Transcriptional Profiling of *Saccharomyces cerevisiae* Reveals the Impact of Variation of a Single Transcription Factor on Differential Gene Expression in 4NQO, Fermentable, and Nonfermentable Carbon Sources

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**ABSTRACT** Cellular metabolism can change the potency of a chemical’s tumorigenicity. 4-nitroquinoline-1-oxide (4NQO) is a tumorigenic drug widely used on animal models for cancer research. Polymorphisms of the transcription factor Yrr1 confer different levels of resistance to 4NQO in *Saccharomyces cerevisiae*. To study how different Yrr1 alleles regulate gene expression leading to resistance, transcriptomes of three isogenic *S. cerevisiae* strains carrying different Yrr1 alleles were profiled via RNA sequencing (RNA-Seq) and chromatin immunoprecipitation coupled with sequencing (ChIP-Seq) in the presence and absence of 4NQO. In response to 4NQO, all alleles of Yrr1 drove the expression of *SNQ2* (a multidrug transporter), which was highest in the presence of 4NQO resistance-conferring alleles, and overexpression of *SNQ2* alone was sufficient to overcome 4NQO-sensitive growth. Using shape metrics to refine the ChIP-Seq peaks, Yrr1 strongly associated with three loci including *SNQ2*. In addition to a known Yrr1 target *SNG1*, Yrr1 also bound upstream of *RPL35B*; however, overexpression of these genes did not confer 4NQO resistance. RNA-Seq data also implicated nucleotide synthesis pathways including the de novo purine pathway, and the ribonuclease reductase pathways were downregulated in response to 4NQO. Conversion of a 4NQO-sensitive allele to a 4NQO-resistant allele by a single point mutation mimicked the 4NQO-resistant allele in phenotype, and while the 4NQO resistant allele increased the expression of the ADE genes in the de novo purine biosynthetic pathway, the mutant Yrr1 increased expression of ADE genes even in the absence of 4NQO. These same ADE genes were only increased in the wild-type alleles in the presence of 4NQO, indicating that the point mutation activated Yrr1 to upregulate a pathway normally only activated in response to stress. The various Yrr1 alleles also influenced growth on different carbon sources by altering the function of the mitochondria. Hence, the complement to 4NQO resistance was poor growth on nonfermentable carbon sources, which in turn varied depending on the allele of Yrr1 expressed in the isogenic yeast. The oxidation state of the yeast affected the 4NQO toxicity by altering the reactive oxygen species (ROS) generated by cellular metabolism. The integration of RNA-Seq and ChIP-Seq elucidated how Yrr1 regulates global gene transcription in response to 4NQO and how various Yrr1 alleles confer differential resistance to 4NQO. This study provides guidance for further investigation into how Yrr1 regulates cellular responses to 4NQO, as well as transcriptomic resources for further analysis of transcription factor variation on carbon source utilization.

**KEYWORDS** Yrr1, 4NQO, RNA-Seq, ChIP-Seq, genetic variation, respiration, fermentation

*Saccharomyces cerevisiae*, baker’s yeast, is a model organism that has been extensively studied to decipher the association between genotypes and phenotypes. In addition to the widely used laboratory strain S288c (Engel et al. 2014), the genomes of other yeast strains have been sequenced, which provides valuable resources to investigate how the genetic differences among strains contribute to phenotypic differences. The YJM789 yeast strain was derived from a clinical isolate from an AIDS patient with *S. cerevisiae* pneumonia, and its draft genome is available (Wei et al. 2007). Genome association comparison of YJM789 to S96, a strain derived from S288c, elucidated the genetic difference...
basis of strain-dependent responses to a toxic chemical, 4NQO (Gallagher et al. 2014).

4NQO is a quinoline-derived carcinogenic drug used for cancer research on animal models because it induces squamous cell carcinoma in oral cavities of mice (Hawkins et al. 1994). YJM789 shows higher resistance to 4NQO treatment than S96 does in Yeast Peptone Dextrose (YPD) medium (Gallagher et al. 2014). Mapping of quantitative trait loci shows that the difference in resistance to 4NQO between the two strains was correlated with variation in the YRR1 gene, which encodes a zinc finger transcription factor. The Yrr1 protein from YJM789, Yrr1Y, possesses a threonine at the position of 775 (T775), while the Yrr1 protein from S96, Yrr1S, possesses an isoleucine at this position (I775). Threonine can be phosphorylated at its hydroxyl functional group, while isoleucine does not possess a hydroxyl group and thus cannot be phosphorylated. However, phosphorylation could not be confirmed, likely due to the technical limitations of detection using mass spectrometry. The peptide containing the potential phosphorylation is 4578 Da and the average weight of the peptides is 1700 Da, and therefore would be considered too large for detection (Rong-Mullins et al. 2017b). Combined with low abundance of transcription factors and the length of the peptide, this precludes the validation of this phosphorylation. Instead, a mutant protein was constructed, Yrr1IE, that mimics the strong negative charge of phosphorylation when I775 of the S96 allele is changed into a glutamate (I775E). The level of 4NQO resistance conferred by Yrr1IE is comparable to that conferred by Yrr1Y and is higher than that conferred by Yrr1S. Conversely, a single point mutation E673G in Yrr1Y can convert the 4NQO-resistant allele to a 4NQO-sensitive allele. However, combining E673G with T775E retains 4NQO resistance conferred by Yrr1Y. This suggests that the potential phosphorylation at T775 of Yrr1Y may play an important role in regulating cellular responses to 4NQO.

YJM789 was isolated from a patient with multiple viral infections being treated for pneumonia with ciproflaxacin, an antibacterial fluoroquinolone (Tawfik et al. 1989). S. cerevisiae is associated with the normal human microbiome and is typically not considered to be pathogenic. However, YJM789’s original heterozygous diploid parent adapted to unique conditions, including a suppressed immune system and a lack of bacterial competition. It is unknown if ciproflaxacin treatment directly contributed to the 4NQO tolerance seen in this strain, if the tolerance preexisted, or if the tolerance came about by adapting to another condition. Several mutations within Yrr1 that changed cellular growth in response to 4NQO also changed cellular growth when cells were forced to respire (Gallagher et al. 2014).

The mechanisms of transcription regulation by Yrr1 have been investigated in earlier studies regarding drug resistance. Yrr1 was shown previously to autoregulate by binding to its own promoter region (Zhang et al. 2001). In addition, Yrr1 is also known to induce higher expression of SNQ2, a gene encoding a multidrug transporter, in response to 4NQO (Cui et al. 1998; Le Crom et al. 2002). However, genome-wide changes of transcription and Yrr1-binding patterns have not been reported to provide a comprehensive view. In this study, we constructed three isogenic strains in the yrr1Δ S96 background carrying three different Yrr1 alleles: Yrr1Y, Yrr1Y and Yrr1IE. We then conducted deep RNA-Seq as well as ChIP-Seq in conditions where Yrr1 regulates the stress response in the presence of 4NQO and glycerol. Here, we discovered that overexpression of SNQ2 bypassed the need for Yrr1 for 4NQO sensitivity conferred by expression of the Yrr1Y allele. While the expression of many genes changed when cells with different alleles of Yrr1 were grown in glycerol, differences in the purine salvage pathway and other antioxidant pathways may quench free radicals generated as yeast shift toward respiration. In the model presented here, 4NQO was actively reduced in the presence of functioning mitochondria to produce free radicals and 4HAQO. 4NQO in the form of 4HAQO interacts directly with DNA to induce DNA oxidation. Our data indicate that genetic variation in a single transcription factor altered the process of oxidative phosphorylation within the cell, which accounted for the aforementioned conversion of 4NQO into a toxic mixture of free radicals and 4HAQO. This study will not only broaden our knowledge of the yeast metabolic response to tumorigenic drugs, but also inform research on the drug resistance of cancer cells for other model organisms. Furthermore, the data presented here provide a resource for the further exploration of the effect of genetic variation in a transcription factor on the utilization of carbon sources.

MATERIALS AND METHODS

S. cerevisiae strains and plasmids

The strains and plasmids used in this study were described in Gallagher et al. (2014) and grown in YPD (1% yeast extract, 2% peptone, and 2% dextrose). Plasmids encoding alleles of YRR1 were under the control of endogenous promoters and terminators in pG35, and were maintained by the addition of G418. The entire coding region of YRR1 was replaced by the hygromycin-resistance gene in S96 (MATα, lys5) and in FY3, an isogenic yeast strain (MATα, ura3) (Winston et al. 1995; Goldstein and McCusker 1999; Gallagher et al. 2014). Petite strains were generated by treating parent strains (FY3 yrr1Δura3, YJM789, YJM789 yrr1ΔhygR, S96, and S96 yrr1ΔhygR) with 1 μg/ml of ethidium bromide for 6 hr in a liquid culture and plating on YPD. After 2 d, the colonies were replica-plated onto YP with 3% glycine-resistance gene in S96 as the sole carbon source, and colonies that failed to grow were tested for loss of the mitochondrial-encoded COX2 gene by PCR amplification. For serial dilution growth assays, saturated cultures were grown in YPD, serially diluted 10-fold, and spotted onto the indicated media (Rong-Mullins et al. 2017a). Because the S96 strain is an isogenic strain to S288c, the sequences and annotations of S288c genome release R64-1-1 (Engel et al. 2014) were used for RNA-Seq and ChIP-Seq analyses. SNQ2 and PDR5 overexpression plasmids were previously published (Tsuimoto et al. 2015). Overexpression plasmids were based on yEP24 with the URA3 selectable marker and were transformed into FY3 yrr1Δ with pG35S. Plasmids were maintained on Yeast Minimal (YM) media with no amino acids and monosodium glutamate as the nitrogen source, so that 500 μg/ml G418 would select for pG35S (Kan8 marker). Overexpression plasmids of all other strains were from the MORF collection (Geperin et al. 2005). Plasmids were maintained in FY3 yrr1Δ by selecting for the URA3 auxotrophy. Expression of the MORF collection is driven by the GAL promoter. Yeast were grown in YM with 2% galactose and 0.1% dextrose to prevent toxicity from overexpression. In YM glycerol (abbreviated as Glyc), 2% galactose and 3% ethanol were also added to the solid media.

RNA-Seq experiment and data analysis

Each of the three untagged isogenic strains with different alleles—Yrr1Y, Yrr1Y and Yrr1IE—were grown as duplicated cultures in liquid YPD.
Table 1. The numbers of loci showing significant (q-value < 0.05) differential expression in RNA-Seq are shown as the total of each functional category and for each comparison of conditions (combination of allele and growth medium).

| Functional Category        | Change | S96, 4NQO | S96, 4NQO + 4NQO | S96, YPD | S96, YPD + 4NQO | Yrr1S, 4NQO | Yrr1S, 4NQO + 4NQO | Yrr1Y, 4NQO | Yrr1Y, 4NQO + 4NQO | Yrr1IE, 4NQO | Yrr1IE, 4NQO + 4NQO | Yrr1IE, YPD | Yrr1IE, YPD + 4NQO |
|----------------------------|--------|-----------|-----------------|---------|-----------------|------------|-------------------|-------------|-------------------|-------------|-------------------|-------------|-------------------|
| All loci tested            | 671    | 16        | 118             | 6       | 108             | 2          | 0                 | 0           | 0                 | 3           | 1                 | 5           | 4                 |
| DNA damage                 | 510    | 6         | 0               | 5       | 10              | 3          | 0                 | 0           | 0                 | 2           | 2                 | 9           | 2                 |
| Oxidative stress           | 609    | 6         | 0               | 5       | 10              | 3          | 0                 | 0           | 0                 | 2           | 2                 | 9           | 2                 |
| Protein folding            | 438    | 6         | 0               | 5       | 10              | 3          | 0                 | 0           | 0                 | 2           | 2                 | 9           | 2                 |
| Purine biosynthesis        | 116    | 6         | 0               | 5       | 10              | 3          | 0                 | 0           | 0                 | 2           | 2                 | 9           | 2                 |
| Pyrimidine biosynthesis    | 19     | 6         | 0               | 5       | 10              | 3          | 0                 | 0           | 0                 | 2           | 2                 | 9           | 2                 |
| Nucleotide salvage         | 18     | 6         | 0               | 5       | 10              | 3          | 0                 | 0           | 0                 | 2           | 2                 | 9           | 2                 |

The condition before "/" is the numerator and the one after is the denominator for differential expression. 4NQO, 4-nitroquinoline-1-oxide; YPD, Yeast Peptone Dextrose; RNR, ribonucleotide-diphosphate reductase.

The genes in specific functional categories were found through Gene Ontology (GO) term search within AmiGO2 (http://amigo2.geneontology.org/amigo): DNA damage: GO:0006974 representing "cellular response to DNA damage"; Protein folding: GO:0006457 representing "protein folding"; and Nucleotide salvage: union of GO:0043173 representing "nucleotide salvage" and GO:0043101 representing "pyrimidine nucleotide biosynthetic process" as well as GO:0008655 representing "pyrimidine-containing compounds salvage."
2.08–4.21 million read pairs were generated per sample and mapped to the S288c genome using Bowtie v2.2.3 (Langmead and Salzberg 2012) with preset “–sensitive,” resulting in alignment rates of 96.1–99.3%. Reanalysis of the data is described below. ChIP-enriched regions (peaks) were identified using CisGenome v2.0 (Ji et al. 2008) and MACS2 v2.1.0 (Zhang et al. 2008), and their outputs were integrated for downstream analyses (see details in the supplemental material). Prediction of DNA motifs at potential Yrr1-binding sites was performed using the Gibbs Motif Sampler in CisGenome with some manual adjustment. Sequence logos of predicted motifs were generated using WebLogo v3.4 (Schneider and Stephens 1990; Crooks et al. 2004). The height of each nucleotide letter represented the posterior mean relative entropy, and the error bars represented Bayesian 95% C.I.s. Pearson’s correlation (r-value) test was performed on ChIP peak metrics of three consolidated peak regions and expression values of their downstream genes using the python scipy package.
**Microscopy**

Cells were grown to midlog in YM and stained live using Mitotracker Green and Rhodamine B hexyl ester (Y7530; Life Technologies) per the manufacturer’s instructions. Cells were imaged for differential interference contrast and fluorescence microscopy using an Eclipse 600-FN Nikon microscope with an Apochromat 100x/1.40 NA oil immersion objective, and a cooled charge-coupled device camera (ORCA-2; Hamamatsu Photonics). Images were processed with MetaMorph v7.0 software (Molecular Devices) and further processed using Image J.

**Data availability**

The S. cerevisiae strains used in this study are available upon request. Supplemental Material, File S1 describes the process of determining confidence in ChIP-Seq peaks based on peak shape metrics, as well as all the supporting figure legends and tables. The RNA-Seq and ChIP-Seq data used in this study are available at NCBI GEO with accession numbers GSE74642 and GSE74700, respectively. The links for the two data sets are: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74642 and https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74700.

**RESULTS AND DISCUSSION**

A global view of RNA-Seq transcriptomic profiles for different YRR1 alleles in the presence or absence of 4NQO

To determine the global transcriptional change due to the presence of different alleles of Yrr1, a transcription factor, the alleles were expressed in the same genetic background and treated with 4NQO. A total of 6717 open reading frames (ORFs) were quantified for FPKM in RNA-Seq. Ten comparisons of conditions were performed to identify differentially expressed ORFs (Table 1, Table S1, and Table S2). The within-allele comparisons between treatments of YPD (in the absence of 4NQO) and 4NQO for each Yrr1 allele were among those showing the largest numbers of significantly differentially expressed loci, with 510–909 loci significantly up- or downregulated (Table 1 and Table S2).

Stress from 4NQO treatment induced the most dramatic transcriptomic changes. Interestingly, the comparison between Yrr1S and Yrr1Y in the presence of 4NQO showed the smallest number of significantly differentially expressed loci, with only six loci significantly up regulated and six downregulated (Table 1 and Table S2). In contrast, there were larger numbers of significantly differentially expressed loci when Yrr1S or Yrr1Y was compared to the phosphomimetic allele Yrr1IE in 4NQO, with 108–143 loci significantly up- or downregulated (Table 1). This suggests that the transcriptomic profile of Yrr1IE was divergent from those of Yrr1S or Yrr1Y in 4NQO. Cells carrying Yrr1IE had similar 4NQO resistance to Yrr1Y cells, both of which were higher than that of Yrr1S cells (Gallagher et al. 2014). Because Yrr1IE was predicted to mimic the charge of phosphorylation at I775 of Yrr1Y in response to 4NQO, the transcriptomic profile of Yrr1IE in YPD was compared to that of Yrr1Y in 4NQO. There were substantial numbers of loci significantly upregulated (671) and downregulated (602) in this comparison. This suggests that despite I775E mimicking the 4NQO resistance of Yrr1Y, Yrr1IE was not simply an activated form of Yrr1Y. Despite the I775E mutation having the same phenotype as Yrr1Y on 4NQO and our prediction that it would behave as an irremovable phosphorylation, the transcriptomic profile of Yrr1IE in YPD was not similar to that of Yrr1Y in 4NQO. Therefore, the phosphomimetic Yrr1IE allele of Yrr1Y in 4NQO could not recapitulate all cellular effects of the YJM789 allele for several possible reasons, such as variation in other proteins, differences in phosphorylation kinetics, or incomplete phosphorylation of the cellular pool of the wild-type allele.

**Genes involved in DNA damage and oxidative stress responses showcase remarkable patterns of differential expression in response to 4NQO**

4NQO and its cellular metabolites are known to cause DNA damage in eukaryotes (Arima et al. 2006; Minca and Kowalski 2011). Among the 328 genes involved in the cellular response to a DNA damage stimulus, 19–31 of them were significantly up- or downregulated in response to 4NQO depending on the Yrr1 allele (Table 1). The upregulated genes

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**Table 2** Three consolidated peak regions with highest confidence of containing functional binding sites of Yrr1

| Identifier | Chromosome | Coordinates | Sequence | Coordinates (Strand) | Sequence | Coordinates (Strand) |
|------------|------------|-------------|----------|----------------------|----------|----------------------|
| 201        | IV         | 454940–465905 | TAAACGGAAATGGG | 465396–465409 (−) | ATATAAAACATAT | 465506–465518 (+) |
| 659        | X          | 606408–607949 | TCTACGGAAATGAG | 607240–607306 (−) | AAATACGCGGAAAT | 607347–607359 (+) |
| 527        | VII        | 893849–894948 | TACACCGAAATAGG | 894482–894495 (−) | AAATACGCAAAAT | 894586–894598 (+) |

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**Table 3** ChIP peak metrics of the three high-confidence regions and expression of their downstream genes for different conditions (combinations of Yrr1 allele and growth medium)

| Consolidated Peak Region ID | Value Type | S96, 4NQO | S96, YPD | S96-1775E, 4NQO | S96-1775E, YPD | YJM789, 4NQO | YJM789, YPD |
|-----------------------------|------------|-----------|---------|----------------|----------------|---------------|---------------|
| 201                         | Summit height | 177.3     | 167.7   | 242.6          | 177.2          | 238.7         | 208.8         |
|                             | Pileup log2fc | 0.900     | 0.756   | 1.084          | 0.991          | 1.012         | 1.167         |
|                             | SNQ2 FPKM   | 144.5     | 64.8    | 177.6          | 77.8           | 199.0         | 83.5          |
| 659                         | Summit height | 267.8     | 330.4   | 132.0          | 100.6          | 260.2         | 232.0         |
|                             | Pileup log2fc | 1.542     | 1.940   | 0.751          | 0.694          | 1.308         | 1.610         |
|                             | RPL43B FPKM | 1599      | 2071    | 2254           | 2143           | 1914          | 1931          |
| 527                         | Summit height | 84.47     | 111.6   | 65.97          | 65.53          | 150.4         | 82.12         |
|                             | Pileup log2fc | 0.509     | 0.553   | 0.582          | 0.570          | 1.042         | 0.556         |
|                             | SNQ1 FPKM   | 27.0      | 22.8    | 34.5           | 26.6           | 34.2          | 28.3          |
|                             | YPP1 FPKM   | 37.8      | 36.8    | 33.8           | 29.8           | 45.6          | 39.2          |

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*Summit height and pileup log2fc: mean summit height and mean pileup log2(fold change) of three biological replicates using two model building options, respectively (Materials and Methods). ID, identifier; 4NQO, 4-nitroquinoline-1-oxide; YPD, Yeast Peptone Dextrose.

FPKM: mean fragments per kilobase of transcript length per million mapped reads of two biological replicates, determined from Rsubread counts of reads.
encode endonucleases, helicases, and proteins involved in DNA mismatch repair. The downregulated genes encode proteins including histones, components of chromatin remodeling complexes, DNA replication proteins, RNA polymerase II subunits, and transcription initiation proteins. Changes in the gene expression of these pathways could compensate for 4NQO-induced damage to DNA.

Metabolic reduction of 4NQO in mammalian cells is known to generate ROS (Varnes and Biaglow 1979; Arima et al. 2006), and therefore imposes oxidative stress on cells. Among the 106 genes involved in the response to oxidative stress, 32–44 of them were significantly upregulated in response to 4NQO, depending on which Yrr1 allele was expressed (Table 1). These genes encode antioxidant proteins such as catalases, superoxide dismutases, peroxidases, peroxiredoxins, and thioredoxins. The higher abundance of these transcripts likely led to more effective reduction of ROS and thus alleviation of 4NQO-induced oxidative stress.

Several differentially expressed genes may contribute to differential 4NQO resistance conferred by different Yrr1 alleles

A tractable set of 12 genes were significantly differentially expressed between the S96 and YJM789 alleles of Yrr1 in the presence of 4NQO. It was hypothesized that these genes may be implicated in the differential resistance of the two alleles, prompting further examination (Figure 1). It is possible that subtle transcriptional differences of other genes between Yrr1S and Yrr1Y in the presence of 4NQO, despite being deemed not significant by our study, also contribute to the differential 4NQO resistance conferred by the two alleles.

When considering the 12 genes with significant expression differences between S96 and YJM789 alleles of Yrr1 under 4NQO treatment, we examined their known functions and potential relationships to 4NQO or other chemical toxins. Several had roles consistent with the putative interplay of resistance and carbon metabolism. Snq2 is an ABC plasma membrane transporter that is required for resistance to many chemicals, including 4NQO (Servos et al. 1993) and ROS (Verwerdis et al. 2001). It is also known to be regulated by Yrr1 (Cui et al. 1998; Le Crom et al. 2002), so its differential expression indicates the detection capability of the study. NDII encodes a NADH:ubiquinone oxidoreductase and is a component of the electron transport chain (ETC) in aerobic respiration. ACH1 encodes a protein with CoA transferase and acetyl-CoA-hydrolase activities that appears in both the cytosol and mitochondria (Lee et al. 1990; Fleck and Brock 2009). Expression of NDII and ACH1 was significantly lower for Yrr1Y than for Yrr1S in the presence of 4NQO, because NDII and ACH1 were significantly upregulated for Yrr1Y but not for Yrr1S in response to 4NQO (Figure 1). Overexpression of NDII is known to increase the accumulation of ROS (Li et al. 2006). Given that metabolic reduction of 4NQO generates ROS, such an expression pattern of NDII may result in less accumulation of ROS, or change oxidative phosphorylation in the cell and decrease the reduction of 4NQO, thus conferring higher 4NQO resistance in cells carrying Yrr1Y (Gallagher et al. 2014). In contrast to NDII, deletion of ACH1 was reported to decrease resistance to oxidative stress caused by 3 mM hydrogen peroxide (Brown et al. 2006), suggesting that higher levels of ACH1 may lead to higher resistance to oxidative stress. Interestingly, our data suggests an association of higher levels of ACH1 with lower resistance to oxidative stress: cells carrying Yrr1Y, where ACH1 expression is higher than in cells carrying Yrr1S, are less resistant to 4NQO.

Nucleotide biosynthetic genes

The genes involved in purine nucleotide biosynthetic processes showed interesting patterns of differential expression. Among the 4NQO genes, ADE genes and ribonucleotide-diphosphate reductase (RNR) genes, displayed relatively consistent patterns within each subgroup but distinct patterns from each other (Figure 1B and Table 1). In addition, null mutations of some genes from the two subgroups have opposite effects on resistance to oxidative stress. The ADE genes (Ade1, Ade2, Ade3, Ade4, Ade5, Ade6, Ade8, Ade16, and Ade17) and from IMP to adenosine monophosphate (IMP) (Ade12 and Ade13) were significantly downregulated in...
response to 4NQO for Yrr1S and Yrr1Y, and significantly lower for Yrr1IE than Yrr1S and Yrr1Y in YPD (Figure 1B). Both Ade16 and Ade17 have 5-aminomidazole-4-carboxamide ribonucleotide transformylase and IMP cyclohydrolase activities (Tibbetts and Appling 1997, 2000). 

**ADE16** is a paralog of **ADE17** with overlapping roles; the expression change of **ADE17** alone may be sufficient for achieving the necessary changes in metabolic activities catalyzed by both **ADE16** and **ADE17**. Like the protein-folding genes discussed above, the expression pattern of **ADE** genes for Yrr1IE in YPD mimicked those of Yrr1S and Yrr1Y in 4NQO. Single null mutations of **ADE1**, **ADE3**, **ADE4**, **ADE5**, **ADE7**, and **ADE8** were shown to increase resistance to oxidative stress caused by 3 mM hydrogen peroxide (Brown et al. 2006). The downregulation of **ADE** genes in response to 4NQO, as shown in our data, may increase resistance to oxidative stress caused by 4NQO.

The RNR complex catalyzes the formation of dNDP from NDP, a rate-limiting step in dNTP synthesis. All four **RNR** genes were significantly upregulated in response to 4NQO for all three Yrr1 alleles (Figure 1B). This is consistent with a previous finding that the mRNA levels of **RNR1** and **RNR3** increase upon treatment with 4NQO, which likely facilitates DNA repair during replication (Elledge and Davis 1990). Deletion of **RNR1** was shown to decrease resistance to oxidative stress caused by 2 mM hydrogen peroxide, 0.5 mM paraquat, and 100% oxygen atmosphere (Outten and Culotta 2005). It is possible that upregulation of **RNR** genes copes with the oxidative stress caused by 4NQO.

![Figure 2](image.png) ChIP peaks shown as ChIP minus input within the three high-confidence regions and corresponding mRNA expression. (A) SNO2 (peak 201), (B) SNG1 and YPP1 (peak 659), and (C) RPL36B (peak 527). The plotted data were mean pileup values of normalized ChIP minus input generated by MACS2, using two model-building options for three biological replicates per combination of the YRR1 allele and growth medium (Materials and Methods). Each plotted region is represented as a black box together with nearby genes shown in the University of California, Santa Cruz Genome Browser (http://genome.ucsc.edu) (Kent et al. 2002). (D) Heatmap showing differential RNA expression for genes SNO2, RPL43B, SNG1, and YPP1 near the three regions (201, 659, and 527) with high confidence of containing functional binding sites for Yrr1, among different conditions. Log2(fold change of FPKM) values calculated by DESeq2 are represented in blue and red. Significant differential expression instances are highlighted based on q-values, where "***" represents between 0.005 and 0.05, and "**" for < 0.005. ChIP, chromatin immunoprecipitation; Chr, chromosome; FPKM, fragments per kilobase gene per million mapped fragments.

**Figure 3** Growth assays of yeast overexpressing ABC transporters. S288c (FY3 yrr1Δ) yeast with different alleles of Yrr1 were transformed with either an empty plasmid (-), or a plasmid overexpressing SNO2 or PDR5. Ten-fold serial dilutions of yeast grown in selective media to maintain both plasmids were spotted onto increasing amounts of 4NQO. Plates were incubated for 3 d and photographed. 4NQO, 4-nitroquinoline-1-oxide; ABC, ATP-binding cassette; YM, Yeast Minimal.

![Figure 3](image.png)
Identification of ChIP-Seq peaks with high confidence of being functional binding sites of Yrr1

In order to investigate binding of Yrr1 to DNA as a transcription factor in association with its impact on gene expression, the published ChIP-Seq data of Yrr1 (Gallagher et al. 2014) were reexamined together with the RNA-Seq data in this study (Table S3). In S. cerevisiae, transcription factors bind to specific recognition sequences upstream of genes, known as upstream activation or repression sequences (Phillips and Hoopes 2008; Hahn and Young 2011). No transcription factor is known to have recognition sequences within gene bodies. However, 1083 out of 1136 narrow peak regions (defined in the Materials and Methods and supplemental material) of Yrr1 ChIP enrichment identified by CisGenome and MACS2 overlap with annotated genes in the published S288c genome. False-positive ChIP peaks have been previously identified within highly expressed genes, i.e., “hyper-ChIPable” regions (Teytelman et al. 2013). Therefore, to examine whether hyper-ChIPability exists in our Yrr1 ChIP-Seq data, expression levels of loci overlapping with narrow peak regions of Yrr1 ChIP were compared to those of all the loci in the RNA-Seq data (Figure S1 in File S1 and Table S1). A considerable number of Yrr1 peaks identified by CisGenome and MACS2 overlap with highly expressed loci, and are possibly false positives due to hyper-ChIPability. Another reason for concern over false positives is the frequent occurrence of negative peaks (enrichment in input over ChIP). Given the concerns, an extra screening procedure is necessary to exclude false-positive peaks from downstream analyses. Therefore, an in silico method was developed in this study in an attempt to identify high-confidence peaks.

Based on their shape metrics, three high-confidence regions were identified in the ChIP data set as binding sites for Yrr1 alleles (Subsection “Determine the confidence in ChIP-Seq peaks based on peak shape metrics,” Figures S2–S4, and Tables S5–S7 in File S1, Table S3, and Table S4). These three ChIP regions were represented using ChIP minus input (Figure S1 in File S1) and log2(fold change) (Figure S4 in File S1). Two DNA motifs at potential Yrr1-binding sites were predicted using the three high-confidence regions (Figure S5 in File S1 and Table 2). Motif 1 shared the consensus sequence “CGGA” (or “TCCG” as reverse complement) with potential Yrr1-binding motifs identified in previous studies (Le Crom et al. 2002; Morozov and Sigsga 2007; Badis et al. 2008; Zhao et al. 2009; Zhu et al. 2009; de Boer and Hughes 2011). Motif 2 did not share a consensus sequence with any previously reported Yrr1-binding motif.

Correlation between high-confidence ChIP peaks and the expression of their nearby genes in 4NQO

Regulation of genes by the binding of a transcription factor is assumed by the mere presence of the transcription factor. The expression of genes downstream of Yrr1 was further investigated. Pearson’s test was performed to investigate the correlation between the ChIP peak strengths in the three high-confidence regions (201, 659, and 527) and the expression levels (represented by FPKM values) of their nearby genes SNQ2, RPL43B, SNG1, and YPP1 (Table 3 and Table 4). Expression of SNQ2 (FPKM values) increased significantly, and Yrr1 binding upstream of SNQ2 (summit height of ChIP peaks in region 201) increased in response to 4NQO for all the three alleles Yrr1E, Yrr1E, and Yrr1Y (Figure 2A, Table 3, and Table S1). In addition, SNQ2 expression in 4NQO was significantly higher for Yrr1Y than for Yrr1E, but less when comparing yeast grown in YPD with the Yrr1E allele to yeast grown in 4NQO with the Yrr1Y allele (Figure 2D). This indicated that the I775E mutation was not sufficient to maximize the expression of SNQ2 on its own. Although the Yrr1E allele could phenocopy Yrr1Y in response to 4NQO, the mutated allele was not the same as the Yrr1Y or Yrr1E allele. There were other variable residues in Yrr1 that were subject to regulation in response to 4NQO, as indicated by the expression of SNQ2 by the Yrr1Y allele in 4NQO being higher than Yrr1E in YPD.

ChIP region 527 is located upstream of two divergent genes, SNG1 and YPP1. The protein structure of Sng1 has not yet been well characterized, but transformation of SNG1 into S. cerevisiae via a multicopy vector conferred resistance to 4NQO (Servos et al. 1993). In addition, a gain-of-function Yrr1 mutant increased expression of SNG1, and Yrr1 directly binds the SNG1 promoter in vitro (Le Crom et al. 2002). Our data connected the contribution of SNG1 to 4NQO resistance and the regulation of SNG1 by Yrr1 by showing increased Yrr1 ChIP peak strength in region 527. The correlation between Yrr1 ChIP peak strength (Table 3) and SNG1 expression was positive but weak in this study (Table 4). The other gene for which ChIP region 527 is upstream, YPP1, is essential to S. cerevisiae and encodes a vesicle-trafficking protein involved in vacuole-targeted endocytosis (Flower et al. 2007). YPP1 has not been reported to contribute to 4NQO responses or to be regulated by Yrr1. There were no significant changes of YPP1 expression in response to 4NQO or among alleles (Figure 2D).

ChIP region 659 is located upstream of RPL43B, which encodes L43B, a protein of the ribosomal 60S subunit. Yrr1E bound the strongest in YPD upstream of RPL43B and Yrr1E bound the weakest in YPD (Figure 2C).
The Yrr1<sup>S</sup> binding decreased in 4NQO compared to YPD, while Yrr1<sup>IE</sup> binding decreased in YPD compared to 4NQO. The binding of Yrr1<sup>IE</sup> was lowest of all the alleles. There was a modest binding increase when yeast were grown in 4NQO. Yrr1 is not known to regulate \textit{RPL43B}. RNA levels of \textit{RPL43B} decreased modestly in response to 4NQO for all three alleles (Figure 2D and Table 3). When all the conditions were included in one test, weak negative correlation was observed with binding and expression; however, the expression of \textit{RPL43B} aligned with the comparisons of the growth of yeast in different conditions (Table 4). For example, growth of yeast with Yrr1<sup>S</sup> was strongly inhibited in 4NQO compared to YPD (Gallagher et al. 2014). It should be noted that expression of \textit{RPL43B} decreased the most when Yrr1<sup>S</sup> was expressed in yeast grown in 4NQO compared to YPD (Figure 2D).

**Impact of overexpression of differentially expressed genes on yeast growth in 4NQO**

Several genes showed increased expression levels in yeast expressing Yrr1<sup>Y</sup> when grown in 4NQO. To assess whether overexpression can bypass the Yrr1<sup>S</sup> 4NQO sensitivity, genes from Figure 1A were overexpressed in yeast along with the different alleles of Yrr1 or a yrr1 knockout. Overexpression plasmids encoding \textit{SNQ2} and the related ABC transporter \textit{PDR5} were transformed into yeast carrying four alleles of Yrr1 including Yrr1<sup>IE</sup>, which is 4NQO-sensitive. In every instance, \textit{SNQ2}, but not \textit{PDR5}, overexpression could rescue growth in the presence of 4NQO, even more than yeast carrying Yrr1<sup>4NQO-resistant</sup> alleles (Figure 3). A selection of diverse genes was placed under a \textit{GAL} promoter to drive overexpression, but only \textit{SNQ2} overexpression could rescue yeast growth on 4NQO (Figure S9 in File S1). While this does not rule out other proteins that may contribute to the 4NQO response, this indicates that higher \textit{SNQ2} expression in 4NQO is the major contributor to higher resistance to 4NQO. The effect of the T775 site in Yrr1 was not sufficient to be the sole regulator of a well-known target of regulation such as \textit{SNQ2}, in contrast to the growth of yeast in different conditions. Transcriptomics and ChIP-Seq are more quantitative and show that the regulation is much more complicated than a simple growth assay previously revealed.

**Impact of Yrr1 on phenotypic expression in nonfermentable carbon sources**

4NQO resistance is linked to poor growth in nonfermentable carbon sources (Gallagher et al. 2014). To metabolize carbon sources such as 4NQO, 4-hydroxyaminoquinoline-1-oxide; GSH, glutathione; YPD, Yeast Peptone Dextrose medium.

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**Figure 5** Live mitochondrial staining of S96 and YJM789 yeast grown in YPD, 4NQO, and glycerol with Mitotracker and Rhodamine B hexyl ester. 4NQO, 4-nitroquinoline-1-oxide; DIC, differential interference contrast; YPD, Yeast Peptone Dextrose medium.

**Figure 6** Growth assays of grande and petite (μ) yeast (FY3 yrr1::URA3) expressing different alleles of Yrr1 on different combinations of carbon source and derivatives of 4NQO. All yeast contained the pYrr1-13xMyc plasmid, and strain labels indicate the allele of Yrr1 encoded on the plasmid. Ten-fold serial dilutions of FY3 yrr1<sup>Δ</sup> yeast grown in YPD or glycerol media with 4NQO or 4HAQO supplemented with the antioxidants GSH. Plates were incubated for 3 d and photographed. 4HAQO, 4-hydroxyaminoquinoline-1-oxide; 4NQO, 4-nitroquinoline-1-oxide; GSH, glutathione; YPD, Yeast Peptone Dextrose medium.

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In comparing the Yrr1<sup>Y</sup> overexpression with the wild-type, the regulation for many genes is not significant. However, the regulation for many genes is not significant. This suggests that the regulation of Yrr1<sup>IE</sup> is more complex than a simple growth assay previously revealed.
However, Yrr1IE was more similar to Yrr1S than Yrr1Y when comparing downregulated genes.

Direct comparisons of transcription and translation between S288c (specifically S96) and YJM789 yeast have consistently found increased expression of YJM789 mRNAs encoding genes involved in respiration (Sun et al. 2016), and relative protein levels between the two strains were also higher when YJM789 yeast were grown in the presence of dextrose (Rong-Mullins et al. 2017). These lines of evidence indicate that YJM789 yeast constitutively upregulates respiration mRNAs and proteins in comparison to S96, regardless of metabolic state, even when cells are grown in dextrose. YJM789 yeast grow slower on nonfermentable carbon sources (Gallagher et al. 2014) and have a lower electron gradient across the mitochondrial membrane than S96 yeast (discussed below). Cox5B expression occurs during anaerobic growth (hypoxic), as compared to its paralog Cox5A, which is expressed during aerobic growth (Burke et al. 1997). COX5B was increased in yeast expressing Yrr1IE and Yrr1Y compared to Yrr1S when grown in dextrose, while COX5A was decreased between Yrr1IE when compared to Yrr1S. Over half of the 452 genes that were downregulated in response to growth on glycerol were involved in ribosome biogenesis, structural ribosomal proteins, and translation (Warner 1999; Woolford and Baserga 2013). Ribosomal mRNAs decreased the most between yeast expressing Yrr1Y, which grow poorly in this condition. Ribosome biogenesis is a costly process, and it is quickly reduced when yeast growth slows (Warner 1999; Woolford and Baserga 2013).

Expression of purine de novo biosynthetic pathway during respiration

Another mRNA that was differentially expressed in yeast expressing Yrr1IE compared to Yrr1S and Yrr1Y was AAH1. Expression of AAH1 in Yrr1IE yeast grown in glycerol compared to YPD decreased 4 log2(fold) compared to yeast expressing other alleles [log2(fold) decrease]. AAH1 is an enzyme that converts adenine to hypoxanthine in the purine salvage pathway (Woods et al. 1984). While all strains irrespective of the allele of Yrr1 downregulated many genes in the purine de novo biosynthetic pathway in both 4NQO and glycerol, these yeast also downregulated IMD3, while IMD4 increased, in glycerol, genes which encode other proteins in the purine salvage pathway (Table S1 and Table S8). Evidence from other systems suggests that the purine salvage pathway can generate purine-based antioxidants in the mitochondria (Becker 1993; Kristal et al. 1999). Respiration is the primary source of endogenous ROS in yeast, and catalase (CTT1) and mitochondrial superoxide dismutase (SOD2) levels were also increased in yeast grown in glycerol.

Shifting yeast to different environmental conditions changed the expression of hundreds of mRNAs. To gain perspective on the role of different alleles of Yrr1, GO term analysis was carried out (Table S9). Changes for each allele were compared between yeast grown in 4NQO or glycerol, and compared to the yeast carrying the same allele of Yrr1 grown in YPD. Representative pathways were graphically represented using the −log of the p-value. Yeast containing Yrr1IE had the fewest GO terms change in either condition (Figure S10 in File S1). Because the Yrr1IE allele is thought to be in part a constitutively active allele, these changes may reflect the phosphorylated state of Yrr1Y. Pathways required for active growth, such as ribosome biogenesis, were consistently downregulated (by strain-specific extents). Yrr1IE downregulated a similar number of genes in the ribosome biogenesis pathway across both conditions, while Yrr1Y showed less downregulation of this pathway by comparing the number of genes in each GO term. Purine biosynthesis, amino acid activation, and IMP biosynthesis were more downregulated in Yrr1Y compared to Yrr1S, while Yrr1IE showed no change. There were also predictive patterns in pathways that were upregulated compared to YPD. By comparing the number of genes in each GO term, yeast carrying the Yrr1S allele had more genes...
involved in cellular respiration upregulated in both 4NQO and glycerol than Yrr1Y and Yrr1IE.

**Yrr1 regulation of metabolism and growth under nonfermentable carbon sources**

Because of the differences in the growth of YJM789 and S96 in glycerol, we assessed the function of the mitochondria from both strains. Glycerol is a nonfermentable carbon source that requires respiration by the mitochondria to metabolize the glycerol. We examined the function of mitochondria using vital stains. Mitotracker stains the mitochondria based on the electron potential across the membrane, and mitochondria from YJM789 were lightly stained compared to those from S96 (Figure 5). In contrast, Rhodamine B hexyl ester, which stains the lipids of the mitochondria, equally stained YJM789 and S96. Taken together with the altered growth on glycerol, this suggested that YJM789 cells have less mitochondria, which were optimized to inhibit growth on glycerol, yet permit growth on YPD. In this case, when grown in the presence of these drugs, yeast were unable to respire and were functionally petite, but the ETC was stopped at different steps based on the target of each drug. If these chemical respiration blockers work at the same step as 4NQO, then no decreased growth inhibition would be seen. Antimycin A blocks the transfer of electrons between cytochrome b and cytochrome c, and decreases oxygen consumption, which occurs at complex IV. Mxyothiazol inhibits cytochrome b1 by competing with ubiquinol, and the effect of mxyothiazol binding induces a red-shift to the visible absorption spectrum of reduced heme b. Oligomycin A is an inhibitor of ATP synthase and blocks proton flow. FCCP breaks the proton gradient because it permeabilizes the mitochondrial membrane and allows electrons to flow around the ATP synthase. Because this occurs after Complex IV and possibly as the cells try to compensate for decreased ATP, the oxygen consumption of FCCP-treated cells increases (Schnellmann 2013). There was no change in growth with antimycin A, mxyothiazol, or oligomycin A with 4NQO, but FCCP exacerbated sensitivity to 4NQO (Figure 7). Blocking the ETC causes electrons to back up, which we posit radicalizes oxygen, increasing the damage from 4NQO treatment. Nevertheless, the addition of GSH rescues all growth inhibition.

**Gene expression patterns in 4NQO and nonfermentable carbon sources**

In the presence of 4NQO, the expression of SNQ2 was significantly higher in yeast with the Yrr1Y allele than with the Yrr1IE allele. For all three alleles, SNQ2 expression was not significantly lower when the yeast cells utilized glycerol when compared to yeast carrying those alleles grown with dextrose as the carbon source. Because cells containing Yrr1Y grow better than those containing Yrr1IE in 4NQO (but grew less in YPglyc media), higher expression of SNQ2 can be associated with higher resistance to 4NQO and with inhibited growth in glycerol media.

**Conclusions**

The role of Yrr1 in 4NQO toxicity may represent the connection between the cell’s responses to various sources of stress, such as nutrient availability (carbon source) and xenobiotics (4NQO), leading us to propose a metabolic model for 4NQO mechanism (Figure 8). In yeast that actively respire, 4NQO is more toxic because of the production of...
both 4HAQO and ROS, and growth can be rescued by the addition of GSH, which quenches the ROS but not the 4HAQO effects. GSH reduces ROS, and can be directly conjugated to 4NQO products (Peklak-Scott et al. 2005) and then transported out of the cell by Ssq2. The addition of 20 times more 4HAQO was required to inhibit yeast growth to the same extent as 4NQO, so most of 4NQO’s toxicity can be traced to the process of conversion, not the 4HAQO metabolite itself. Petite yeast cannot respire and hence were more resistant to 4NQO because 4NQO was not converted. Conversely, forcing yeast to respire increased the toxicity of 4NQO. The unexpected relative resistance to 4NQO of petite yeast illustrated that shifting the carbon metabolism and by proxy the redox state of yeast altered 4NQO response. In other words, repressing respiration increased 4NQO resistance, whether that was by actually preventing respiration in petite cells or expressing alleles of Yrr1 that cause yeast to respire less. Because petite 4NQO-resistant yeast could not grow on a nonfermentable carbon source, it is a most extreme example of reduced growth on a nonfermentable carbon source compared to the only slowed growth of Yrr1Y-carrying yeast in similar conditions. The variation in gene expression among yeast with different Yrr1 alleles was a combination of direct regulation and indirect change. The indirect change in gene expression involved general pathways, which were downregulated in response to stress.

In an effort to correlate ChIP-Seq and transcriptomics in a variety of environmental conditions, we assessed changes in gene expression induced by different alleles of a single transcription factor. While a single polymorphism can flip a 4NQO-sensitive allele to a 4NQO-resistant allele by inducing the transcription of Ssq2, the simplicity of this model did not translate well to the growth of yeast in glycerol. The expression of Yrr1Y conferred a productive transcriptional response to 4NQO, but slowed the growth of yeast in nonfermentable carbon sources. The Yrr1Y allele increased the 4NQO resistance more than either wild-type allele; however, examination of the transcriptomic data revealed significant differences in genome-wide expression between the Yrr1IE and Yrr1Y alleles, despite similar phenotypes in 4NQO. There are likely additional modes of Yrr1 regulation, in particular when cells are grown in glycerol, which precluded the correlation of ChIP-Seq of a single transcription factor and changes in the transcriptome. The changes in the transcriptome between three different conditions with the different alleles of Yrr1 combine not only inputs from Yrr1, but also regulators that respond to these conditions. The transcriptomic data set produced here can be further used for the analysis of expression variation in different carbon sources due to genetic variation in a single transcription factor.

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