

Genome-Wide Transcriptional Analysis of Aerobic and Anaerobic Chemostat Cultures of *Saccharomyces cerevisiae*

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The yeast *Saccharomyces cerevisiae* is unique among eukaryotes in exhibiting fast growth in both the presence and the complete absence of oxygen. Genome-wide transcriptional adaptation to aerobiosis and anaerobiosis was studied in assays using DNA microarrays. This technique was combined with chemostat cultivation, which allows controlled variation of a single growth parameter under defined conditions and at a fixed specific growth rate. Of the 6,171 open reading frames investigated, 5,738 (93%) yielded detectable transcript levels under either aerobic or anaerobic conditions; 140 genes showed a >3-fold-higher transcription level under anaerobic conditions. Under aerobic conditions, transcript levels of 219 genes were >3-fold higher than under anaerobic conditions.

Aerobic organisms have evolved a multitude of defense mechanisms to protect themselves against oxygen, which is highly toxic to obligately anaerobic organisms. In addition to its role as electron acceptor in aerobic respiration, aerobes require molecular oxygen for various biosynthetic reactions (e.g., the oxygenase reactions involved in the synthesis of sterols and unsaturated fatty acids) (1, 2). Anaerobes have to bypass these oxygen-requiring reactions, either by acquiring their products from the environment or by using alternative pathways.

Most identified yeast species can ferment sugars to ethanol and carbon dioxide (24). Thus, they do not depend on oxygen for their dissimilatory metabolism and grow rapidly under oxygen-limited conditions. Only very few species can grow in the complete absence of oxygen (27). In fact, the most important yeast species in fundamental and applied research, *Saccharomyces cerevisiae*, stands out among yeasts and among eukaryotes with respect to its rapid growth under aerobic as well as strictly anaerobic conditions (27). In combination with the availability of its complete genome sequence (13), this makes *S. cerevisiae* an ideal model organism with which to study physiological adaptation to aerobiosis and anaerobiosis in eukaryotes. Research into the physiological mechanisms that enable *S. cerevisiae* to grow anaerobically is not only of fundamental scientific interest: oxygen requirement is a key factor in the application of yeasts in the production of alcoholic beverages and fuel ethanol (7, 22).

Genome-wide transcription analysis is a powerful tool for determining the complete set of mRNAs and their relative expression levels as a function of growth conditions. All studies published to date on genome-wide transcription in *S. cerevisiae* rely on the use of batch cultures (6, 10, 14, 30). The inherent drawback of this cultivation method is that it does not allow studies of the effect of individual cultivation parameters. For example, in standard shake flask cultures of yeasts, essential culture parameters such as pH, dissolved-oxygen concentra-

tion, and concentration of nutrients change continuously during growth. Even when pH and dissolved-oxygen concentrations are controlled (e.g., by using fermentor cultures), physical and chemical culture parameters cannot be manipulated independent of the specific growth rate. Since the specific growth rate has a drastic impact on the regulation of gene expression (11, 21), this readily obscures interpretation of the transcriptional patterns derived from such batch cultures.

Chemostat cultivation allows reproducible steady-state cultivation of microorganisms (20). In chemostat cultures, important parameters such as the specific growth rate, culture pH, and dissolved-oxygen concentration can be accurately controlled. Thus, chemostat cultivation allows physiological studies in which a single culture parameter is varied while all other conditions are kept constant (20, 29). This makes chemostat cultivation a virtually indispensable technique for genome-wide expression studies.

In this study, glucose-limited chemostat cultures of *S. cerevisiae*, grown at a fixed specific growth rate, pH, and temperature, were used to compare the aerobic and anaerobic transcript profiles of this yeast.

MATERIALS AND METHODS

Strain and growth conditions. The prototrophic laboratory strain *S. cerevisiae* CEN.PK113-7D (*MATa*) was kindly provided by P. Kötter (J.-W. Goethe Universität, Frankfurt, Germany). Steady-state chemostat cultures were grown in 1-liter working-volume Applikon laboratory fermentors as described in detail elsewhere (23). In brief, the cultures were fed with a defined mineral medium containing glucose as the growth-limiting nutrient (25). The dilution rate (which equals the specific growth rate) in the steady-state cultures was 0.10 h^{-1} , the temperature was 30°C , and the culture pH was 5.0. Aerobic conditions were maintained by sparging the cultures with air ($0.5 \text{ liter} \cdot \text{min}^{-1}$). The dissolved-oxygen concentration, which was continuously monitored with an Ingold model 34 100 3002 probe, remained above 80% of air saturation. For anaerobic cultivation, the reservoir medium was supplemented with Tween 80 and ergosterol as described by Verduyn et al. (26). Anaerobic conditions were maintained by sparging the medium reservoir and the fermentor with pure nitrogen gas ($0.5 \text{ liter} \cdot \text{min}^{-1}$). Furthermore, Norprene tubing and butyl rubber septa were used to minimize oxygen diffusion (27). Residual glucose concentrations in the aerobic and anaerobic chemostat cultures, assayed after rapid sampling in liquid nitrogen (11), were 0.17 and $0.40 \text{ mmol} \cdot \text{liter}^{-1}$, respectively.

mRNA isolation and cDNA preparation. Cells for RNA isolation were harvested by a rapid sampling procedure, as induction of aerobic genes has been shown to occur within minutes after exposure of anaerobic cultures to oxygen

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TABLE 1. Comparison of chip results to Northern data

Gene	Chip (arbitrary units)		Chip ratio, anaerobe/aerobe	Northern ratio ^a	Comment
	Anaerobe	Aerobe			
<i>CYC1</i>	1,283	3,621	1:2.8	1:7.3	Same RNA sample (this study)
<i>ROX1</i>	289	1,237	1:4.3	1:9.8	Same RNA sample (this study)
<i>DAN1</i>	5,726	143	40:1	>40:1 ^b	Same RNA sample (this study)
<i>MAE1</i>	1,709	392	4.4:1	3-4:1	Same strain, same conditions, different samples (4)
<i>ACSI</i>	340	2,180	1:6.4	>6.4 ^b	Different strain, same conditions (23)
<i>ACS2</i>	2,895	2,755	1.1:1	2.8:1	Different strain, same conditions (23)

^a From phosphorimager read-outs normalized to the *ACT1* or *PDA1* mRNA signal.

^b Anaerobic signal was 0.

(reference 5 and our unpublished results). Due to the large volumes, samples from the fermentor could not be harvested directly in liquid N₂. We therefore collected 50-ml samples in a predetermined amount of ice in a centrifuge bucket placed in an ice-salt bath at -5°C such that the temperature of the sample dropped to 2°C within 15 s. The mixtures were centrifuged at the same temperature, and the pellets were frozen in liquid N₂.

For total RNA extraction, the frozen pellet of 50 ml of culture was resuspended in 15 ml of phenol (pH 8.0), 15 ml of bead buffer (75 mM ammonium acetate, 10 mM EDTA), and 1 ml of 10% sodium dodecyl sulfate. After the addition of 5 g of glass beads (425- to 600- μ m diameter, acid washed), the sample was vortexed twice for 1 min, incubated at 65°C in a water bath for 15 min, and vortexed again for 1 min. The upper phase was extracted with phenol-chloroform (50:50) and subsequently precipitated by adding 1/10 volume of 7.5 M ammonium acetate and 2 volumes of absolute ethanol. Poly(A)⁺ RNA was purified by using Oligotex-dT (Qiagen).

First-strand cDNA synthesis was performed by mixing 20 μ g of poly(A)⁺ RNA with 3,000 pmol of dT₂₁ in a final volume of 200 μ l of 1 \times first-strand buffer (Gibco). The mixture was incubated at 65°C for 10 min and subsequently cooled on ice; 12 μ l of 100 mM dithiothreitol, 4 μ l of 20 mM deoxynucleoside triphosphates (Pharmacia), and 20 μ l of Superscript II (200 U/ μ l; Gibco) were added, and reverse transcription was carried out at 42°C for 60 min, followed by the addition of 3 μ l of bacterial DNA control mix to enable assessment of variation in the effectiveness of the labeling procedure. The mixture was extracted with phenol-chloroform (50:50), and cDNA was precipitated by adding 0.5 volume of 7.5 M ammonium acetate and 2.5 volumes of absolute ethanol.

Hybridization and data processing. The cDNA was fragmented to an average size of approximately 50 bp by DNase I in 1 \times One-phor-all buffer (Pharmacia) containing CoCl₂. After incubation at 37°C for 5 min, the DNase I was inactivated by incubation in a boiling water bath for 10 min. cDNA fragments were 3' end labeled by a 60-min incubation at 37°C in the presence of biotinylated ddATP and terminal transferase (Boehringer). The hybridization mixture was prepared by adding 125 μ l of 2 \times ST-T (2 M NaCl, 20 mM Tris [pH 7.6], 0.010% Triton X-100), 2 μ l of herring sperm DNA (10 mg/ml), and 1 μ l of control DNA (strain 948B; 5 nM in 6 \times SSPE-T [6 \times SSPE-T contains 0.9 M NaCl, 60 mM NaH₂PO₄, 6 mM EDTA, and 0.005% Triton X-100, adjusted to pH 7.6]) to the biotinylated cDNA, and the volume was adjusted to 250 μ l. After incubation in a boiling water bath for 10 min followed by chilling on ice for 5 min, 200 μ l of the target preparation was transferred into a prewetted chip chamber. Hybridizations were carried out at 43°C for 16 h, rotating at 60 rpm. Subsequently the hybridization mixture was collected and stored at -80°C for further use. The chip was rinsed with 6 \times SSPE-T, washed on a fluidics station (10 washes with 6 \times SSPE-T and 2 washes with 0.5 \times SSPE-T), rotated with SSPE-T at 43°C for 15 min, and then rinsed with 6 \times SSPE-T. The hybridized chips were stained by rotation with 0.4 μ l of a 1-mg/ml streptavidin-phycoerythrin solution (Molecular Probes) and 10 μ l of a 20-mg/ml bovine serum albumin solution in 190 μ l of 6 \times SSPE-T at 43°C for 10 min. Prior to scanning, the chip was rinsed with 6 \times SSPE-T and washed on a fluidics station (five washes with 6 \times SSPE-T).

After subtraction of the values of the mismatch oligonucleotides, intensities of the signals were normalized to the total intensities of the chips. For the eight chips used (two sets of four), these values varied between 979,735 and 1,908,932 arbitrary units.

RESULTS AND DISCUSSION

Genome-wide transcription patterns were analyzed in aerobic and anaerobic, steady-state chemostat cultures of the prototrophic laboratory strain *S. cerevisiae* CEN.PK113-7D (*MATa*) (9) using Affymetrix Ye6100 gene chips, which represent a DNA array encompassing virtually the entire *S. cerevisiae* genome. After scanning the arrays, data analysis was performed with Affymetrix GeneChip software. Transcript levels in aerobic and anaerobic cultures (which were hybridized to

different gene chips) were compared after normalization. This involved division of individual fluorescence intensities through the fluorescence of the entire chip. The complete data set is available online (15).

Reliability of the DNA array analysis was evaluated by comparing transcript levels of three reference genes in the aerobic and anaerobic cultures with classical Northern data from the same RNA samples (Table 1). In addition, commonly used mRNA loading standards such as *ACT1* (18), *PDA1* (28), and *HHO1* (21) exhibited the same transcript levels (<10% difference) in aerobic and anaerobic cultures. The measured aerobic/anaerobic values were 3,669/3,839 for *ACT1*, 2,561/2,687 for *PDA1*, and 2,083/2,071 for *HHO1*. Mating type a-specific genes (*MFA1*, *MFA2*, and *STE2*) were expressed in both cultures, whereas only low transcript levels of α -specific genes (*MF α 1*, *MF α 2*, and *STE3*) were detected. Few data are available from conventional Northern studies on transcription in aerobic and anaerobic chemostat cultures. However, published data from Northern studies for *MAE1* (three- to fourfold-higher level in the anaerobic cultures) and *ACSI* (present only under aerobic conditions) agreed well with our data (4, 23). For *ACS2* (similar levels in aerobic and anaerobic cultures), a slight increase was previously reported (23).

In the glucose-limited chemostat cultures, 5,738 (93%) of 6,171 open reading frames (ORFs) from the *S. cerevisiae* genome were transcribed at a detectable level under either aerobic or anaerobic conditions (Fig. 1). This fraction is higher than reported for previous genome-wide transcription studies

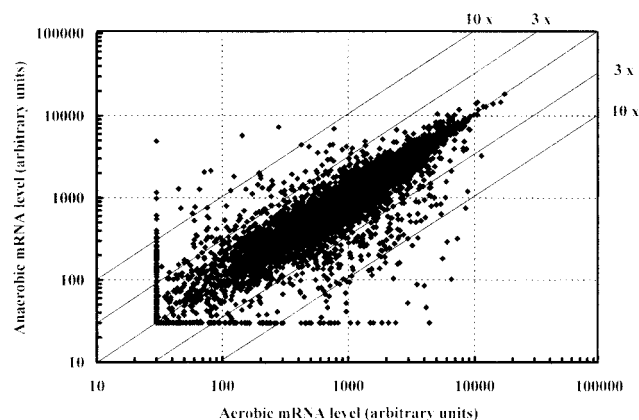


FIG. 1. Transcript levels of 6,171 yeast ORFs represented on the Affymetrix Ye6100 gene chips in aerobic and anaerobic chemostat cultures (dilution rate = 0.10 h⁻¹; pH 5.0; temperature = 30°C) of *S. cerevisiae* CEN.PK113-7D. Transcripts that were considered absent by the Affymetrix software are set at a value of 30 to allow calculation of a ratio. The diagonal lines indicate various ratios between aerobic and anaerobic transcript levels.

TABLE 2. Aerobic genes

ORF ^a	Gene name	mRNA level (arbitrary units)		Ratio, aerobic/anaerobic	Similarity
		Anaerobic	Aerobic		
YOR388C	<i>FDH1</i>	A ^b	4,609		
YPL275W		A	4,389		YOR388C
YPL276W		A	2,368		YOR388C
YDR256C	<i>CTA1</i>	A	2,076		
YHR096C	<i>HXT5</i>	A	1,846		
YNL195C		A	1,578		Unknown
YGR110W		A	1,497		Unknown
YCR010C		A	1,489		Unknown
YDL218W		A	1,189		Unknown
YPL223C	<i>GRE1</i>	102	6,535	64	
YJR095W	<i>ACR1</i>	52	2,956	57	
YMR303C	<i>ADH2</i>	85	3,992	47	
YGR236C		88	3,491	40	
YHR139C	<i>SPS100</i>	125	4,477	36	
YPR151C		182	5,560	31	Unknown
YMR107W		320	7,282	23	Unknown
YMR118C		104	2,273	22	Unknown
YLR174W	<i>IDP2</i>	291	6,026	21	
YPL201C		162	2,545	16	Unknown
YDR380W		156	2,456	16	Unknown
YMR058W	<i>FET3</i>	114	1,538	13	
YBR047W		138	1,772	13	Unknown
YML054C	<i>CYB2</i>	262	3,202	12	
YLR205C		162	1,968	12	Unknown
YPL147W	<i>PXA1</i>	133	1,520	12	
YDR070C		749	8,479	11	Unknown
YPR001W	<i>CIT3</i>	130	1,452	11	
YER065C	<i>ICL1</i>	136	1,527	11	
YKR009C	<i>FOX2</i>	325	3,546	11	
YLL053C		94	1,022	11	Unknown
YGR256W	<i>GND2</i>	189	1,948	10	

^a ORF with an aerobic/anaerobic ratio of at least 10 and with an aerobic expression level of 1,000 or more.

^b A, absence of transcription according to the Affymetrix software.

on batch cultures of *S. cerevisiae* (30) and may reflect the alleviation of glucose catabolite repression that occurs as a result of the low residual glucose concentration in glucose-limited chemostat cultures (9, 21).

The majority of the yeast genes showed similar transcript levels under aerobic and anaerobic conditions (Fig. 1). Only 219 genes showed a >3-fold-higher transcription level under aerobic conditions. Under anaerobic conditions, transcript levels of 140 genes were >3-fold higher than aerobically. Only a very small number of genes exhibited a >10-fold difference between aerobic and anaerobic mRNA levels (examples given in Tables 2 and 3).

Surprisingly, the majority of genes involved in respiratory sugar metabolism (e.g., those encoding enzymes of the tricarboxylic acid cycle or proteins involved in respiration) showed little or no repression under anaerobic conditions. This result appears to contradict earlier work by DeRisi et al. (10), who found that transcription of most genes involved in respiration was strongly induced upon a switch from fermentative growth to respiratory growth. However, this contradiction is only apparent. In the experiments of DeRisi et al., the shift from fermentative metabolism to respiratory metabolism was accomplished by growing *S. cerevisiae* on glucose in batch cultures. This results in a typical diauxic pattern because initially, the high sugar concentration in the medium causes glucose catabolite repression of respiratory enzymes (12, 16). Only when glucose is exhausted and cells start consuming ethanol this repression is relieved. In our experiments, aerobic and anaerobic growth were studied in glucose-limited chemostat

cultures in which the low residual glucose concentrations alleviated glucose repression. Apparently, under these conditions, the flux through the tricarboxylic acid cycle and respiration is primarily regulated posttranscriptionally (e.g., by concentrations of intracellular metabolites and effectors).

The physiological functions of several of the 53 genes which exhibited a strongly (>10-fold) elevated transcript level under aerobic conditions could be directly linked to typical aerobic processes. This group includes genes involved in respiration [e.g., *NDE2*, encoding an isoenzyme of the mitochondrial external NADH dehydrogenase; YMR118c, encoding a succinate dehydrogenase; and *CYB2*, encoding a cytochrome *b*-(L-lactate cytochrome *c* oxidoreductase)], protection against oxygen toxicity (*CTA1*, encoding the peroxisomal isoenzyme of catalase), and β oxidation (*PXA1*, encoding a transporter involved in translocation of long-chain fatty acids across the peroxisomal membrane; and *FOX2*, encoding 3-hydroxyacyl coenzyme A epimerase). For some other genes that were specifically expressed under aerobic conditions, the role in aerobic metabolism was less obvious, either because they encode proteins with unknown function (Table 2) or because the known functions of their protein products could not be clearly correlated with aerobic growth. This holds, for example, for the sporulation-specific gene *SPS100* (Table 2), which exhibited a 36-fold-higher transcript level in aerobic cultures, even though sporulation did not occur in these cultures. Also, the high expression of three genes presumed to encode formate dehydrogenases (Table 2) in aerobic cultures is unclear.

A comparison of the aerobic and anaerobic transcript pro-

TABLE 3. Anaerobic genes

ORF ^a	Gene name	mRNA level (arbitrary units)		Ratio, anaerobic/aerobic	Similarity
		Anaerobic	Aerobic		
YMR319C	<i>FET4</i>	1,159	A ^b		
YPR194C		982	A		Oligopeptide transporter
YIR019C	<i>STA1/FLO11</i>	981	A		
YHL042W		608	A		Unknown
YHR210C		552	A		Gal10p
YGL162W	<i>SUT1/STO1</i>	371	A		
YHL044W		334	A		Unknown
YOL015W		320	A		Unknown
YJR047C	<i>ANB1/TIF51B</i>	4,901	21	231	
YJR150C	<i>DAN1</i>	5,726	143	40	
YML083C		2,056	72	29	Unknown
YBR085W	<i>AAC3</i>	1,281	45	28	
YOR010C	<i>TIR2</i>	2,140	81	27	
YER011W	<i>TIR1</i>	7,262	278	26	
YKR053C	<i>YSR3/LBP2</i>	1,268	66	19	
YER188W		746	47	16	Unknown
YCL025C	<i>AGP1</i>	1,817	123	15	
YPL265W	<i>DIP5</i>	772	61	13	
YDL241W		645	58	11	Unknown
YBL029W		1,223	116	11	Unknown
YDR044W	<i>HEM13</i>	4,420	422	11	
YLR413W		2,006	194	10	Unknown

^a ORF with an anaerobic/aerobic ratio of at least 10 and an anaerobic expression level of 300 or more.

^b A, absence of transcription according to the Affymetrix software.

files of wild-type *S. cerevisiae* does not by itself allow conclusions about the molecular mechanisms of transcriptional regulation. However, the methodology used for this study is, in principle, well suited for disentangling the regulatory network via comparison of transcript profiles in wild-type strains and strains with defined modifications in regulatory genes. Some indication as to the involvement of known regulatory mechanisms can be obtained from the presence of consensus sequences in the promoters of aerobically and anaerobically induced genes. For example, 7 of the 11 previously identified Rox1-binding-site-containing hypoxic genes (*SUT1*, *ANB1*, *HEM13*, *HMG2*, *AAC3*, *ROX1*, and *COX5b*) (8, 17) showed elevated transcript levels under anaerobic conditions. It is not clear whether the marginal (1.3-fold) increases of the *CPhi/CPR1* and *OLE1* and the unchanged *ERG11* and *CYC7* mRNA levels are caused by the stringent anaerobic conditions in the fermentor cultures used in this study.

The functions of some of the genes exhibiting a strongly elevated transcript level under anaerobic conditions (Table 3) could be directly linked to anaerobic metabolism. For example, the anaerobic induction of *SUT1*, encoding a protein involved in sterol uptake (Table 3), can be directly linked to the strict requirement for uptake of exogenous sterols in anaerobic cultures. Similarly, the requirement of mitochondrial ATP under anaerobic conditions is reflected by the strong (28-fold) induction of *AAC3*, encoding a mitochondrial ATP/ADP translocator. The high transcript level of *FET4*, which encodes a low-affinity ferrous iron transporter, is probably related to the fact that in anaerobic cultures, iron is predominantly present as Fe(II). Indeed, aerobic cultivation resulted in the strong (13-fold) induction of *FET3*, encoding a cell surface ferroxidase required for high-affinity ferrous iron uptake. As for the aerobic genes of *S. cerevisiae*, the physiological role of many of the anaerobically induced genes remains unclear. This does not only hold for the substantial fraction of these genes that encode proteins with completely unknown function. For example, the roles in anaerobic metabolism of several genes implicated

in stress response (*DAN1*, *TIR1*, *TIR2*, and *YSR3/LBP2*) or amino acid transport (*AGP1* and *DIP5*) remain to be elucidated.

In quantitative terms, the aerobic and anaerobic transcript profiles of *S. cerevisiae* exhibit little difference. This observation can be interpreted in two ways. One possibility is that only few genes contribute to this eukaryote's unique ability to grow rapidly under both aerobic and anaerobic conditions. Alternatively, genes with similar aerobic and anaerobic transcription levels may contribute to this metabolic flexibility. Discrimination between these possibilities requires a combination of the results from this study with investigations, under well-defined aerobic and anaerobic conditions, of the fitness of defined null mutants in all yeast genes. In principle, competition experiments with large sets of defined yeast mutants (3) in aerobic and anaerobic chemostat cultures should present an excellent tool for such studies (19).

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