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). Acclimation of Saccharomyces cerevisiae to low temperature: a chemostat-based transcriptome analysis. *Molecular biology of the cell, 18*(12), 5100-5112.

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Outline

- Background Information
- Goal of the Present Study
- Methods and Procedures
- In chemostat based cultures, nutrient limitation affected the transcriptional regulation of genes at low temperatures.
- Comparing this chemostat study to other batch culture studies revealed inconsistent gene expression.
- Is the environmental stress response (ESR) an obligatory response to growth at low temperatures?

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Background Information

- Temperature fluctuations are an evitable environmental factor that affects all organisms.
- Sudden exposure to environmental changes will trigger a dynamic stress-response called adaptation.
- Prolonged exposure to nonlethal stimuli leads to acclimation.
- Chemostat cultures allow for accurate control of specific growth rate, independent of other culture conditions.
- Ms2p/msn4p has been previously suggested to be a transcriptional factor in cold temperature.

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Goals of the Present Study

- **Tai et al. (2007) aimed to investigate steady-state, acclimatized growth of *S. cerevisiae* at colder temperatures.**
  - **Emphasized transcriptional regulation of the genome.**
- **They compared their chemostat culture results with those from previous studies in batch cultures and demonstrated the differences between the two.**
- **They also aimed to further describe the role of ESR genes at low-temperature in chemostat cultures.**

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Methods and Procedures

- ***S. cerevisiae*** **strain: CEN.PK113-7D (MATa)**
- **Grown in a chemostat culture (D = 0.03 h^{-1})**
- **Four Growth Conditions:**
  - **12°C, glucose-limited**
  - **12°C, ammonium-limited**
  - **30°C, glucose-limited**
  - **30°C, ammonium-limited**
- **Utilized various analytical methods and microarray analysis and compared their data to other *S. cerevisiae* low-temperature transcriptome datasets.**

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Growth-Efficiency Was Not Affected by Growth Temperature

Table 1. Physiological characteristics of *S. cerevisiae* grown in ammonium- and glucose-limited anaerobic chemostat cultures

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

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

[a] Values expressed as mmol $\cdot\ g_{DW}^{-1} \cdot h^{-1}$.

- **Biomass was similar at both temperatures in both nutrient conditions.**
- **In the 12°C cultures, the residual concentrations of glucose and ammonia were higher than the 30°C cultures.**
  - **Residual nutrient concentration is temperature dependent.**

Less *S. cerevisiae* Growth on Glucose-Limited Media

Table 2. Protein and storage carbohydrates contents of *S. cerevisiae* biomass grown in ammonium- and glucose-limited anaerobic chemostat cultures

Limiting nutrient	Growth temperature (°C)	Biomass dry weight ($g_{DW} \cdot l^{-1}$)	Whole cell protein ($g_{protein} \cdot g_{DW}^{-1}$)	Biomass nitrogen content ($mg_{nitrogen} \cdot g_{DW}^{-1}$)	Trehalose ($g_{equivalent\ glucose} \cdot g_{DW}^{-1}$)	Glycogen ($g_{equivalent\ glucose} \cdot g_{DW}^{-1}$)
Glucose	12	1.71 ± 0.09	0.40 ± 0.01	nd	<0.005	0.06 ± 0.01
Glucose	30	1.89 ± 0.06	0.43 ± 0.01	nd	0.02 ± 0.00	0.04 ± 0.00
Ammonium	12	2.27 ± 0.05	0.47 ± 0.03	63 ± 3	<0.005	0.02 ± 0.00
Ammonium	30	3.53 ± 0.01	0.34 ± 0.01	41 ± 2	0.04 ± 0.00	0.05 ± 0.01

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

- **There was more yeast growth on the ammonium-limited media.**
- **Amount of trehalose was equal in the 12°C media and slightly higher in the 30°C ammonium-limited media.**
- **Overall, yields and fermentation rates were similar in all growth conditions.**
  - **This indicates that the growth temperature did not affect the growth efficiency.**

Higher Transcription Levels in Ammonium-limited Cultures

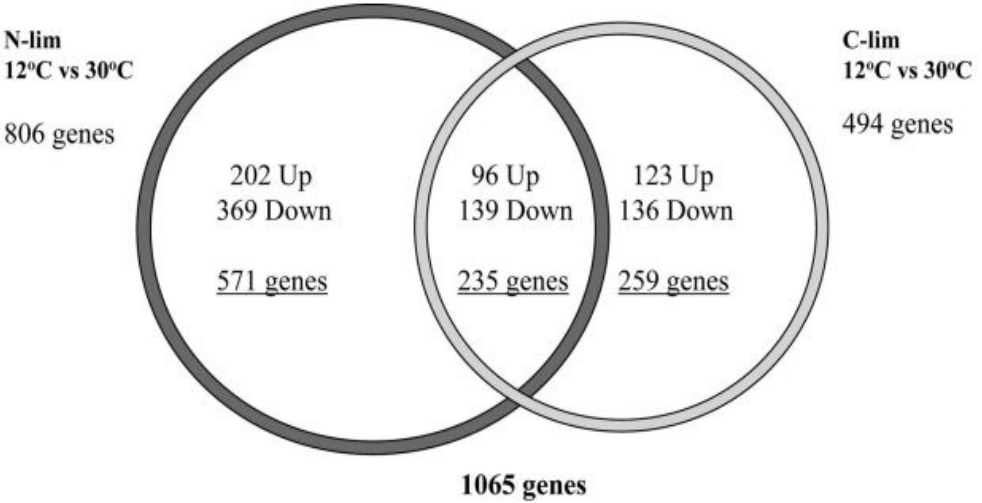
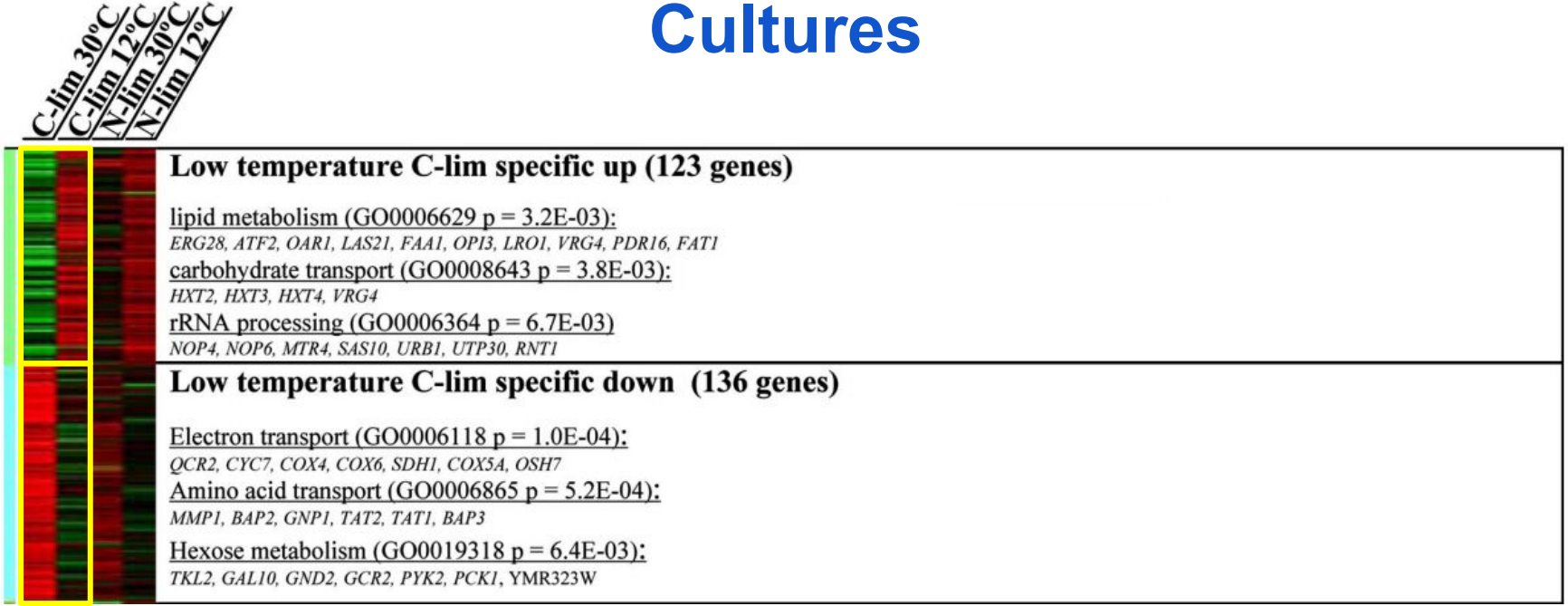


Figure 1. Global transcriptome responses to anaerobic growth at 12 and 30°C in anaerobic glucose- and ammonium-limited chemostat cultures (D = 0.03 h^{-1}). The Venn diagram shows the number of significant differentially expressed genes between 12 and 30°C in both C and N limitations.

- **Of the 1065 genes, 644 genes were down-regulated and 421 up-regulated genes.**
- **235 genes showed a consistent down-regulation and up-regulation in the glucose-limited and ammonium-limited conditions**

More Genes Were Induced in the Glucose-Limited Cultures



- **Temperature-responsive genes were screened for enrichment of specific functional categories.**
- **C-lim cultures showed the most activity in these categories**

Greater Transcriptional Regulation in N-Lim

Low temperature N-lim specific up (202 genes)

Protein synthesis (GO0006412 p = 9.8E-06)/protein complex assembly (GO0006461 p = 6.2E-06):
RPL43B, CAX4, ECM39, GCD10, GYP5, IMP3, MEF1, MRPL20, MRPL3, NIP1, OST2, RPL12A,, RPL13A, RPL15A, RPL17B, , RPL3, RPL40B, RPL41B, RPL8B, RPL9A, RPL9B, RPS10A, RPS16B, RPS21A, RPS21B, RPS26B, RPS28A, RPS9B, RSM26, SUI1, TEF4, TIF5, YGL068W, YFL034C

Ribosome biogenesis and assembly (GO0042254 p = 2.0E-25)/RNA processing (GO0006396 p = 7.4E-11)/ rRNA processing (GO0006364 p = 1.0E-18):
ARX1, BRX1, CBF5, CIC1, DBP3, DRS1, ENP1, ERB1, GAR1, HAS1, HCA4, IMP3, IMP4, LTV1, MAK5, MRT4, MTR3 ,NIP7, NOB1, NOC2, NOC4, NOP1, NOP2, NOP58, NOP7, NUG1, POP3, PWP1, RCL1, REX4, RIX1, RIX7, RLP24, RPL12A, RPL3, RPL40B, RRB1, RRP12, RSA3, SIK1, SNU13, TIF5, UTP15, UTP21, UTP23, UTP9, YTM1

Low temperature N-lim specific down (369 genes)

Nitrogen compound metabolism (GO0006807 p = 5.4E-04) and catabolism (GO0044270 p = 2.8E-05))/amine transport (GO0015837 p = 1.6E-03) / allantoin metabolism (GO0000256 p = 8.5E-05):
ALD2, ALD3, ADY2, DAL3, DAL1, DUR1,2, CAR2, CHA1, CHA4, SHC1, EKI1, GAT1, PUT4, MET4, DAL7, DAL80, ARG1, CAR1, DCG1, ASP3-1, GLT1, CPS1, ARO10, YIL167W, *GLN3, PUT1, DAL2, DTR1, GAP1, AVT6, CAN1, PTK2,* YMR088C, *DUR3, MEP1, MEP2*

Polysaccharide (GO0005976 p = 3.1E-03) and trehalose metabolism (GO0005991 p = 1.1E-03) :
SHC1, GSY2, GSY1, PCL7, PCL10, PIG2, GSC2, GAC1, TPS2, NTH1, TPS1, TSL1

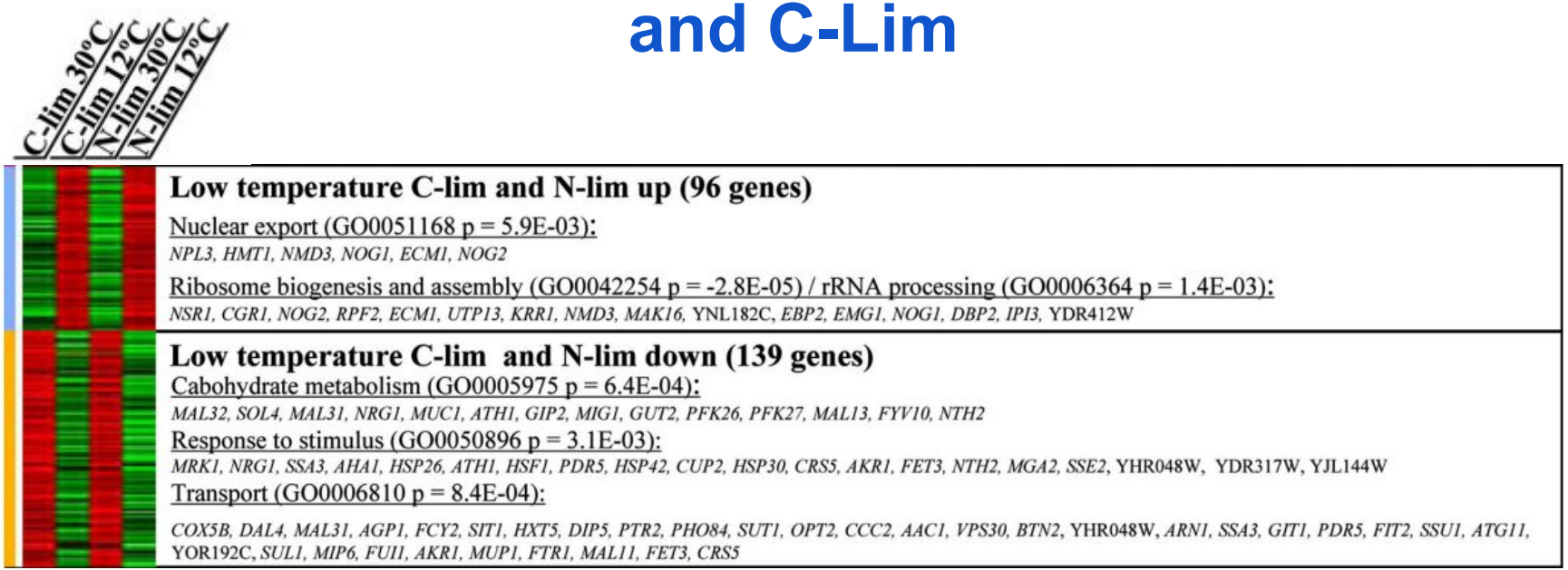
M phase of mitotic cell cycle (GO000087 p = 5.5E-05) and chromosome segregation (GO0007059 p = 2.8E-03):
CDC16, PDS5, ALK1, SIC1, PDS1, TOP1, IRR1, SMC1, SMC3, SUM1, KAR3, RTT101, KEL1, YCG1, TFB1, ASE1, CTF4, GAC1, KIP2, YNL116W

Cellular morphogenesis (GO0000902 p = 2.8E-03):
TCO89, KCC4, TOR1, DOP1, TAO3, TOR2, LGE1, SLA1, KEL1, WHI4, BOI2, SCH9, VRP1, RAX1, RNY1, GIN4, WHI5, ZDS1, ABP1

Response to stimulus (GO0050896 p = 3.5E-05):
ABF1, ALD3, ASP3-1, BDF1, CAD1, CDC39, CIN5, CTF4, DNA2, FAB1, FAR11, FRT2, FYV6, GAC1, GRE3, HSP104 , HSP78, IRE1, ISW2, ITC1, MDG1, MET4, MGT1, MLF3, MLH3, MSH6, NCE4, NTH1, PDS1, POL1, POL2, PRB1, PTR3, RAD28, RPN4, RRD1, RRD2, SAN1, SCH9, SHU2, SIN3, SMC1, SPL2, SSA4, TFB1, TPS1, TPS2, TSL1, UBA4, VRP1, WSC4, WWM1, YGK3, YKL075C

- **S. cerevisiae in N-lim cultures showed stronger transcriptional regulation in these categories.**

Strong Transcriptional Regulation in Both N-Lim and C-Lim



- **C-lim and N-lim cultures showed similar regulation trends in nuclear export, ribosome biogenesis/assembly, carbohydrate metabolism, and other categories.**

Overall, More Transcription Factors were Overrepresented

Table 3

(A) 5′ upstream cis-regulatory motif

Regulatory cluster	Motif name	Putative-binding protein	Promoter element	occ[a]	Expected occ[b]	occ E[c]
Low Temperature C-lim Up			—			
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[d]	F[e]
Low Temperature C-lim Up	Mbp1p	1.6E-03	10	65
Low Temperature C-lim Down	Hap2-Hap1	3.9E-05	3	4
	Hap3-Hap1	9.9E-06	3	3
Low Temperature N-lim Up	Fhl1p	3.4E-05	19	203
	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

(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.

[a] 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.

[c] 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.

[e] Number of genes in category in genome.

- **There were significantly overrepresented cis-regulatory binding motif in the 5’ upstream regions.**
- **There were significantly overrepresented promoter elements that bind known transcription factors or transcription factor pairs according to the ChiP-on-chip analysis.**

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259 Genes Found in Common in Three Batch-Culture Studies

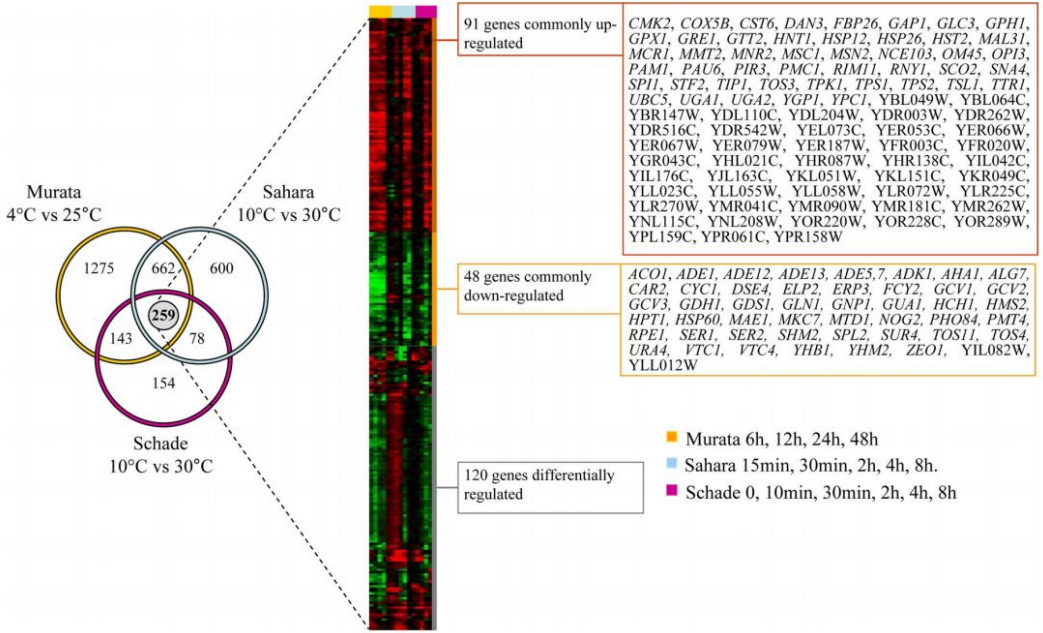
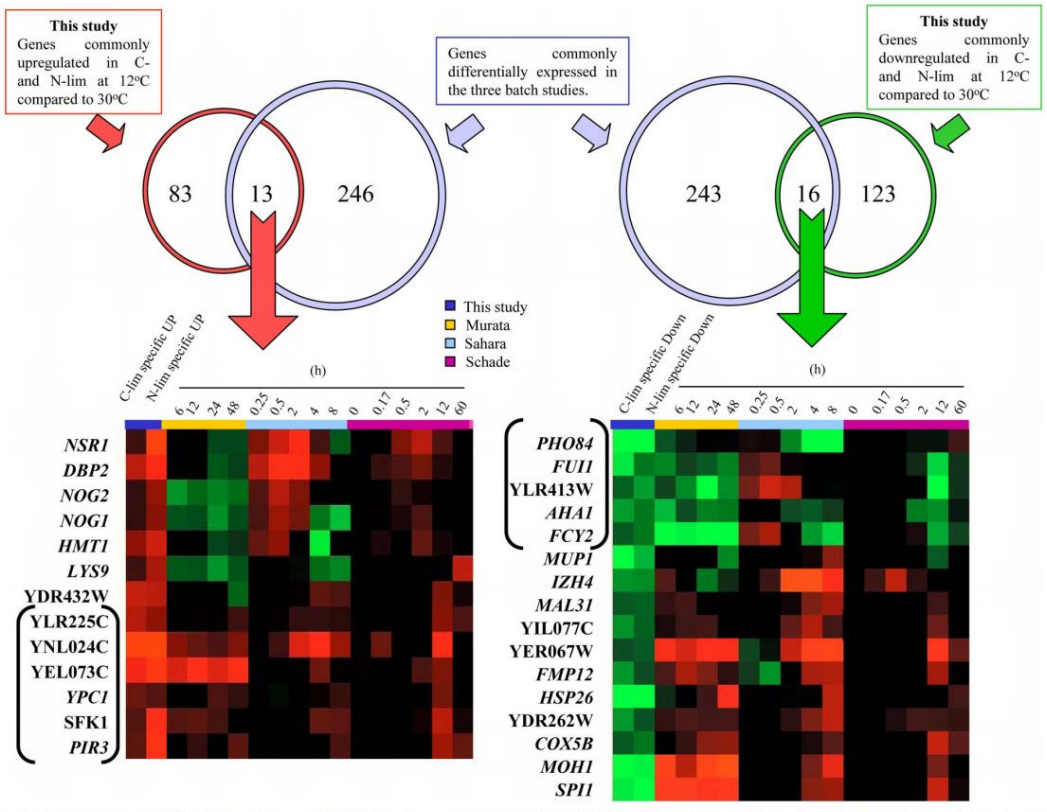


Figure 3. Genes differentially expressed in batch cultures during adaptation to low temperature. (A) Venn diagram showing the number of genes that are common to three batch-culture studies on low-temperature transcriptional adaptation (Sahara *et al.*, 2002; Schade *et al.*, 2004; Murata *et al.*, 2006). (B) Heat map representing the transcript ratio of 259 genes found in common in the three batch-culture low-temperature transcriptome datasets.

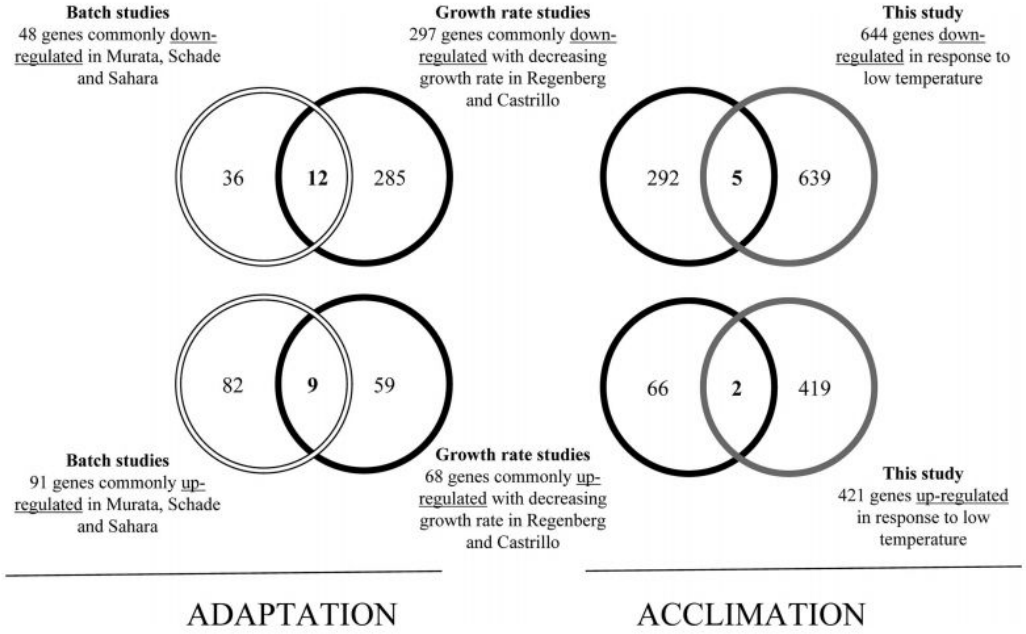
- Venn diagram compares the genes from the three different batch culture studies.
- Responses of genes was not consistent.
- The heat map shows the transcript ratio of the 259 genes found in common.

11 Genes Show Consistent Regulation



- Three genes that were consistently up-regulated are involved in lipid metabolism.
  - SFK1, YPC1, and YEL073C
- Three genes that were consistently down-regulated encode transporters.

More Genes Regulated in Chemostat-Cultures



- Genes were taken from Castrillo et al (2007) and Regenberg et al (2006).
- 25% of low-temperature down-regulated genes had altered transcript levels.
- 10% of the low-temperature up-regulated genes had altered transcript levels.

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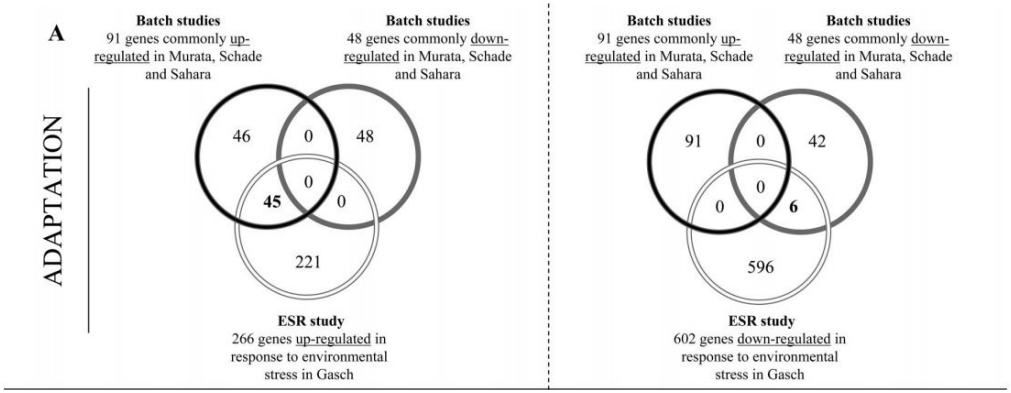
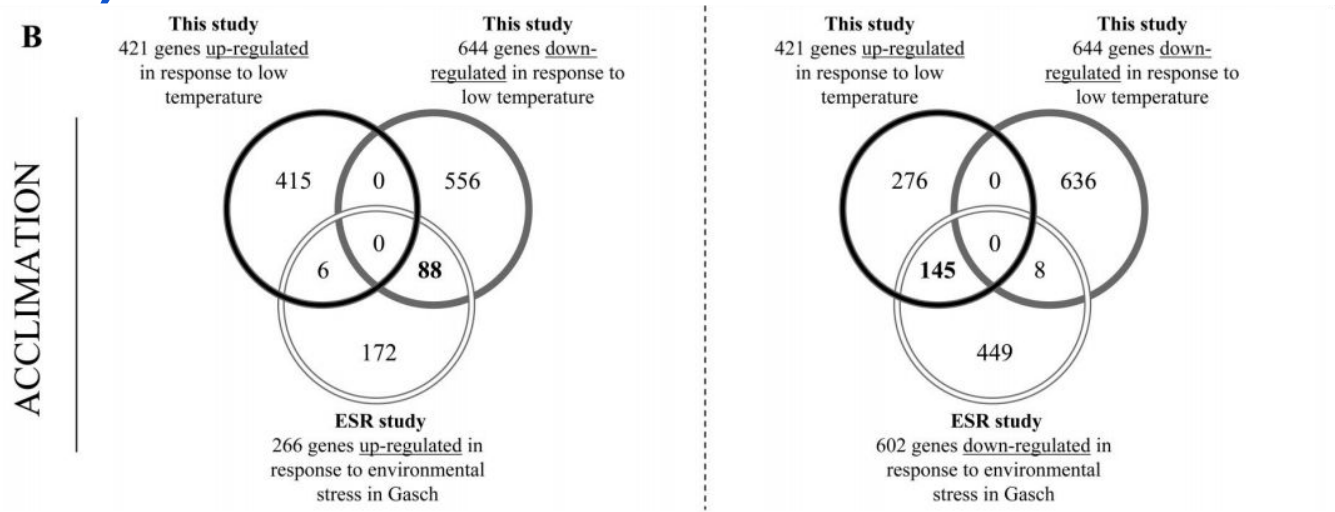
Extensive Overlap between the ESR Genes and the Genes in the Batch-Cultures



- **50% of consistently low-temperature up-regulated genes and 13% of low-temperature down-regulated genes found in the batch-culture studies were found in the ESR genes from Gasch et al. (2000)**
- **Proposed that the ESR mechanism becomes induced with the growth temperature is decreased.**

Comparison of Gene Regulation in the Gasch et al. (2000) and the Chemostat Cultures



- **233 genes up-regulated and down-regulated in the study by Gasch et al. (2000) had an opposite transcriptional response in the low-temperature chemostat cultures.**

Chemostat Cultures Offer a New Perspective on Transcriptional Regulation at Low Temperatures

- Genes that were consistently up- or down-regulated in both the batch cultures and the chemostat cultures coded for crucial cell functions
- Prior studies had different results because they used batch-cultures and Tai et al. (2007) used chemostat-cultures.
- ESR is not the main response to low temperatures.
- Transcriptional responses to low-temperature and low specific growth rate can be dissected by the use of chemostat cultures.

Works Cited

- **Tai, S. L., Daran-Lapujade, P., Walsh, M. C., Pronk, J. T., & Daran, J. M. (2007). Acclimation of Saccharomyces cerevisiae to low temperature: a chemostat-based transcriptome analysis. *Molecular biology of the cell, 18*(12), 5100-5112.**

Acknowledgments

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