Physiological and Transcriptional Responses of Anaerobic Chemostat Cultures of *Saccharomyces cerevisiae* Subjected to Diurnal Temperature Cycles

Hebly, M., de Ridder, D., de Hulster, E. A., de la Torre Cortes, P., Pronk, J. T., & Daran-Lapujade, P. (2014). Physiological and transcriptional responses of anaerobic chemostat cultures of Saccharomyces cerevisiae subjected to diurnal temperature cycles. *Appl. Environ. Microbiol.*, *80*(14), 4433-4449.

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Outline

1. Diurnal temperature fluctuations occur in natural environments
2. Yeast respond to temperature fluctuations through gene expression
3. Temperature fluctuations cause physiological and transcriptional responses in cells
4. Presence of glucose triggers concentration-dependent transcriptional response
5. Temperature fluctuations impact the cell cycle in yeast
6. Carbohydrate concentrations fluctuate during DTC
7. Steady-state and DTC experiments showed similar results

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Temperature changes affect cellular functions

- Temperature affects the cellular processes in microorganisms, leading to decreased transcription and translation (Feller et al., 2003)
  - Cells must adapt metabolically and physiologically in order to maintain homeostasis (Madigan et al., 2006)
  - Changes to membrane fluidity and induction of temperature-related proteins (Thieringer et al., 1998)
- Chemostat and batch culture studies performed to study **acclimation** to cold shock or heat shock

Diurnal temperature fluctuations are prevalent in natural environments

- In natural environments:
  - Temperature dynamics in a circadian changes, with high temperatures during day and low temperatures during night
  - Rate of adaptation is temperature-dependent
- **Does the 24-hour cycle allow for cell acclimation to temperature, or is there constant temperature shock?**

Yeast must respond to diurnal temperature cycles

- *Saccharomyces cerevisiae* CEN.PK113-7D
  - Optimal growth temperature is 30℃, with range of 4°- 40°C (Salvado et al., 2011)
  - Often grows in exposed environments (Lodolo et al., 2008)
- Previous studies focus on acclimation to fixed temperature and the global transcriptional response
  - This study will focus on **diurnal temperature cycles (DTC)** (24h sinusoidal temp cycle)

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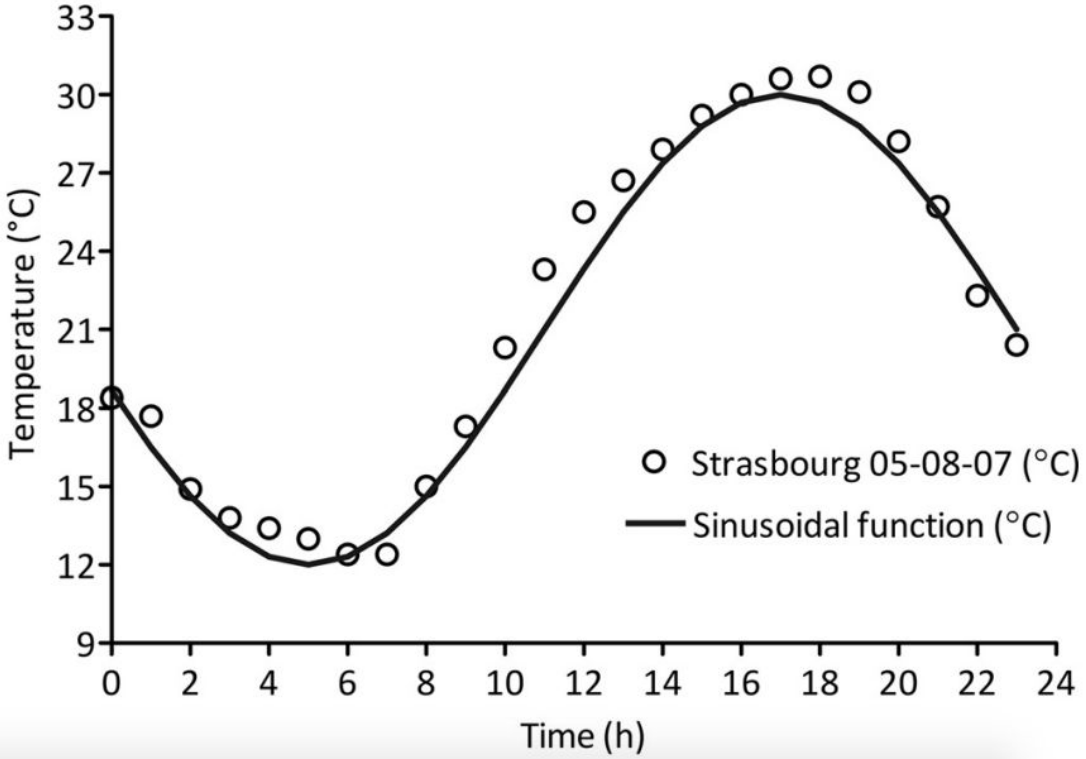
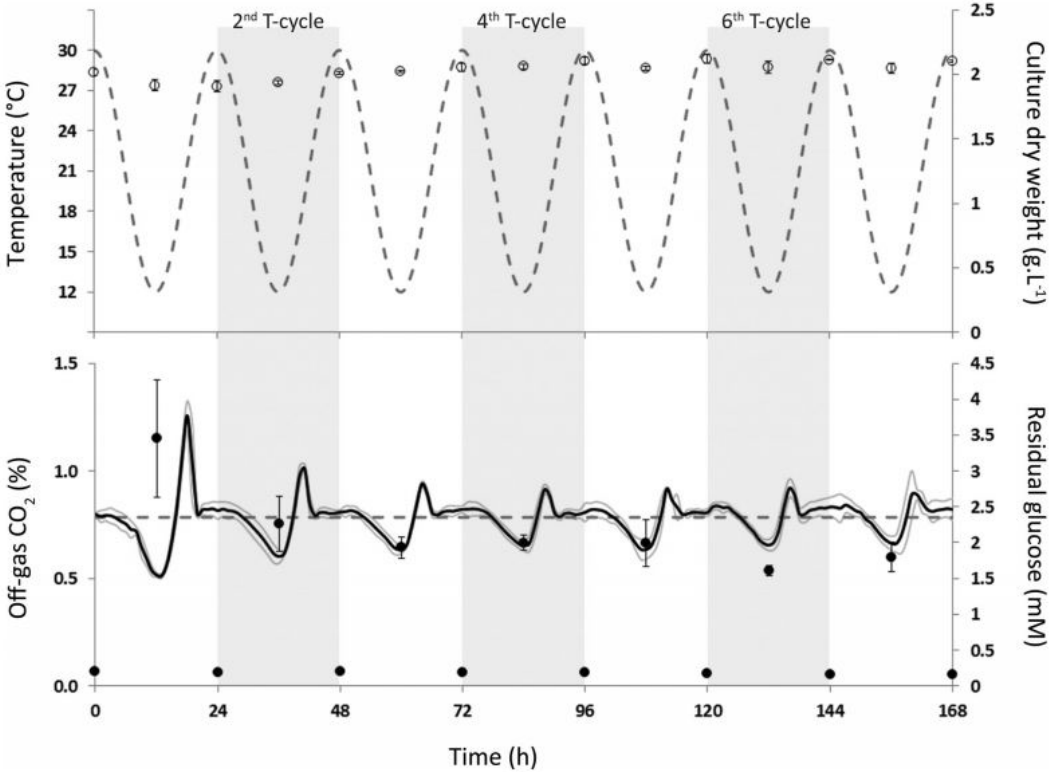
Figure 1: Experimental temperature follows a diurnal sinusoidal function



- Diurnal temperature based on sinusoidal wave
- Temperature range: 12°- 30°C
- Samples were taken every 5-6 cycles
- o = experimental temperature

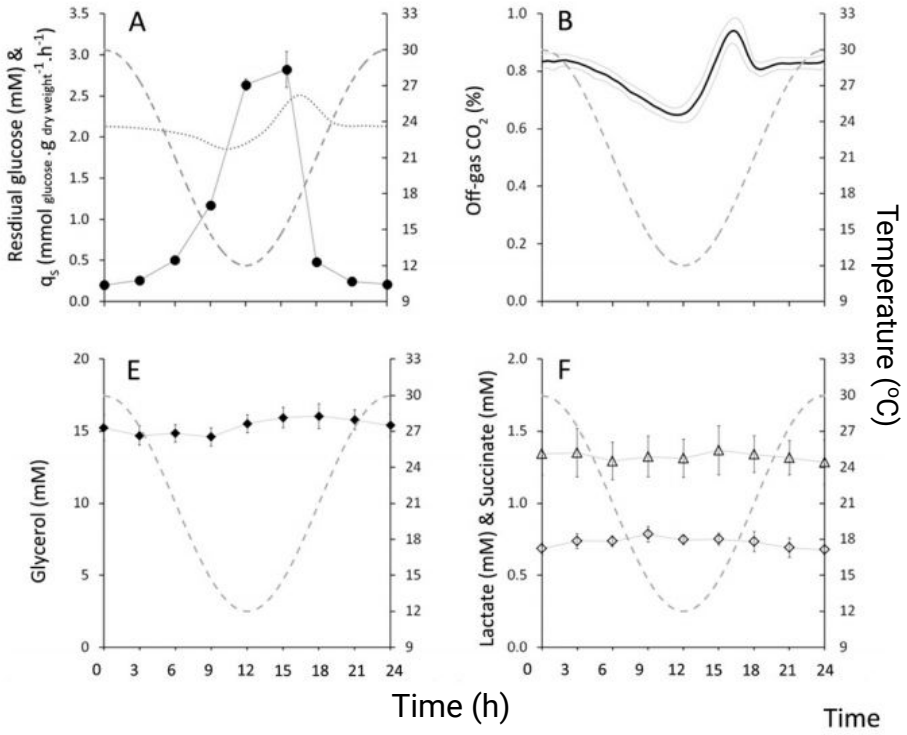
  --- = sinusoidal function

Figure 2: Biomass remains constant while CO_2 and glucose levels fluctuate based on temperature



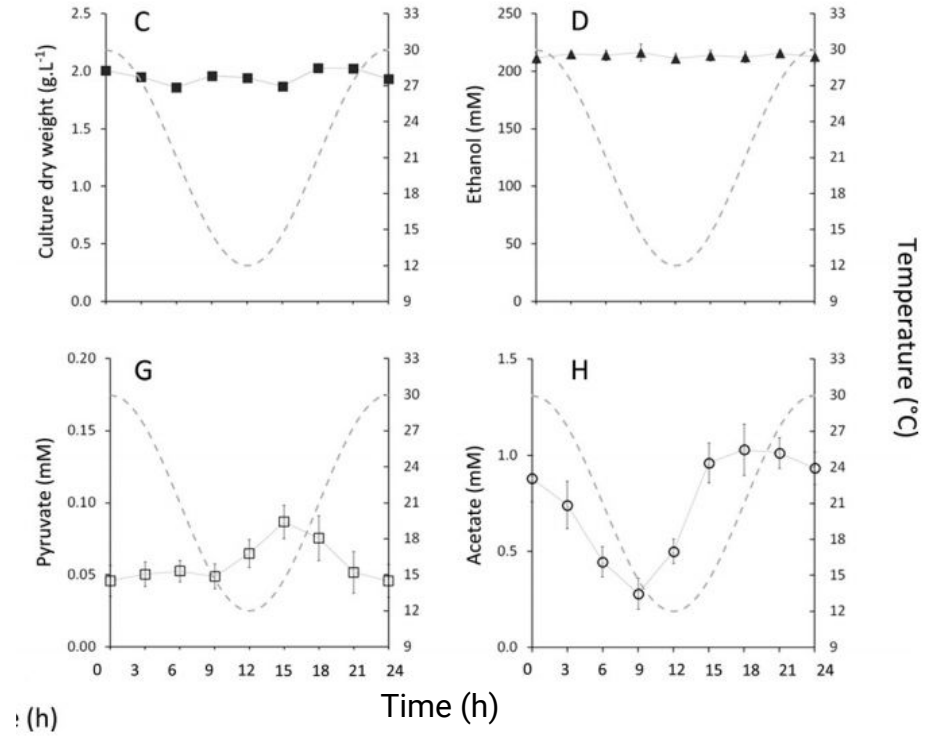
- Chemostat culture:
  - Biomass remained constant
  - Temperature changed cyclically
  - CO_2 decreases in low temperature, increase in high temperature
  - Residual glucose was lowest at high temperature

Figure 3: Temperature fluctuations cause changes in glucose and CO_2, while glycerol, lactate, and succinate remain constant



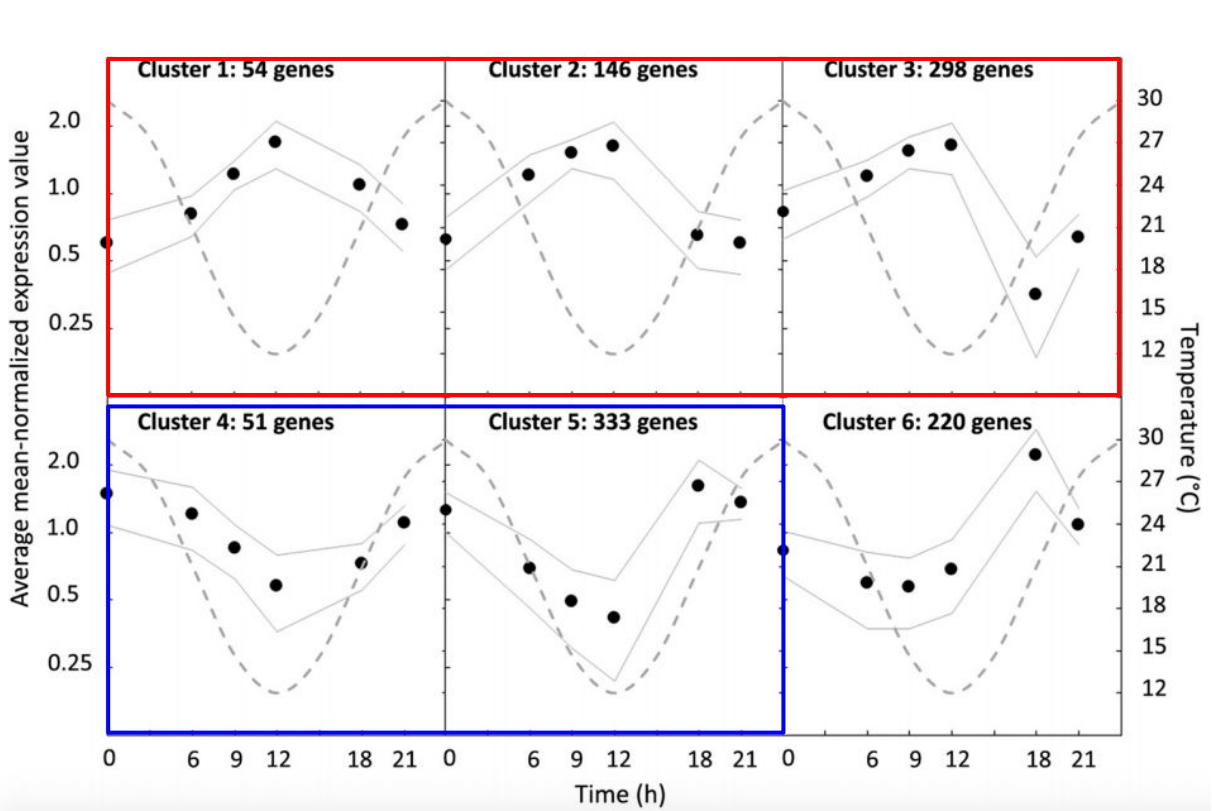
- **A**: Residual glucose concentration is inversely proportional to temperature
- **B:** CO_2 decreased as temperature decreased, with sharp increase once the temp increases
- **E:** Glycerol remained constant
- **F:** Lactate (Δ) and Succinate (◊) remained constant

Figure 3: Temperature fluctuations cause changes in acetate, while biomass, ethanol, and pyruvate remain unchanged



- **C:** Biomass of culture remained constant throughout with a deviation of < 5%
- **D:** Ethanol levels remained constant
- **G:** Pyruvate levels were unaffected by temperature
- **H:** Acetate levels rhythmically varied
  - Decreased concentration compared to glycerol and ethanol

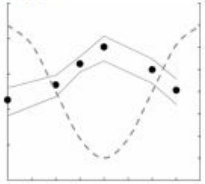
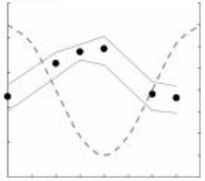
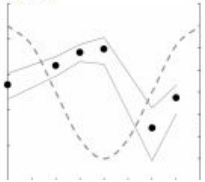
Figure 4: Shifts in expression for different clusters of genes showed downregulation and upregulation



- **1,102** genes showed significant ($p<0.002$) changes
- 498 genes peaked at low temperature
- 384 genes decreased at low temperature
- 220 genes were unaffected by decrease in temp, but showed major increase after temp increased
- Clusters 3 and 5 showed a decrease and increase, respectively, compared to initial transcript levels

Table 1: Overrepresented functional categories of the clusters reveal differences in genes

TABLE 1 Functional enrichment analysis[a]

Cluster (no. of genes) and profile	Enriched functional category (MIPS functional category)	No. of significantly changed genes in enriched functional category/total no. of genes in functional category	*P* value
1 (54)	Cell wall (42.01)	8/215	4.0×10^{-4}
			
2 (146)	**Mbp1**	14/165	2.5×10^{-5}
	Ino4	6/33	8.6×10^{-5}
	Swi6	12/160	2.7×10^{-4}
	Swi4	11/144	4.2×10^{-4}
	Ino2	5/31	6.1×10^{-4}
			
3 (298)	Protein synthesis (12)	108/511	3.8×10^{-46}
	Transcription (11)	113/1,036	1.5×10^{-20}
	Protein with binding function or cofactor requirement (16)	81/1,049	1.1×10^{-6}
	Fhl1	48/208	2.0×10^{-21}
	Rap1	27/145	4.0×10^{-10}
	Sfp1	14/50	3.5×10^{-8}
			

- Overrepresented functional categories
  - Cluster 1: cell wall organization
  - Cluster 2: targets for transcription factors Swi4, Swi6, Mbp1
  - Cluster 3: protein synthesis, ribosome biogenesis, mRNA processes

Table 1: Overrepresented functional categories of the clusters reveal differences in genes

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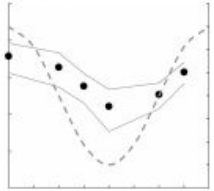
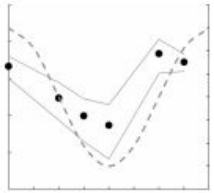
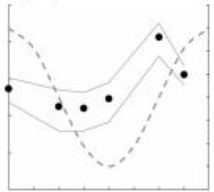
4 (51) 	**Bas1**	4/39	2.4×10^{-4}
	Gcn4	7/182	5.5×10^{-4}
5 (333) 	Energy (2)	80/360	4.1×10^{-31}
	Mitochondrion (42.16)	31/170	5.5×10^{-10}
	C compound and carbohydrate metabolism (1.05)	53/510	6.0×10^{-7}
	Transported compounds (substrates) (20.01)	56/585	3.9×10^{-6}
	Fe/S binding (16.21.08)	4/5	3.5×10^{-5}
	Protein folding and stabilization (14.01)	15/95	1.0×10^{-4}
	Mitochondrial transport (20.09.04)	15/100	1.9×10^{-4}
	Hap4	19/57	2.7×10^{-11}
	Hsf1	15/55	7.5×10^{-8}
	Hap3	9/26	3.6×10^{-6}
	Cin5	24/180	1.8×10^{-5}
	Ume6	17/131	4.2×10^{-4}
6 (220) 	Metabolism (1)	87/1,530	1.3×10^{-7}
	Cell rescue, defense, and virulence (32)	41/558	2.0×10^{-6}
	Lysosomal and vacuolar protein degradation (13.13.04)	7/27	2.6×10^{-5}
	Energy (2)	27/360	9.7×10^{-5}
	Msn2	15/122	1.7×10^{-5}
	Msn4	14/121	6.3×10^{-5}

- Overrepresented functional categories
  - Cluster 4: targets of Bas1, target for transcription factor for Gcn4
  - Cluster 5: energy metabolism/ conservation
  - Cluster 6: cellular functions and protections mechanisms

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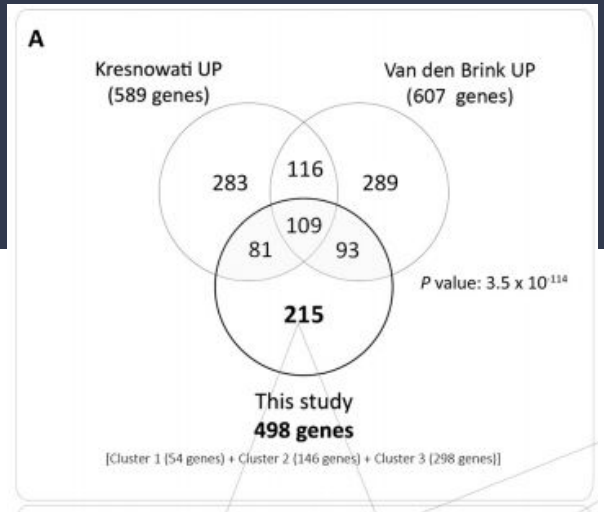
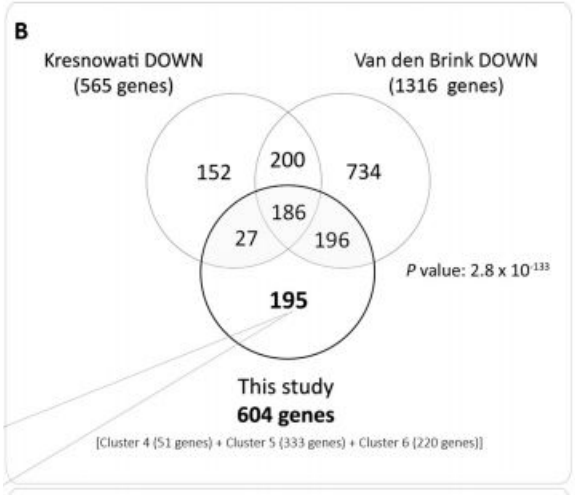
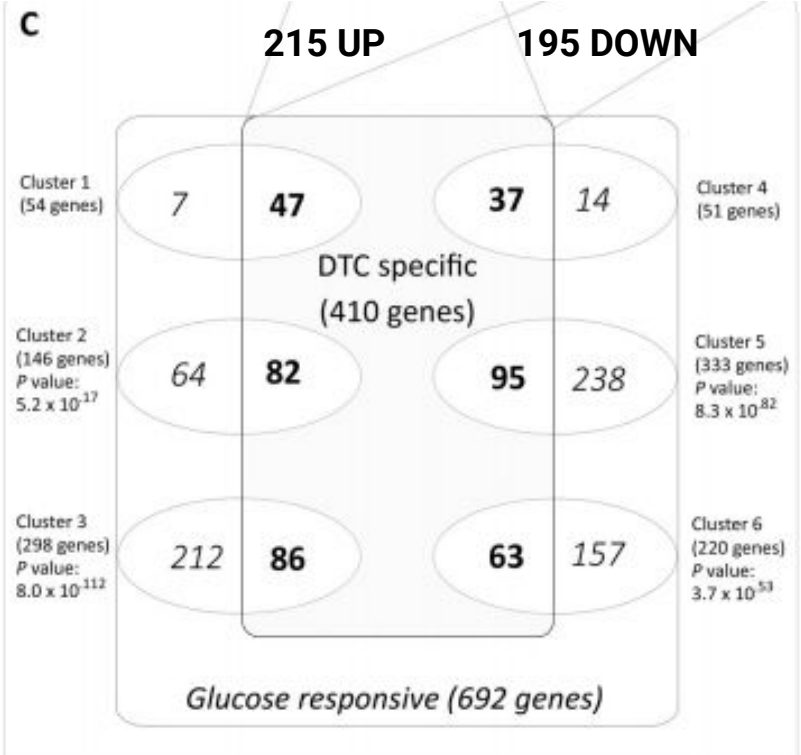
Figure 5a,b: Upregulated and downregulated genes overlap with previous glucose concentration studies





- Presence of glucose triggers concentration-dependent transcriptional response (Gancendo et al., 2008)
- Studies: Kresnowati et al. and Van den Brink et al.
- Half of upregulated genes were common to the studies
  - **109** genes consistently upregulated
- One-third of downregulated genes were common to the studies
  - **186** genes consistently downregulated

Figure 5c: Glucose-responsive genes are not uniformly distributed to clusters



- Glucose-responsive genes are not uniformly distributed to clusters
  - Overrepresented functional categories include response to glucose excess
- 215 upregulated, 195 downregulated DTC specific genes
  - Separated from glucose-responsive genes

Figure 5d: Overrepresented functional categories of DTC specific genes overlap with glucose-responsive genes

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		

- 410 DTC specific genes
  - Overrepresented functional categories include response to glucose excess, lipid metabolism, intracellular transport
  - Protein synthesis

Table 2: Reducing p-value stringency narrowed DTC specific genes and functional categories to six major cellular processes

TABLE 2 Functional enrichment analysis of DTC-specific genes[a]

Cluster	Enriched functional category(ies) (MIPS functional category or GO category)	Genes	No. of significantly changed genes in enriched functional category/ total no. of genes in functional category	*P* value
1	None			
2	ER-to-Golgi transport (20.09.07.03)	*ERP2, ERP1, YIP3, RER1, SHR3, CHS7*	6/72	8.1×10^{-5}
	Membrane lipid metabolism (01.06.02)	*PLB2, DPM1, CDS1, CST26, CHO1, OPI1*	6/83	1.8×10^{-4}
	RNA polymerase III transcriptional preinitiation complex assembly (GO:0070898)	*NHP6A, NHP6B, YBR090C*	3/13	2.7×10^{-4}
	Swi4	*MNN5, CIS3, PCL1, SVS1, HTA1/ HTA2, PCL2, YHP1, GIC1*	8/144	9.4×10^{-5}
	Swi6	*MNN5, CIS3, PCL1, NRM1, SVS1, PCL2, YHP1, GIC1*	8/160	2.0×10^{-4}
	Mbp1	*SEN34, MNN5, SEC14, PCL1, NRM1, HTA1/HTA2, YHP1, GIC1*	8/165	2.4×10^{-4}
	Ino2	*FAS2, CDS1, KNH1, OPI1*	4/31	2.5×10^{-4}
	Ino4	*YIP3, FAS2, CDS1, CHO1*	4/33	3.2×10^{-4}
	Tec1	*MNN5, PCL1, SVS1, PCL2, CHS7*	5/64	4.5×10^{-4}
3	None			
4	Degradation of glycine (01.01.09.01.02)	*GCV2, GCV1*	2/5	2.7×10^{-4}
	C-1 compound catabolism (01.05.05.07)	*GCV2, GCV1*	2/6	4.1×10^{-4}
	Bas1	*MTD1, ADE17, GCV2, GCV1*	4/39	4.8×10^{-5}
	Gcn4	*SNO1, GCV2, HIS3, ICY2, YMC1, GNP1*	6/182	3.4×10^{-4}
5	Metabolism of arginine (01.01.03.05)	*ARG7, ARG1, CPA1*	3/20	4.4×10^{-4}
	Arg81	*ARG1, CPA1, CUP9*	3/22	5.9×10^{-4}
6	**Opi1**	*DAK1, YOP1*	2/23	1.7×10^{-4}

- Number of DTC specific genes was narrowed to 253
- Functional categories:
  - Phospholipid metabolism
  - ER-to-Golgi transport
  - RNA polymerase III transcription
  - One-carbon metabolic processes
  - Amino acid metabolism
  - Cell cycle progression

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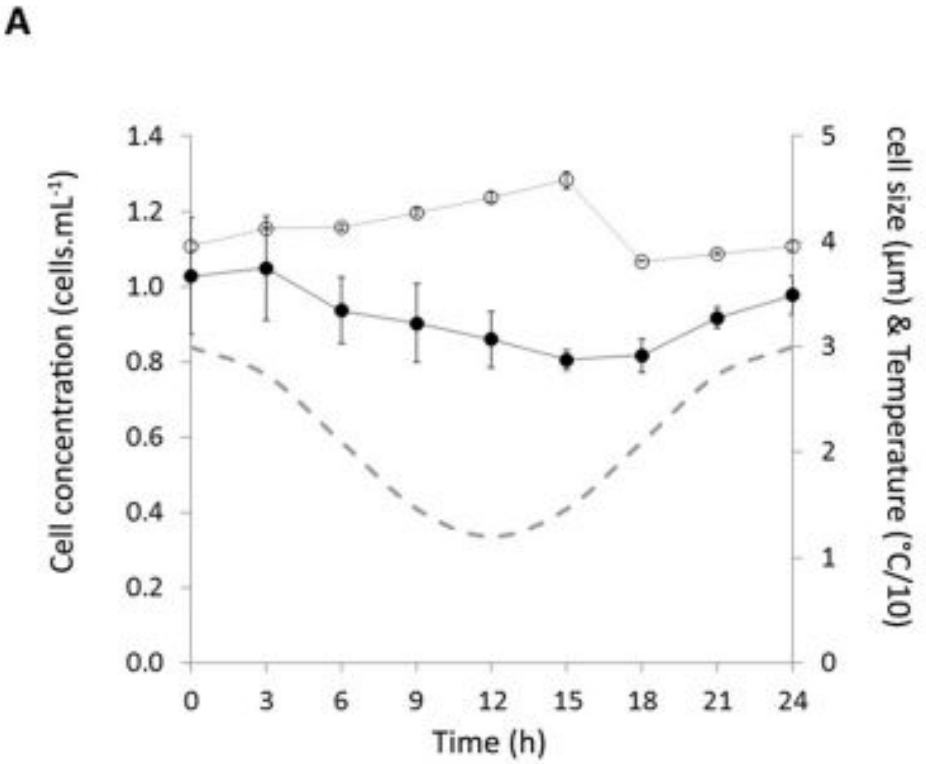
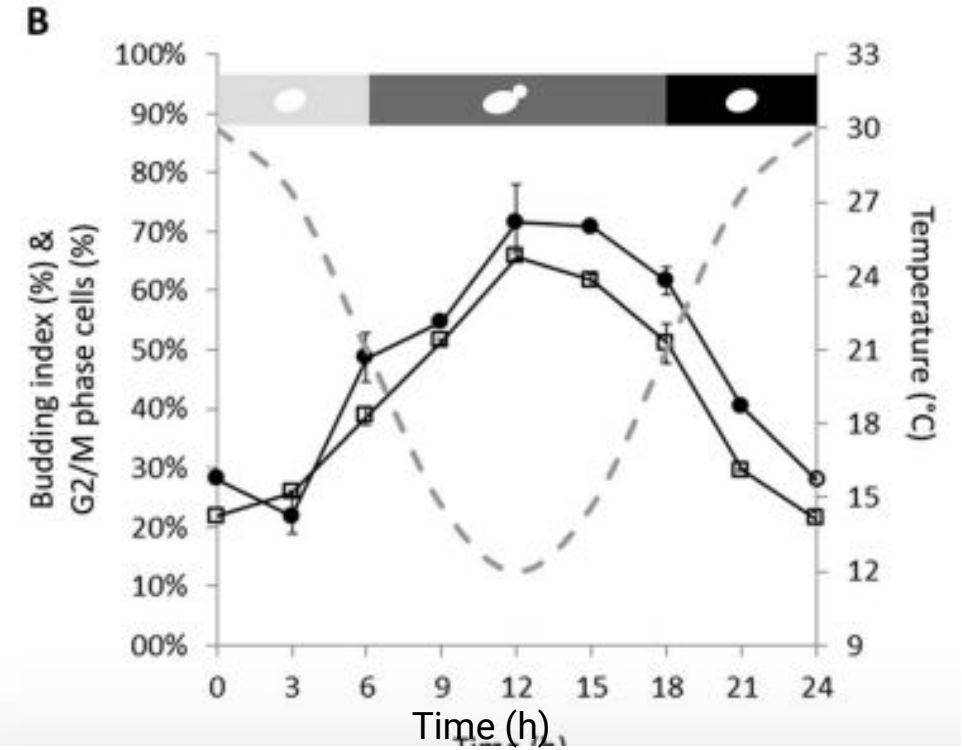
Figure 6a: At low temperatures, cells stop dividing, but continue to grow in size



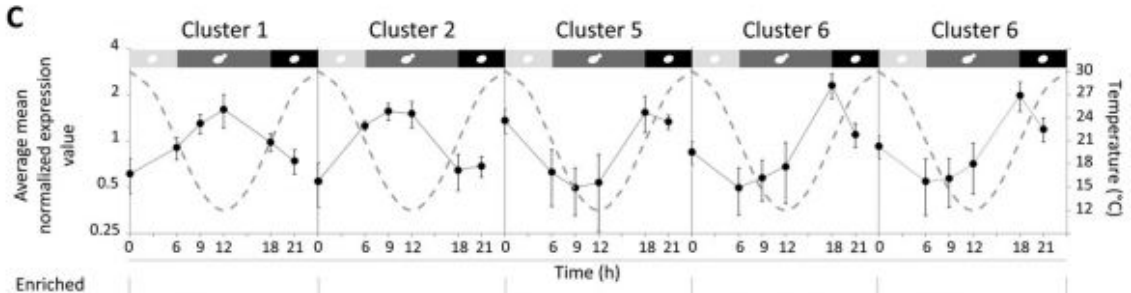
- Temperature decrease:
  - 22% decrease in cell number (●) and 16% increase in cell size (○)
- Temperature increase:
  - Cell number increase, cell size decreases
- Cells stop dividing, but continue growth at low temp

Figure 6b: Accumulation of budding cells occurs at low temperatures



- Low temperature:
  - 70% of the cells are arrested at the G2/M phase as buds
  - Budding index (●) and flow cytometric (⧅)
- Release of the buds once the temperature is increased

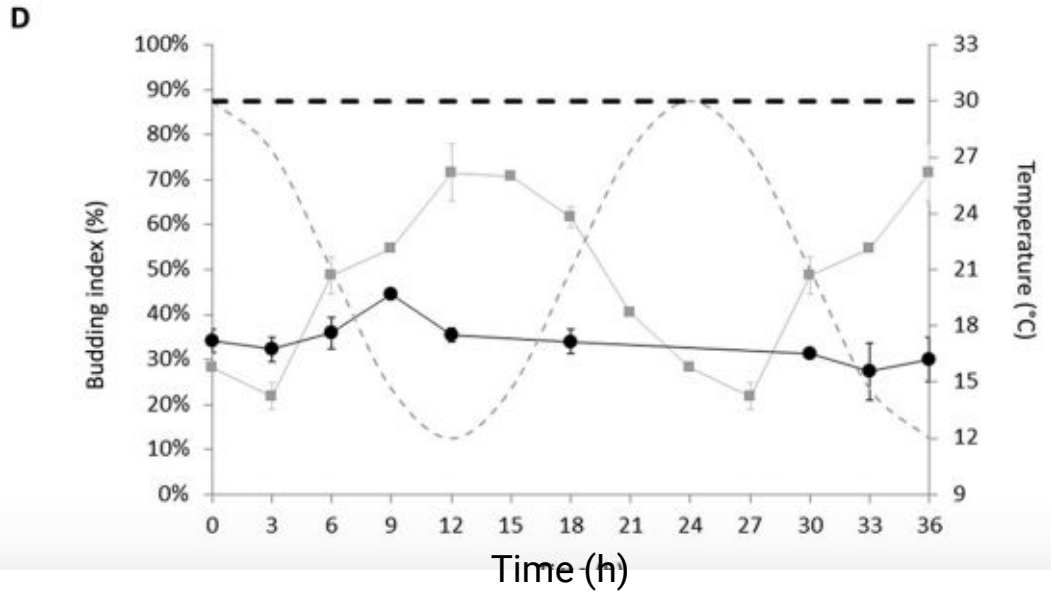
Figure 6c: Changes in expression of genes involved in cell cycle control occurred across timepoints



Enriched custom-made categories	S/G2 phase (Spellman *et al.* 1998)	S phase (Spellman *et al.* 1998)	Early G1 phase (Cho *et al.* 1998)	Early G1 phase (Cho *et al.* 1998)	M/G1 phase (Spellman *et al.* 1998)
(a/b)*	(7/116)	(6/70)	(12/67)	(7/67)	(12/108)
P value	4.8×10^{-5}	5.1×10^{-3}	1.5×10^{-4}	7.9×10^{-3}	3.1×10^{-4}
Genes	*YLR225C*	*YJL118W*	*YBR053C* *GLK1*	*EIS1*	*YKL151C* *PIL1*
	BUB2	*GIC1*	*AIM44* *INH1*	*COQ5*	*AFR1* *PIR3*
	CWP1	*HTA1///HTA2*	*CIT1* *MRH1*	*GSY2*	*COQ5* *PNC1*
	GAS3	*NRM1*	*CYT1* *NDL1*	*LSP1*	*GSY1* *ROD1*
	GAS5	*VRG4*	*DSE3* *QCR9*	*MCR1*	*ICR24* *UGX2*
	MXR1	*YPH1*	*DSE4* *YRO2*	*PIR3*	*NFT1* *YGP1*
	TUB2			*VPS13*	

- **S/G2 phase** genes were enriched at 12ºC
- Upregulation of genes from **early G1** and **M/G1 phases** after the temperature increased

Figure 6d: Budding index levels for cells in a fixed temperature environment



- Black line:
  - Budding index of cells remained constant after being placed in fixed 30ºC
- Gray line:
  - BI of cells in diurnal temperature environment

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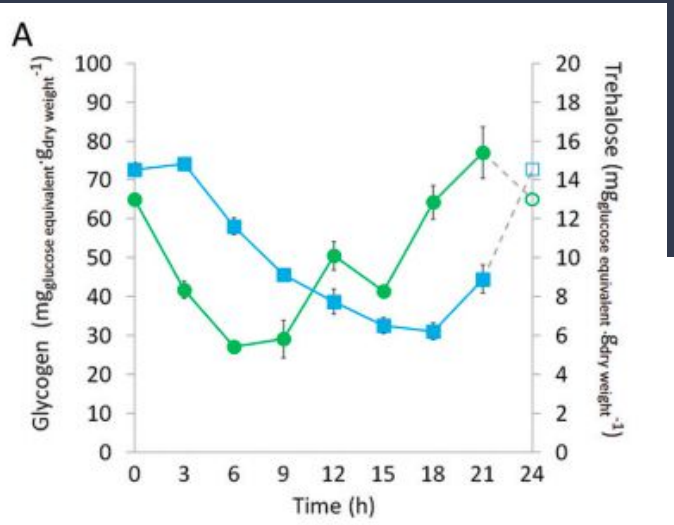
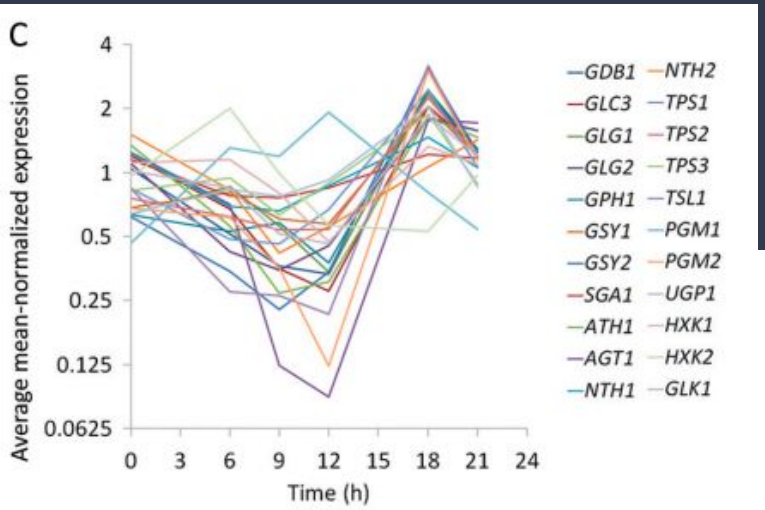
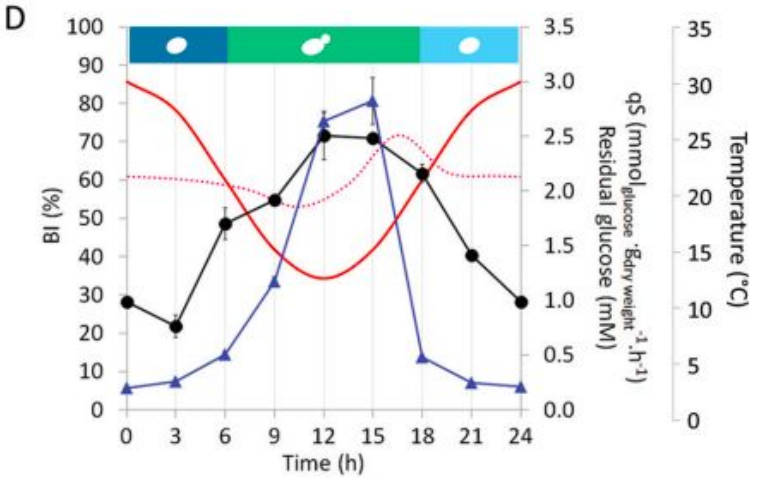
Figure 7a,b: Trehalose and glycogen levels fluctuate, while precursors are temp dependent



- Metabolism of trehalose and glycogen is temperature dependent
- Trehalose and glycogen concentrations fluctuated throughout DTC
  - At 6 hours, glycogen increased and trehalose decreased
- UDP-glucose and trehalose-6-phosphate were unaffected by temperature
- Glucose-1-phosphate and glucose-6-phosphate were inversely proportional to temperature

Figure 7c,d: Genes involved with trehalose and glycogen showed similar expression





- Genes involved in the synthesis or degradation of trehalose or glycogen had similar expression changes
  - Downregulation at low temp, upregulation at high temp
- Residual glucose concentration

  Budding Index

  Temperature

  Glucose consumption q_s (- - -)

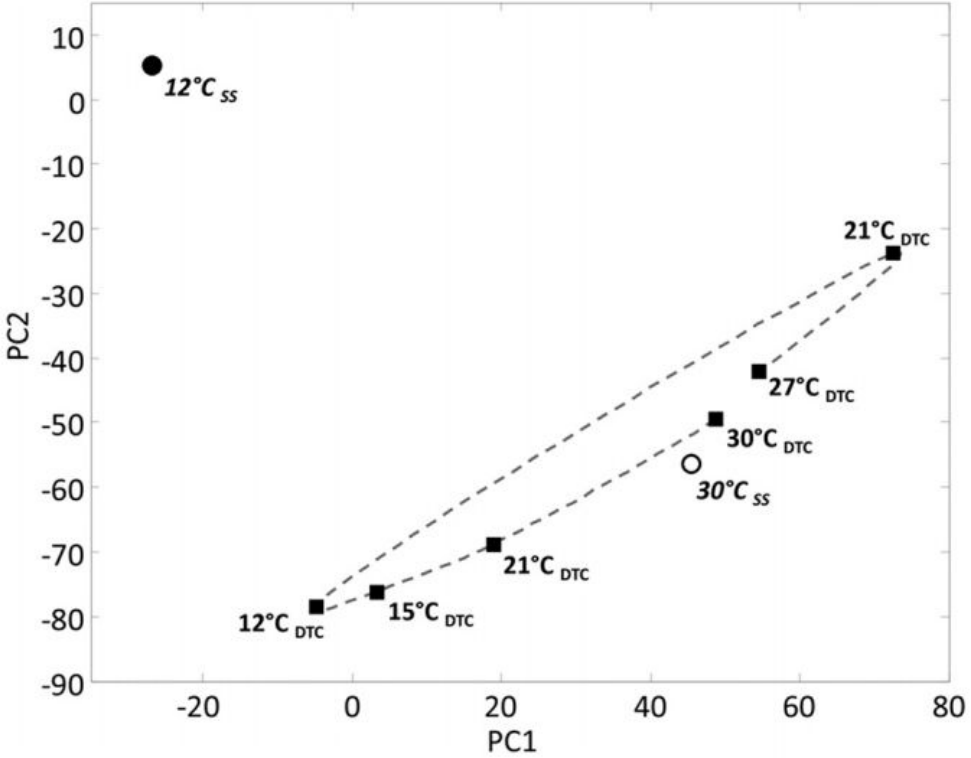
Table 3: Physiological characteristics were similar between steady state and DTC cultures, except for glycogen concentration

TABLE 3 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

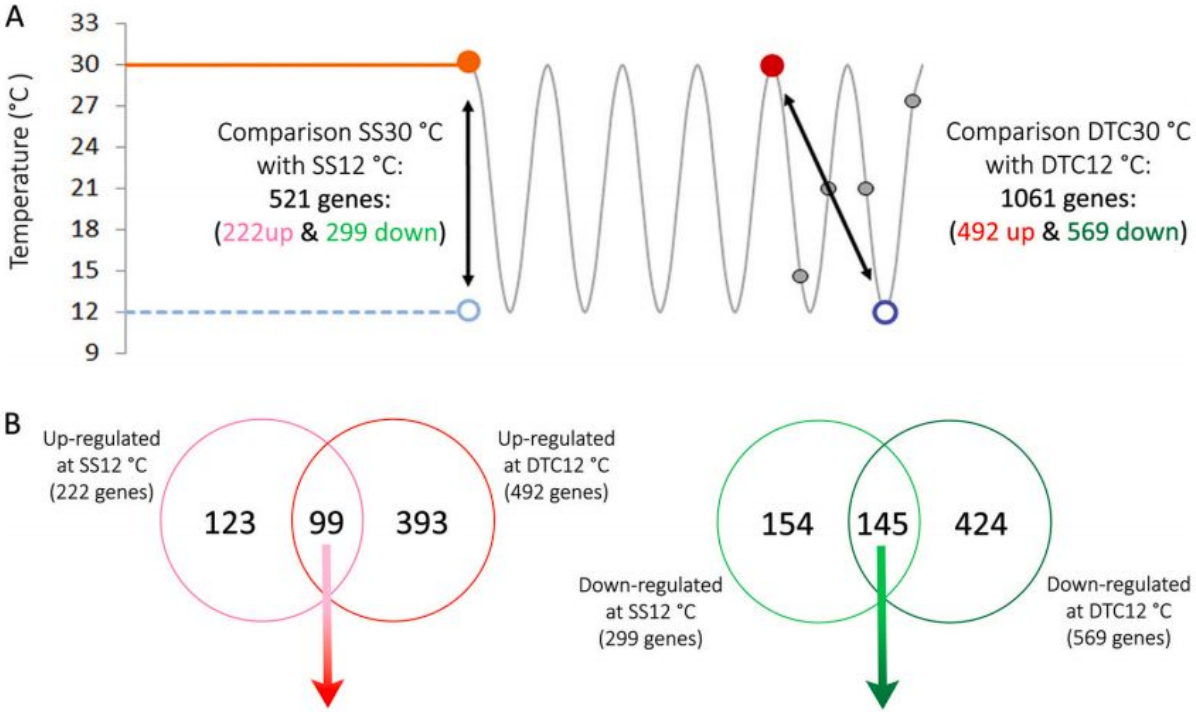
- Cultures grown at 12°C or 30°C fixed temperature (steady-state (SS))
- The values for biomass yield, specific uptake and production rates, and residual glucose concentration were similar between the two experiments
- Glycogen concentration varied
  - Higher glycogen than trehalose in steady state cultures
- Increase in BI for DTC culture at 12°C

Figure 8: Principal component analysis reveals similarity between SS and DTC at 30ºC, and a distinction at 12 ºC



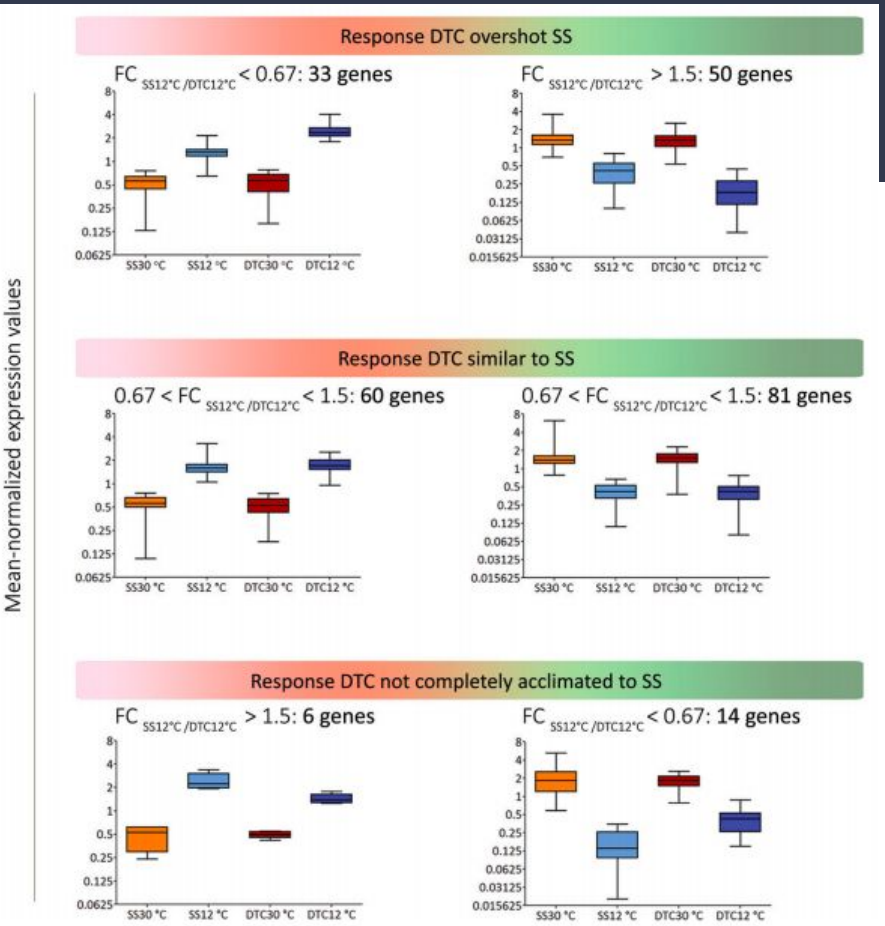
- Principal component analysis
  - Transcript levels are similar between SS and DTC at 30ºC
    - 94 genes were different
  - Clear distinction between SS and DTC at 12ºC

Figure 9a,b: Half of the number of genes showed significant change in expression in SS cultures



- Genes with significant change
  - SS: 521 genes
  - DTC: 1061 genes
- 99 genes were consistently upregulated (44%)
- 145 genes consistently downregulated (48%)
- 393 up, 424 downregulated genes respond to DTC not acclimation

Fig 9c: Differences between the upregulated and downregulated genes in each study



- 99 upregulated genes
  - 33 genes overshot SS
  - 60 genes similar to SS
  - 6 genes not completely acclimated to SS
- 145 downregulated genes
  - 50 genes overshot SS
  - 81 genes similar to SS
  - 14 genes not completely acclimated

Discussion & Conclusion

- Rhythmic variation of residual glucose concentration impacted transcriptional response
- Functional categories of genes with significantly changed expression levels include phospholipid metabolism (Tronchoni et al., 2012) , cell wall synthesis
    - Cyclic variation of acetate → acetyl coenzyme A, a precursor to fatty acid synthesis (Kozak et al., 2014)
    - Intracellular transport, Swi4 and Swi6 → constant cell wall maintenance
- Cell cycle was affected by temperature, contrary to previous studies (Vanoni et al., 1984)
- Temperature was likely not a main factor in glycogen and trehalose concentrations
- Steady-state and diurnal temperature cycle cultures were similar physiologically
- Future study: Mimic the variance in growth rate that occurs in natural environments where DTC occurs.

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