

# Metabolic Adaptation to Nutrients Involves Coregulation of Gene Expression by the RNA Helicase Dbp2 and the Cyc8 Corepressor in *Saccharomyces cerevisiae*

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**ABSTRACT** Cells fine-tune their metabolic programs according to nutrient availability in order to maintain homeostasis. This is achieved largely through integrating signaling pathways and the gene expression program, allowing cells to adapt to nutritional change. *Dbp2*, a member of the DEAD-box RNA helicase family in *Saccharomyces cerevisiae*, has been proposed to integrate gene expression with cellular metabolism. Prior work from our laboratory has reported the necessity of *DBP2* in proper gene expression, particularly for genes involved in glucose-dependent regulation. Here, by comparing differentially expressed genes in *dbp2Δ* to those of 700 other deletion strains from other studies, we find that *CYC8* and *TUP1*, which form a complex and inhibit transcription of numerous genes, corepress a common set of genes with *DBP2*. Gene ontology (GO) annotations reveal that these corepressed genes are related to cellular metabolism, including respiration, gluconeogenesis, and alternative carbon-source utilization genes. Consistent with a direct role in metabolic gene regulation, loss of either *DBP2* or *CYC8* results in increased cellular respiration rates. Furthermore, we find that corepressed genes have a propensity to be associated with overlapping long noncoding RNAs and that upregulation of these genes in the absence of *DBP2* correlates with decreased binding of *Cyc8* to these gene promoters. Taken together, this suggests that *Dbp2* integrates nutrient availability with energy homeostasis by maintaining repression of glucose-repressed, *Cyc8*-targeted genes across the genome.

## KEYWORDS

helicase  
DEAD-box  
metabolism  
*Cyc8*  
*Dbp2*

Rapidly proliferating cells, such as exponentially growing budding yeast *Saccharomyces cerevisiae*, must meet high demands on both energy production and biosynthesis of anabolic precursors. Budding yeast thrive in a wide range of environments with different nutrient availability. In

order to optimize cell growth, yeast can adjust their metabolism in response to nutrient availability to balance the energy production and biomass synthesis. Signaling pathways such as protein kinase A (PKA)/Ras, AMP-activated protein kinase (AMPK)/*Snf1*, and target of rapamycin (TOR) pathways are involved in sensing nutrient availability and regulating metabolic decisions at transcriptional, post-transcriptional, translational, and post-translational levels (Broach 2012; Kayikci and Nielsen 2015). How cells integrate information about environmental nutrients with the gene expression program has long been an active area of research as it not only has huge implications on the chemical industry, but also sheds light on metabolism in human diseases (Diaz-Ruiz *et al.* 2011; Borodina and Nielsen 2014).

The budding yeast *S. cerevisiae* preferentially uses glucose as a carbon source. In the presence of high levels of glucose, yeast cells prefer to ferment glucose into ethanol instead of respiration despite the presence of oxygen (Broach 2012). In the presence of glucose, genes involved in gluconeogenesis, respiration, and utilization of alternative carbon sources are repressed; a process referred to as carbon catabolite or glucose

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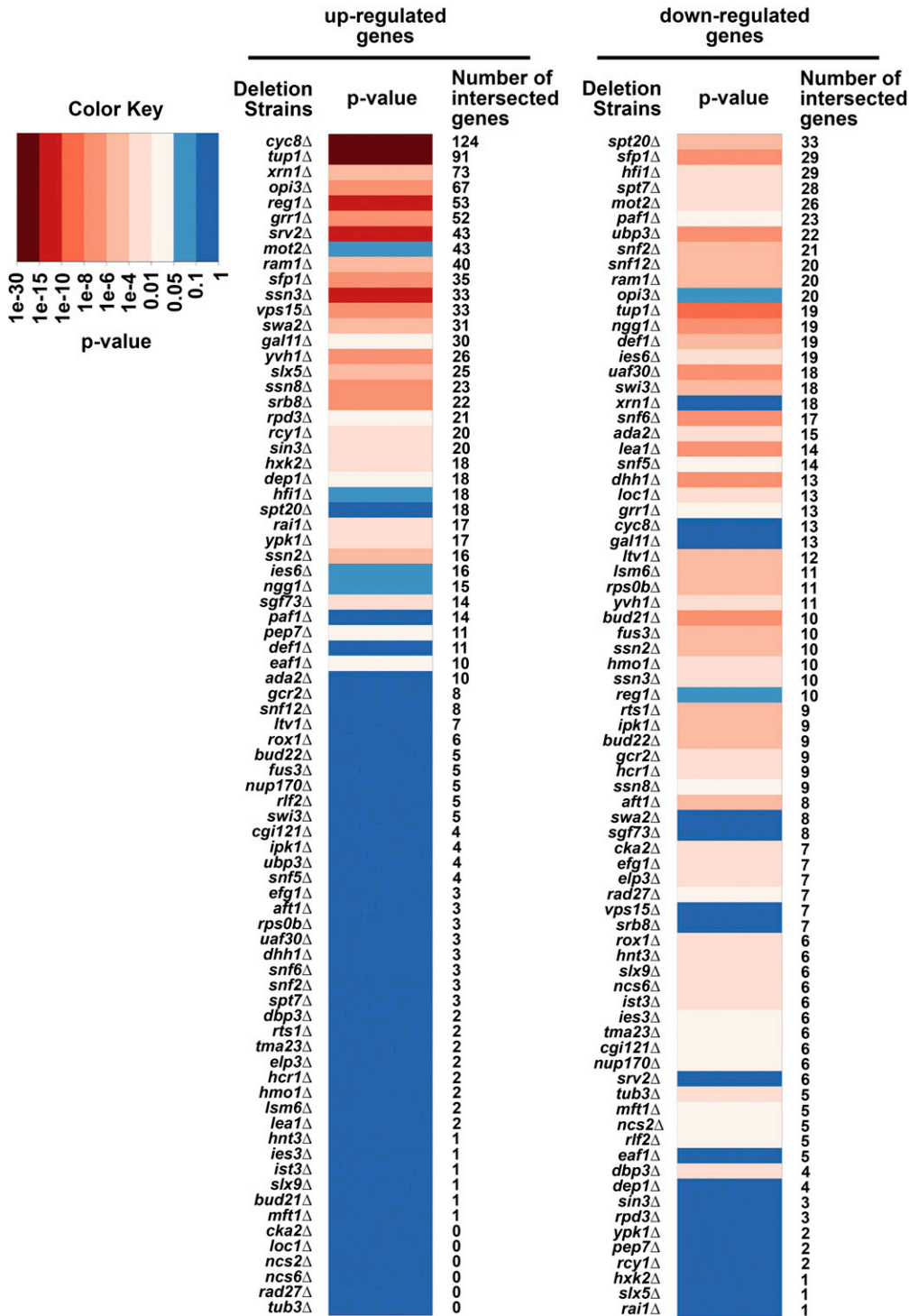
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## Deletion strains sharing common regulated genes with *dbp2Δ*



**Figure 1** *Dbp2* and other gene regulatory factors share coregulated genes. A heatmap of *P*-values of the transcriptional profile comparisons between *dbp2Δ* and other deletion strains is shown and sorted by the number of intersected genes. Lists of DEGs in *dbp2Δ* were compared to that of 700 deletion strains (Beck *et al.* 2014; Kemmerer *et al.* 2014). One-sided Fisher's exact test was used to estimate the significance of the observed intersection (Bonferroni correction). Common genes between the two studies (Beck *et al.* 2014; Kemmerer *et al.* 2014) were used as background. SGGs (O'Duibhir *et al.* 2014) were filtered from the DEG lists to help identify more direct effects of the deleted genes. A heatmap of the *P*-values indicating significant similarities ( $P \leq 0.05$ , light to dark red; color key) or no similarities ( $P > 0.05$ , light to dark blue; color key) of the coregulated genes between deletion strains and *dbp2Δ* was generated. Deletion strains sharing significant up- and/or down-regulated genes with *dbp2Δ* are included in the heatmap. The heatmap was sorted by the number of intersected up- (left) or down-regulated (right) genes between *dbp2Δ* and the number of intersected genes in each comparison is listed on the right-hand side of each heatmap.

repression (Gancedo 1998; Kayikci and Nielsen 2015). Glucose repression depends on transcription factor Mig1 and the *Cyc8-Tup1* corepressor complex as well as the inactivation of the kinase *Snf1* (Gancedo 1998; Kayikci and Nielsen 2015). However, budding yeast has the capability to use alternative fermentable carbon sources, such as galactose and sucrose; or nonfermentable carbon sources, such as

ethanol and acetate (Schüller 2003; Turcotte *et al.* 2010). A shift from glucose to a nonfermentable carbon source leads to reprogramming of gene expression such that gluconeogenic, respiratory, and alternative carbon-source utilization genes are induced (DeRisi *et al.* 1997; Soontornngun *et al.* 2007). This switch is achieved through transcriptional activators such as *Cat8* and the *Hap2/3/4/5* complex as well as

■ **Table 1** Yeast strains

Strains	Genotype	Source
Wild type (BY4741)	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Open Biosystems
<i>dbp2Δ</i>	MATa <i>dbp2::KanR ura3Δ0 leu2Δ0 his3Δ0 TRP1 met- lys?</i>	Cloutier et al. (2012)
CYC8-3xFLAG	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 CYC8-3xFLAG</i>	Cloutier et al. (2013)
<i>dbp2Δ</i> CYC8-3xFLAG	MATa <i>dbp2::HygB his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 CYC8-3xFLAG</i>	Cloutier et al. (2013)
<i>cyc8Δ</i>	MATa <i>cyc8::KanR his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Open Biosystems
<i>tup1Δ</i>	MATa <i>tup1::KanR his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Open Biosystems
<i>dbp3Δ</i>	MATa <i>dbp3::KanR his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Open Biosystems

the activation of the kinase Snf1 (Schüller 2003; Broach 2012). Activated Snf1 influences transcription in many aspects, including phosphorylating the repressor Mig1 (Treitel et al. 1998; Broach 2012). The phosphorylation of Mig1 leads to its relocalization from the nucleus to the cytoplasm, thus derepressing its target, glucose-repressed genes (DeVit et al. 1997; DeVit and Johnston 1999).

Dbp2, a member of the DEAD-box RNA helicase family, functions as a key player in gene expression programs related to cellular metabolism in *S. cerevisiae* (Cloutier et al. 2012; Beck et al. 2014). We have previously reported that the nuclear localization of Dbp2 is dependent on glucose availability, suggestive of a broad role in regulating gene expression in response to nutrient availability (Beck et al. 2014). However, the mechanism of Dbp2 in regulating a specific cellular pathway was unknown. Herein, we show that Dbp2 corepresses Cyc8-targeted genes, representing a major branch of the metabolic pathway, and that loss of *DBP2* correlates with derepression of respiration and gluconeogenesis. This suggests that Dbp2 integrates nutrient availability with metabolic adaptation through regulation of Cyc8-targeted genes.

## MATERIALS AND METHODS

### Bioinformatics analyses

**RNA sequencing data processing and differential gene expression analyses:** RNA sequencing (RNA-seq) data were previously deposited in the Gene Expression Omnibus (GSE58097; Beck et al. 2014). Color space reads were mapped to the yeast reference genome (R64-2-1) using the SHort Read Mapping Program 2 (SHRIMP2, version 2.2.3) with the option “-single-best-mapping” (David et al. 2011). The aligned reads were processed as previously described (Cloutier et al. 2016) with the following modifications. Briefly, the aligned reads were imported into R using Rsamtools and GenomicAlignments (Morgan et al. 2010; Lawrence et al. 2013; R Development Core Team 2016). The first mapped read in a proper pair (flags = 83, 99) with a mapping quality (mapq)  $\geq 40$  was retained. Reads overlapping rDNA regions were excluded from the analysis. Remaining reads were counted against the genomic features using GenomicAlignments from Bioconductor to determine the number of reads for sense RNA transcripts (Gentleman et al. 2004; Lawrence et al. 2013). The mode of “IntersectionNotEmpty” was used for the summarizeOverlaps function. EdgeR was used to determine differential gene expression (Robinson et al. 2010; McCarthy et al. 2012; GSE99097). Genes with very low counts (<10) across all samples were filtered from the analysis. Transcripts with gene expression change >2.2-fold and FDR  $\leq 0.01$  were determined as differentially expressed.

**Comparison of differentially expressed genes in *dbp2Δ* and other strains with genetic perturbations:** The gene expression profiles of 700 responsive deletion mutants identified in Kemmeren et al. (2014) that have at least four differentially expressed transcripts compared to

the wild-type strain were used for transcription profile analysis. As previously described, a cutoff of fold change (FC) > 1.7 and  $P < 0.05$  was applied to determine the list of significantly changed genes of each of the 700 deletion mutants. To look for the genes that may act in concert with *DBP2* in gene expression, the list of up- and down-regulated genes in each of the 700 deletion mutants was compared to the up- and downregulated sense (protein-coding) transcripts in *dbp2Δ*. In addition, the *dbp2Δ* strain has a slow growth phenotype. This phenotype was also observed for many other deletion mutants, and a slow growth signature was identified (O’Duibhir et al. 2014). The slow growth-related genes (SGGs) ( $n = 633$ ) identified from O’Duibhir et al. (2014) were filtered from the lists of differentially expressed genes (DEGs) to avoid identifying genes that are affected by the slow growth phenotype. One-sided Fisher’s exact tests were conducted for each comparison using R. Bonferroni correction was applied to correct for multiple comparisons. Lists of genes that have an effect similar to *DBP2* ( $P \leq 0.05$  after correction) in repressing (share common upregulated genes between *dbp2Δ* and the deletion mutant) or activating (share common downregulated genes between *dbp2Δ* and the deletion mutant) gene expression were extracted and  $P$ -values from the Fisher’s exact tests are presented as a heatmap (Figure 1).

**Comparison of DEGs in *dbp2Δ* and upon glucose deprivation:** RNA-seq data of wild-type cells cultivated in the presence or absence of glucose were retrieved from the NCBI’s Gene Expression Omnibus (GSE43747; Freeberg et al. 2013). Data were processed as previously described (Freeberg et al. 2013) with the following modifications. First, nucleotides with low quality (<30) were trimmed from the end of the reads using FASTX-Toolkit ([http://hannonlab.cshl.edu/fastx\\_toolkit/](http://hannonlab.cshl.edu/fastx_toolkit/)). Next, sequences of the barcodes and adaptors were trimmed from the reads using Cutadapt (Martin 2011). Reads <18 nt were removed at the same time. The processed reads were mapped to the reference yeast genome (R64-2-1) using Tophat and Bowtie2 (Trapnell et al. 2009; Langmead and Salzberg 2012). The aligned reads were read in R using Rsamtools (Morgan et al. 2010). Only reads with mapq  $\geq 40$  were kept. Read counts per feature were determined using GenomicAlignments from Bioconductor (Gentleman et al. 2004; Lawrence et al. 2013). EdgeR was used to determine differential gene expression between strains grown in the glucose-replete or -deprived media (Robinson et al. 2010; McCarthy et al. 2012). Genes with very low counts (<10) across all samples were filtered. A cutoff of FC > 2.2 and FDR  $\leq 0.01$

■ **Table 2** Oligos used for plasmid construction

	Sequence
SmaI-CYC8 F	TCCCCGGGGCTAAAGCACATCCGATCTGAG
BamHI-CYC8 R	CGCGGATCCCGTAGAACCCAAAGCATTAGG
SmaI-TUP1 F	TCCCCGGGGTAAGTCTGCGGAATCGATCTG
SpeI-TUP1 R	GGACTAGTAAGTGCAGCTGGACGAATC

■ **Table 3** Oligos used for Northern blotting (dsDNA probes)

	Sequence
PCK1 F	ATGGTCTATCAACCGTGAAGAGC
PCK1 R	TCAACAATCTATGTGGTCTGCG
SFC1 F	CATCCAGCCATCAATCTCATGG
SFC1 R	TAGTATCCAATGGAGCGTTGGA
JEN1 F	CTGGTGAACAGCAACAACCTG
JEN1 R	GGTGCATCTTCAATCGCTGTT
YAT1 F	ATACATCGAGCAGTTCTGGTATGAC
YAT1 R	GGATCGAGTCTGTGTAGATGTCTG
SCR1 F	GGATACGTTGAGAATTCTGGCCGAGG
SCR1 R	AATGTGCGAGTAAATCCTGATGGCACC

was used to determine the significant change in gene expression. In addition, as the cells used for the RNA-seq above were treated with 4sU, the effects of 4sU were removed by removing genes that are significantly changed upon 4sU treatment ( $FC > 2.2$  and  $FDR \leq 0.01$ ,  $n = 41$ ) from the DEG lists upon glucose deprivation (Freeberg *et al.* 2013). SGGs were then filtered from the DEG lists. One-sided Fisher's exact tests were used to estimate the significance of the intersection between the lists of DEGs in *dbp2Δ* and that upon glucose deprivation. Venn diagrams were generated by the online program BioVenn (Hulsen *et al.* 2008).

**Functional annotation clustering analysis:** Functional annotation clustering of biological processes was conducted using DAVID (version 6.7) (Huang *et al.* 2009a,b). Functional clusters with enrichment score  $\geq 1.3$  (equivalent to  $P \leq 0.05$ ) are shown.

## Experimental procedures

**Plasmids and strains:** Yeast strains were constructed using classical yeast genetics and are listed in Table 1. To construct the *CYC8/CEN* plasmid, a DNA fragment with 888 bp upstream and 460 bp downstream of the *CYC8* open reading frame (ORF) was produced with 5' *Sma*I and a 3' *Bam*HI restriction sites flanking the fragment using genomic DNA as templates. The fragment was then cloned into *pRS315* using the standard molecular cloning method. The *TUP1/CEN* plasmid was constructed in the same way except that the inserted DNA fragment was flanked with 1000 bp upstream and 499 bp downstream of the *TUP1* ORF with 5' *Sma*I and 3' *Spe*I restriction sites. Oligos used for cloning are listed in Table 2.

**Respiration profile measurements:** The oxygen consumption assays were conducted as described (Yao *et al.* 2016). Briefly, yeast strains were grown to log phase in YPD and shifted to assay medium (1.67% yeast nitrogen base, 0.5% ammonium sulfate, and 2% ethanol or glucose). Cells were seeded in the 24-well microplate (Seahorse Bioscience) at  $5 \times 10^5$  cells per well and incubated at 30° for 1 hr. The oxygen consumption rates were measured using the Seahorse XF<sup>e</sup>24 analyzer. The *cyc8Δ* and *tup1Δ* strains were confirmed with plasmid-based complementation.

■ **Table 4** PrimeTime assays for ChIP

Name	Forward	Reverse	Probe
PCK1	ATTATGGATAGCGGATAAAGGG	ACGTACCATTGTCCAACCAG	CCCAAACAGGATTGTAAAGCTTAGACGC
SFC1	CTGCTCGAGGTGCTATCTTTT	TGTGACATTACGGTTTGATAAAGG	CTTTGAGATTCTTGTGCGCCACGGAGT
YAT1	GCGGAATGACAAAACCATCAG	TCCTTGCCCTTTACTTTGG	CACGATCTCTCCAGTCACAATGGCAA
JEN1	CTTCTGCACACCATCCGG	ACCAGGTAACGTTCTAATGCAT	TCTTCACTTAACGGTCTTTTGCCCC

Briefly, yeast cells were grown in SC-LEU media to midlog phase and spotted on the SC-LEU agar plates in fivefold serial dilutions. Plates were incubated at 37° until wild-type cells were fully grown.

**Gene induction analyses:** Yeast cells were first grown at 30° to an  $OD_{600}$  of 0.4–0.6 in SC with 2% glucose medium. The cells were then spun at 3000 rpm, washed twice with SC and transferred to SC medium containing 1% potassium acetate and grown for another 6 hr. 15 OD units were collected prior to (0 min) and at 30, 60, 90, 120, 240, and 360 min after the nutritional shift. Cells were flash frozen and stored at –80° before use.

**RNA isolation and Northern blotting:** RNA was isolated from cells using standard acid phenol purification. 30 μg total RNA was resolved on a 1.2% formaldehyde-agarose gel and transferred to a nylon membrane (Brightstar Hybond N<sup>+</sup>, Invitrogen). Northern blotting was performed as previously described (Cloutier *et al.* 2012). Radiolabeled double-stranded DNA (dsDNA) probes were generated using PCR products using wild-type genomic DNA as a template. Oligos used for Northern blotting probes are listed in Table 3. Target transcripts were visualized using a PhosphorImager (GE Healthcare) and quantified by densitometry using ImageQuant (GE Healthcare).

**Chromatin immunoprecipitation:** Chromatin immunoprecipitation (ChIP) was performed as described previously (Cloutier *et al.* 2013) with the following modifications. Chromatin from  $\sim 5 \times 10^6$  cells was immunoprecipitated with 2 μl of FLAG M2 monoclonal antibody (F3165; Sigma) and 24 μl of Protein G Dynabeads (30 mg/ml; Invitrogen) at 4° for 2 hr. Immunoprecipitated DNA was isolated and quantitative PCR (qPCR) was performed using PrimeTime Assay primers listed in Table 4 and TaqMan qPCR mix (Invitrogen). All ChIP experiments were performed with three biological replicates. Error bars represent the SD from the mean.

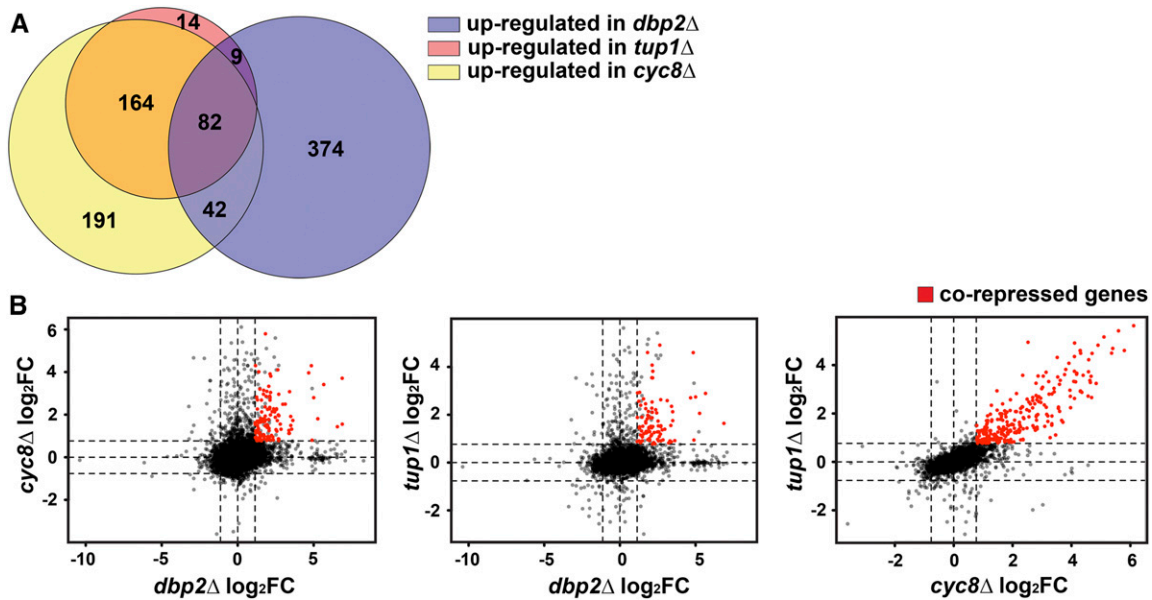
## Data availability

Data reference numbers deposited in the Gene Expression Omnibus are as follows: GSE58097 (Beck *et al.* 2014), GSE42527 (Kemmeren *et al.* 2014), and GSE43747 (Freeberg *et al.* 2013), GSE99097.

## RESULTS

### Analysis of transcription profiles reveals similarities between DBP2 and other gene regulatory factors

Nutritional status and gene expression programs are highly integrated in yeast (Broach 2012). Gene expression profiling has revealed that genes necessary for nutrient utilization and metabolism require *DBP2* for normal expression (Beck *et al.* 2014). This specificity suggests that *Dbp2* may function similar to or in concert with known transcription factors involved in nutrient utilization. A recent study of 1484 yeast deletion strains used gene expression profiling by microarray to identify 700 genes that affect gene expression in *S. cerevisiae* (Kemmeren *et al.* 2014). To test our hypothesis, we compared lists of DEGs in *dbp2Δ* to



**Figure 2** *TUP1* and *CYC8* share corepressed genes with *DBP2*. (A) Venn diagram showing the numbers of genes upregulated upon loss of *CYC8*, *TUP1*, and *DBP2*. The Venn diagram was generated by BioVenn (Hulsen *et al.* 2008). SGGs were removed from the gene lists. (B) *CYC8* and *TUP1* repress a common subset of genes with *DBP2*, but to a different extent. The  $\log_2FC$  of all genes except SGGs in *cyc8Δ* and *tup1Δ* was plotted against that of *dbp2Δ*, as well as of each other. Genes that are significantly upregulated between *dbp2Δ* and *cyc8Δ* (left), *dbp2Δ* and *tup1Δ* (middle), or *cyc8Δ* and *tup1Δ* (right) are colored in red. Dashed lines mark the  $\log_2FC$  cutoff in *dbp2Δ* [ $\pm\log_2(2.2)$ ], *cyc8Δ*, and *tup1Δ* [ $\pm\log_2(1.7)$ ].

those for each of the 700 strains from this study. Importantly, as the *dbp2Δ* strain has a slow growth phenotype that is also observed for other deletion strains (O'Duibhir *et al.* 2014), we removed SGGs from the DEG lists to avoid analysis of indirect effects from the growth defects (O'Duibhir *et al.* 2014). The remaining DEGs were then compared and one-sided Fisher's exact tests were used to determine the significance of the observed intersection of the lists between *dbp2Δ* and other deletion strains. This analysis identified 77 deletion strains that have similar up- or downregulated genes to *dbp2Δ* (Figure 1,  $P$  value  $\leq 0.05$  after a Bonferroni correction). Interestingly, strains harboring the deletion of *CYC8* or *TUP1* share the most upregulated genes with *dbp2Δ*, with *cyc8Δ* and *tup1Δ* sharing 124 and 91 upregulated ones with *dbp2Δ*, respectively (Figure 1, left). *Cyc8* and *Tup1* form a heteropentameric complex required for transcriptional repression of various genes, including glucose-repressed genes, cell-type-specific genes and hypoxic genes (Williams and Trumbly 1990; Keleher *et al.* 1992; Balasubramanian *et al.* 1993; Varanasi *et al.* 1996; Smith and Johnson 2000). Whereas *tup1Δ* significantly shares both up- and downregulated

genes with *dbp2Δ*, *cyc8Δ* only significantly shares upregulated genes. This suggests that *TUP1* and *CYC8* may not always act in concert.

Besides *tup1Δ*, only a small subset of deletion strains that share similar upregulated genes with *dbp2Δ* also share downregulated genes. These include strains harboring deletions of the RNA polymerase II (RNAPII) mediator complex (*SSN2*, *SSN3*, *SSN8*), a component of SCF ubiquitin-ligase complex (*GRR1*), ribosome biosynthesis genes (*SFP1*, *YVH1*), and a component of the CAAX farnesyltransferase complex (*RAM1*) (Figure 1, left and right) (He *et al.* 1991; Hengartner *et al.* 1995; Skowrya *et al.* 1997; Marion *et al.* 2004; Kemmler *et al.* 2009; Lo *et al.* 2009). We also observed common upregulated genes between *dbp2Δ* and strains with deletions in components of the Rpd3 histone deacetylase complex, such as *RPD3*, *SIN3*, and *DEP1*, as well as RNA decay factor *XRN1* (Figure 1, left) (Larimer and Stevens 1990; Hengartner *et al.* 1995; Carrozza *et al.* 2005). This suggests that *DBP2* represses gene expression through regulating transcription and/or RNA stability. Interestingly, *SSN3*, *SSN8*, and *SRB8* are involved in glucose repression (Balciunas and Ronne 1995). Several other genes required for glucose

■ **Table 5** Gene corepressed by *CYC8*, *TUP1*, and *DBP2* (82)

Annotation Cluster	Enrichment Score <sup>a</sup>	Description of GO Terms <sup>b</sup>	Unique Genes <sup>c</sup>	Gene Count <sup>d</sup>
1	2.35	Monosaccharide metabolic process	<i>GAC1</i> , <i>ERR3</i> , <i>GAL3</i> , <i>YHR210C</i> , <i>NDE2</i> , <i>YNR071C</i> , <i>PCK1</i> , <i>SGA1</i>	8
2	2.07	Regulation of transcription	<i>ROX1</i> , <i>GAC1</i> , <i>YOR338W</i> , <i>RPM2</i> , <i>CSR2</i> , <i>VHR1</i> , <i>YAP6</i> , <i>REG2</i> , <i>SMP1</i> , <i>MIG1</i> , <i>THI2</i> , <i>RGM1</i> , <i>SUT1</i> , <i>HAL1</i> , <i>GAL3</i> , <i>YPR196W</i> , <i>ACA1</i> , <i>CUP9</i> , <i>GIS1</i> , <i>POG1</i> , <i>ASK10</i>	21

Table of GO term clusters of *CYC8*, *TUP1*, and *DBP2* commonly repressed genes. Functional annotation clustering analysis was conducted with the 82 genes corepressed by *CYC8*, *TUP1*, and *DBP2* in Figure 2A.

<sup>a</sup>GO clusters with significant enrichment score of at least 1.3 ( $P \leq 0.05$ ).

<sup>b</sup>GO term descriptions for the corresponding functional annotation cluster.

<sup>c</sup>Unique genes included in each GO cluster.

<sup>d</sup>Number of unique genes in each cluster.

■ **Table 6 Genes corepressed by *CYC8* and *DBP2* (124)**

Annotation Cluster	Enrichment Score	Description of GO Terms	Unique Genes	Gene Count
1	2.31	Phosphate metabolic process/oxidative phosphorylation/ion transport	<i>ATP1, ATP6, COX9, CYC1, COX8, QCR6, ATP19, ATP18, GAL3, MRK1, VHS1, KIN82, FRK1, SKS1, SFC1, JEN1, FRE7</i>	17
2	1.87	Generation of precursor metabolites and energy/cell death	<i>ATP1, ATP6, COX9, CYC1, COX8, QCR6, ATP19, ATP18, GAC1, YBR238C, ERR3, ISF1, NDE2, YJL045W, SGA1, FRE7</i>	16
3	1.64	Regulation of transcription	<i>ROX1, GAC1, YOR338W, RPM2, CSR2, VHR1, YAP6, REG2, SMP1, MIG1, THI2, MIG2, RGM1, SUT1, HAL1, SKS1, GAL3, YPR196W, ACA1, SOK2, CUP9, MET32, GIS1, POG1, ASK10</i>	25
4	1.63	Sulfur metabolic process	<i>THI2, MET17, THI22, SNZ1, VHR1, MET32, ADI1, PET18, YAT1</i>	9
5	1.58	Carbohydrate transport	<i>MAL11, STL1, HXT4, HXT1, SKS1</i>	5
6	1.55	Monosaccharide metabolic process	<i>GAC1, ERR3, GAL3, YHR210C, NDE2, YNR071C, PCK1, SGA1</i>	8

Table of GO term clusters of *CYC8* and *DBP2* commonly repressed genes. Functional annotation clustering analysis was conducted with the 124 genes corepressed by *CYC8* and *DBP2* in Figure 2A.

repression, such as *REG1*, *GRR1*, and *HXX2*, were also identified as similar to *DBP2* (Figure 1, left) (Flick and Johnston 1991; Gancedo 1998). The overlap between genes repressed by *DBP2* and those repressed by factors involved in glucose repression is consistent with a link between *DBP2* and glucose metabolism (Beck *et al.* 2014).

#### ***CYC8* and *TUP1* share corepressed genes with *DBP2***

The *GAL* protein-coding genes are part of the galactose metabolic switch that allows yeast cells to use galactose when glucose is absent (Sellick *et al.* 2008). Our prior studies demonstrated that *Dbp2* maintains *Cyc8*-dependent repression of the *GAL* cluster genes (Cloutier *et al.* 2013). The fact that deletion of *CYC8* or *TUP1* also results in a similar set of derepressed genes as loss of *DBP2*, however, suggests that this role may be more widespread than just at this locus. To see how many genes are commonly upregulated between *dbp2Δ*, *cyc8Δ*, and *tup1Δ*, a Venn diagram was plotted using the lists of upregulated genes in each deletion strain. This revealed that 82 genes are corepressed by *CYC8*, *TUP1*, and *DBP2* (Figure 2A). To determine if loss of *CYC8* or *TUP1* affects gene expression to a similar extent as loss of *DBP2*, we plotted the  $\log_2FC$  of all genes in *dbp2Δ* against those of *cyc8Δ* and *tup1Δ* (Figure 2B). Consistent with a function in gene repression, the majority of the common DEGs in *dbp2Δ* and *cyc8Δ* (Figure 2B, left) or *dbp2Δ* and *tup1Δ* (Figure 2B, middle) are upregulated in the mutants. However, the common upregulated genes between *dbp2Δ* and *tup1Δ* or *dbp2Δ* and *cyc8Δ* do not show a linear relationship. This is in contrast to

*TUP1* and *CYC8*, whose corepressed genes show a linear relationship in the deletion strains (Figure 2B, right). This suggests that even though *TUP1* and *CYC8* share corepressed genes with *DBP2*, *Dbp2* and *Cyc8* or *Tup1* act in a biochemically distinct manner. Alternatively, the lack of correlation could be due to the different methods of gene expression profiling, *i.e.*, microarray *vs.* RNA-seq (Zhao *et al.* 2014). We also observed that many genes are specifically repressed by *CYC8* but not *TUP1* [Figure 2B, right; *cyc8Δ*  $\log_2FC > \log_2(1.7)$  and *tup1Δ*  $\log_2FC < \log_2(1.7)$ ], suggesting that these two factors may not always function in concert. This is consistent with a prior study showing additive effects from simultaneous deletion of *CYC8* and *TUP1* (Williams and Trumbly 1990).

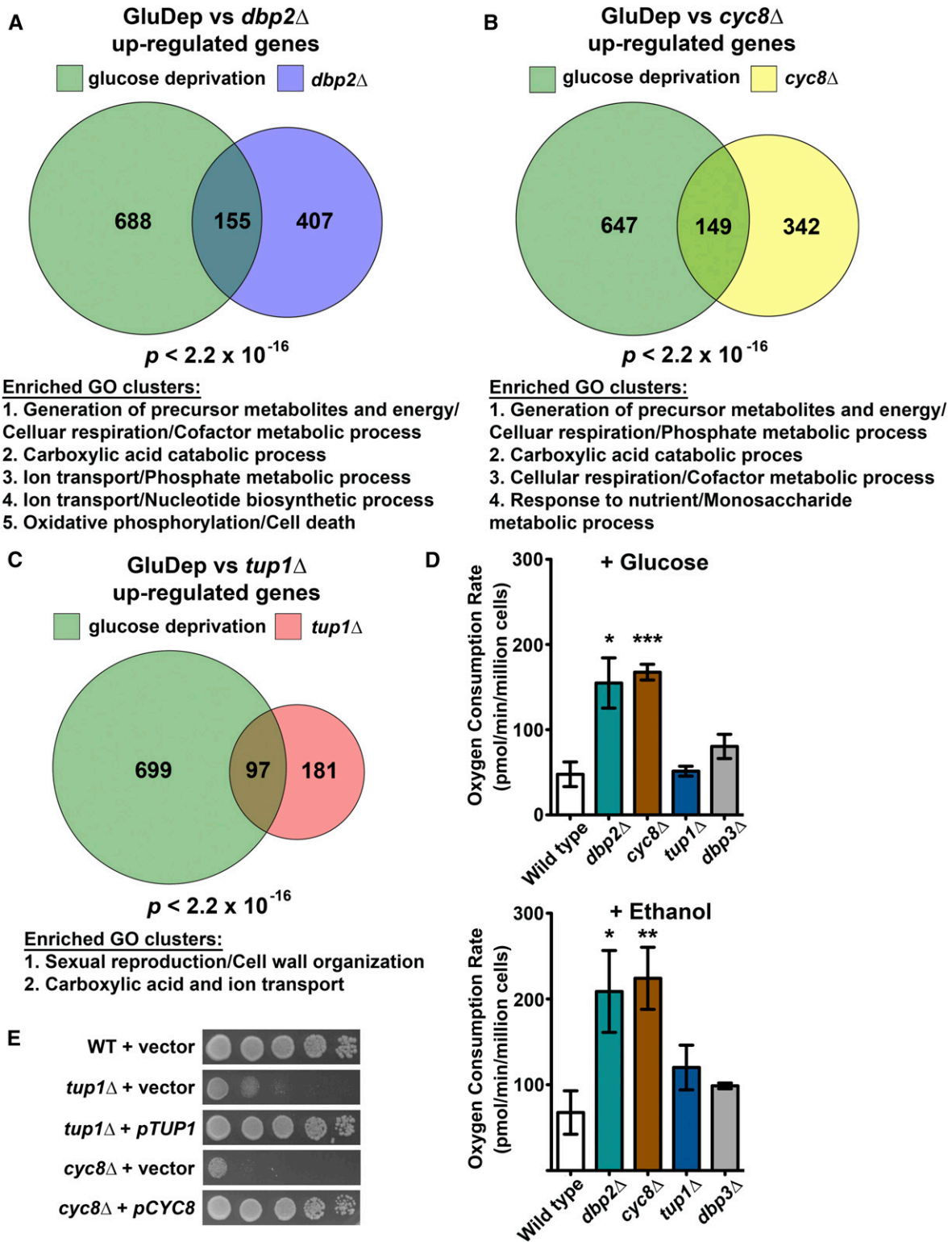
#### ***CYC8*, *TUP1*, and *DBP2* corepressed genes are enriched in monosaccharide metabolism and transcriptional regulation processes**

To determine if genes corepressed by *CYC8*, *TUP1*, and *DBP2* fall into common biological processes, we conducted functional annotation clustering analyses using DAVID (Huang *et al.* 2009a,b). This revealed enrichment of transcripts whose products function in monosaccharide metabolic processes or regulation of transcription (Table 5 and Supplemental Material, Table S1). Interestingly, *MIG1*, *THI2*, *ROX1*, and *SMP1*, which have been shown to regulate genes involved in metabolism and stress response, were also identified (Nehlin *et al.* 1991; Zitomer and Lowry 1992; de Nadal *et al.* 2003; Nosaka *et al.* 2005).

■ **Table 7 Genes corepressed by *TUP1* and *DBP2* (91)**

Annotation Cluster	Enrichment Score	Description of GO Terms	Unique Genes	Gene Count
1	3.30	Monosaccharide metabolic process	<i>GAC1, ERR3, TOS3, GAL3, YHR210C, NDE2, PFK27, YNR071C, PCK1, SGA1</i>	10
2	3.12	Regulation of transcription	<i>ROX1, GAC1, YOR338W, RPM2, CSR2, VHR1, YAP6, REG2, SMP1, MIG1, THI2, RGM1, SUT1, HAL1, GAL3, ZNF1, YPR196W, ACA1, CUP9, GIS1, POG1, ASK10</i>	22
3	1.55	Regulation of transcription from RNAPII promoter	<i>ROX1, GAC1, YOR338W, RPM2, CSR2, VHR1, YAP6, REG2, SMP1, MIG1, THI2, RGM1, SUT1, HAL1, GAL3</i>	15

Table of GO term clusters of *TUP1* and *DBP2* commonly repressed genes. Functional annotation clustering analysis was conducted with the 91 genes corepressed by *TUP1* and *DBP2* in Figure 2A.



**Figure 3** *DBP2* and *CYC8*, but not *TUP1*, are linked to cellular respiration. (A–C) Glucose repressed genes are enriched in *DBP2* (A), *CYC8* (B), and *TUP1* (C) targets. A Venn diagram showing the number of genes upregulated in *dbp2*Δ, *cyc8*Δ, *tup1*Δ, and upon glucose deprivation for 2 hr is shown (Freeberg et al. 2013). The *P*-value originates from one-sided Fisher's exact tests conducted to estimate the significance of the intersection. Descriptions of enriched GO clusters for the intersected genes in each Venn diagram are listed below each of the Venn diagrams. See Table S4, Table S5, and Table S6 for complete results of the functional annotation clustering analysis. (D) Loss of *DBP2* or *CYC8* leads to increased respiration, even in the presence of glucose. Oxygen consumption in wild-type cells and indicated mutant cells was measured using the Seahorse XF<sup>24</sup> analyzer in the presence of 2% glucose or 2% ethanol. The *dbp3*Δ strain harbors a deletion of the DEAD-box protein Dbp3 involved in rRNA processing, and serves as a negative control. Data show the mean ± SD of three independent biological replicates. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001. (E) Ectopic

This suggests that *CYC8*, *TUP1*, and *DBP2* may regulate cellular metabolism and response to stress, possibly through regulating gene expression.

*DBP2* also shares unique corepressed genes with *CYC8* and *TUP1*, individually (Table 6 and Table 7 and Table S2 and Table S3). Out of the 124 genes corepressed by *CYC8* and *DBP2*, we observed enrichment of six functional annotation clusters including oxidative phosphorylation and ion transport, generation of precursor metabolites and energy and cell death, regulation of transcription, carbohydrate transport, and transmembrane transport (Table 6 and Table S2). The *TUP1* and *DBP2* corepressed genes ( $n = 91$ ), on the other hand, showed enrichment of clusters including monosaccharide metabolic processes and regulation of RNAPII transcription (Table 7 and Table S3). This suggests that *Cyc8* and *Tup1*, although thought to act exclusively as a complex, may have different functions individually in gene regulation. This is consistent with our prior observation that loss of *CYC8* and *TUP1* has different effects at some different gene loci (Figure 2B, right).

### DBP2 and CYC8, but not TUP1, repress cellular respiration

Yeast cells preferentially use glucose as a carbon source for energy production through aerobic fermentation (Broach 2012). The presence of glucose suppresses respiration, gluconeogenesis, and usage of non-fermentable carbon sources, referred to as carbon catabolite or glucose repression (Gancedo 1998; Broach 2012). It is known that *CYC8* and *TUP1* function as key components in glucose repression (Gancedo 1998). To determine if *DBP2* is also required for glucose repression, we estimated the degree of overlap between genes upregulated in *dbp2Δ* and upon glucose deprivation by comparing the list of genes from our *dbp2Δ* RNA-seq to a previously published study of glucose-deprived wild-type cells (Freeberg *et al.* 2013). This revealed an overrepresentation of glucose-repressed genes in *dbp2Δ* cells (Figure 3A, one-sided Fisher's exact test,  $P < 2.2 \times 10^{-16}$ ). We also compared upregulated genes in *cyc8Δ* or *tup1Δ* to those in glucose-deprived cells (Figure 3, B and C). In agreement with roles for *CYC8* and *TUP1* in glucose repression, carbon catabolite-repressed genes are also overrepresented in both *cyc8Δ* and *tup1Δ* cells (Figure 3, B and C). Interestingly, functional annotation clustering revealed several commonly repressed gene categories between glucose-deprived cells and *dbp2Δ* (Figure 3A and Table S4) or *cyc8Δ* (Figure 3B and Table S5), including generation of precursor metabolites and cellular respiration. In contrast, genes upregulated in *tup1Δ* and glucose-deprived cells are enriched in sexual reproduction, cell wall organization, and carboxylic acid and ion transport categories (Figure 3C and Table S6). This suggests that *Cyc8* and *Dbp2* may modulate glucose repression of different cellular pathways than *Tup1*.

To test whether *DBP2*, *CYC8*, and/or *TUP1* repress respiration in the presence of glucose, we measured the oxygen consumption rate in wild-type, *dbp2Δ*, *cyc8Δ*, and *tup1Δ* cells as a readout for cellular respiration (Figure 3D). Strains harboring the *CYC8* or *TUP1* deletion were obtained from the null collection and were confirmed by PCR (data not shown) and plasmid-based complementation (Figure 3E). We also assayed the oxygen consumption in the absence of *DBP2*, an unrelated DEAD-box protein involved in rRNA processing (Weaver *et al.* 1997). Briefly, yeast cells were cultivated in media with glucose to log phase and shifted to assay medium with 2% ethanol or 2% glucose to

promote respiration or fermentation, respectively. Cells were then seeded in a microplate, incubated at 30° for 1 hr, and the oxygen consumption rates were measured using a Seahorse XF<sup>24</sup> analyzer to measure metabolic flux. Interestingly, the oxygen consumption rate in both *dbp2Δ* and *cyc8Δ* was higher than in wild-type cells in the presence of glucose, indicating active respiration (Figure 3D, top). We then measured the oxygen consumption rate in the presence of ethanol to stimulate cellular respiration (Figure 3D, bottom). Similar to glucose, both *dbp2Δ* and *cyc8Δ* showed increased oxygen consumption as compared to wild type. In contrast, neither *tup1Δ* nor *dbp3Δ* showed differences as compared to wild type in either carbon source (Figure 3D), suggesting that *Dbp2* and *Cyc8* play specific roles in glucose repression of respiration, independent of *Tup1*.

### DBP2, CYC8, and TUP1 corepressed genes are enriched in overlapping long noncoding RNAs

Besides respiration, genes involved in gluconeogenesis and utilization of nonfermentable carbon sources are also repressed in the presence of glucose and are corepressed by *DBP2* and *CYC8* or *TUP1* (Table 5, Table 6, and Table 7) (Gancedo 1998; Broach 2012). These include *PCK1*, *SFC1*, *YAT1*, and *JEN1* (Valdés-Hevia *et al.* 1989; Schmalix and Bandlow 1993; Palmieri *et al.* 1997; Casal *et al.* 1999; Akita *et al.* 2000; Turcotte *et al.* 2010). Work from our laboratory has shown that *DBP2* promotes *Cyc8*-dependent repression at the *GAL* genes by antagonizing long noncoding RNAs (lncRNAs) that would otherwise promote induction (Cloutier *et al.* 2013, 2016). Interestingly, annotated lncRNAs are found at the promoter and/or 5' regions of glucose-repressed genes in *dbp2Δ* cells including *PCK1*, *SFC1*, *YAT1*, and *JEN1* (Figure 4, A–D). For example, the 5' region of *PCK1* is overlapped with XUT1463 in *dbp2Δ* (Figure 4A, *dbp2Δ* – strand) whereas this transcript is not readily detected in wild type (Figure 4A, WT – strand). Similarly, the promoter and/or 5' regions of *SFC1*, *YAT1*, and *JEN1* are overlapped with annotated lncRNAs including CUT696 (*SFC1*; Figure 4B, *dbp2Δ* – strand), XUT0944 (*YAT1*; Figure 4C, WT and *dbp2Δ* – strands) and SUT657 (*JEN1*; Figure 4D, WT and *dbp2Δ* – strands). To determine if there is a propensity for lncRNAs to cover the promoter and/or 5' regions of genes corepressed by *DBP2*, *CYC8*, and *TUP1*, we analyzed the distribution of lncRNAs across the corepressed genes in comparison to unaffected genes. This revealed that ~40% of the corepressed genes are overlapped with annotated lncRNAs within the promoter and/or 5' end (Figure 4E). This ratio is significantly higher than that observed in genes not corepressed by *CYC8*, *TUP1*, and *DBP2* (Figure 4E, proportion test,  $P = 2.146 \times 10^{-7}$ ). This suggests that the expression of *CYC8*, *TUP1*, and *DBP2* corepressed genes may be regulated by lncRNAs similarly to the *GAL* gene cluster (Cloutier *et al.* 2016).

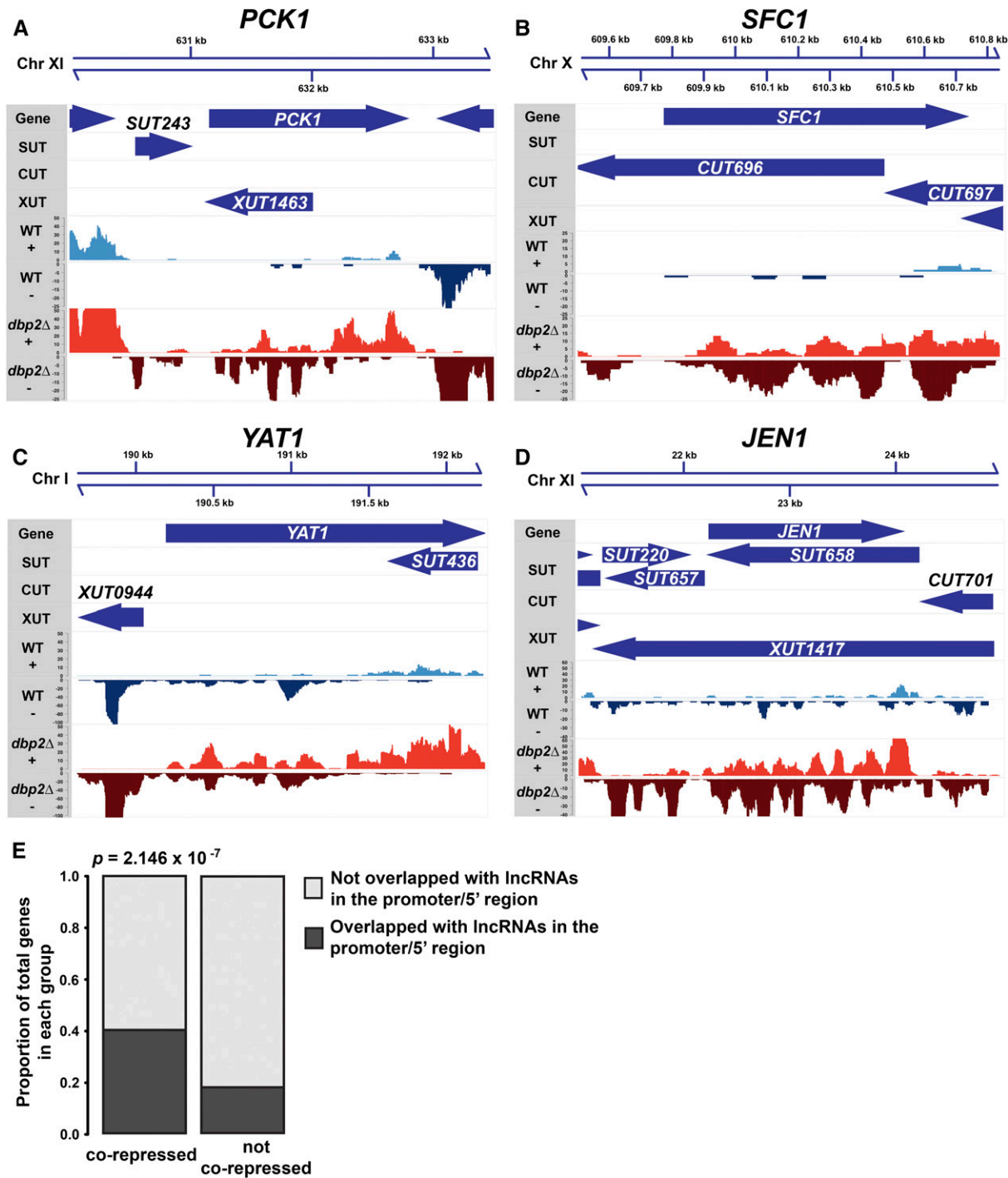
### Loss of DBP2 results in increased expression of nonfermentable carbon utilization genes upon a nutrient shift

Upregulation of *GAL* lncRNAs in the absence of *DBP2* causes reduced *Cyc8* binding and derepression of the *GAL* cluster genes (Cloutier *et al.* 2013, 2016). To determine if these corepressed genes are also derepressed in the absence of *DBP2*, we conducted transcription induction assays prior to and at different time points following a nutritional shift from glucose to the nonfermentable carbon source acetate (Figure 5, A–D). This revealed

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expression of *CYC8* or *TUP1* from a CEN plasmid rescues the heat sensitivity of *cyc8Δ* and *tup1Δ*, respectively. Serial dilutions of wild-type (WT), *cyc8Δ*, and *tup1Δ* strains harboring an empty vector (*pRS315*), *cyc8Δ* strain harboring *pCYC8*, and *tup1Δ* strain harboring *pTUP1* were spotted on SC-LEU plates and incubated at 37°.



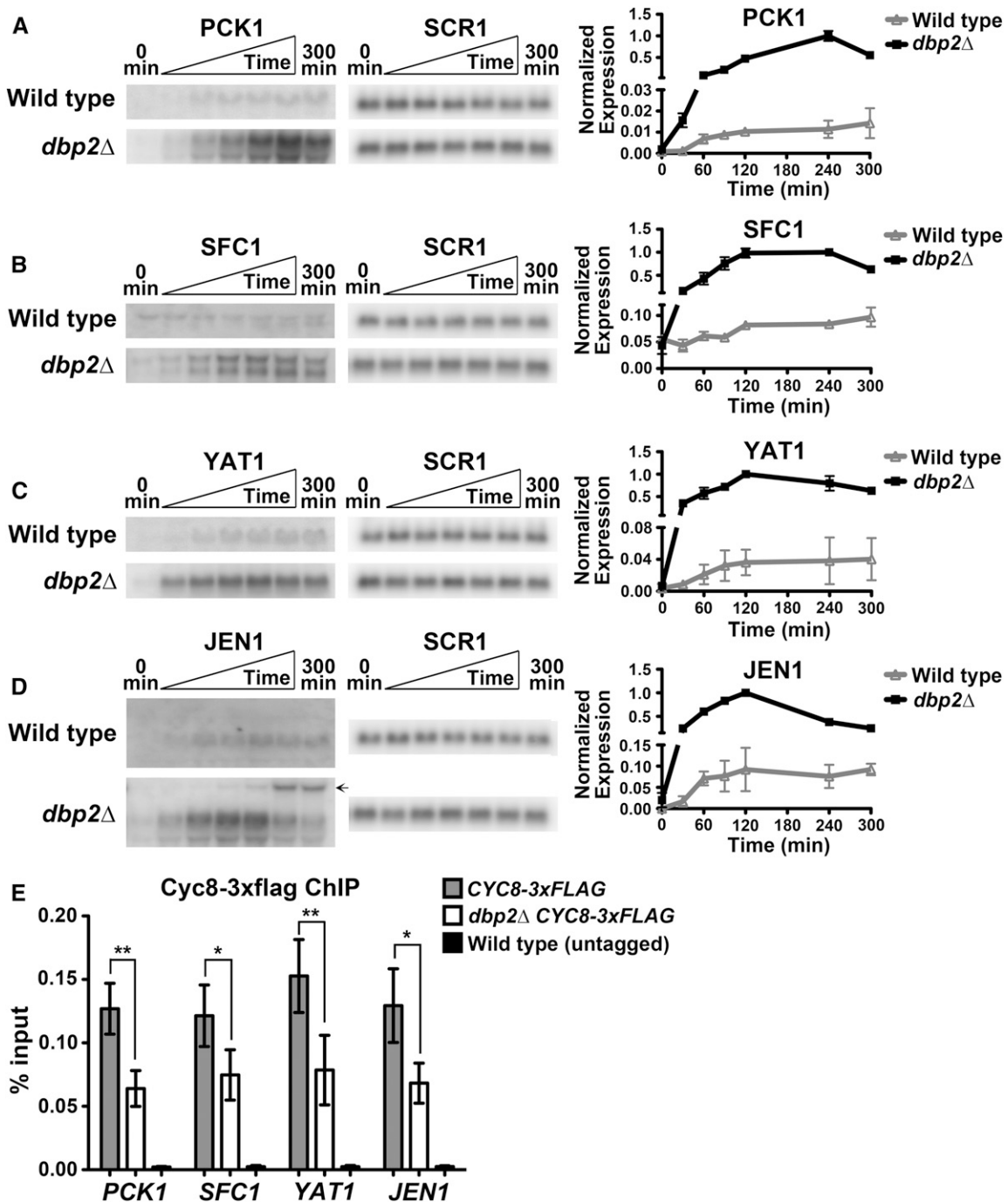


**Figure 4** *DBP2/CYC8/TUP1* corepressed genes are overlapped by lncRNAs. (A–D) Genomic tracks of *PCK1*, *SFC1*, *YAT1*, *JEN1*, and associated lncRNAs from *dbp2Δ* and wild type (WT). Normalized strand-specific genome tracks of *PCK1* (A), *SFC1* (B), *YAT1* (C), and *JEN1* (D) in wild type and *dbp2Δ* are presented at the bottom (GSE99097). Gene and annotated lncRNA (CUTs, SUTs, and XUTs) tracks (Xu et al. 2009; Wery et al. 2016) are shown at the top. The names of short transcripts are listed above the corresponding arrow. (E). Gene start region of corepressed genes are frequently overlapped by lncRNAs. Gene start region is defined as the region of 100 bp upstream plus the first 100 bp of ORF. The *P*-value indicates significant enrichment of genes overlapped with annotated lncRNAs among all of the corepressed genes (proportional test, one-sided). Chr, chromosome.

increased expression of all four genes in *dbp2Δ* cells as compared to wild type, consistent with derepression. We also observed induction of a larger transcript overlapping *JEN1* in *dbp2Δ* cells, presumably corresponding to XUT1417 (Figure 5D, arrow; see Figure 4D). Interestingly, levels of this

transcript are inversely proportional to *JEN1* mRNA, suggesting that transcription of these two RNAs is mutually exclusive.

To determine if loss of *Cyc8* at gene promoters could account for derepression, ChIP of *Cyc8*-3xFLAG was conducted in wild type and



**Figure 5** Loss of *DBP2* results in reduced Cyc8 binding and rapid induction of genes involved in utilizing nonfermentable carbon source. (A–D) Transcriptional induction assays of *PCK1* (A), *SFC1* (B), *YAT1* (C), and *JEN1* (D) were conducted by shifting wild-type and *dbp2Δ* cells from SC+2% glucose to SC+1% potassium acetate media. RNA was collected from cells prior to (0 min) and at 30, 60, 90, 120, 240, and 300 min following nutritional shift. Transcripts were detected by Northern blotting using <sup>32</sup>P-labeled, dsDNA probes. *SCR1* serves as an internal control. Resulting transcript profiles from two biological replicates (error bars indicate ± SD) were normalized to *SCR1* and plotted over time (right). The normalized expression of the time points with maximum transcript levels in each induction profile was set to one. The transcript, presumably *XUT1417*, is labelled ←. (E) ChIP of Cyc8-3xFLAG in wild-type and *dbp2Δ* cells. Wild-type and *dbp2Δ* strains harboring 3xFLAG-tagged *CYC8* at the endogenous locus were cultivated in YP+2% glucose. ChIP was conducted using an anti-FLAG antibody. A wild-type strain with untagged *CYC8* was used as negative control. Results were represented as percentage of immunoprecipitated DNA over input and shown as mean ± SD of three independent biological replicates. \**P* < 0.05, \*\**P* < 0.01.

*dbp2Δ* (Figure 5E). Consistent with our prior findings at the *GAL* gene cluster (Cloutier *et al.* 2016), we observed reduced Cyc8 binding to the promoters of *PCK1*, *SFC1*, *YAT1*, and *JEN1* in *dbp2Δ* cells as compared

to wild type (Figure 5E). This suggests that *Dbp2* promotes efficient Cyc8 binding at target genes, providing a mechanism for coregulation of glucose repression. In conjunction with our prior studies showing

that Dbp2 localization is responsive to nutrient availability (Beck *et al.* 2014), we propose that Dbp2 integrates nutritional status with metabolic gene regulation by maintaining Cyc8-dependent repression of lncRNA-targeted genes in the presence of glucose. This provides a putative mechanism for regulation of cellular metabolism by an RNA helicase, suggestive of a unique mechanism to regulate energy homeostasis in rapidly growing cells.

## DISCUSSION

Cells fine tune their metabolic programs according to nutrient availability in order to maintain homeostasis. Here, we demonstrate that the DEAD-box RNA helicase Dbp2 is a key component of this metabolic network, mediating glucose repression of metabolic genes through modulating Cyc8 association to the chromatin, and this role may involve lncRNAs. DEAD-box proteins are the largest group of RNA helicases in the helicase enzyme family and have been found in all domains of life (Linder and Jankowsky 2011). Dbp2, a DEAD-box protein in *S. cerevisiae*, functions as a key player of proper gene expression (Cloutier *et al.* 2012; Beck *et al.* 2014). It is proposed that Dbp2 promotes assembly of proteins onto nascent RNA during transcription (Ma *et al.* 2013), a role that may involve active remodeling of the RNA secondary structure and/or RNA-protein complexes. Interestingly, Dbp2 is actively shunted to the cytoplasm upon glucose deprivation or a switch to another carbon source (Beck *et al.* 2014), suggesting that Dbp2 may have a much more widespread role in metabolism, and its effects allow yeast cells to rapidly adapt to the environmental change.

The Cyc8-Tup1 complex in *S. cerevisiae* represses hundreds of genes including glucose-repressed genes, oxygen-regulated genes, and cell-type-specific genes (DeRisi *et al.* 1997; Green and Johnson 2004). Mutation or deletion of *CYC8* or *TUP1* leads to pleiotropic effects including defects in carbon catabolite repression (Gancedo 1998; Smith and Johnson 2000). Interestingly, our study shows that *CYC8* and *DBP2*, but not *TUP1*, repress respiration; one of several pathways that is repressed in the presence of sufficient extracellular glucose. This is surprising as Cyc8 and Tup1 are thought to function primarily as a complex. This suggests that Cyc8 may have functions outside of Tup1, a role supported by the unique distribution of transcript abundance between *cyc8Δ* and *tup1Δ* cells outside of those identified as coregulated (Figure 2B, right). A previous study has shown that simultaneous deletion of *CYC8* and *TUP1* results in higher invertase activity, as a result of *SUC2* derepression, than either of the single deletions alone (Williams and Trumbly 1990). This suggests that these two factors have independent as well as shared activities.

lncRNAs are RNAPII products that lack an ORF and are >200 nt in length (Kornienko *et al.* 2013). Emerging evidence suggests many lncRNAs function in gene regulation of protein-coding gene transcripts by acting as decoy, guide, or scaffold of other regulatory factors or through transcription interference (Wang and Chang 2011; Kornienko *et al.* 2013). We have previously reported that Dbp2-dependent repression of the *GAL* cluster genes is dependent on expression of lncRNAs associated with the *GAL* cluster (Cloutier *et al.* 2016). By analyzing the presence of lncRNAs at corepressed genes by *DBP2* and *CYC8*, we now show that a significant fraction of these genes are overlapped by lncRNAs encoded in *cis*; suggesting a genome-wide link between Dbp2, lncRNAs, and glucose repression. Interestingly, recent studies have connected several lncRNAs to glucose homeostasis in mammalian cells (Sun and Wong 2016). For example, H19 functions in glucose homeostasis through the bioavailability of microRNA (miRNA) let-7 that inhibits the expression of insulin receptor and lipoprotein lipase genes (Kallen *et al.* 2013; Gao *et al.* 2014). Another

example is the lncRNA UCA1, which has been shown to promote glycolysis by activating mTOR-STAT3 and repressing miRNA143 in cancer cells (Li *et al.* 2014). A recent genome study has revealed >350 metabolically sensitive lncRNAs in mice (Yang *et al.* 2016). This suggests that lncRNAs can act as key regulators in metabolism.

Taken together, our study suggests that Dbp2 integrates nutritional availability with metabolic adaptation. This may also be the case for DDX5, the mammalian ortholog of Dbp2, as knockdown affects glucose metabolic genes, reduces glycolysis, and facilitates respiration in mammalian cells (Mazurek *et al.* 2014; Xing *et al.* 2017). Future studies are necessary to determine if the role of Dbp2 in cellular metabolism is conserved in mammalian cells. High fermentation rate suppresses the respiratory network, a phenomenon referred to as the Crabtree effect (De Deken 1966). Many tumor cells, on the other hand, exhibit enhanced glycolytic activities and impaired respiratory activities, similar to the preference of *S. cerevisiae* in undergoing aerobic fermentation (Diaz-Ruiz *et al.* 2011). This drastic change in metabolic activities, which is referred to as the Warburg effect, is a hallmark of many cancer cells (Diaz-Ruiz *et al.* 2011). Thus, understanding how yeast cells control their metabolism has great implications on human diseases.

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