## Acclimation of Saccharomyces cerevisiae to Low Temperature: A Chemostat-based Transcriptome Analysis

Tai, S. L., Daran-Lapujade, P., Walsh, M. C., Pronk, J. T., & Daran, J. M. (2007) *Molecular Biology of the Cell, 18,* 5100-5112.

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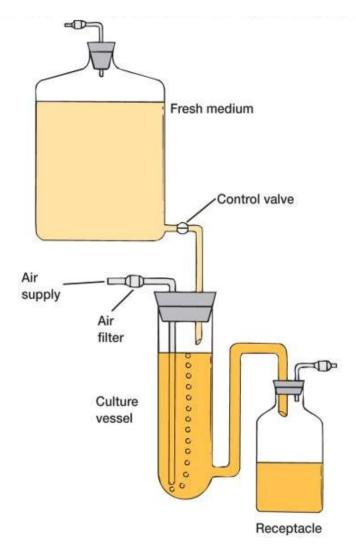
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- S. cerevisiae was grown in four different experimental conditions.
- DNA microarray analysis was used to analyze growth limitations applied to S. cerevisiae.
- *S. cerevisiae* stores carbohydrates during chemostat culture for unknown reasons.
- Promoter analysis reveals regulation trends in gene regulation.
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# Chemostat culture allows for fixed experimental conditions

- Chemostat culture involves the continuous addition of fresh medium.
- Allows for control of growth factors.
- Specific growth rate can be measured accurately.
- Batch culture is a closed system which does not allow for accurate measurement of specific growth rate.



# Low-temperature acclimation and cold shock have differing results

- Cold shock entails a rapid decrease to near freezing temperatures.
- Low-temperature acclimation is the biological response of cells to steady decreases to near freezing temperatures.
- Trehalose is required for transcription in low temperature situations.
  - Not present in low temperature acclimation results.

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# S. Cerevisiae grown in varying conditions of temperature and nutrient content

Limiting nutrient	Growth temperature (°C)	$Y_{Glu/X} (g_{DW} \cdot g_{glucose}^{-1})$	q <sub>Glu</sub> a	q <sub>etOH</sub> <sup>a</sup>	q <sub>CO2</sub> <sup>a</sup>	Carbon recovery (%)	Residual glucose (mM)	Residual ammonia (mM)
Glucose	12	$0.07 \pm 0.01$	$-2.5 \pm 0.2$	$3.8 \pm 0.3$	$4.4 \pm 0.3$	$100 \pm 3$	$2.8 \pm 1.1$	65.2 ± 2.2
Glucose	30	$0.07 \pm 0.00$	$-2.3 \pm 0.0$	$3.5 \pm 0.0$	$3.8 \pm 0.2$	$95 \pm 1$	$0.3 \pm 0.1$	$61.3 \pm 4.5$
Ammonium	12	$0.05 \pm 0.00$	$-3.6 \pm 0.2$	$6.1 \pm 0.3$	$6.0 \pm 0.6$	$97 \pm 4$	$90.0 \pm 9.8$	$1.5 \pm 0.2$
Ammonium	30	$0.04 \pm 0.00$	$-4.0 \pm 0.1$	$6.8 \pm 0.2$	$7.4 \pm 0.2$	$97 \pm 2$	$85.1 \pm 8.2$	$0.2 \pm 0.1$

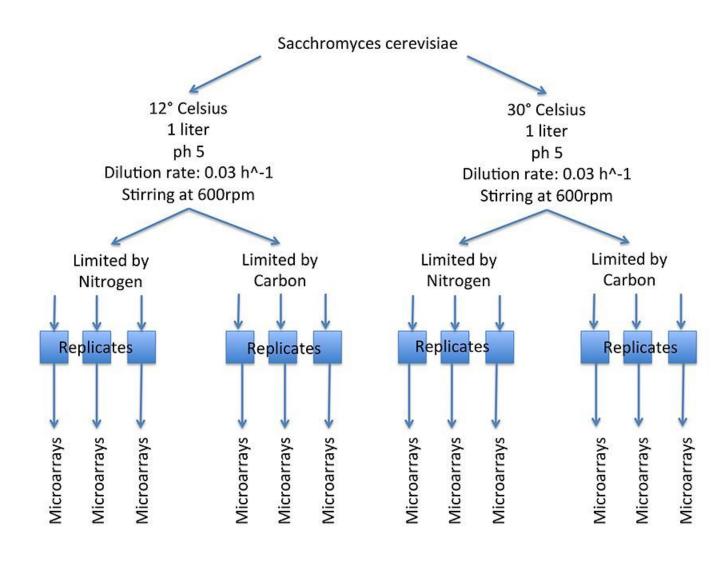
Cultures were grown at 30 and 12°C (D =  $0.03 \, h^{-1}$ ). Values represent the mean  $\pm$  SD of data from three independent steady-state chemostat cultivations.  $Y_{Glu/X}$ , biomass yield on glucose; DW, dry weight.

#### Table 1

- S. Cerevisiae is minimally affected by variation in temperature.
- Higher temperatures correlated with lesser amounts of a limiting nutrient corresponding residual residue.
  - Larger amounts of residual glucose at 12°C connected to possible contamination of gene sets.

<sup>&</sup>lt;sup>a</sup> Values expressed as mmol  $\cdot g_{DW}^{-1} \cdot h^{-1}$ .

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# Temperature and nutrient limitations were analyzed using DNA microarray analysis

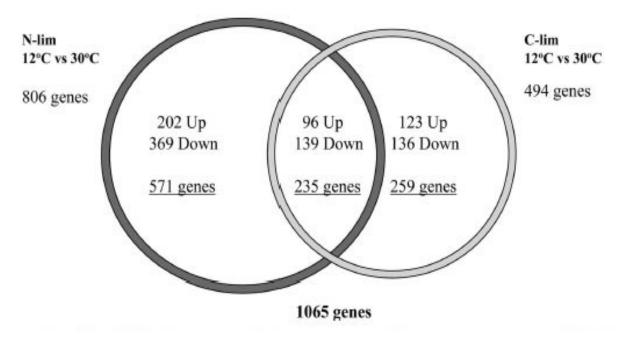


Figure 1

• 1065 represents the number of temperature responsive genes in *S. cerevisiae* genome.

# Heat mapping was used to identify transcription level of temperature responsive genes

#### Low temperature C-lim specific up (123 genes)

lipid metabolism (GO0006629 p = 3.2E-03); ERGIS, ATY2 CARL LAST, FAM. OPP. LEO., 1904. PORIS, FATY carbohydrate transport (GO0008643 p = 1.8E-03); BTY2, BTY3, BTY4, 1904. (BNA processing (GO0006364 p = 6.7E-03)

#### Low temperature C-lim specific down (136 genes)

Electron transport (GO0006118 p = 1.0E-04); gct2 ctrc? cons.cons.stwn.const.outr Amino acid transport (GO0006865 p = 5.2E-04); seare, sur2 ctrc. zurz.zurz.surz.

WORK NORK MERK SAND CHRI, CEPTE RIVEL

Hosese metabolism (GO0019318 p = 6.4E-03); 7XL2 GALIS GADE GCR2 PTEL PCR1, YMR323W

#### Low temperature N-lim specific up (202 genes)

Protein synthesis (GO0006412 p = 9.8E-06)/protein complex assembly (GO0006461 p = 6.2E-06);

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Ribosome biogenesis and assembly (GO0042254 p = 2.0E-25)/RNA processing (GO0006396 p = 7.4E-11)/rRNA processing (GO0006364 p = 1.0E-18°c

#### Low temperature N-lim specific down (369 genes)

Nirrogan, compared metabolism (GC0004607 p = 5.8E-04) and estabolism (GC0004427) p = 2.0E-05)/amina transport (GC00015637 p = 1.6E-03) / allastoin regulacijsm (GC0000256 p = 8.5E-05);

ALDO ALDO ADVE DALE DALL DEBLE CARE CARE CARE, CHAI, ANCE, BEEL DATE MOVE DALE DALED ANCE CARE DECIL DEBLE DEFL DESC ANCES.
VILLEYN, GLEG POTE DALE DERL GARE AFTE CARE PERC, VANORING, BORE MEDEL MEDEL.

Polysaccharide (GO0005976 p = 3.1E-03) and trebalose metabolism (GO0005991 p = 1.1E-03);

SMCV, GREZ GSTV, PCL2, PCL10, PIG2, GSC2, G4CV, 7PS2, 9700, 7PSV, 7SC1

M phase of mitotic cell cycle. (G0000087 p. = 5.5E-05) and chromosome segregation (G00007059 p. = 2.8E-05); CECNI PRIS ALC: SCI. ASSI SOF, BRI SMC, SMCE SUM, EAST STEEL, EGG. 1931, SSI; CTP4 GACT, EFE, VALLEGO

Cellular morphogenesis (GO0000002 p = 2.8E-03);

PECORR BETCA FORE, DORY, TAKES, TORZ, LOZE, SEAV, KEET, WINA BORZ SCHR, FRYY, BAIT, BYTT, GERA WHIS ZOSY, ARP)

Response to stimulus (GO0050896 p = 3.5E-05);

4897, ALDIS ARPLA, BENG, CARN, CECON, CECON, CECON, CECON, CARL, FARLE, FREZ, FEFER, GLACI, GREZ, HERPLAN, FREY, GREZ, HERY, ARDIS, MICEL, MICHA, MIC

- Goal was to identify regulatory networks involved in acclimation of S. cerevisiae to low temperature.
- Observed changes in
  - Transporters of limiting nutrient
  - Translational machinery
- NCR genes and catabolite repression

#### Low temperature C-lim and N-lim up (96 genes)

Nuclear export (GO0051168 p = 5.9E-03);

MPLS MMTY, NWISE HOGE ECHE, NOGE

Ribosome biogenesis and assembly (GO0042254 p = -2.8E-05) / rRNA processing (GO0006364 p = 1.4E-03);

#### Low temperature C-lim and N-lim down (139 genes)

Cabobydrate metabolism (GO0005975 p = 6.4E-04);

MALJZ SOLA MALJY, NBGJ, MOCY, ATWI, GIFZ, MIGT, GUTZ, PFK26, PFK27, MALJY, FTF/10 HTRZ

Response to stimulus (OC00150696 p. = 3.1E-03); HORL NIGH STAL ARM, MEDI ARM, MEDI PORS MEDI, CUPE REPO, CRIS, ARM, PETS NIME, MEAS SEED, YHRIMEN, YERSHEN, YRIMAN

COUSA, DALA MALIF, AGP., PCT2, STC, MITS, DIPS, FTR2, PWONE SUTJ. OPT2, CCC2, AACT, FFEN, BTHC, YHRANEW, ABAT, SSAU, GITJ. FORE, PTT2, SSET, ATG/J. FOR FOC., SULJ, MITS PT23, AGP., MCP1, PTR), BALLT, FET3, CESS Tai et al., 2007

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# S. cerevisiae stores protein and carbohydrate in chemostat culture

Limiting nutrient	Growth temperature (°C)	Biomass dry weight (g <sub>DW</sub> ·l <sup>-1</sup> )	Whole cell protein (g <sub>protein</sub> · g <sub>DW</sub> <sup>-1</sup> )	Biomass nitrogen content (mg <sub>nitrogen</sub> • g <sub>DW</sub> <sup>-1</sup> )	Trehalose (gequivalent glucose • g <sub>DW</sub> -1)	Glycogen (gequivalent glucose • g <sub>DW</sub> <sup>-1</sup> )
Glucose	12	$1.71 \pm 0.09$	$0.40 \pm 0.01$	nd	< 0.005	$0.06 \pm 0.01$
Glucose	30	$1.89 \pm 0.06$	$0.43 \pm 0.01$	nd	$0.02 \pm 0.00$	$0.04 \pm 0.00$
Ammonium	12	$2.27 \pm 0.05$	$0.47 \pm 0.03$	$63 \pm 3$	< 0.005	$0.02 \pm 0.00$
Ammonium	30	$3.53 \pm 0.01$	$0.34 \pm 0.01$	$41 \pm 2$	$0.04 \pm 0.00$	$0.05 \pm 0.01$

Cultures were grown at 30 and 12°C (D =  $0.03 \, h^{-1}$ ). Values represent the mean  $\pm$  SD of data from three independent steady-state chemostat cultivations. DW, dry weight; nd, not determined.

#### Table 2

- Trehalose and glycogen biosynthesis occurs under stress conditions.
  - Biosynthesis varies with temperature.
- Reason for storage of carbohydrates in nonfreezing temperature in unclear.
  - Trehalose was not produced in tps1 and tps2 strains, yet did not affect viability.

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## Promoter Analysis Reveals Regulation Trends in Gene Regulation

(A) 5' upstream cis-regulatory motif

Regulatory cluster	Motif name	Putative-binding protein	Promoter element	occa	Expected occ <sup>b</sup>	occ E
Low Temperature C-lim Up			000			
Low Temperature C-lim Up			_			
Low Temperature N-lim Up		_	TGAAAAA	206	113.04	2.30E-11
	PAC	_	CGATGAG	57	17.49	6.1E-14
		_	TGAGATG	49	16.3	4.1E-07
Low Temperature N-lim Down	GATAA	Gln3/Gat1/Dal80/Gzf3	AGATAAG	203	102.57	3.1E-15
	STRE	Msn2/Msn4	ACCCCTT	29	8.73	1.6E-03
Low Temperature C- and N-lim Up	PAC		CGATGAG	30	8.39	5.0E-05
Low Temperature C- and N-lim Down	_	_	CGTCCAC	13	2.85	7.8E-03

(B) Overrepresentation of transcription factors (TF) binding targets

Regulatory cluster	Factor	p value	K <sup>d</sup>	Pe
Low Temperature C-lim Up	Mbp1p	1.6E-03	10	65
Low Temperature C-lim Down	Hap2-Hap1	3.9E-05	3	4
101-00-101-10-10-10-10-10-10-10-10-10-10	Hap3-Hap1	9.9E-06	3	3
Low Temperature N-lim Up	Fhl1p	3.4E-05	19	203
The state of the s	Sfp1p	1.3E-03	7	51
Low Temperature N-lim Down	Gln3p	2.1E-07	20	92
	Gln3-Dal82	5.5E-07	8	15
	Hap2-Dal82	6.8E-05	5	9
Low Temperature C- and N-lim Up				
Low Temperature C- and N-lim Down	Aft2p	7.5E-04	10	34
	Hsf1p	3.0E-08	16	133
	Nrg1p	7.6E-07	14	128
	Phd1p	3.3E-04	9	99
	Rcs1p	1.1E-04	9	86
	Rox1p	6.0E-05	8	62
	Sok2p	5.7E-05	9	79
	Nrg1-Aft2	6.0E-05	5	20
	Phd1-Nrg1	1.4E-05	7	37
	Rox1-Phd1	7.8E-05	5	21
	Sok2-Nrg1	4.3E-07	8	33

<sup>(</sup>A) Significantly overrepresented cis-regulatory binding motifs in 5' upstream regions. (B) Significantly overrepresented promoter elements that bind known transcription factors (TF) or TF pairs according to ChiP-on-chip analysis (Harbison et al., 2004). C-Lim, glucose-limited; N-Lim, ammonium-limited.

- STRE elements exist in promoters of downregulated genes in N-limited cultures
- Upregulation in both conditions show PAC regulatory motifs in promoters
- Common promoter sequences show possible regulation relationships

<sup>\*</sup> The number of occurrences of promoter element in the regulatory cluster.

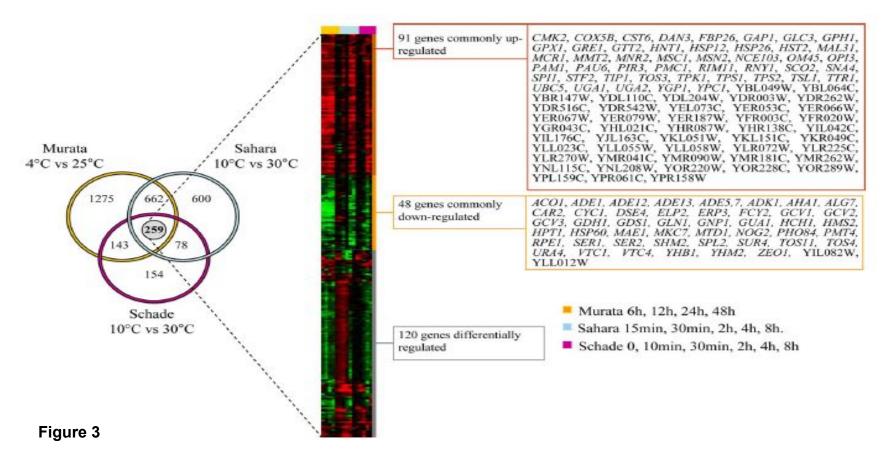
b Expected number of occurrences of the promoter element in a randomly chosen cluster of genes of the same cluster size.

The probability of finding the number of patterns with the same level of overrepresentation, which would be expected by chance alone.

d Number of genes in category in cluster.

<sup>&</sup>quot; Number of genes in category in genome.

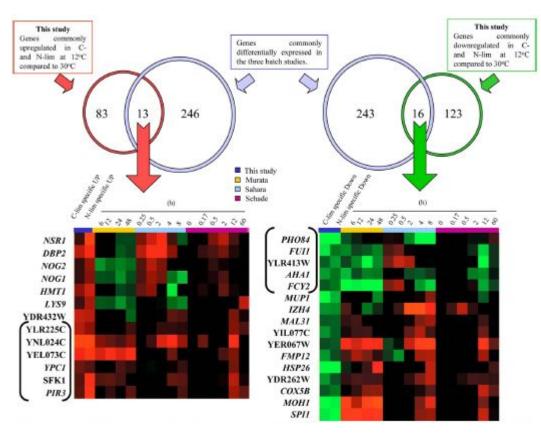
## **Genes Common to All Researchers Were Categorized According to Regulation**



- Sahara and Schade tested cold shock, dropping temperature to between 10 and 20 degrees Celsius
- Murata tested cold shock at temperatures below 10 degrees Celsius, which caused cell growth to cease.

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## Overlapping Genes Showing the Same Temperature Response



- Chemostat study compared with Sahara et al. 2002,
   Schade et al. 2004, and
   Murata et al. 2006
- 29 genes total were in common, only 11 showed consistency

Figure 4

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## Few Genes Simultaneously Respond to Temperature and Growth Rate

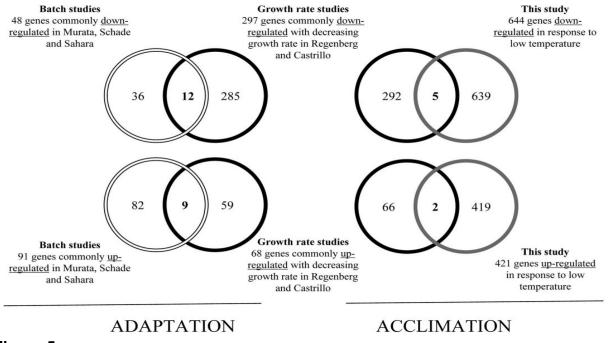
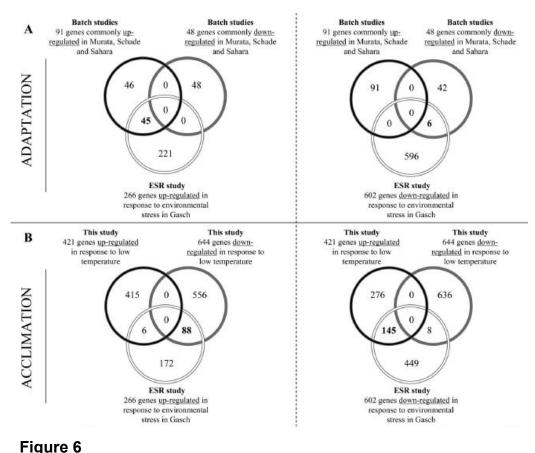


Figure 5

- Studies examined changes in gene regulation in response to a decrease in growth rate compared to both the batch studies and study data for overlap in gene regulation.
- A very small number of genes overlap in response to both temperature decrease and growth rate decrease.

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## One Third of Low Temperature Response Genes in the Batch Studies are Attributed to ESR



- ESR: environmental stress response
  - General mechanism responds to multiple stimuli
- Batch and chemostat genes compared with Gasch et al. 2000
- Some genes showed opposite results in response to low temperatures

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## **Acknowledgments**

Dr. Dahlquist
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