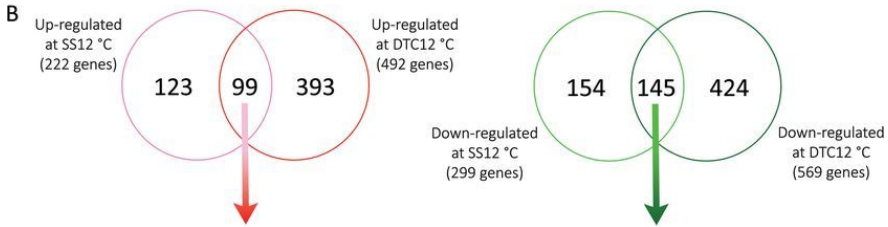
Pairwise Transcriptome Analysis Shows Differences in Gene Expression Between DTC and Steady State

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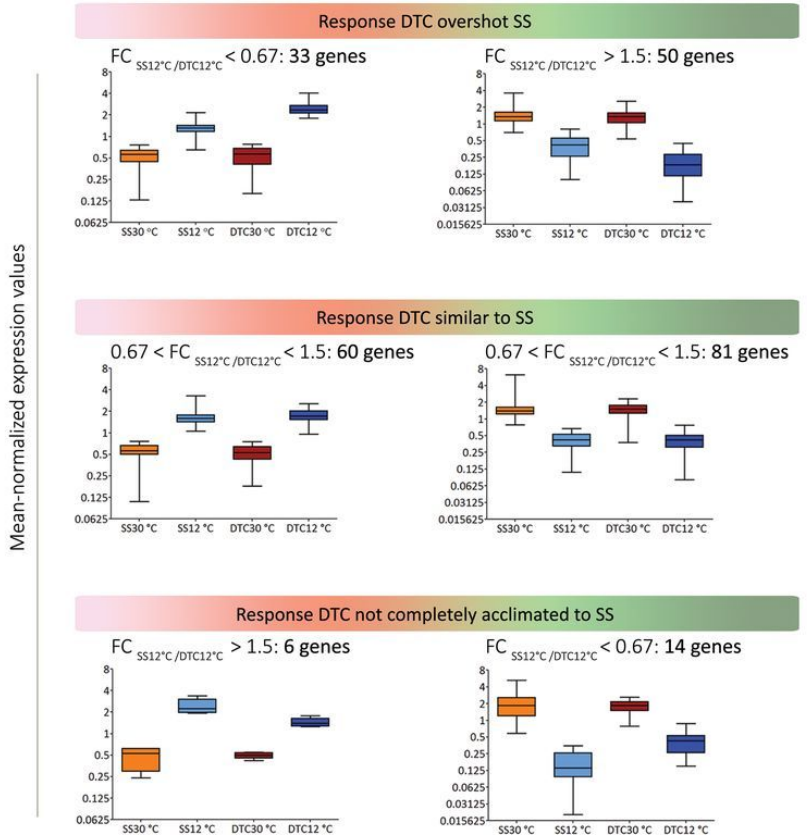
Clustering of DTC and SS Responses at 12ºC Show Differences in Gene Expression Magnitude

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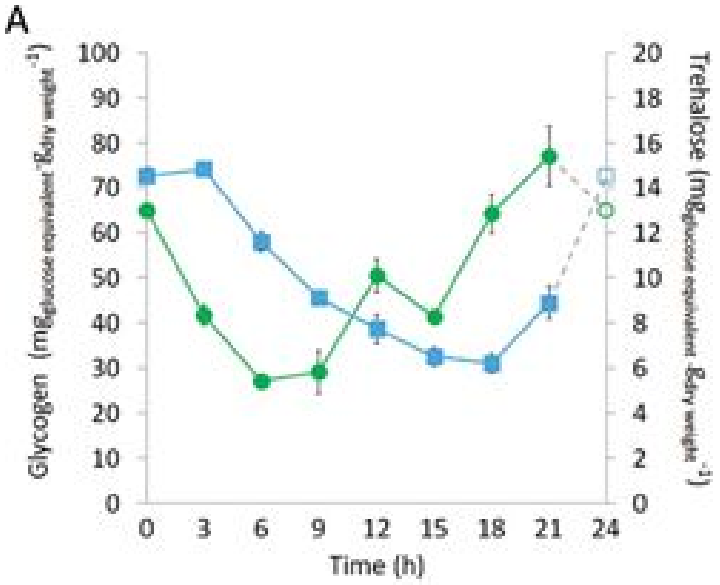
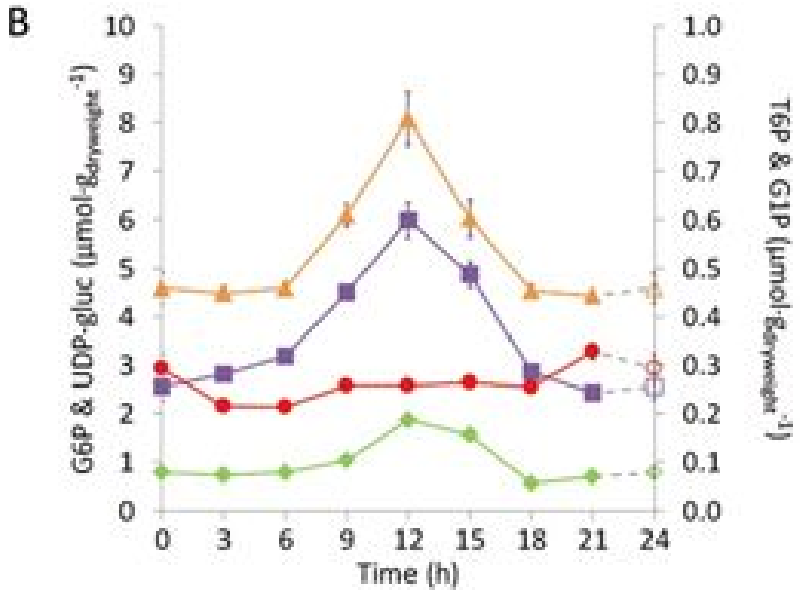
Physiological-Transcriptional Comparison Shows Carbohydrate Reserve is not a Direct Result of Temperature

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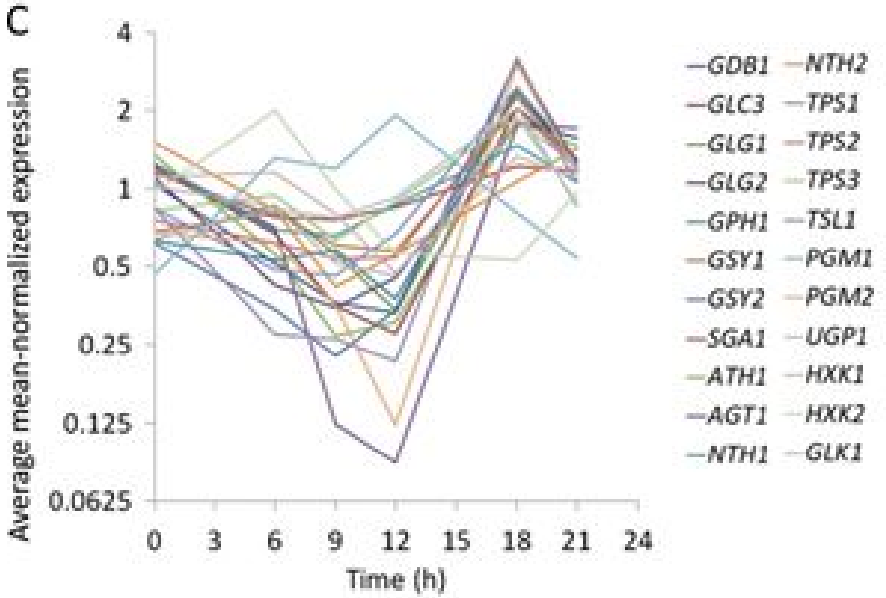
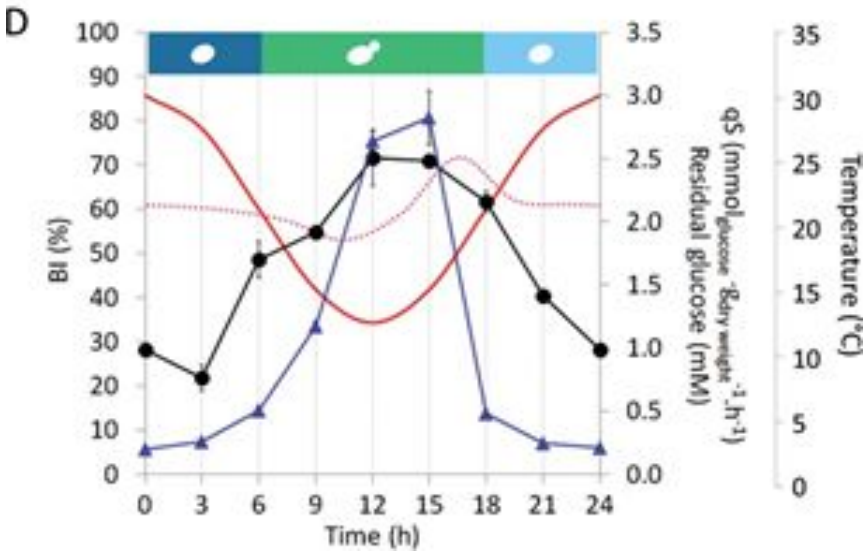
Carbohydrate Profile of *S. cerevisiae* is Related to Imposed Fluctuation in Growth Rate and the Cell Cycle

- **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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- **Future studies should allow for growth rates independent of temperature cycles.**

Future Studies Should Account for Variables that Occur in Nature

- **Fluctuations in temperature did not directly induce cyclic responses in gene expression.**
  - **Change in transcriptome were caused by alterations to the cell cycle.**
  - **This study maintained specific growth rates at a relatively constant level.**
  - **Future studies should use auxostats or fed-batch cultures to mimic natural growth dynamic**
- **Future studies might should also evaluate effectiveness of Monod kinetics under these “natural” conditions.**

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.
- Future studies should better replicate growth conditions found in nature.

Acknowledgments

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LMU Department of Biology

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