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Transcriptome profiling of *Saccharomyces cerevisiae* during a transition from fermentative to glycerol-based respiratory growth reveals extensive metabolic and structural remodeling

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Abstract Transcriptome analyses using a wild-type strain of *Saccharomyces cerevisiae* were performed to assess the overall pattern of gene expression during the transition from glucose-based fermentative to glycerol-based respiratory growth. These experiments revealed a complex suite of metabolic and structural changes associated with the adaptation process. Alterations in gene expression leading to remodeling of various membrane transport systems and the cortical actin cytoskeleton were observed. Transition to respiratory growth was accompanied by alterations in transcript patterns demonstrating not only a general stress response, as seen in earlier studies, but also the oxidative and osmotic stress responses. In some contrast to earlier studies, these experiments identified modulation of expression for many genes specifying transcription factors during the transition to glycerol-based growth. Importantly and unexpectedly, an ordered series of changes was seen in transcript levels from genes encoding components of the TFIID, SAGA (Spt-Ada-Gcn5-Acetyltransferase), and SLIK (Saga LIKe) complexes and all three RNA polymerases, suggesting a modulation of structure for the basal transcriptional machinery during adaptation to respiratory growth. In concert with data given in earlier studies, the results presented here highlight important aspects of metabolic and other adaptations to respiratory growth in yeast that are common to utilization of multiple carbon sources. Importantly, they also identify aspects specific to adaptation of this organism to growth on glycerol as sole carbon source.

Keywords Respiratory growth · Metabolic reprograming · Metabolic shift · Environmental adaptation · *Saccharomyces cerevisiae* · Microarray analysis

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Introduction

The yeast *Saccharomyces cerevisiae* must adapt to a wide variety of growth conditions in its natural environment, and to do so the organism has evolved flexible controls to adjust its metabolic and other systems rapidly and appropriately. One important aspect of normal yeast growth is the ability to utilize a variety of different carbon sources for growth. Many studies have examined transcriptional and metabolic patterns and controls operating during growth of this organism on various carbon sources (e.g., Schüller 2003 for review). However, despite information provided by those studies, relatively little currently is known concerning the details of global gene expression and metabolic restructuring during the crucial period of transition from growth on one carbon source to growth on another. We therefore used microarrays to perform transcriptome profiling of yeast during the transition from catabolite-repressed fermentative growth using glucose as carbon source to respiratory growth using

glycerol as sole carbon source. Further, we defined the transcriptome profile of yeast growing continuously in medium with glycerol or ethanol as sole carbon source.

Glucose is a fermentative carbon source for yeast; i.e., in the presence of this sugar, yeast metabolism is primarily (although not exclusively) fermentative, and the product of that fermentative process is ethanol. Further, glucose is the preferred carbon source for *S. cerevisiae*, and cells growing in medium containing only this sugar repress expression of genes encoding products required for metabolism of other carbon sources; this is referred to glucose or catabolite repression. (Gancedo 1998; Carlson 1999 for review; see also Rolland et al. 2002). Yeast growing in glucose-based medium eventually exhaust that carbon source, at which point the cells' doubling rate is attenuated somewhat while they undergo the transition from fermentative to respiratory growth using ethanol as carbon source. This period of metabolic adjustment is designated the diauxic shift. One microarray-based study of the global pattern of gene expression during the diauxic shift showed that transcript levels from more than 700 genes were increased twofold or more, and those from more than 1,000 genes were attenuated twofold or more, during the shift (DeRisi et al. 1997). At the time of that study, ~ 50% of the affected genes specified products of unknown function. Many genes encoding products involved in metabolic adaptation showed increased expression during the shift, including *ALD2*/YMR170C, encoding a cytoplasmic aldehyde dehydrogenase, and *ACS1*/YAL054C, which encodes an acetyl-coA synthetase isoform. These two enzymes direct the products of alcohol dehydrogenease into the TCA and glyoxylate cycles. Increased expression of *PCK1*/YKR097W, which encodes phosphoenolpyruvate carboxykinase, and *FBP1*/YLR377C, specifying fructose bisphosphatase was also observed. These enzymes reverse the direction of metabolites within the glycolytic pathway to favor production of glucose-6-phosphate (DeRisi et al. 1997). Cells double more slowly during ethanol-based growth than during growth in glucose-based medium, and this was reflected during the shift by attenuation of expression of genes encoding ribosome proteins, initiation factors for the protein synthetic system, and others. As expected, nuclear genes specifying products that function in the electron transport-oxidative phosphorylation systems, mitochondrial translation system, etc. showed transcriptional up-regulation during the shift from glucose- to ethanol-based growth. Interestingly, this study also demonstrated increased transcript levels from genes involved in the general stress response during the shift (DeRisi et al. 1997; see below).

More recently, a microarray-based analysis of the diauxic shift performed on a finer temporal scale revealed even more extensive metabolic remodeling than was identified in the earlier study (Brauer et al. 2005). In the newer study, RNA samples were prepared at 15 min intervals before, during, and after the shift, and transcript changes observed at each time point were compared to those in the same wild-type strain growing continuously in a chemostat at low glucose concentration. As in the earlier study, expression of genes encoding ribosome proteins and other components of the translation system was attenuated during and after the shift, as was expression of genes specifying products involved in amino acid metabolism; also as in the earlier study, transcription of genes whose products function in the electron transport-oxidative phosphorylation systems and other energy transduction pathways was up-regulated, as was that of genes encoding components of the general stress response (Brauer et al. 2005). The temporal resolution employed in this study allowed identification of a relatively orderly increase in transcript levels from genes encoding hexose transporters of increasing affinity over time during the shift. These results indicated that, as glucose concentration decreased in the growth medium during the transition, the increasingly starved cells attempted to scavenge the remaining glucose before undergoing the metabolic transition to respiratory growth. Importantly, this study indicated that as starvation for glucose, the preferred carbon source, began, a signal was elicited that initiated the metabolic change from primarily fermentative to respiratory growth.

Yeast cells can respire on carbon sources other than ethanol, of course, and one of these is glycerol. This short chain alcohol enters the cell and is phosphorylated by glycerol kinase, the product of the *GUT1*/YHL032C (glycerol utilization) gene (Pavlik et al. 1993). The product of the reaction, glycerol-3-phosphate, is then converted to dihydroxyacetone phosphate by the product of *GUT2*/YIL155C, glycerol-3-phosphate dehydrogenase, and this intermediate eventually is converted to pyruvate (Rønnow and Kielland-Brandt 1993; Schüller 2003; see also below); interestingly Gut2p functions in mitochondria, although the gene encoding it is nuclear. In our hands and those of others, wild-type yeast cells grow about as well in glycerol-based medium as they do in ethanol-based medium. The purpose of the present study was to assess whether the global pattern of gene expression during the metabolic transition from glucose-based, primarily fermentative growth of yeast to glycerol-based respiratory growth reflects the patterns estab-

lished by others for the diauxic shift transition to ethanol-based growth. The data presented identify patterns of gene expression that appear to be general features of the adaptation to respiratory growth, but they also highlight aspects of the adaptation process which are carbon-source specific.

Materials and methods

Yeast strains, culture conditions

Yeast strain BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) was obtained from the ATCC and used for all experiments. Rich medium (YP, 2% peptone, 1% yeast extract) plus 2% glucose (YPD), 2% glycerol (YPG), or 2% ethanol (YPE) was used for growth as required (Sherman 2002). For transcriptome analysis of cells growing continuously in medium with glucose, glycerol, or ethanol as sole carbon source, cultures were grown to early log phase (A_{600}=0.6) in YPD, YPG, and YPE, then harvested for nucleic acid preparation. For transcriptome analysis of cells transitioning from glucose- to glycerol-based growth, cultures were grown to A_{600}=0.6 in YPD, collected and washed two times in YPG, resuspended in YPG, then returned to growth and harvested for nucleic acid preparation at 15, 30, 60 min after the medium change; in the text following, these samples are designated YPG15, YPG30, and YPG60, respectively. Cells grown continuously in YPD served as the reference for all analyses; i.e., all fold-change values are given with respect to congruent transcript levels in cells grown with glucose as sole carbon source. For all experiments, cells were grown in 500 ml flasks in 50 ml culture volume at 30°C, with shaking at 250 rpm.

Nucleic acid preparation for microarray analyses

Total nucleic acids were prepared from yeast cell pellets as described (McEntee and Hudson 1989; Lu et al. 2005). Poly-A+ RNA was isolated from total nucleic acids using the Oligotex mRNA kit (Qiagen, Valencia, CA, USA) by two rounds of binding to oligotex beads according to manufacturer's recommendations. cDNA, cRNA synthesis and purification were performed according to the Affymetrix users' manual (Affymetrix 2000; Affymetrix, Santa Clara, CA, USA) from approximately 1 μg mRNA. cRNA quality was confirmed by gel electrophoresis (Sambrook and Russell 2001). Nucleic acids from three biological replicates each of strain BY4741 harvested 30 min after medium change, and during continuous glycerol-based growth, were prepared, and the resulting labeled cRNA was hybridized to separate arrays; all other samples were from a single replicate. Complete original array data are available upon request to A.P. Hudson.

Probe hybridization

Affymetrix YGS98 microarrays were hybridized as instructed (Affymetrix 2000) with 15 μg cRNA. cRNA fragmentation, probe hybridization, and probe level analysis using Microarray Suite 4.0/5.0 (MAS) (Affymetrix) were done at the Michigan State University Genomics Technology Support Facility. Quality of hybridized cRNA was confirmed by determination of 3′:5′ ratios (MAS 4.0/5.0).

Data analysis

Data analyses were performed using GeneSpring 7.2 (Agilent Technologies, Palo Alto, CA, USA). All probeset fluorescence intensities less than 0.01 were set to 0.01. Median of each array was set to 500 and other values scaled accordingly. Finally, each probeset was divided by the fluorescence value of that probeset under fermentative growth conditions. Differentially regulated transcripts were selected from an initial pool of 6,397 probesets corresponding to ORFs from the total 9,335 probe sets on the YGS98 array. Except as noted, differentially regulated transcripts were further restricted to the set flagged as present by MAS 4.0/5.0 under both conditions being compared and passing an initial twofold cutoff. Transcripts that are referred to as "twofold up", "twofold down", or "twofold differentially regulated" were selected from the pool of 6,397 probesets, flagged present in at least one of the two conditions being compared and which passed the initial twofold cutoff as appropriate. *T* test *P* values were calculated assuming equal variances for YPG30 and YPG. The GeneSpring analysis option "all available error estimates" was used for YPG15, YPG60 and YPE conditions. Enrichment *P* values for specific gene ontology (GO) terms were calculated using the binomial distribution without multiple testing correction using GO term finder at the *Saccharomyces* genome database (SGD) website (http://www.yeastgenome.org). Further annotation was derived from TRANSFAC (http://www.gene-regulation.com/pub/databases.html#transfac) and KEGG (http://www.genome.jp/kegg). Transcription factor activity was inferred by statistical enrichment of differentially regulated transcripts by T-profiler (Zakrzewska et al. 2005; Boorsma and Bussemaker 2005; http://www.t-profiler.org).

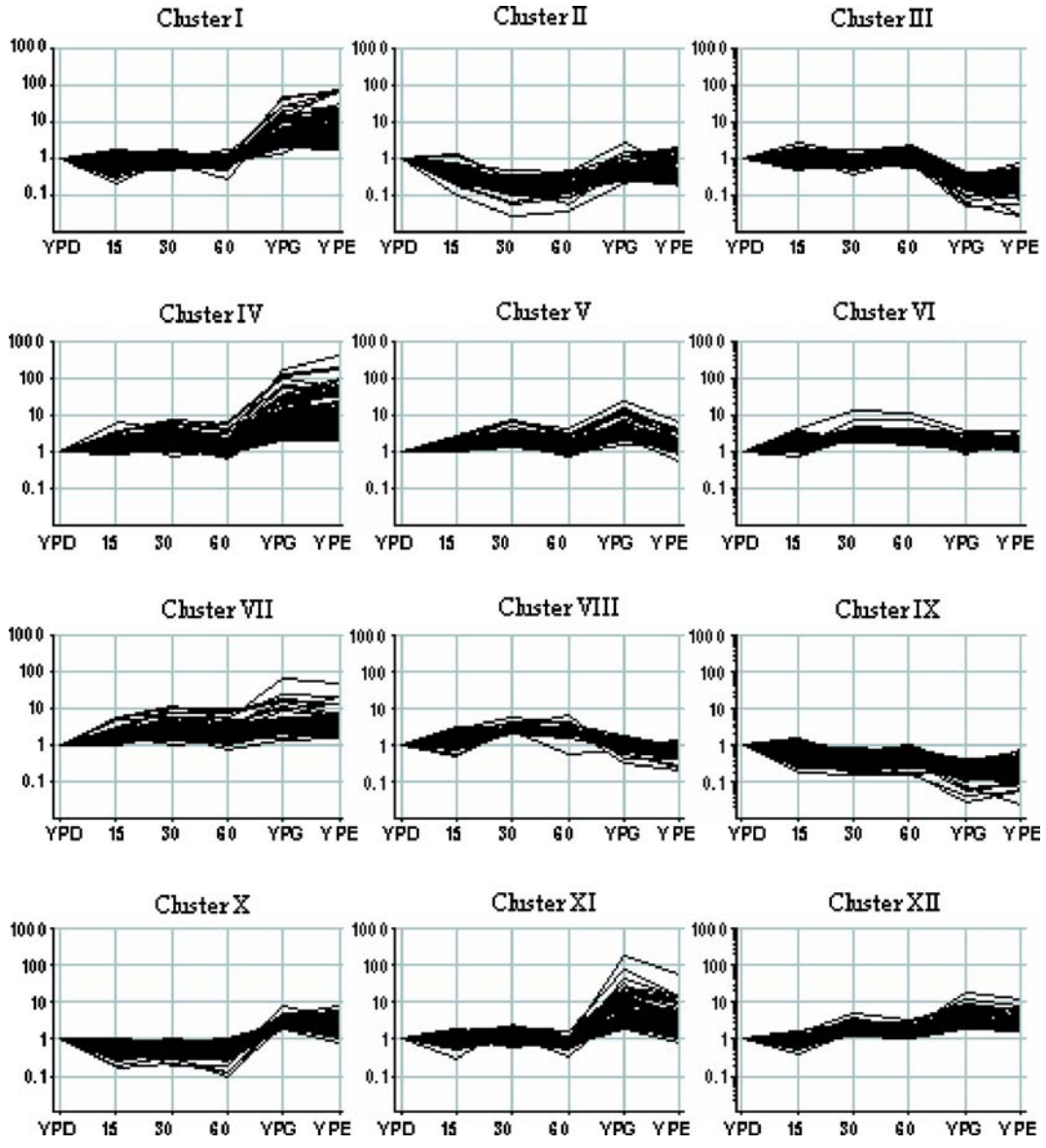

Fig. 1 *K* means clustering analysis showing gene expression during the transition from growth on YPD to growth on YPG. Expression values are indexed relative to expression during growth on YPD. *15*, *30*, and *60* indicate cells grown to early log phase in YPD then transferred to YPG for 15, 30 or 60 min, respectively. *YPG* and *YPE* indicate values for cells continuously grown to early log phase on those media

Results

Overview of transcript profile changes using *k* means clustering

The transcriptome profile of wild-type yeast cells grown to early log phase in glucose-based medium, then transferred for 15, 30, or 60 min to medium containing glycerol as sole carbon source was determined using Affymetrix GeneChip microarrays. For comparison, the transcriptome profile of cells of the same strain grown to early log phase in medium containing either glycerol or ethanol as sole carbon source also was defined. Analyses of gene expression patterns revealed a large panel of transcriptional changes supporting the transition from fermentative to glycerol-based respiratory growth. Hierarchical clustering indicated that definition of 12 coherent gene expression clusters would be the most informative, and we therefore used *k* means clustering to produce those 12 clusters (Fig. 1). GO analysis allowed assignment of functional annotations to each cluster (Table 1).

Cluster I (see Fig. 1 for graphic display of this and all other clusters)

Enriched for genes encoding components of the electron transport chain (Table 1 for GO analysis of genes in each cluster). Expression of genes in this cluster was

Table 1 Gene ontology (GO) enrichment among *k* means clusters

Cluster		Size	Gene ontology terms[a]
I	Respiration	141	Oxidative phosphorylation (1.20E-19, 19 of 46) Mitochondrial electron transport chain (5.71E-12, 11 of 26)
II	Transport	38	Cell wall (2.9E-04, 5 of 113) Organic cation transport (4.6E-4, 2 of 6) Ion transport (0.019, 3 of 110)
III	Protein synthesis	210	Ribosome biogenesis and assembly (3.66E-15, 36 of 254) Ribosome biogenesis (3.55E-14, 32 of 214) Transcription from RNA polymerase I promoter (2.02E-05, 7 of 28) Transcription from RNA polymerase III promoter (1.3E-04, 7 of 38) Lipid biosynthesis (1.8E-05, 14 of 124)
IV	Respiration	139	Generation of precursor metabolites and energy (4.91E-10, 22 of 228) Energy derivation by oxidation of organic compounds (1.51E-09, 20 of 198) Carbohydrate biosynthesis (4.55E-06, 9 of 64) Cation transport (7.63E-05, 9 of 92) Response to osmotic stress (5.7E-3, 5 of 59) Response to stress (0.01, 15 of 407) SLIK complex (0.033, 2 of 15)
V	Carbohydrate and fatty acid metabolism	48	Carbohydrate metabolism (9.1E-05 8 of 221) Fatty acid metabolism (1.9E-04, 4 of 43) Fatty acid β-oxidation (1.6E-03, 2 of 9)
VI	Stress	80	Trehalose metabolism (2.7E-03, 2 of 7) Response to stimulus (7.9E-03, 13 of 561) Response to stress (0.013, 10 of 407) Ribosome export from nucleus (0.013, 10 of 407)
VII	Endocytosis, actin, stress	108	Endocytosis (1.15E−06, 9 of 70) Vesicle-mediated transport (2.3E−05, 15 of 287) Actin filament organization (3.7E−04, 6 of 63) Response to stress (0.001, 15 of 407) Chromatin modification (0.0015, 9 of 181) Transport (9.7E-03, 23 of 937)
VIII	Protein localization	44	Protein localization (3E-04, 8 of 290) Ribosomal protein import into the nucleus (8.6E-03, 2 of 23) snRNP protein import into nucleus (8.6E-03, 2 of 23)
IX	Membrane and sphingolipid metabolism	157	Membrane lipid metabolism (6.3E-07, 13 of 106) Sphingolipid metabolism (3.81E-06, 7 of 29) Membrane lipid biosynthesis (3.92E-06, 10 of 71) Ribosome biogenesis and assembly (1.06E-05, 18 of 254) rRNA processing (3.15E-05, 14 of 176)
X	Stress, electron transport	56	Age-dependent response to oxidative stress (1.3E-03, 2 of 7) Mitochondrial electron transport QH_2 to cytochrome *c* (2.2E-03, 2 of 9) Peroxisome organization and biogenesis (0.034, 2 of 38) Sporulation (0.035, 3 of 94)
XI	Miscellaneous	74	α-glucoside transport (1.2E-03, 2 of 5) Generation of precursor metabolites and energy (2.2E-03, 8 of 228) Positive regulation of transcription (4.6E-03, 4 of 66) Vacuolar protein catabolism (4.7E-03, 2 of 10) Energy derivation by oxidation of organic compounds (0.015, 6 of 198) Response to stimulus (0.02, 11 of 561)
XII	Miscellaneous	52	Disaccharide metabolism (0.017, 2 of 28) Protein amino acid phosphorylation (0.03, 3 of 91) Meiotic recombination (0.03, 2 of 40)

1,154 genes whose expression in at least one growth condition analyzed was twofold differentially regulated, statistically significant ($P = 0.05$; binomial distribution), and called present by MAS 5.0 were clustered by *k* means ($k = 12$)

[a] Significantly enriched GO terms. Parentheses contain enrichment *P* value followed by the number of genes in the GO classification which were differentially regulated in the cluster and finally the total number of genes in the GO classification in the entire genome

decreased slightly during the transition from YPD to YPG; expression was strongly induced in cells undergoing continuous growth in glycerol- or ethanol-based medium.

Cluster II

Enriched for genes encoding proteins localized to the cell wall and those specifying transporter proteins.

Expression of genes in this cluster was strongly down-regulated during the transition to respiratory growth; expression of these genes in cells growing continuously in YPG or YPE was nearly equivalent to levels in YPD-grown cells.

Cluster III

Enriched for genes encoding enzymes involved in ribosome biogenesis and those encoding proteins involved in transcription from RNA polymerases I and III promoters. Expression of genes in this cluster was relatively unchanged during the transition to respiratory growth but was strongly down-regulated in cells growing continuously on YPG or YPE.

Cluster IV

Included many genes encoding products involved in energy derivation by oxidation of organic compounds, genes specifying TCA cycle enzymes, and those encoding components of the SLIK (see below) complex. Expression of genes in this cluster showed moderate increase at 15, 30, 60 min after the change from YPD to YPG. Expression was high in cells growing continuously on YPG and YPE.

Cluster V

Enriched in genes specifying products involved in carbohydrate and fatty acid metabolism. Expression of these genes showed strong increase after transition from YPD to YPG, and in cells grown to early log phase in YPG. Expression of genes in Cluster V was less strongly induced in cells grown to early log phase in YPE.

Cluster VI

Enriched in genes encoding products involved in the response to stimuli and certain types of stress. Expression of genes in this cluster was most strongly increased in cells during the time course following the change from YPD to YPG. Expression was less highly up-regulated in cells grown to early log phase in YPG or YPE.

Cluster VII

Enriched in genes encoding products involved in several processes including endocytosis, actin-filament organization, and certain responses to stress. Expression of genes in this cluster increased relatively steadily after the change from YPD to YPG; they were most highly expressed in cells grown to early log phase in YPG or YPE.

Cluster VIII

Enriched in genes specifying products involved in protein localization and in protein import into the nucleus. Expression of genes in this cluster was increased during the transition from fermentative to respiratory growth, but expression was not up-regulated in cells grown in YPG or YPE.

Cluster IX

Enriched for genes encoding enzymes involved in membrane lipid and sphingolipid metabolism. Expression of genes in this cluster steadily decreased in cells after the shift from YPD to YPG; expression was down-regulated in cells grown to early log phase in medium containing glycerol or ethanol as sole carbon source.

Cluster X

Enriched for genes encoding components of complex III of the electron transport system and genes encoding stress response proteins. Expression of genes in this cluster was steadily down-regulated during the transition from glucose- to glycerol-based growth. Expression was induced in cells grown continuously in YPG or YPE.

Clusters XI and XII

Enriched for genes encoding products involved in diverse processes, including disaccharide metabolism, vacuolar protein catabolism, and others. Expression of genes in these clusters did not increase much over the times assessed following the change from YPD to YPG, but expression was elevated in cells growing continuously in YPG or YPE. Genes in cluster XI were more highly expressed on average during glycerol- and ethanol-based growth than those in cluster XII.

Features of transcriptional adaptation to glycerol-based respiratory growth

Genes in Clusters III and IX displayed some level of down-regulation of expression during the transition from growth in YPD to YPG, and they displayed consistently low-level expression during continuous growth in YPG and YPE (Fig. 1, Tables 1, 2); these

clusters were enriched in genes encoding products required for rapid growth, such as those involved in protein synthesis, stable RNA and membrane lipid synthesis; down-regulation of expression from genes encoding enzymes required for lipid synthesis occurs during stationary phase (Backhus et al. 2001). Similar to results in the diauxic shift studies (DeRisi et al. 1997; Brauer et al. 2005), by 30 min after transfer of cells from YPD to YPG expression was down-regulated from genes encoding products required for biosynthesis, including transcription by RNA polymerases I and III and ribosome biogenesis/assembly. However, the unexpected observation of differential regulation of many genes specifying products present at the plasma membrane and in the cell cortex prompted further analysis of expression from these groups of genes. In cells growing continuously in YPG or YPE, the functions of all the most highly up-regulated genes were related to mitochondrial function or energy generation; the functions of the most severely down-regulated genes were related to the attenuated growth rate. Genes encoding peroxisomal enzymes were highly enriched among those whose expression was up-regulated in each condition tested (Table 2).

Continuity of gene expression profiles throughout the adaptation to glycerol-based respiratory growth

To differentiate genes involved at multiple stages in the process of adaptation to glycerol-based growth from those involved only at a single stage, sets of genes differentially expressed under each condition tested were compared pairwise to identify those differentially expressed in two or more conditions (Table 3; part A). In each of the possible pairwise comparisons, the extent of overlap between gene sets differentially expressed in both conditions was highly significant ($\chi^2 \leq 0.01$). Further, a high degree of similarity was found among transcript profiles from cells harvested at the three time points assessed and between the transcript profiles of cells grown continuously in YPG and YPE (Table 3; part B). A GO analysis of genes differentially regulated in pairs of conditions was performed to determine which gene classes were regulated similarly throughout the adaptation process. This revealed enrichment of genes encoding proteins localized to the plasma membrane or those involved in transport in each comparison of 15 min to other conditions (Table 3; part C). As expected, cells grown in YPG or YPE shared a large number of differentially regulated genes (1,574, χ^2=1 E-103). GO analysis of genes differentially expressed in both media revealed that much of the overlap was due to transcriptional down-regulation of genes involved in biosynthesis (e.g., ribosome biogenesis and assembly, RNA polymerase complex formation, etc.) and up-regulation of expression from genes encoding products involved in mitochondrial processes (e.g., i respiration) (Table 3; part C). SRP (signal recognition particle)-dependent co-translational targeting to membranes was highly enriched in several of the pairwise comparisons. To our knowledge, differential regulation of genes encoding components of the SRP under respiratory conditions has not been reported previously.

Similarities in the response to environmental stress, aerobiosis, the diauxic shift, and growth on glycerol revealed by transcript profile comparison

Gene expression under a variety of conditions relevant to glycerol-based respiratory growth has been reported. Overall, we identified a high degree of similarity among the gene sets differentially expressed in this study and those showing differential expression during the diauxic shift (DeRisi et al. 1997), under aerobic versus anaerobic growth conditions (ter Linde et al. 1999), during growth on glycerol as sole carbon source (Ohlmeier et al. 2004), and during elicitation of the environmental stress response (Gasch et al. 2000) (Table 4). Most of the latter gene set, and genes whose expression is Msn2/4p dependent, overlapped with coding sequences in Clusters IV, V, and XI; these clusters displayed average expression levels that were elevated during continuous growth on YPG or YPE. About half the genes whose expression was increased under aerobic conditions in a previous study (ter Linde et al. 1999) were found in the present study in Clusters I, IV, XI, whose average expression level was high during continuous growth in YPG or YPE; these clusters all displayed average expression levels that were elevated during continuous growth in YPG. Clusters I and IV were enriched for genes whose products are involved in respiration. About 10% of genes whose expression was increased under anaerobic conditions in the previous study overlapped with clusters identified here. Each gene was from a cluster whose average expression was either attenuated or relatively unchanged during growth in YPG or YPE.

Metabolic restructuring of glycerol metabolism

Transcript levels from *GUT1* and *GUT2* increased strongly over time following the change to growth in glycerol-based medium (Fig. 2). As expected, mRNA levels from these two genes were high during continuous growth on glycerol-based medium; interestingly,

Table 2 Functional (GO) characterization of genes whose expression was twofold or more differentially regulated during the transition from glucose- to glycerol-based growth

Condition	Number[a]	Gene ontology terms[b]
15 min	Up (262)	Fatty acid metabolism (3.07E-05, 9 of 43)
		Cell cortex (3.36E-03, 10 of 99)
		Peroxisome degradation (5.35E-03, 2 of 3)
		Glutamate catabolism (2.43E-03, 2 of 2)
	Down (397)	Plasma membrane (8.76E-09, 38 of 242)
		Interphase (2.89E-06, 18 of 89)
		Integral to membrane (1.4E-04, 29 of 250)
		Cyclin-dependent kinase holoenzyme complex (2.4E-04, 5 of 10)
30 min	Up (310)	Catabolism (7.33E-07, 38 of 373)
		Energy reserve metabolism (2.99E-05, 9 of 36)
		Protein serine/threonine phosphatase complex (8.8E-4, 5 of 17)
		Retromer complex (1.35E-03, 7 of 38)
	Down (607)	Membrane (1.09E-11, 146 of 1,007)
		Transcription from RNA polymerase I promoter (1.03E-06, 13 of 28)
		Transcription from RNA polymerase III promoter (5.63E-06, 14 of 38)
		Ribosome biogenesis and assembly (2.85E-05, 42 of 254)
		Regulation of cell cycle (5.3E-04, 25 of 143)
60 min	Up (527)	Establishment of cellular localization (4.32E-05, 62 of 507)
		Peroxisomal matrix (4.2E-04, 6 of 13)
		Cell cortex (4.1E-04, 18 of 99)
		Nucleus (1.1E-04, 182 of 1,978)
	Down (571)	Membrane (1.42E-15, 151 of 1,007)
		Ion transport (3.19E-07, 27 of 110)
		Localization (4.26E-06, 118 of 1,005)
		Regulation of cell cycle (5.1E-04, 24 of 143)
YPG	Up (750)	Generation of precursor metabolites and energy (6.67E-24, 85 of 228)
		Energy derivation by oxidation of organic compounds (3.45E-19, 71 of 198)
		Mitochondrion (3.68E-14, 184 of 1032)
		Mitchondrial envelope (8.39E-09, 52 of 208)
		Peroxisome (7.66E-09, 23 of 51)
	Down (1,227)	Ribosome biogenesis and assembly (8.27E-28, 129 of 254)
		Nucleolus (6.19E-21, 127 of 298)
		Nuclear lumen (5.03E-14, 177 of 586)
		Endoplasmic reticulum (4.57E-12, 113 of 334)
		RNA polymerase complex (2.91E-09, 24 of 32)
YPE	Up (971)	Mitochondrion (1.21E-14, 227 of 1032)
		Mitochondrial envelope (2.25E-13, 73 of 208)
		Mitochondrial inner membrane (1.34E-12, 61 of 162)
	Down (1,275)	Ribosome biogenesis and assembly (1.17E-25, 129 of 254)
		Nucleolus (5.18E-17, 122 of 298)
		Nuclear lumen (2.87E-12, 178 of 586)
		Endoplasmic reticulum (1.37E-11, 116 of 334)
		Endomembrane system (5.02E-09, 89 of 258)

[a] Refers to number of genes differentially regulated under the specified condition relative to expression in cells continuously growing in YPD, and which are in the SGD website

[b] Significantly enriched GO terms. Enrichment *P* value followed by the number of genes in the GO classification which were differentially regulated in the cluster and in the genome

GUT1 and *GUT2* transcript levels were relatively high in cells growing on YPE, although they did not reach levels seen in glycerol-grown cells. A corresponding decrease in transcript levels from genes encoding products with activities that oppose those of Gut1p and Gut2p (e.g., *GPP1*/YIL053W and *GPP2*/YER062C, encoding somewhat redundant glycerol-3-phosphatases, and *GPD2*/YOL059W, encoding the mitochondrial/cytoplasmic isoform of glycerol-3-phosphate dehydrogenase) (Valadi et al. 2004) was identified over time following medium change, illustrating the metabolic switch from glycerol production to glycerol utilization; mRNA levels from *GPD1*, encoding a cytoplasmic/peroxisomal glycerol-3-phosphate dehydrogenase, increased somewhat after the change to YPG, although increased production of this enzyme is unlikely to be relevant directly to the adaptation to glycerol utilization. The substantial transcript levels

Table 3 Strong correlation between transcript profiles of cells grown continuously on glycerol and ethanol and moderate correlation among transcript profiles of cells at 15, 30 and 60 minutes following transition to medium containing glycerol as sole carbon source. In all panels, 15, 30, 60 refer to samples taken at those times after medium change; YPG YPE refer to cells continuously grown in glycerol- or ethanol-based medium

Condition	Genes	Condition				
		15	30	60	YPG	YPE
		659	935	1100	1980	2270
A) Number of genes 2-fold or more differentially expressed in common between conditions examined						
15	659	–	–	–	–	–
30	935	311	–	–	–	–
60	1100	345	586	–	–	–
YPG	1980	256	498	427	–	–
YPE	2270	256	495	475	1574	–

Condition	15	30	60	YPG	YPE
B) Similarity of transcript profiles between conditions expressed as Pearson correlation coefficient (r^2)					
15	–	–	–	–	–
30	0.35	–	–	–	–
60	0.41	0.68	–	–	–
YPG	0.06	0.12	0.05	–	–
YPE	0.03	0.04	0.02	0.73	–

seen from *GUT2* and *GPD1* during ethanol-based growth (Fig. 2) may result from high level activity of the glycerol-3-phosphate shuttle (see Larsson et al. 1998); more study will be required to elucidate this issue. Transcripts from *STL1*/YDR536W (named as a sugar transporter-like protein), encoding a glycerol/H^+ symporter (Ferreira et al. 2005), were strongly induced during the shift and were induced several hundred-fold during continuous growth in both YPG and YPE (see also below).

Adaptation to glycerol-based growth involves apparent structural changes

Twenty-three of 99 genes encoding products directly annotated to the cell cortex were differentially regulated twofold or more ($P \leq 0.05$; binomial distribution) under at least one condition tested (Table 5). Clusters VI and VII were significantly enriched for genes encoding proteins localized to the cell cortex ($P = 0.023$, 1.83E-05, respectively; binomial distribution) (data not shown). Depolarization of the actin cytoskeleton occurs in cells under circumstances in which glucose is suddenly unavailable, and it occurs without *de novo* protein synthesis (Uesono et al. 2004). Together, these observations suggest that the cortical actin cytoskeleton undergoes a biphasic response in which it is depolarized by existing proteins upon removal of glucose; it presumably has a somewhat different composition when rebuilt. Moreover, 51/232 genes encoding products localized to the plasma membrane were differentially regulated under at least one condition tested. The greatest enrichment was found in clusters II, IX, and X (P=0.001, 0.025, 0.021, respectively; binomial distribution) (data not shown). Genes in these clusters exhibited down-regulation at the early time points examined after the change from glucose- to glycerol-based medium. However, genes in these clusters showed no transcriptional change, down-regulation, and up-regulation, respectively, during continuous growth on YPG or YPE compared to their levels in continuously glucose-grown cells (Fig. 1).

Expression of genes encoding components of transcriptional regulatory systems during the transition to glycerol-based respiratory growth

In contrast to data from the diauxic shift studies, changing cells from glucose- to glycerol-based growth elicited differential expression of a large suite of genes specifying transcription factors and other transcription-related proteins starting soon after medium change; these changes continued through the time course examined and were observed in cells grown to early log phase in the latter medium (Table 6). mRNA levels from 18 genes encoding transcription factors or cofactors which function at RNA polymerase II promoters were twofold or more lower at 15 min after transfer of cells to YPG. Genes encoding subunits of RNA polymerases I, II, and III, as well as polymerase I

Table 3 continued

Condition	15	30	60	YPG
C) Biological function of genes which were differentially expressed in common between conditions				
30	Membrane (8.23E-07, 75 of 1007) Plasma membrane (2.42E-06, 28 of 242) Ammonium transport (1.4E-04, 4 of 6) Monocarboxylic acid transport (4.2E-04, 4 of 8) Establishment of localization (1.39E-03, 60 of 956) SRP-dependent cotranslational protein targeting to membrane (1.46E-03, 5 of 19)	–	–	–
60	Membrane (2.19E-07, 83 of 1007) Plasma membrane (2.35E-07, 32 of 242) Ammonium transport (2.1E-04, 4 of 6) Organic cation transport (2.1E-04, 4 of 6) SRP-dependent cotranslational protein targeting to membrane (3.2E-04, 6 of 19) Establishment of localization (3.5E-04, 68 of 956)	Establishment of localization (1.08E-05, 114 of 956) Transport (1.31E-05, 112 of 939) Localization (3.74E-05, 116 of 1005) Plasma membrane (9.93E-11, 53 of 242)	–	–
YPG	Peroxisomal matrix (1.23E-03, 4 of 13) Bud (1.28E-03, 13 of 137) Monocarboxylic acid transport (2E-04, 4 of 8) Transport (5.7E-04, 52 of 939)	Transcription from RNA pol I promoter (5.10E-06, 11 of 28) Transcription from RNA pol III promoter (1.67E-05, 12 of 38) SRP-dependent cotranslational protein targeting to membrane (6.11E-05, 8 of 19) RNA polymerase complex (4.98E-07, 13 of 32)	Monocarboxylic acid transport (1.2E-04, 5 of 8) Transport (1.4E-04, 82 of 939) SRP-dependent cotranslational protein targeting to membrane (1.5E-04, 7 of 19) Establishment of localization 2.5E-04, 82 of 956) Transcription from RNA polymerase I promoter (2.9E-04, 8 of 28)	–
YPE	establishment of localization (2.9E-04, 59 of 956) membrane (6.4E-04, 60 of 1007) monocarboxylic acid (3.1E-04 4 of 8) transport (3.3E-04, 58 of 939)	Transcription from RNA polymerase I promoter (7.40E-07, 12 of 28) Transcription from RNA polymerase III promoter (3.01E-06, 13 of 38) Monocarboxylic acid transport (2.4E-04, 9 of 31) Protein targeting to membrane (3.2E-04, 9 of 31) RNA polymerase complex (6.99E-08, 14 of 32)	Transport (8.16E-06, 95 of 939) Establishment of localization (9.78, 996 of 956) Localization (2.52E-05, 98 of 1005) Monocarboxylic acid transport (2E-04, 5 of 8)	Ribosome biogenesis and assembly (1.27E-13, 116 of 254) Nucleolus (6.98E-08, 110 of 298) Endoplasmic reticulum (7.57E-06, 111 of 334) RNA polymerase complex (11.2E-05, 21 of 32) Mitochondrial electron transport chain (2.4E-04, 16 of 26)

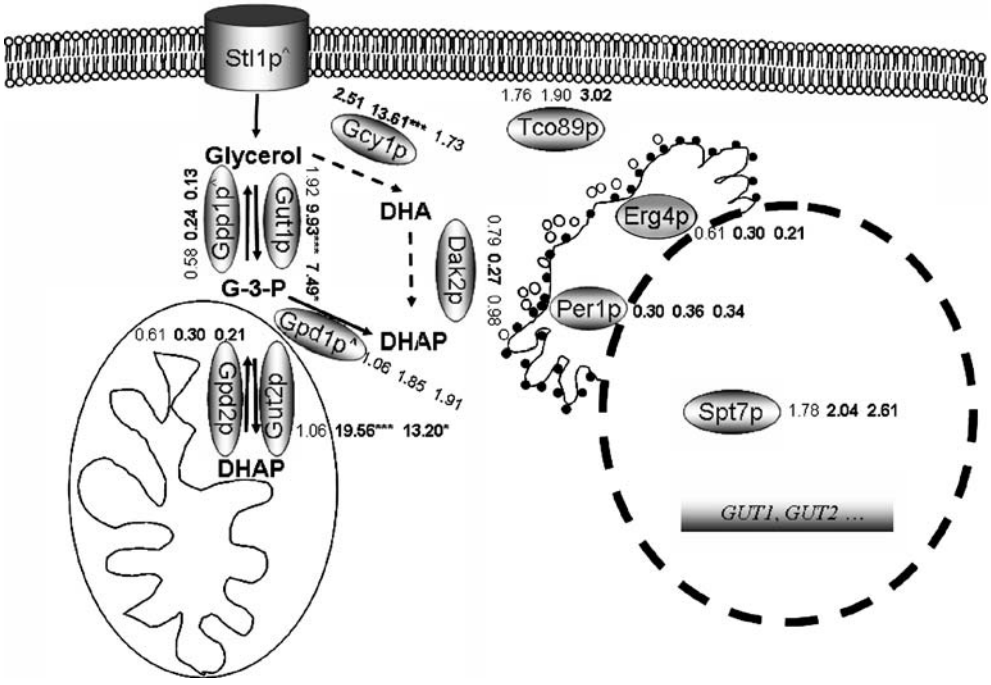

Fig. 2 Restructuring of glycerol metabolism during the transition from YPD to YPG. Transcript levels relative to levels during growth on YPD are depicted near each gene product (YPG30, YPG, YPE). Values twofold or greater are highlighted in *bold*. Statistical significance using the *t* test is denoted by *asterisks*; $P \leq 0.05$*, $P \leq 0.01$**, $P \leq 0.001$***. *Barrel* indicates the glycerol symporter. Enzymes are diagrammed as *ovals* and are paired with the reaction catalyzed where appropriate. Genes whose expression is down-regulated by loss of Hog1p (Rep et al. 2000) are denoted by "^ " next to the corresponding enzyme

transcription factors, were statistically over-represented in the group of down-regulated genes starting at 30 min after medium change (data not shown). The number of transcription factors affected by the change to growth on YPG, and the extent of differential regulation of the genes encoding them, increased over time. The greatest differential in these transcript levels was found in cells growing continuously in YPG, relative to their levels of expression during continuous glucose-based growth; in YPG, expression of genes encoding transcription factors was up-regulated as strongly as 13-fold (YJL103C) and as strongly down-regulated as 6.5-fold (*HAC1*/YFL031W). In glycerol-grown cells, 203/742 genes annotated as transcription-related by GO or elsewhere displayed twofold or greater differential expression, compared their level in glucose-based medium. Similarly, differential expression of genes encoding transcription factors in continuously ethanol-grown cells was extensive, with expression of 235/742 genes annotated as transcription-related at least twofold differentially regulated compared to results in glucose-grown cells (Table 6). The mediator complex, which functions as a transcriptional co-regulator, contains 24 subunits, four of which were twofold down- and one twofold up-regulated in cells grown in YPG. Genes encoding components of the CCR4-NOT complex were enriched in Cluster I ($P = 0.023$; binomial distribution) (data not shown).

Adjustment of basal transcriptional machinery during the transition to respiratory growth

Results summarized above indicate a significant number of changes in expression from transcription-related genes during adaptation to glycerol-based growth. We assessed alterations in transcript levels from genes specifying components of the basal transcriptional machinery during and after transition to YPG. Analysis of data from cells harvested over time identified a suite of transcript changes consistent with a slower growth rate over this period, as expected and consistent with data above for attenuated expression of genes encoding ribosome proteins, etc. Interestingly, genes encoding components of the basal transcriptional apparatus did not display universal down-regulation of expression (Table 6). For example, expression of components of the SLIK (Saga LIKe) complex, which shares many components with SAGA (Spt-Ada-Gcn5-Acetyltransferase) and TFIID, was statistically enriched in Cluster IV ($P = 3.3E\text{-}02$; binomial distribution) containing genes displaying highly induced expression during continuous growth in YPG or YPE.

Table 4 Similarity of differential gene expression during the transition to glycerol-based respiratory growth in this study and in previously published studies

Cluster (total)	Oxygen[a]				Diauxic[b]				Stress[c]				Glycerol[d]			
	Aerobic		Anaerobic		2 × Up		2 × Down		ESR		Msn2/4p dependent		2 × Up		2 × Down	
I (142)	4	**2.8%**	0		70	49.3%	2	1.4%	17	12.0%	4	2.8%	83	**58.5%**	2	1.4%
II (39)	0		0		3	7.9%	13	**34.2%**	0		0		2	5.3%	20	**52.6%**
III (210)	0		1	**0.5%**	5	2.4%	95	**45.2%**	0		0		1	0.5%	149	**71.0%**
IV (139)	7	**5.0%**	0		65	46.8%	6	4.3%	33	**23.7%**	21	**15.1%**	68	48.9%	3	2.2%
V (49)	1	2.1%	0		32	**66.7%**	1	2.1%	23	**47.9%**	18	**37.5%**	38	**79.2%**	0	
VI (80)	0		1	**1.3%**	21	26.3%	0		13	16.3%	8	10.0%	15	18.8%	7	8.8%
VII (108)	0		0		41	38.0%	4	3.7%	22	20.4%	11	10.2%	36	33.3%	4	3.7%
VIII (44)	0		0		11	25.0%	3	6.8%	4	9.1%	1	2.3%	11	25.0%	4	9.1%
IX (159)	0		1	**0.6%**	4	2.5%	59	**37.6%**	0		0		0		102	**65.0%**
X (57)	0		0		11	19.6%	3	5.4%	5	8.9%	1	1.8%	32	57.1%	4	7.1%
XI (75)	4	**5.4%**	0		42	**56.8%**	1	1.4%	24	**32.4%**	17	**23.0%**	54	**73.0%**	1	1.4%
XII (52)	0		0		30	**57.7%**	1	1.9%	8	15.4%	6	11.5%	28	53.8%	2	3.8%
Intersection	16		3		335		188		149		87		368		298	
Universe	30		22		821		800		282		181		1,490		798	
	53.3%		13.6%		42.0%		23.1%		52.8%		48.1%		24.7%		37.5%	

Genes whose expression was differentially regulated in both this study and in the specific previous study are grouped by cluster. Percentages reported are percent of cluster which was also differentially expressed in the specified condition. The top three percent values in each column are highlighted in bold. Universe refers to the number of genes differentially expressed in the original reference and which were also present on the YGS98 array. Intersection refers to the number of those genes from the universe that were differentially expressed in this study. The bottom row gives percent of the intersection from the universe

[a] Genes whose expression is 10-fold induced under anaerobic or aerobic conditions (ter Linde et al. 1999)

[b] Genes whose expression is twofold up- or down-regulated in at least one condition (DeRisi et al. 1997)

[c] Genes whose expression was twofold up-regulated under the specified condition: (ESR-entire Environmental Stress Response) (Gasch et al. 2000).

[d] Genes whose expression was twofold up or down-regulated and flagged "present" in both measurements (Ohlmeier et al. 2004)

Importantly, about equal numbers of genes showed transcriptional up- and down-regulation among those encoding components of SLIK, SAGA and TFIID, suggesting that the composition of each complex was undergoing adjustment during the transition from fermentative to respiratory growth. This suggests that some aspects of transcript initiation may be subtly different between respiratory growth and fermentative, catabolite-repressed growth. Genes encoding components of the SAGA and SLIK complexes showed more differential regulation than did those specific to TFIID (Table 6; see also Discussion).

Assessment of transcription factor activity with T-profiler

T-profiler is an application that allows estimation of transcription factor activity based on differential expression of genes which possess a consensus sequence for, or are known to be bound by, a particular transcription factor (Zakrzewska et al. 2005). T-profiler analysis of differentially regulated genes at 30 min after the change from YPD to YPG, and in cells grown to early log phase on YPG, revealed a suite of transcriptional effects. These included an increase for many transcription factors that activate expression from genes encoding products involved in respiration or glucose repression (Table 7). Analysis of genes whose expression was down-regulated under these two conditions revealed that most transcription factors which were apparently responsible for these gene expression changes fell into two groups: those recognizing genes encoding rRNA processing enzymes (possessing rRPE (Ribosomal RNA Processing Element) and PAC motifs in their 5' regions) and those functioning during the cell-cycle at START (Mbp1p, Stb1p, Swi4/6p) or G2/M (Fkh2p, Mcm1p, Ndd1p).

Glycerol- and ethanol-based growth induces multiple stress responses

In cells grown to early log phase in glycerol-based medium, genes displaying twofold or more up-regulation of expression were enriched for several GO terms relating to the stress response, including: "response to stress" ($P = 4.46E-05$; binomial distribution), "age-dependent response to oxidative stress" ($P = 6.31E-03$; binomial distribution), "response to salt stress" ($P = 8.6E-03$; binomial distribution). Studies of global gene expression during the diauxic shift demonstrated a

Table 5 Changes in relative transcript levels from selected genes encoding products localized to the cell cortex and plasma membrane during and after the transition from glucose- to glycerol-based medium

Transcript	YPG30[a]	YPG[b]	YPE[c]	Function
Cell cortex				
YNL194C	139.6	348.3***	376.9**	Hypothetical ORF
NUM1	2.29*	4.10***	5.32	Nuclear migration
FMP45	2.55	13.72*	3.25	Found in mitochondrial proteome
Actin cap				
GSC2	1.39	5.32***	7.68*	1,3-β-D-glucan synthase catalytic component
Actin cortical patch (endocytosis)				
YAP1801	1.88	2.12*	2.24	Yeast assembly polypeptide; clathrin cage assembly
INP53	1.49	2.25*	4.40	Inositol polyphosphate 5-phosphatase
YSC84	2.37**	2.73**	3.29	SH3 domain in C-terminus
MYO5	1.58	2.86**	4.53	Myosin I
EDE1	2.38	3.19**	4.92	EH domain protein involved in endocytosis
Plasma membrane				
HXT10	0.41	3.66**	3.70	Putative hexose transporter
HXT5	5.15	166.59**	287.80***	Moderate affinity hexose transporter
STL1	3.48*	561.65***	942.11***	Sugar transporter-like protein
PHO89	3.44	5.04**	7.05*	Na^+/Pi symporter (putative)
GAP1[d]	0.71	7.83*	3.46	General amino acid permease
ARN2[d]	1.18	8.13****	6.94*	Triacetylfusarinine C transporter
HSP30	10.07*	8.82**	20.59**	Induced by heat shock, ethanol and stationary phase
ALP1	0.98	25.10**	16.42*	Basic amino acid permease
HSP12	3.64	28.19*	46.56**	Heat shock protein 12
PUT4	0.60	44.61*	67.03**	Proline specific permease
JEN1	5.23	132.53**	188.25***	Carboxylic acid transporter homolog

Genes listed immediately under "cell cortex" are annotated directly to that GO category; "actin cap" and "actin cortical patch" are child terms of "cellular cortex". All genes encoding products localized to the actin cortical patch are involved in endocytosis (transcript levels given relative to the level in steady-state glucose-based growth)

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$

[a] Relative transcript level at 30 min post-shift from glucose- to glycerol-based medium

[b] Relative transcript during steady-state growth on glycerol-based medium

[c] Relative transcript during steady-state growth on ethanol-based medium

[d] Localized to the endosome

transcript pattern consistent with elicitation of a general stress response (DeRisi et al. 1997; Brauer et al. 2005). Not surprisingly, the rapid shift of yeast from fermentative to glycerol-based growth produced a similar general stress response in our experiments (Table 8). Of genes associated with this response, 12 with verified STRE (STress Response Element) elements were at least twofold up-regulated at 30 min after medium change and during continuous growth in both YPG and YPE. Interestingly, transfer of yeast to YPG also elicited up-regulation of many genes associated with the oxidative and osmotic stress responses. That is, expression of 4/50 oxidative stress genes was induced twofold or more at 30 min after the medium change; expression of 10 of these 50 genes was up-regulated during continuous growth in YPG. Transcripts from 12/59 osmotic stress genes ($P = 0.021$; binomial distribution) were twofold or greater up-regulated, and seven were twofold or more down-regulated, in glycerol-grown cells, with *SIP18*/YMR175W (encodes a protein of unknown function, induced by osmotic stress) transcripts induced 185-fold over the levels during fermentative growth; not all these genes were up-regulated at 30 min after the change to YPG. Although the osmotic and oxidative stress pathways are linked through Skn7p (*SKN7*/YHR206W), a transcription factor which can be activated through either osmotic stress by the HPt protein, the Ypd1p protein kinase (*YPD1*/YDL235C), or by oxidative stress, the osmotic stress response seen during and after transition to YPG did not appear to be an artifact of the strong oxidative stress response. Of the three genes annotated to both osmotic and oxidative stress responses, only one (*HSP12*/YFL014W) was induced during or after the transition to YPG. Striking transcript level changes also were observed from genes whose expression is apparently controlled by Hog1p (*HOG1*/YLR113W; high osmolarity glycerol, protein kinase) throughout the time course and in continuously glycerol- and ethanol-grown cells; i.e., of 47 genes whose expression was fourfold or more down-regulated

Table 6 Changes in relative transcript levels for selected genes encoding products associated with the transcription process during and after the transition from glucose- to glycerol-based medium

Transcript	YPG30[a]	YPG[b]	YPE[c]	Function
Transcription factors				
RRN7	0.26	0.40	0.26	RNA polymerase I transcription
HAC1	0.48	0.15	0.09	Membrane biogenesis
ROX1	0.67	4.98*	7.59*	Repressor of hypoxic genes
GAL4	1.05	5.30***	6.00	Galactose transcriptional activator
POG1	0.93	5.69***	4.76	G0/G1 transition
MAL33	1.26	6.56**	8.10*	Maltose transcriptional activator
GAL3	2.59	8.32****	16.12*	Relieves Gal80 mediated repression
YGR067C	0.60	9.46***	20.60**	Zn^{2+} finger domain is identical to Adr1p
HAP4	1.55	9.75**	18.80**	Positive regulator of respiratory chain
YJL103C	1.13	13.11**	11.92*	Contains a cytochrome *c* binding domain
RNA polymerase subunits				
RPA34	0.43	0.25	0.23	RNA polymerase subunit
RPB10	0.41	0.23	0.19	RNA polymerase subunit ABC10-beta
RPB9	0.50	0.25	0.34	RNA polymerase II subunit B12.6
RPC25	0.44	0.23	0.13	RNA polymerase III subunit C25
Transcriptional coactivators				
GAL11	0.47	2.08*	1.54	Mediator complex
CAF120	0.95	4.50**	5.90*	CCR4-NOT complex
TAF2	1.39	2.50*	2.90	TFIID
SPT15	0.50	0.35	0.34	TFIID
ADA2	1.09	1.82	4.89	SAGA and SLIK complex
HFI1	0.65	0.39	0.22	SAGA and SLIK complex
SPT7	1.78	2.04	2.61	SAGA and SLIK complex
TRA1	1.65	2.16*	2.69	SAGA and SLIK complex
SPT20	1.16	2.43*	3.44	SAGA and SLIK complex
TAF6	1.07	0.43	0.26	TFIID, SAGA, and SLIK complex
TAF9	0.72	0.35	0.41	TFIID, SAGA, and SLIK complex

Transcript levels given relative to the level in steady-state glucose-based growth

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$

[a] Relative transcript level at 30 min post-shift from glucose- to glycerol-based medium

[b] Relative transcript during steady-state growth on glycerol-based medium

[c] Relative transcript during steady-state growth on ethanol-based medium

in a *hog1* mutant, 31 were twofold up-regulated in cells continuously grown in glycerol (Rep et al. 2000); of 20 genes whose expression is decreased in a *hog1* mutant, 14 were twofold down-regulated, and only one 2-fold up-regulated, in cells continuously grown on YPG. Differential expression of most genes that were induced/repressed in cells continuously grown on YPG was apparent by 30 min. Thus, Hog1p appears to be active during the transition to, and continuous growth in, glycerol-based medium. Expression profiles from stress response genes during growth on YPG and YPE were similar (Pearson r^2 = 0.86, 0.94, 0.76, 0.61 for oxidative, osmotic, general, Hog1p-mediated stress response genes, respectively).

Discussion

In the present study, microarray analysis was used to investigate the global pattern of gene expression underlying the transition of the yeast *S. cerevisiae* from glucose-based fermentative growth to growth on glycerol, a respiratory carbon source. Earlier work from other groups had characterized the transcriptional alterations undergone by this organism as it undergoes the diauxic shift, a transition from fermentative growth to respiratory growth using the ethanol produced by the fermentation process (DeRisi et al. 1997; Brauer et al. 2005). The observations presented here demonstrate that many of the transcriptional patterns identified in cells undergoing the diauxic shift are shared by cells adapting to growth on glycerol, another short-chain alcohol. That is, expression of genes encoding proteins that function in translation, DNA replication, and other general systems were down-regulated to reflect the generally slower growth rate of respiring cells compared to that of fermenting cells. Transcriptional up-regulation was identified in gene sets encoding products for the electron transport system and related respiratory energy production systems. Importantly,

Table 7 Identification of transcription factors of putatively higher or lower activity using T-profiler

TF	30 min	YPG	YPE	Function
Associated with up-regulated genes				
Adr1p	NS	1.6E-10	1.1E-07	Activates transcription of *ADH2* gene
Aft2p	NS	1.4E-03	6.0E-05	Iron-regulated transcriptional activator
Cat8p	NS	1.1E-11	1.0E-15	Binds to CSRE of gluconeogenic genes
Hap4p	NS	1.6E-03	6.7E-05	Respiratory chain, TCA, mt r-proteins
Hsf1p	8.6E-06	NS	NS	Heat shock response
Mig1p	NS	6.6E-10	3.4E-03	Glucose repression
Msn2/4p	< 1.0E-15	< 1.0E-15	< 1.0E-15	General stress response
Sip4p	NS	4.7E-02	8.8E-05	Binds to CSRE of gluconeogenic genes
Sko1p	NS	NS	3.6E-02	Osmotic and oxidative stress response
Tbp1p	NS	5.8E-03	3.9E-02	TATA-binding protein
Ume6p	NS	4.9E-06	6.5E-04	Unscheduled meiotic gene expression
Yap5p	NS	4.2E-04	2.9E-02	YAP5 transcription is activated by SBF (G1/S)
Associated with down-regulated genes				
Fkh2p	9.8E-05	NS	NS	G2/M transition
Gln3p	4.3E-03	NS	NS	Glutamate synthesis
Mbp1p	1.3E-04	NS	NS	MBF component, START (G1/S)
Mcm1p	4.2E-03	NS	NS	G2/M transition
Ndd1p	6.4E-05	NS	NS	G2/M transition
PAC	4.8E-04	2.7E-12	4.0E-12	rRNA processing *cis*-element
Reb1p	NS	4.5E-02	NS	RNA pol I enhancer binding protein, cell cycle
Rlm1p	9.3E-03	NS	NS	Cell wall biogenesis
rRPE	6.5E-04	< 1.0E-15	< 1.0E-15	rRNA processing *cis*-element
Stb1p	1.9E-02	NS	NS	Positive regulator of MBF, START (G1/S)
Ste12p	6.0E-05	8.1E-03	2.2E-02	Invasive and pseudohyphal growth
Swi4p	2.7E-09	NS	NS	SBF component, START (G1/S)
Swi6p	2.6E-07	NS	NS	SBF/MBF component, START (G1/S)
Tec1p	3.3E-02	NS	NS	Invasive and pseudohyphal growth

Transcription factors which were associated with genes whose expression is twofold up- or down-regulated 30 min following transition to glycerol-based medium or during continuous growth on glycerol or ethanol are listed. Only significant E values are reported. *NS* not significant

however, and in contrast to the earlier diauxic shift studies, our results indicated that transition of cells from glucose- to glycerol-based growth engendered modulation in the components required for transcriptional preinitiation complex formation, and it elicited differential expression from many genes specifying transcription factors and other transcription-related proteins. Further, the transition to glycerol-based growth involved significant up-regulation of expression from genes encoding products associated not only with the general stress response, as seen in the diauxic shift studies, but also those involved in the osmotic and oxidative stress responses. Thus, the observations presented here identify important new functional groups of genes whose expression is modulated during the transition from fermentative to respiratory growth.

The results presented here allow comparison of the transcriptional processes underlying the adaptation of yeast to respiratory growth on ethanol versus glycerol as sole carbon source. As might be expected, growth in medium containing one of these two short-chain alcohols compared to that of the same strain in medium containing the other elicited modulation of expression in largely overlapping gene sets. Indeed, quantitative analysis of the transcript profile of cells adapting to growth on glycerol as sole carbon source compared to that of cells undergoing the diauxic shift revealed a high degree of similarity among genes differentially expressed under both conditions (up-regulated χ^2=4.14E-125; down-regulated χ^2=1.02E-71). However as noted above, meaningful differences in the transcriptional responses to ethanol- versus glycerol-based growth were identified here, and we reanalyzed the earlier published diauxic shift data to determine whether those differences were present but simply not commented upon in the earlier publications. The reassessment indicated that changes in expression similar to those given here for many genes encoding osmotic stress-related proteins were identifiable in the earlier data sets. However, fewer osmotic stress-related genes were involved in the earlier studies than we identified, and the differential expression values were less extreme than those seen from the same genes in our cells undergoing continuous growth in YPE or YPG. Transcript induction from genes encoding proteins of the osmotic stress response may be a relatively late

Table 8 Changes in relative transcript levels from genes encoding selected components of the general, oxidative, and osmotic stress response

Transcript	YPG30[a]	YPG[b]	YPE[c]	Function
General stress response				
TPS2[f]	1.87	4.04***	2.30	Trehalose-6-phosphate synthase/phosphatase
GSY2	1.89	4.08**	5.31	Glycogen synthase
TSL1[f]	2.16	4.1***	5.07	Regulatory subunit of trehalose-6-phosphate synthase/phosphatase
PGM2	3.54	4.61***	3.02	Phosphoglucomutase
GPH1	1.51	5.82*	6.33*	Glycogen phosphorylase
CTT1[f]	5.03	10.97*	2.51	Catalase T
HSP26	7.71*	17.50*	13.94*	Heat shock protein 26
GAC1	1.87	21.51**	13.96*	Glc7p regulatory subunit
HSP12[d,e,f]	3.64	28.19*	46.56**	Heat shock protein 12
Oxidative stress response				
SCH9	0.52	2.94**	4.43	Controls PKA activity and G1 progression
RIM15	0.73	2.25*	2.89	Cell proliferation in response to nutrients
MCR1	1.22	2.48*	1.81	Involved in ergosterol biosynthesis
GAD1	3.66	8.63**	5.60	Glutamate decarboxylase
GPX1	3.22	20.48**	13.48*	Induced by glucose starvation
Osmotic stress response				
MYO1	3.47**	3.22*	3.95	Myosin type II
HAL1	1.07	3.48***	2.82	Decreases intracellular Na^+ and increases intracellular K^+
AGP2	2.34	4.27*	11.34	Carnitine transporter
SIP18[f]	1.18	184.87*	121.13**	Salt-induced protein

Transcript levels given relative to the level in steady-state glucose-based growth

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

[a] Relative transcript level at 30 min post-shift from glucose- to glycerol-based medium

[b] Relative transcript during steady-state growth on glycerol-based medium

[c] Relative transcript during steady-state growth on ethanol-based medium

[d] Also a member of the oxidative stress response

[e] Also a member of the osmotic stress response

[f] Genes whose expression is down-regulated by loss of Hog1p (Rep et al. 2000)

development during the diauxic shift, and the effect therefore may not have been pronounced enough at the time points examined in the earlier studies to be readily noticeable (DeRisi et al. 1997; Brauer et al. 2005). Overlapping sets of oxidative stress genes were induced in our experiments and in one diauxic shift study (DeRisi et al. 1997); however, while expression from several of the same oxidative stress genes was induced in both, this response was less pronounced than was that of the general stress response. Regardless, the high degree of similarity in expression from genes encoding components of these stress responses between ethanol and glycerol-grown cells suggests that induction of those responses is common to respiratory growth in general and not to growth on, or adaptation to, a specific carbon source.

In contrast to data from the two diauxic shift studies, the experiments presented here identified significant changes in expression from genes encoding components of the TFIID, SAGA, and SLIK complexes. The large number of transcriptional adjustments for genes encoding these proteins in our data set cannot be due solely to the carbon source change from glucose to glycerol, since we saw virtually the same panel of transcript differences in cells grown to early log phase in both YPG and YPE versus those grown in YPD (Pearson r^2=0.75, 0.73, and 0.88 for SAGA, SLIK, and TFIID, respectively). We suspect that the alterations in the composition of these complexes identified here reflect a subtle but real adjustment of the basal transcriptional machinery, an adjustment required for the adaptation to respiratory growth. Transcript level changes in components of the SLIK complex are of particular interest in this respect, since two of those components, Rtg2p and Spt7p (*RTG2*/YGL252C, encodes a sensor of mitochondrial dysfunction; *SPT7*/YBR081C, encodes a component of the SAGA complex) have been linked to aspects of the respiration process; i.e., deletion of the genes encoding these proteins effects retrograde signaling from mitochondria to nucleus and growth on glycerol, respectively (Jazwinski 2005). Clearly, more study will be required to determine whether the subunit composition of SLIK and the other two complexes is different during respiratory growth versus fermentative growth on YPD, and if

so, what differential functions the various subunits play under each growth condition.

The timescale required for adaptation to environmental change was addressed in the studies given here. Importantly, the transcript profile in cells harvested at 60 min after the change from glucose- to glycerol-based medium did not closely resemble that of cells continuously growing in YPG. This clearly indicates that, while dramatic transcript profile changes can and do occur within minutes, the final adaptation to a carbon source change, and presumably to other significant environmental alterations as well, occurs over a much longer time course; i.e., even though the carbon source change was essentially instantaneous in our experiments, transcriptional adaptation to growth on glycerol must require hours as it does during the diauxic shift, where the ethanol concentration in the environment builds gradually. In relation to this contention,our experiments identified many changes in the expression of genes encoding transcription factors, and other proteins related to the transcriptional control system, in cells undergoing the transition from fermentative growth to glycerol-based respiratory growth. More study will be required to define how the order of production of transcriptional governance-related proteins controls the adaptation process.

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