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Acclimation of *Saccharomyces cerevisiae* to Low Temperature: A Chemostat-based Transcriptome Analysis[D]

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Effects of suboptimal temperatures on transcriptional regulation in yeast have been extensively studied in batch cultures. To eliminate indirect effects of specific growth rates that are inherent to batch-cultivation studies, genome-wide transcriptional responses to low temperatures were analyzed in steady-state chemostats, grown at a fixed specific growth rate (0.03 h^{-1}). Although in vivo metabolic fluxes were essentially the same in cultures grown at 12 and at 30°C, concentrations of the growth-limiting nutrients (glucose or ammonia) were higher at 12°C. This difference was reflected by transcript levels of genes that encode transporters for the growth-limiting nutrients. Several transcriptional responses to low temperature occurred under both nutrient-limitation regimes. Increased transcription of ribosome-biogenesis genes emphasized the importance of adapting protein-synthesis capacity to low temperature. In contrast to observations in cold-shock and batch-culture studies, transcript levels of environmental stress response genes were reduced at 12°C. Transcription of trehalose-biosynthesis genes and intracellular trehalose levels indicated that, in contrast to its role in cold-shock adaptation, trehalose is not involved in steady-state low-temperature adaptation. Comparison of the chemostat-based transcriptome data with literature data revealed large differences between transcriptional reprogramming during long-term low-temperature acclimation and the transcriptional responses to a rapid transition to low temperature.

INTRODUCTION

Temperature fluctuations are an inevitable aspect of microbial life in exposed natural environments in which diurnal and/or seasonal temperature changes are not buffered, such as the surfaces of leaves, fruits, and flowers. *Saccharomyces cerevisiae*, a yeast whose natural environment is often associated with sugar-rich, plant-related environments, exhibits an array of cellular responses to temperature changes. Temperatures below its optimum range for growth (25–35°C; Watson, 1987) slow down enzyme kinetics and, consequently, cellular processes (MacDonald and Storey, 2002, 2005; Tai *et al.*, 2007). In addition, suboptimal temperatures affect a variety of cellular characteristics and processes, including growth phase (Werner-Washburne *et al.*, 1993), respiration (Lewis *et al.*, 1993), lipid composition of membranes (Hunter and Rose, 1972; Gelinas *et al.*, 1991), and trehalose content (Van Dijck *et al.*, 1995; Rodriguez-Vargas *et al.*, 2002).

In interpreting effects of low temperature and other environmental parameters on microbial physiology, the time scale of exposure is essential. Sudden exposure to environmental changes (e.g., cold shock) is likely to trigger rapid, highly dynamic stress-response phenomena (adaptation). Prolonged exposure to nonlethal stimuli subsequently leads to acclimation, i.e., establishment of a physiological state in which regulatory mechanisms have resulted in full adaptation of genome expression to the (suboptimal) environmental conditions. On an even longer time scale, mutational changes may lead to evolutionary adaptation of the genome itself (Brown *et al.*, 1998).

Recent studies on the genome-wide transcriptional responses of *S. cerevisiae* to low temperatures have mainly focused on cold shock (Sahara *et al.*, 2002; Homma *et al.*, 2003; Schade *et al.*, 2004; Murata *et al.*, 2006). Transcriptional responses during adaptation to suboptimal temperatures that still allow for growth ("cold shock" at 10 to 20°C; Sahara *et al.*, 2002; Schade *et al.*, 2004) were shown to differ from those observed after exposure to temperatures below 10°C ("near freezing"), at which cell growth ceases (Homma *et al.*, 2003; Murata *et al.*, 2006). These studies enabled the identification of two distinct phases during cold-shock response: 1) an early cold response (ECR) occurring within the first 12 h after exposure to low temperature and 2) a late cold response (LCR) occurring later than 12 h after exposure to low temperature (Schade *et al.*, 2004).

Transcriptional induction of the trehalose-biosynthesis genes *TPS1* and *TPS2* is consistently observed in cold-shock studies and after exposure to near-freezing conditions. Several of the other genes that have been consistently associated with cold shock (*HSP12*, *HSP26*, *HSP42*, *HSP104*, *YRO2*, and

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SSE2) are also induced after heat shock (Schade *et al.*, 2004; Murata *et al.*, 2006). Other cold-induced genes encode three cell-wall mannoproteins (Tip1p, Tir1p, and Tir2p; Kondo and Inouye, 1991; Kowalski *et al.*, 1995; Fujii *et al.*, 1999; Cohen *et al.*, 2001), a fatty-acid desaturase (Ole1p) that influences membrane fluidity (Nakagawa *et al.*, 2002), and Nsr1p, a nucleolar protein required for pre-rRNA processing and ribosome biogenesis (Lee *et al.*, 1992; Yan and Melese, 1993). The stress response element (STRE) binding factors Msn/Msn4 have been implicated in coordinate regulation of low-temperature-responsive genes (Kandror *et al.*, 2004; Schade *et al.*, 2004). Consistently, many genes induced upon a temperature downshift were also induced under a variety of other stress conditions (Schade *et al.*, 2004).

Despite the plethora of low-temperature transcriptome datasets, major questions still have to be addressed. First of all, the low-temperature transcriptome data already published (Sahara *et al.*, 2002; Homma *et al.*, 2003; Schade *et al.*, 2004; Murata *et al.*, 2006) reveal major discrepancies. For instance inconsistencies were observed in the expression ribosomal protein (RP) genes. Although Sahara *et al.* (2002) reported an increased transcription of many RP genes during a temperature downshift to 10°C, a similar temperature downshift resulted in a totally different transcriptional response in the study by Schade *et al.* (2004). Second, although the induction of genes involved in reserve carbohydrate seems to be a consistent feature of cold-shock, trehalose accumulation is only indispensable for survival in near-freezing conditions. Above 10°C, a *tps1*Δ *tps2*Δ double null mutant showed no growth defects or viability loss (Kandror *et al.*, 2004; Panadero *et al.*, 2006). Third, although the Msn2p/Msn4p complex has been suggested to be involved in cold-shock response (Kandror *et al.*, 2004; Schade *et al.*, 2004), no clear low temperature–specific transcriptional network has been identified so far. Finally, the differences in transcriptional response to adaptation and acclimation to low temperature have never been thoroughly investigated. All hitherto published low-temperature transcriptome studies on *S. cerevisiae* have been performed in batch cultures. Although this culture mode is well suited to study the dynamics of adaptation to low temperature, it is poorly adapted for the study of prolonged exposure to low temperature. In such cultures, the specific growth rate (μ) is strongly affected by temperature, which makes it impossible to dissect temperature effects on transcription from effects of specific growth rate. This is relevant because specific growth rate as such has a strong impact on genome-wide transcript profiles (Regenberg *et al.*, 2006; Castrillo *et al.*, 2007). Furthermore, all culture variables (e.g., intra- and extracellular metabolites, pH, and oxygen availability) evolve in time and result in complex data patterns that obscure data interpretation and make the identification of temperature-specific responses very difficult.

In contrast to batch cultures, chemostat cultures enable accurate control of specific growth rate, independent of other culture conditions. In chemostat cultures, the dilution rate (D) is defined as the ratio of the flow rate of the ingoing medium (f, $l \cdot h^{-1}$) and the culture volume (V, l). When the culture volume is kept constant by continuous removal of culture broth, a steady-state will be reached in which the specific growth rate (μ, h^{-1}) is equal to the dilution rate (provided that D is smaller than the maximum specific growth rate). In steady-state chemostat cultures, the identity of the growth-limiting nutrient (e.g., carbon or nitrogen source) can be chosen by accurate design of the reservoir medium. Because concentrations of all metabolites and substrates in steady-state chemostat cultures are constant in time, these cultures provide a flexible and reproducible platform for studies on microbial physiology and gene expression in fully acclimatized cultures (Piper *et al.*, 2002; Hoskisson and Hobbs, 2005). Evolutionary adaptation phenomena can be minimized by limiting the number of generations of cultivation (Novick and Szilard, 1950; Jansen *et al.*, 2005).

The goal of the present study is to investigate steady-state, acclimatized growth of *S. cerevisiae* at suboptimal temperatures, with emphasis on genome-wide transcriptional regulation. To eliminate interference by specific growth rate, *S. cerevisiae* was grown at 12 to 30°C in anaerobic chemostat cultures, at a fixed specific growth rate of 0.03 h^{-1}. Because transcriptional responses can be highly context dependent (Tai *et al.*, 2005), transcription was analyzed in both glucose- and ammonium-limited chemostat cultures grown at these two temperatures. The results of this steady-state analysis of yeast transcription at suboptimal growth temperature were compared with those from previous studies in batch cultures (Sahara *et al.*, 2002; Schade *et al.*, 2004; Murata *et al.*, 2006).

MATERIALS AND METHODS

Strain and Growth Conditions

The prototrophic, haploid reference *S. cerevisiae* strain CEN.PK113-7D (MAT**a**) provided by P. Kötter (Institut für Mikrobiologie, J. W. Goethe Universität Frankfurt, Frankfurt, Germany), was grown at a dilution rate (D) of 0.03 h^{-1} at both 12 or 30°C in 2.0 l chemostats (Applikon, Schiedam, The Netherlands) with a working volume of 1.0 l as described previously (Tai *et al.*, 2005). A temperature probe connected to a cryostat controlled cultures grown at 12°C. Cultures were grown in a defined synthetic medium that was limited by carbon or by nitrogen with all other growth requirements in excess as previously described (Tai *et al.*, 2005). The dilution rate was set at 0.03 h^{-1} with pH measured on-line and kept constant at 5.0 by automatic addition of 2 M KOH using an Applikon ADI 1030 Biocontroller. The stirrer speed was set to 600 rpm. Anaerobic growth and steady-state conditions were maintained as previously described (Tai *et al.*, 2005). Biomass dry weight, metabolites, dissolved oxygen, and gas profiles were constant for at least three volume changes before sampling.

Analytical Methods

Culture supernatants were obtained with the rapid sampling method described in Mashego *et al.* (2003). Concentrations of glucose and metabolites were analyzed by high-performance liquid chromatography on an AMINEX HPX-87H ion exchange column using 5 mM H_2SO_4 as the mobile phase. Ethanol evaporation from cultures was determined as described in Kuyper *et al.* (2003). Residual ammonium concentrations were determined using cuvette tests from DRLANGE (Dusseldorf, Germany). Culture dry weights were determined as described in Postma *et al.* (1989) and whole cell protein contents as described in Verduyn *et al.* (1990). Trehalose and glycogen measurements were performed as described in Parrou and Francois, (1997). Trehalose was determined in triplicate measurements for each chemostat. Glycogen was determined in duplicate for each chemostat. Glucose released by glycogen and trehalose breakdown was determined using the UV method based on Roche kit no. 0716251 (Almere, The Netherlands).

The elemental composition of the yeast biomass grown under nitrogen limitation was analyzed using the Carlo Erba elemental analyzer (PerkinElmer Life and Analytical Sciences, Monza, Italy) following the BN211 protocol from ECN (Petten, The Netherlands).

Microarray Analysis

Sampling of cells from chemostats, probe preparation, and hybridization to Affymetrix Genechip microarrays (Santa Clara, CA) were performed as previously described in Piper *et al.* (2002). RNA quality was determined using the Agilent 2100 Bioanalyzer (Wilmington, DE). The results for each growth condition were derived from three independently cultured replicates. The average coefficient of variation for the triplicate transcriptome analyses for each of the four growth conditions was below 0.20. In addition, the level of the *ACT1* transcript, a common loading standard for conventional Northern analysis, varied <12% over the four growth conditions.

For additional statistical analyses, Microsoft Excel running the significance analysis of microarrays add-in (SAM Version 1.12; Tusher *et al.*, 2001) was used for pair-wise comparisons. Statistical significance of differences in transcript levels between cultivation conditions was assessed using a threshold fold difference of 2 and a median false discovery rate of 1%. Venn diagrams and heat-map visualizations of transcript data were generated with Expressionist Analyst version 3.2 (Genedata, Basel, Switzerland).

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 $mmol \cdot g_{DW}^{-1} \cdot h^{-1}$.

Promoter analysis was performed using the web-based software Regulatory Sequence Analysis (RSA) Tools (van Helden *et al.*, 2000) as described in Tai *et al.* (2005).

The statistical assessment of overrepresentation of GO biological processes categories (http://www.geneontology.org/; Eilbeck *et al.*, 2005) among sets of significantly changed transcripts was achieved using the Database for Annotation, Visualization and Integrated Discovery (DAVID) 2006 (Huang *et al.*, 2007). Overrepresentation of transcription-factor binding sites as defined by chromatin immunoprecipitation (ChIP)-on-chip analysis (http://jura.wi.mit.edu/fraenkel/download/release_v24/bound_by_factor/ORFs_bound_by_factor_v24.0.p005b_041213.txt) was assessed by Fisher's exact test, employing hypergeometric distribution with a Bonferroni correction and a p value threshold of 0.01 (Kresnowati *et al.*, 2006). The probability was calculated as follows: the p value of observing z genes, belonging to the same functional category is as follows:

$$p = \sum_{x=z}^{\max(N,M)} \frac{\binom{N}{x} \cdot \binom{G-N}{M-x}}{\binom{G}{M}}$$

where N is the total number of genes where a transcription factor can bind upstream (Harbison *et al.*, 2004), M is the total number of genes in the cluster, and G is the total number of yeast gene features on the YG98S array (6383 probesets).

The microarray data have been deposited at Genome Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) under the series number GSE6190.

Comparison with Other S. cerevisiae Low-Temperature Transcriptome Datasets

The batch transcriptome datasets that were used for comparison in this study can be downloaded from the following: Beltran *et al.*, 2006, http://biopuce.insa-toulouse.fr/jmflab/winegenomics; Murata *et al.*, 2006, http://kasumi.nibh.jp/~iwahashi/; Sahara *et al.*, 2002, http://staff.aist.go.jp/t-sahara/; Schade *et al.*, 2004, http://cbr-rbc.nrc-cnrc.gc.ca/genetics/cold/; and Gasch *et al.*, 2000, http://genome-www.standford.edu/yeast_stress/. For other low-temperature transcriptome studies (Sahara *et al.*, 2002; Beltran *et al.*, 2006; Murata *et al.*, 2006), only average ratios were provided. Because this precluded statistical analysis, only genes with an average fold difference above 2 were considered to be significantly changed. This amounted to 1609 genes for Sahara *et al.* (2002) and 2339 genes for Murata *et al.* (2006). As for Schade *et al.* (2004), only the statistically significant changed genes were provided, and hence these 634 transcripts were used. The set of environmental-stress–responsive genes (Gasch *et al.*, 2000) was downloaded from the website http://genome-www.stanford.edu/yeast_stress/.

The growth rate related dataset from Regenberg *et al.* (2006) and Castrillo *et al.* (2007) were download from the following URLs: http://genomebiology.com/content/supplementary/gb-2006-7-11-r107-s3.txt and http://jbiol.com/content/6/2/4/additional/ (see Supplementary Table S3 for up-regulated genes and Supplementary Table S4 for the down-regulated genes), respectively.

RESULTS

Overview

To compare the physiology and transcriptome of *S. cerevisiae* grown at 12 and 30°C without interference by the different maximum specific growth rate at these two temperatures, the present study was based on chemostat cultures grown at a fixed dilution rate of $0.03\ h^{-1}$. This dilution rate, which was chosen based on batch culture experiments at different temperatures (Tai *et al.*, 2007), allowed for steady-state growth at both temperatures. To prevent possible effects of temperature-dependent oxygen solubility and of temperature-dependent distribution of sugar metabolism over respiration and alcoholic fermentation, the cultures were grown anaerobically. Biomass yields and fermentation rates were similar at 12 and 30°C in both carbon- and nitrogen-limited chemostat cultures, indicating that growth efficiency was not severely affected by the growth temperature (Table 1).

DNA microarray analysis was used to analyze effects of growth temperature on gene expression in the glucose- and ammonium-limited chemostat cultures. In the glucose-limited cultures, 494 genes yielded a significantly different transcript level at the two temperatures, compared with 806 in the nitrogen-limited cultures (Figure 1). The total number of temperature-responsive genes was 1065, representing 16% of the *S. cerevisiae* genome (Figure 1). This temperature response showed a strong context dependency with respect to the nutrient limitation regime. Indeed, only 235 genes showed a consistent up- or down-regulation under both nutrient-limitation regimes (Figure 1). To identify regulatory networks involved in the acclimation of *S. cerevisiae* to low temperature, the temperature-responsive genes were screened for enrichment of specific functional categories (Figure 2), and their promoter regions were searched for *cis*-regulatory motifs (see *Material and Methods*).

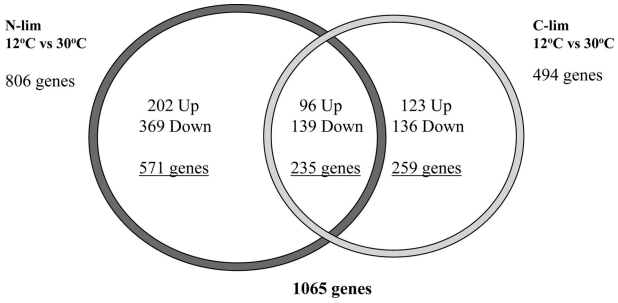

Figure 1. Global transcriptome responses to anaerobic growth at 12 and 30°C in anaerobic glucose- and ammonium-limited chemostat cultures ($D = 0.03\ h^{-1}$). The Venn diagram shows the number of significant differentially expressed genes between 12 and 30°C in both C and N limitations.

Figure 2. Heat map representing the transcript level ratio of 1065 differentially expressed genes in anaerobic glucose- and ammonium-limited chemostat cultures (D = 0.03 h^{-1}) grown at 12 and 30°C. The genes indicated in the figure belong to the enriched GO categories. Nineteen NCR-responsive genes are underlined.

Low-Temperature Chemostat Cultivation Results in Altered Uptake Kinetics for the Limiting Nutrient and Enhanced Catabolite Repression

In the chemostat cultures grown at 12°C, the residual concentrations of the growth-limiting nutrients glucose and ammonium were 7.5–10-fold higher than in the cultures grown at 30°C (Table 1). The resulting higher degree of substrate saturation of the membrane transporters for these nutrients is likely to partly compensate for their reduced capacity at 12°C. Change in transport kinetics was also reflected in the transcript levels of genes involved in the uptake of the growth-limiting nutrients (Figure 2). This adaptation was most striking under glucose limitation, where the low-affinity *HXT3* and the intermediate-affinity *HXT2* and *HXT4* hexose-transporter genes (Reifenberger *et al.*, 1997) showed increased transcript levels at 12°C compared with 30°C (+2.6, +3.7, and +33.5-fold increases, respectively), whereas *HXT5* and *HXT16* were expressed at reduced levels at 12°C (−40 and −10-fold difference, respectively). Transcription of *HXT6* and *7*, encoding the main high-affinity glucose transporters (Reifenberger *et al.*, 1997), was not significantly affected by the growth temperature. The effect of the transcriptional remodeling of glucose transport under these conditions on glucose-uptake kinetics was recently confirmed by zero trans-influx transport assays (Tai *et al.*, 2007). In the ammonium-limited cultures, the three genes encoding ammonia permeases (*MEP1*, *MEP2*, and *MEP3*) were differentially transcribed at 12 and 30°C. *MEP1* and *MEP2*, which encode high-affinity permeases showed reduced transcript levels at 12°C (−2.8-fold and −4-fold, respectively), whereas transcription of the low-affinity permease *MEP3* (Marini *et al.*, 1997) was moderately increased (1.8-fold).

In chemostat cultures grown at specific growth rates that are much lower than μ_{max}, the residual concentration of the limiting nutrient is typically sufficiently low to prevent catabolite repression (Silver and Mateles, 1969). For example, glucose repression is virtually absent in glucose-limited, aerobic chemostat cultures grown at a dilution rate of 0.10 h^{-1} (25% of the maximum specific growth rate μ_{max}), thus enabling the simultaneous consumption of glucose and alternative carbon sources (Jong-Gubbels *et al.*, 1995). The observed temperature dependency of the residual concentrations of growth-limiting nutrients (Table 1) is consistent with the fact that the specific growth rate of 0.03 h^{-1} used in this study is much closer to μ_{max} at 12°C (ca. 0.04 h^{-1}; Tai *et al.*, 2007) than at 30°C (ca. 0.33 h^{-1}; Bakker *et al.*, 2000). The increased concentration of the residual limiting nutrients at 12°C resulted in a higher degree of catabolite repression as revealed by the down-regulation of eight typical target genes for glucose catabolite repression (*QCR2, CYC7, COX4, COX6, SDH1, COX5A, OSH7, GRX1*) and at least 19 nitrogen catabolite repression (NCR) target genes (Figure 2). This down-regulation of targets of the NCR was supported by the enrichment of Gln3 and Gln3/Dal82 pair targets and by the overrepresentation of a protein-binding motifs (AAGATAAG) similar to the GATAAG factor binding motif involved in the NCR mechanism (Cunningham and Cooper, 1991; Cooper, 2002; Table 3). This effect, which contributes to the observed context dependency of the transcriptional responses to low temperature (Figure 1), underlines the importance of a combinatorial design of transcriptome experiments (Knijnenburg *et al.*, 2007).

Acclimation to Nonfreezing Low Temperature Does Not Require a High Storage Carbohydrate Content

Transcriptional induction of genes involved in the metabolism of storage carbohydrates, and in particular of trehalose, is consistently observed after cold shock and exposure to near-freezing conditions. Trehalose accumulation is therefore considered to be a typical feature of low-temperature adaptation (Kandror *et al.*, 2004). However, during steady-state chemostat cultivation at 12°C, transcriptional upregulation of genes involved in trehalose and glycogen metabolism was not observed. Instead, transcript levels of these genes were not affected by the growth temperature in glucose-limited cultures and, in many cases, even showed a decreased transcript level at 12°C in the ammonium-limited cultures (Figure 1). Specifically *TPS1*, *TPS2*, *TSL,1* and *NTH1*, which encode enzymes involved in trehalose metabolism, and *GSY1*, *GSY2*, *GAC1*, *PIG2*, *PCL10*, and *PCL7*, which are involved in glycogen metabolism, showed a −2.0 to −4.1-fold lower transcript level in ammonium-limited cultures grown at 12°C than in similar cultures grown at 30°C. Analyses of intracellular storage carbohydrate contents were consistent with the transcript profiles. In the ammonium-limited chemostat cultures, trehalose and glycogen contents were significantly lower at 12 than at 30°C (Table 2). In the glucose-limited cultures, trehalose contents were also lower at 12°C, whereas the glycogen content was 50% higher than in glucose-limited cultures grown at 30°C. These results show that accumulation of glycogen and trehalose and the transcriptional induction of the genes involved in the synthesis of these compounds is not a prerequisite for yeast acclimation to low temperature.

Key genes in storage carbohydrate synthesis have been shown to be regulated by Msn2/Msn4 via STREs in their promoters (Kandror *et al.*, 2004). Promoter analysis revealed an overrepresentation of STRE elements in the upstream regions of genes that showed a reduced transcript level at 12°C in the nitrogen-limited cultures (Table 3). These results are consistent with a situation in which glycogen and trehalose biosynthesis genes are induced by stress resulting from a sudden change from optimal to low growth temperature. The

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.

Table 3. Identification of (A) significantly overrepresented cis-regulatory binding motifs in 5′ upstream regions and (B) significantly overrepresented promoter elements that bind known transcription factors (TF) or TF pairs according to ChiP-on-chip analysis (Harbison *et al.*, 2004) in low-temperature up- and down-regulated gene clusters derived from C-Lim and N-Lim chemostat experiments

(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.

present study indicates that, once cells are physiologically adapted to low temperature, the stress response and, consequently, the up-regulation of storage carbohydrate synthesis recedes. The exact physiological relevance of storage carbohydrate metabolism during adaptation to nonfreezing low temperatures remains unclear, as *tps1*Δ *tps2*Δ strains defective in trehalose biosynthesis show no growth defect or viability loss after cold shock when the temperature remains above 10°C (Kandror *et al.*, 2004; Panadero *et al.*, 2006).

Up-Regulation of the Translation Machinery at Low Temperature

In chemostat cultures of *S. cerevisiae*, grown at a fixed specific growth rate and at 30°C, transcription of genes involved in protein synthesis is virtually constant over a wide range of growth conditions (Daran-Lapujade *et al.*, 2004; Saldanha *et al.*, 2004; Tai *et al.*, 2005). Strong transcriptional responses in genes belonging to this functional category were observed when chemostat cultures were grown at different temperatures. Sixteen genes involved in ribosome biogenesis and assembly showed higher transcript levels at 12°C than at 30°C in both glucose- and ammonium-limited cultures (Figure 2). This response was most pronounced in the ammonium-limited cultures, where an additional 80 genes involved in protein synthesis (including 22 genes encoding ribosomal proteins) showed increased transcript levels at 12°C (Figure 2). Among the genes that showed an increased transcript level at 12°C, analysis of 5′ upstream sequences revealed a

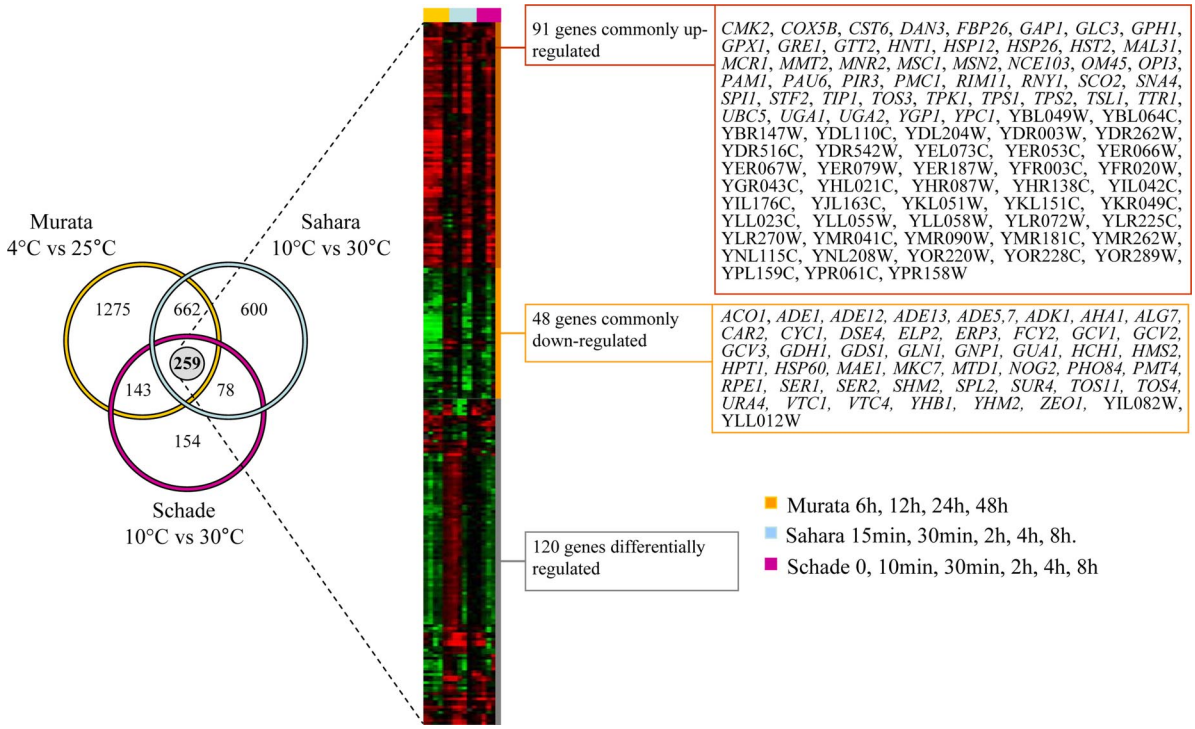

Figure 3. Genes differentially expressed in batch cultures during adaptation to low temperature. (A) Venn diagram showing the number of genes that are common to three batch-culture studies on low-temperature transcriptional adaptation (Sahara *et al.*, 2002; Schade *et al.*, 2004; Murata *et al.*, 2006).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

clear enrichment of PAC *cis*-regulatory motifs (Table 3). This motif is involved in transcriptional regulation of ribosomal protein encoding genes (Marion *et al.*, 2004; Martin *et al.*, 2004).

Low temperature is known to affect the stability of RNA secondary structures and, consequently, the kinetics of translation initiation (Farewell and Neidhardt, 1998). The *NSR1* and *DBP2* genes, which encode two proteins involved in RNA processing that have previously been identified as low-temperature markers (Kondo *et al.*, 1992; Schade *et al.*, 2004), were up-regulated at low temperature under both nutrient limitation regimes.

The increased expression of genes involved in protein synthesis did not lead to any clear effect on biomass composition in the glucose-limited chemostat cultures grown at 12°C. However, in the ammonium-limited chemostat cultures, the cellular protein and nitrogen contents were substantially higher at 12 than at 30°C (Table 2). The difference of the nitrogen content was larger than that of the protein content (52 vs. 38%), which may be due to an increased rRNA level. A possible increase of the rRNA content at low temperature in the ammonium-limited cultures was supported by increase transcript levels of four genes that encode subunits of polymerase I (*RPA12*, *RPA49*, *RPA135*, and *RPC40*) and 33 genes involved in rRNA processing (Figure 2). In nitrogen-limited cultures, the cellular protein content is typically lower than under nitrogen excess conditions (Tai *et al.*, 2005; Kolkman *et al.*, 2006). At 12°C, the protein content in the ammonium-limited cultures was as high as that in the nitrogen-excess (glucose-limited cultures). This increased protein content may contribute to a compensation for impaired enzyme kinetics at low temperature.

Transcriptional Responses to Low Temperature: Adaptation versus Acclimation

Steady-state chemostat cultivation at low temperature describes acclimation to this suboptimal condition. To investigate how transcriptional reprogramming during low-temperature acclimation differs from dynamic transcriptional adaptation to a temperature decrease, the chemostat-based transcriptome data were compared with transcriptome datasets from earlier low-temperature adaptation studies. Initially, four published transcriptome datasets obtained during adaptation of *S. cerevisiae* to low, nonfreezing temperatures (Sahara *et al.*, 2002; Schade *et al.*, 2004; Beltran *et al.*, 2006) were included in this analysis. Beltran *et al.* (2006) studied temperature downshifts from 25 to 13°C, Sahara *et al.* (2002) and Schade *et al.* (2004) studied downshifts from 30 to 10°C, and Murata *et al.* (2006) downshifts from 25 to 4°C. Because a first comparison revealed little overlap between the dataset of Beltran and the three other datasets, the former was not included in the subsequent analysis. The reasons for these differences may reside in the very specific experimental set-up used by Beltran *et al.* (2006), who monitored 100-l grape must fermentations at 13 and 25°C. Indeed grape must-based wine fermentations present many specificities such as low pH (~3), high mixed sugar (glucose, fructose) concentration (160–200 g/l), low mixed nitrogen sources (amino acids) concentrations, and static growth with a first aerobic phase followed by an anaerobic fermentation phase.

Comparison of the three remaining datasets yielded a group of 259 genes that responded to temperature downshifts in all three studies, although their response was not always consistent (Figure 3). Indeed, of these 259 genes, as few as 91 were consistently up-regulated at low tempera-

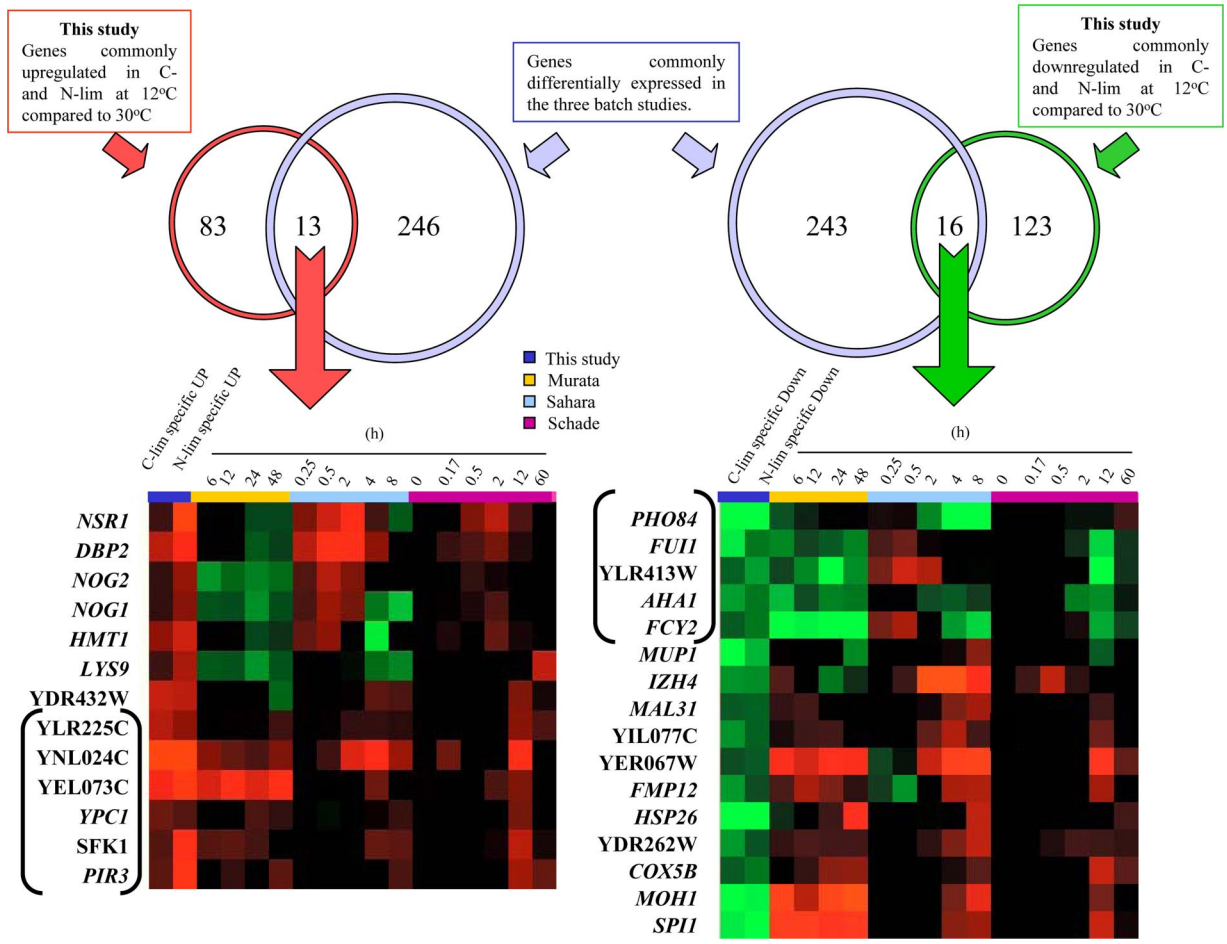

Figure 4. Comparison of the transcript ratio of 259 genes common to three batch-culture low-temperature transcriptome datasets with the 12°C/30°C ratio of the genes specifically up- (n = 96) and down-regulated (n = 139) in anaerobic glucose- and ammonium-limited chemostat cultures (D = 0.03 h^{-1}). Venn diagrams show the number of low-temperature–responsive genes common to the batch-culture and chemostat-based datasets. The heat map shows the expression ratios of the genes common to batch and chemostat datasets. The genes indicated between brackets show a consistent transcriptional regulation at low temperature in all datasets.

ture, and only 48 were consistently down-regulated (Figure 3; see *Material and Methods*). This set of 259 "low-temperature adaptation" genes was then compared with the 235 genes that exhibited a consistently lower or higher transcript level in glucose- and ammonium-limited chemostat cultures grown at 12°C compared with chemostat cultures grown at 30°C. Twenty-nine genes were transcriptionally regulated during both adaptation and acclimation to low temperature (Figure 4). However, only 11 genes showed a consistent pattern of regulation in all four situations: *PIR3*, *SFK1*, *YPC1*, YEL073C, YNL024C, and YLR225C were consistently up-regulated at low temperature, whereas *PHO84*, *FUI1*, *AHA1*, *FCY2*, and YLR413W were consistently down-regulated (Figure 4). Gasch *et al.* (2000) reported that upon heat shock from 25 to 37°C the changes in transcript abundance were higher than comparing cultures grown at constant temperatures (25 or 37°C). This preliminary observation could reflect the differences recorded in this study between batch- and chemostat-derived data.

Three of the genes that were consistently up-regulated at low temperature (*SFK1*, *YPC1*, and YEL073C) are involved in lipid metabolism. Temperature is well known to affect fluidity of lipid bilayers, and homeoviscous adaptation mostly occurs through the modification of membrane lipid composition (Hunter and Rose, 1972). Sfk1p is required for the localization to the plasma membrane of Stt4p, an essential protein in *S. cerevisiae*. Together with Stt4p, Skf1p is involved in a pathway required to generate phosphatidylinositol, as well as in other cellular processes (Audhya and Emr, 2002). YEL073C, which encodes an uncharacterized protein, is controlled by Ino2/Ino4 activators (Hoppen *et al.*, 2005) that regulate phospholipid biosynthesis genes. *YPC1* encodes an alkaline ceramidase involved in the synthesis of ceramides, which form the backbone of sphingolipids, important modulators of stress responses in mammalian cells (Mao *et al.*, 2000).

Three genes encoding transporters were found among the five consistently down-regulated genes (Figure 4). Low temperature can affect transport and protein translocation, also through modification of membrane fluidity (Baker *et al.*, 1988). Indeed, in the chemostat-based comparison, genes involved in membrane transport processes were strongly overrepresented among the genes that were down-regulated at low temperature, irrespective of the nutrient limitation regime (Figure 2). Among seven genes that were commonly up-regulated at low temperature in the chemostat-based dataset as well as in the datasets of Schade *et al.* (2004) and Sahara *et al.* (2002) but not in the data set of Murata *et al.* (2006), five were involved in protein translocation across the nuclear envelope (*NOG1*, *NOG2*, *HTM1*, *NPL3*, and *NSR1*;

Figure 4). *NSR1* has previously been identified as a cold-shock marker (Kondo *et al.,* 1992). Also *DBP2*, up-regulated at low temperature in three of the four datasets, encodes a well-known low-temperature marker (Figure 4). Dbp2p is an ATP-dependent RNA helicase of DEAD box family. Prokaryotic orthologues have been shown to unwind cold-stabilized mRNA secondary structures to increase translation efficiency (Jones and Inouye, 1994; Thieringer *et al.,* 1998).

Context Dependency of Temperature Response

The small number of genes that showed a consistent transcriptional response to low temperature during acclimation and adaptation (Figure 3) can at least partly be attributed to the context-dependency of transcriptional regulation. Most genes are not regulated by a single environmental parameter, but respond to multiple stimuli. The most obvious difference between batch (shake-flask) and chemostat cultivation is the specific growth rate. Although in batch cultures a temperature decrease automatically causes a decrease of the specific growth rate, this parameter is kept constant in chemostat cultures. Two recent chemostat studies have systematically explored the effect of specific growth rate on transcriptional regulation in *S. cerevisiae* (Regenberg *et al.,* 2006; Castrillo *et al.,* 2007). To investigate whether a substantial part of the transcriptional responses to low temperature observed in previous batch culture studies may in fact have been related to differences in specific growth rate, the 365 genes that showed a consistent (up- or down-) transcriptional response to specific growth rate in the datasets of Castrillo *et al.* (2007) and Regenberg *et al.* (2006) were compared with the set of 139 genes that showed a consistently (up- or down-) transcriptional response to low temperature in published batch culture studies (Figures 3 and 5). This comparison revealed that the altered transcript levels of 25% of the low-temperature down-regulated genes (12 of 48) and 10% of the low-temperature up-regulated genes (9 of 91) are likely to have been primarily related to specific growth rate, rather than to temperature per se. When a similar comparison was performed with the temperature-responsive genes identified in the present chemostat study, a negligible overlap ($<$0.7%) with the growth-rate–responsive genes was observed (Figure 5).

Another possible explanation for the discrepancies between previous low- temperature studies the present study is oxygen availability. In shake-flask cultures exposed to air, the dissolved oxygen concentration is likely to be strongly temperature dependent as a result of a temperature dependency of both oxygen solubility and respiration rate. To eliminate interference by these effects, the chemostat cultures used in the present study were sparged with high-grade nitrogen gas to ensure anaerobicity. *TIP1, TIR1,* and *TIR2*, which encode cell wall mannoproteins, have been characterized as markers for cold shock in batch studies (Kondo and Inouye, 1991; Schade *et al.,* 2004). The transcript levels of these genes did not exhibit a temperature dependency in the chemostat cultures. This is probably due to the fact that even at 30°C, these genes are already strongly induced under anaerobic conditions. *TIR1* deletion even abolishes anaerobic growth of *S. cerevisiae* (Abramova *et al.,* 2001). Under anaerobic conditions, *S. cerevisiae* requires the supply of exogenous ergosterol and unsaturated fatty acids (Andreasen and Stier, 1953, 1954). In anaerobic cultures, *OLE1*, required for synthesis of unsaturated fatty acids and previously described as a low-temperature marker, is repressed (Stukey *et al.,* 1990). This repression of *OLE1* occurred similarly at 30 and 12°C, disqualifying this gene as a low-temperature marker in anaerobic cultures. These examples stress the necessity of taking into account the context dependency and hierarchical nature of transcriptional regulation.

Environmental Stress Response, a Low-Temperature Adaptation-specific Response

A general environmental stress response (ESR) mechanism has been implicated in the regulation of *S. cerevisiae* genes whose transcription shows a consistent response to multiple environmental stress parameters (Ruis and Schuller, 1995). Based on previous low-temperature transcriptome experiments, it has been proposed that a reduction of the growth temperature transcriptionally induces a set of ESR genes (Kandror *et al.,* 2004; Schade *et al.,* 2004). In transcriptome studies analysis that explored a wide range of growth conditions and environmental stresses (Gasch *et al.,* 2000), a set of coregulated genes was identified that showed a similar a response to all tested stress stimuli (266 up-regulated and 602 down-regulated genes). When the set of ESR genes defined by Gasch *et al.* (2000) was compared with sets of genes that were consistently up- or down-regulated at low temperature in batch culture (see a combination of datasets from Sahara *et al.,* 2002, Schade *et al.,* 2004, and Murata *et al.,* 2006; and see above), an extensive overlap was revealed (Figure 6). Fifty percent of the consistently low-temperature up-regulated genes and 13% of the low-temperature down-regulated genes found in the batch-culture studies were also found in the ESR genes identified by Gasch *et al.* (2000). In total, a third of the low-temperature–responsive genes found in the three batch-culture studies could be linked to ESR (Figure 6A). When a similar comparison with the ESR genes was done with the 1065 genes that showed a different transcript level at low temperature in the anaerobic glucose- and/or ammonium-limited chemostat cultures, only 14 genes showed a consistent response in the two categories. Interestingly, several genes (233 genes) up- or down-regulated in the study by Gasch *et al.* (2000) showed an opposite transcriptional response in the low-temperature chemostat cultures (Figure 6B), suggesting an alleviation of the environmental stress at low temperature. These results clearly demonstrate that ESR is not an obligatory response to growth at low temperature, but rather occurs during adaptation upon a sudden exposure to suboptimal temperatures.

DISCUSSION

Chemostat-based Low-Temperature Transcriptomics: Experimental Design

In its most rudimentary form, transcriptome analysis involves a single, pair-wise comparison in batch cultures of two experimental conditions or strains, in which one of the situations is labeled as the reference. This simple approach does not take into account the context dependency of transcriptional responses. As many genes are controlled by multiple transcriptional regulation systems that act in a hierarchical manner, the transcriptional response to a stimulus may be strongly dependent on other environmental parameters (i.e., on the experimental context).

Context dependency is enhanced by the fact that, even in well-controlled chemostat cultures, it is impossible to change a single cultivation parameter without any impact on others. For example, in the present study, growth of *S. cerevisiae* at 12 and 30°C was studied at the same specific growth rate of 0.03 h^{-1}. However, the inevitable consequence of this choice was a higher residual glucose concentration in the cultures grown at 12°C. Assuming simple

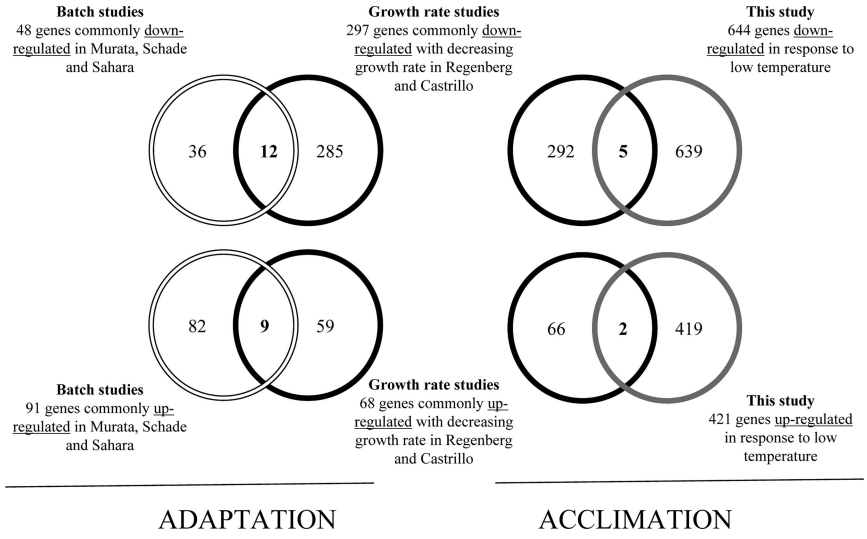

Figure 5. Comparison of the genes specifically up- or down-regulated during acclimation (this study) or adaptation (overlap of the datasets from Sahara *et al.*. 2002, and Schade *et al.,* 2004, Murata *et al.,* 2006) to low temperature with the growth rate–dependent genes identified by Castrillo *et al.* (2007) and Regenberg *et al.* (2006).

Monod kinetics for growth (Monod, 1949), this difference was at least partly due to the fact that a specific growth rate of 0.03 h^{-1} represents ca. 75% of μ_{max} at 12°C, but only ca. 10% of μ_{max} at 30°C. Consequently, a comparison of glucose-limited cultures only would have led to a "contamination" of temperature-responsive gene sets with genes whose transcription is influenced by glucose. Although the same applies for ammonium-limited cultivation, a combinatorial experimental design involving both glucose- and ammonium-limited cultures enabled us to reduce the impact of such

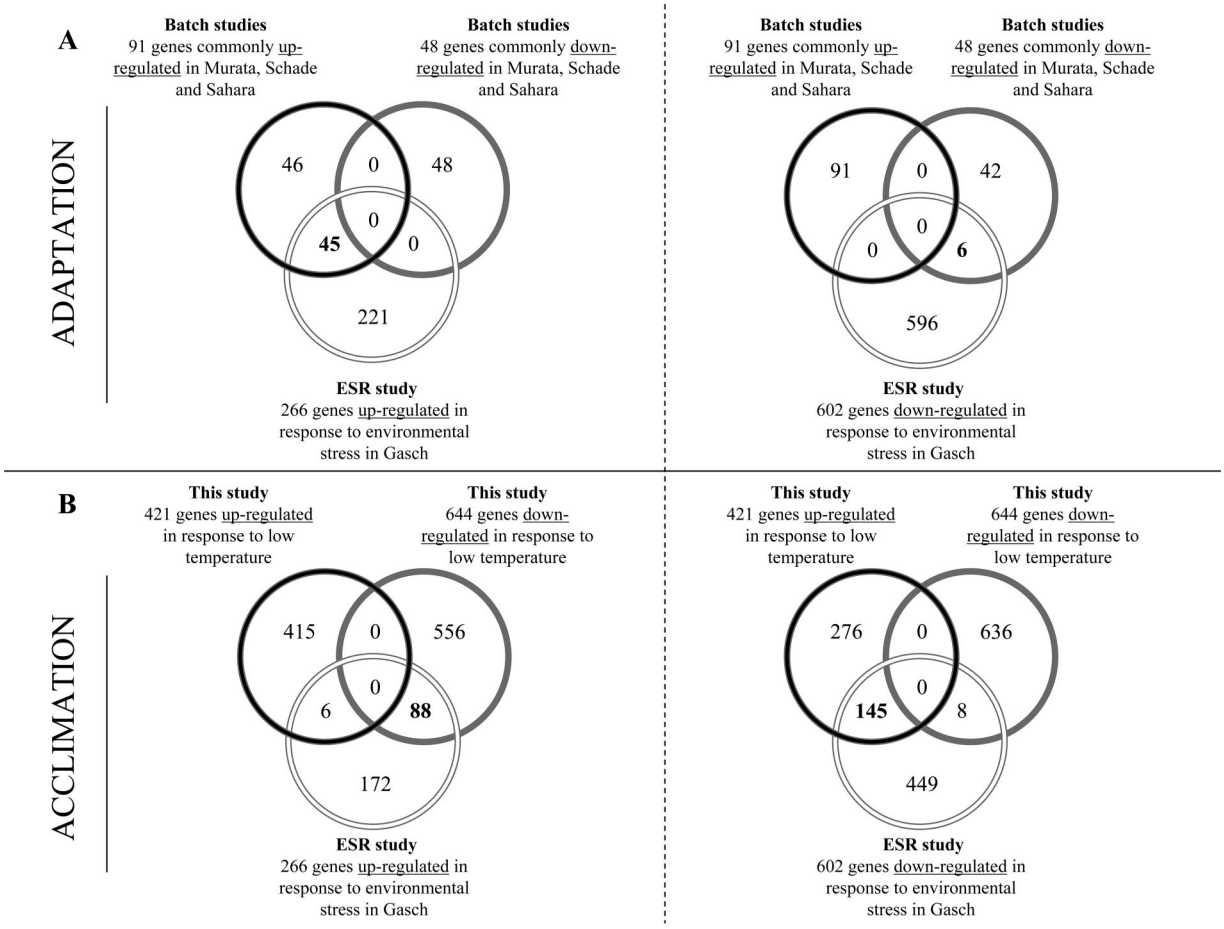

Figure 6. Comparison of the genes specifically up- or down-regulated during acclimation (this study) or adaptation (overlap datasets from Sahara *et al.*. 2002, and Schade *et al.,* 2004, Murata *et al.,* 2006) to low temperature with the ESR genes identified by Gasch *et al.* (2000).

secondary effects. A similar approach has recently been followed in a recent analysis of the combinatorial effects of oxygen status and nutrient limitation regimes in *S. cerevisiae* (Tai *et al.*, 2005; Knijnenburg *et al.*, 2007).

Using combinatorial approaches, robust "core" sets of genes can be defined that show an essentially context-independent response to single environmental stimuli. This should, however, not detract attention from the fact that context-dependent responses can be extremely relevant in natural environments and industrial environments. For example, it will be of interest to investigate how temperature responses in *S. cerevisiae* are influenced by oxygen availability. This is even more important because, in the present study, processes involved in homeoviscous adaptation of membrane composition (Hunter and Rose, 1972) may have been masked by the inclusion of the anaerobic growth factors oleate (Andreasen and Stier, 1954) and ergosterol (Andreasen and Stier, 1953) in the growth media (see *Results*).

Specific Growth Rate and ESR

Changes in experimental conditions may affect the specific growth rate. At the temperatures of 12 and 30°C analyzed in this study, the specific growth rate of *S. cerevisiae* in batch cultures differs by almost an order of magnitude (Tai *et al.*, 2007). There is now a growing body of evidence that specific growth rate itself has a strong effect on genome-wide transcription (Regenberg *et al.*, 2006; Castrillo *et al.*, 2007). Chemostat cultivation provides an opportunity to compare different cultivation conditions and/or microbial strains at a fixed specific growth rate. The relevance of this approach for studies on low-temperature physiology of *S. cerevisiae* is underlined by the observation that 15% of the genes that showed a consistent transcriptional response in three previous batch-culture studies on cold adaptation (Sahara *et al.*, 2002; Schade *et al.*, 2004; Murata *et al.*, 2006) were also identified in chemostat studies on growth-rate–dependent transcription performed at 30°C (Regenberg *et al.*, 2006; Castrillo *et al.*, 2007). Conversely, less than 1% of the temperature-responsive genes identified in the present chemostat study showed a growth-rate–dependent transcript level. This difference appeared to be strongly linked to the observed differences in storage carbohydrate metabolism and environmental stress responses in the two cultivation systems.

In batch cultures of *S. cerevisiae*, exposure to low temperatures invariably induces an increased synthesis of storage carbohydrates (in particular trehalose) and transcriptional up-regulation of genes involved in storage carbohydrate metabolism (Kandror *et al.*, 2004). In the chemostat cultures, neither of these effects was observed. Transcriptional regulation of storage carbohydrate metabolism involves the Msn2/Msn4 complex (Boy-Marcotte *et al.*, 1998; Francois and Parrou, 2001). Together with Hsf1, this complex is also involved in transcriptional regulation of *HSP* genes (Amoros and Estruch, 2001). The Msn2/Msn4 complex binds to a conserved STRE motif (Gorner *et al.*, 1998) in promoter regions of its target genes and has also been implicated in the broad ESR (Gasch *et al.*, 2000). In contrast to the situation in batch cultures, where low-temperature adaptation is accompanied by a strong ESR response (29% of the differentially expressed genes in batch at low temperature respond to ESR as well), only three ESR-induced genes (*YCP1, VPS73,* and *EMI1*) showed a higher transcript at 12 than at 30°C in the chemostat cultures. Conversely, 88 ESR-induced genes showed a consistently lower transcript level at 12°C. These results clearly indicate that low-temperature acclimatized growth does not involve a Msn2/Msn4-complex regulatory role but seems to be based on a different regulation mode not identified until now.

Regenberg *et al.* (2006) demonstrated that, *in S. cerevisiae*, transcription of ESR genes is negatively correlated with specific growth rate. Comparison of low-temperature responses in chemostat cultures with those in batch cultures, strongly support the notion that transcription of ESR genes is elicited primarily be a reduced specific growth rate, rather than by low temperature per se. This mechanism is likely to apply to ESR responses to other environmental stresses, as conditions that induce ESR generally also lead to a reduced specific growth rate (Fernandez-Ricaud *et al.*, 2007). A specific growth-rate–mediated general stress response, controlled by the concentration of the RNA-polymerase subunit *RpoS*, has also been reported in *Escherichia coli* (Ihssen and Egli, 2004). A direct relation with specific growth rate provides a plausible explanation for the extensive similarities observed in the responses of *S. cerevisiae* to a broad range of thermal and chemical stresses, e.g., Gasch *et al.* (2000) and Regenberg *et al.* (2006) data.

Transcriptional Responses to Low Temperature: Acclimation versus Adaptation

Chemostat-based transcriptome analysis at 12 and 30°C yielded a set of 235 genes that showed a consistent transcriptional response to low temperature, irrespective of the growth limiting nutrient (glucose or ammonium; Figure 1). These genes represent the transcriptional acclimation, i.e., physiological adaptation to steady-state growth under a set of environmental conditions, of *S. cerevisiae* at low temperature. A comparison of this low-temperature acclimation gene set with published datasets on the initial, dynamic transcriptional adaptation of *S. cerevisiae* to a rapid decrease of the cultivation temperature in batch cultures, revealed limited correspondences as well as important differences.

The only clearly defined group of genes that was commonly regulated in low-temperature chemostats and batch-culture studies on low-temperature adaptation (Sahara *et al.*, 2002; Schade *et al.*, 2004) was involved in lipid metabolism (Figure 4, see *Results*). This is consistent with the notion that after a temperature downshift homeoviscous adaptation of the membrane composition is essential for growth (Hunter and Rose, 1972; Torija *et al.*, 2003; Beltran *et al.*, 2006). For some gene sets, an overlap over the chemostat results with only some of the batch-culture cold-adaptation studies (Sahara *et al.*, 2002) was observed. Our chemostat expression set provided clear-cut data on the transcriptional up-regulation of the translational machinery at low temperature. Although these data were in agreement with some batch-culture studies (Sahara *et al.*, 2002), they were in conflict with others (Schade *et al.*, 2004). Up-regulation of the ribosomal-protein–encoding genes at low temperature would fit with a translational compensation for the underlying low-temperature–induced problems.

Studies on cold adaptation in batch cultures of *S. cerevisiae* revealed a clear transcriptional up-regulation at low temperature of chaperone-encoding genes such as *HSP26* and *HSP42* (Homma *et al.*, 2003; Schade *et al.*, 2004). The proteins encoded by these genes have been shown to prevent aggregation of cytosolic proteins during heat shock (Haslbeck *et al.*, 1999). Conversely, these genes were transcriptionally down-regulated at low temperature in the chemostat cultures. In contrast to their well-established role under heat shock conditions, a protein-folding activity of these proteins has not been demonstrated during cold shock. The currently available transcriptome data indicate that accumulation of incorrectly folded proteins may occur during a rapid tem-

perature decrease, but is not a persisting problem in fully low-temperature acclimatized yeast cultures.

The present study demonstrates that transcriptional responses to low temperature and low specific growth rate, two parameters that are intrinsically linked in batch cultures, can be dissected by the use of chemostat cultures. Furthermore, the marked differences between the transcript profiles of low-temperature–acclimatized chemostat cultures and those observed in low-temperature–adaptation studies in batch cultures underline the importance of discriminating different phases in the physiological adaptation to environmental change. It should be stressed that low-temperature acclimation in *S. cerevisiae* does not solely involve transcriptional reprogramming. Indeed, it has recently been demonstrated that compensation for the decreased capacity of glycolytic enzymes at low temperature is predominantly accomplished via changes in intracellular metabolite levels (Tai *et al.*, 2007). The experimental approach described in this study should also be applicable to analyze posttranscriptional modes of cellular regulation.

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