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In *Saccharomyces cerevisiae* ρ^0 Cells, *UME6* Contributes to the Activation of ABC Transporter Genes and Pleiotropic Drug Resistance via *RPD3* and *PDR3*

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Abstract: In *Saccharomyces cerevisiae*, the Rpd3L complex includes the histone deacetylase Rpd3 and the DNA binding proteins Ume6 and Ash1 and serves as a transcriptional silencer or enhancer. In *S. cerevisiae*, the transcription of *PDR5*, which encodes a major drug efflux pump, and pleiotropic drug resistance (PDR) are hyperactivated by the transcription factor Pdr3 in $\rho^{0/-}$ cells, which lack mitochondrial DNA. We previously showed that *RPD3* and *UME6* are required for the activation of *PDR5* transcription and PDR in *S. cerevisiae* ρ^0 cells. Here, using real-time PCR analysis, we revealed that *RPD3* and *UME6* are responsible for the activated basal expression of the ABC transporter-encoding genes *SNQ2*, *PDR15*, and *PDR5* in *S. cerevisiae* ρ^0 cells. Furthermore, using real-time PCR analysis and a spot dilution assay, we found that Ume6 increases the basal expression of *PDR5* and *PDR15* and induces PDR in a manner dependent on *RPD3* and *PDR3* in ρ^0 cells. This finding may contribute to the elucidation of the relationships between the molecules required for the activation of ABC transporter genes in *S. cerevisiae* $\rho^{0/-}$ cells and in pathogenic *Candida* species.

Keywords: *Saccharomyces cerevisiae*; *UME6*; *RPD3*; *PDR3*; *PDR5*; pleiotropic drug resistance

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1. Introduction

Multidrug resistance refers to the acquired resistance of cancer cells and microorganisms to a wide variety of chemotherapeutic drugs [1,2]. Therefore, multidrug resistance is a serious concern in the treatment of cancer and microbial infections. In mammalian cells, overexpression of the *MDR1* gene, which encodes P-glycoprotein, an ATP-binding cassette (ABC) transporter, is a major mechanism underlying multidrug resistance in cancer cells [3–5]. Yeast *Candida* species *Candida albicans* and *Candida glabrata* are opportunistic pathogenic microorganisms [1]. In pathogenic *Candida* species, the most prevalent mechanism of multidrug resistance involves increased activity of ABC transporters and major facilitator superfamily pumps [6–8]. Therefore, the elucidation of multidrug resistance mechanisms in cancer cells and *Candida* species is needed to develop anticancer and antifungal drugs that overcome multidrug resistance [3,9,10].

The yeast *Saccharomyces cerevisiae* is used as a model organism for studies of multidrug resistance in pathogenic *Candida* species [11]. The resistance phenotype of *S. cerevisiae* to a broad range of cytotoxic compounds is referred to as pleiotropic drug resistance (PDR) [12]. PDR in *S. cerevisiae* is often associated with the overexpression of plasma membrane ABC transporters [13–15]. Plasma membrane ABC transporters, such as Pdr5, Snq2, and Yor1, export a variety of functionally and structurally unrelated compounds from cells [13,16,17]. *PDR5* encodes a major efflux pump of structurally and functionally unrelated drugs and xenobiotics, such as fluconazole and cycloheximide [18,19].

The transcription of the ABC transporter genes *PDR5*, *SNQ2*, *YOR1*, *PDR10*, and *PDR15* in *S. cerevisiae* is controlled by the paralogous transcription factors Pdr1 and/or

Pdr3 [13,17,20]. DNA consensus motifs, termed pleiotropic drug response elements (PDREs), are located in the promoter regions of *PDR5*, *SNQ2*, *YOR1*, *PDR10*, and *PDR15* and are recognised by Pdr1 and/or Pdr3 [13,17,20,21]. Two PDREs are located in the promoter region of *PDR3* and are recognised by Pdr3 and Pdr1 [22]. Thus, Pdr3 positively autoregulates the transcription of *PDR3* via these two PDREs [22]. Although the functions of Pdr1 and Pdr3 in PDR are partially redundant, Pdr1 plays a major role in PDR and basal *PDR5* expression [17,23]. Gain-of-function mutations in *PDR1* and *PDR3* (e.g., *pdr1-3* and *pdr3-7*) increase the expression of *PDR3*, *PDR5*, *SNQ2*, *YOR1*, *PDR10*, and *PDR15* to activate PDR [14,15,24].

The retrograde signalling pathway is strongly activated in *S. cerevisiae* and *C. glabrata* $\rho^{0/-}$ cells, which have mitochondrial DNA defects [25,26]. Deletion of mitochondrial DNA in *C. glabrata* cells results in increased expression of multidrug resistance genes, including *CgCDR1*, and increased PDR. Pdr3 but not Pdr1 is strongly activated in *S. cerevisiae* $\rho^{0/-}$ cells; therefore, the transcription of ABC transporter genes such as *PDR5* and PDR is hyperactivated [27]. It has previously been reported that the Hsp70 chaperone Ssa1, the mitochondrially localised phosphatidylserine decarboxylase Psd1, the subunit of the RNA polymerase II mediator complex Med12, and the histone H2 ubiquitination enzyme Lge1 are required for the activation of ABC transporter gene transcription and PDR in *S. cerevisiae* $\rho^{0/-}$ cells. Pdr3 is negatively regulated by the Hsp70 protein Ssa1 through a direct interaction [28]. Furthermore, less Ssa1 is bound to Pdr3 in ρ^0 cells than in ρ^+ cells, which contain mitochondrial DNA, suggesting the release of Pdr3 from the negative regulation of Ssa1 in ρ^0 cells [28]. Deletion of Med12 completely suppresses the induction of *PDR5* expression in ρ^0 cells but not in ρ^+ cells [29]. Psd1 is involved in phosphatidylethanolamine (PE) synthesis, and loss of the *PSD1* gene from ρ^0 cells prevents the normal activation of *PDR5* [30]. In addition, the expression of a catalytically inactive form of Psd1 induces *PDR5* transcription in ρ^0 cells [30]. Lge1 is indispensable for the induction of *PDR5* transcription in ρ^0 cells [31,32]. However, the relationships among these molecules required for the activation of ABC transporter genes in ρ^0 cells are unknown.

Hda1 and Rpd3 are the key lysine deacetylases for antifungal resistance in *S. cerevisiae* ρ^+ cells [33]. Although the histone deacetylase Rpd3 is required for Hsp90-dependent antifungal resistance, it is not required for Pdr5-mediated PDR in *S. cerevisiae* ρ^+ cells [33]. In *S. cerevisiae*, the Rpd3L complex participates in chromatin remodelling and transcriptional repression [34–36]. The Rpd3L complex contains Rpd3, the transcriptional scaffold and corepressor Sin3, the histone chaperone Ume1, and the sequence-specific DNA binding proteins Ume6 and Ash1 [36,37]. The Rpd3L complex is recruited to target gene promoters by Ume6 or Ash1 [38]. For example, Ume6 is known to bind to upstream repressor sequence 1 (URS1) in many early meiotic genes (EMGs) [39]. The Rpd3L and Isw2 chromatin remodelling complexes are recruited to EMG promoters by Ume6 [35,40]. Thus, the Rpd3L and Isw2 chromatin remodelling complexes repress EMG expression by hypoacetylation of histone H3 and histone H4 during mitotic growth [34,38,41]. In addition to their roles as transcriptional repressors, Ume6 and Rpd3 play roles as transcriptional activators. The meiosis-specific transcriptional activator Ime1 binds to Ume6 and converts Ume6 from a transcriptional repressor to a transcriptional activator to release EMGs from repression [42]. Rpd3 functions as a transcriptional activator for the anaerobic genes *DAN/TIR* and osmoresponsive genes [43,44].

We previously showed that *RPD3* and *UME6* are required for the hyperactivation of *PDR5* transcription and PDR via retrograde signalling in *S. cerevisiae* ρ^0 cells [45]. In contrast, we also previously reported that Ume6 suppresses the basal transcription of ABC transporters, including *PDR5*, and the PDR in ρ^+ cells; however, *RPD3* is required for drug resistance but does not alter the basal *PDR5* mRNA level [46].

Here, we show that *RPD3* and *UME6* are responsible for the activated basal expression of not only *PDR5* but also *SNQ2* and *PDR15* in *S. cerevisiae* ρ^0 cells. We also show that *UME6* increases the basal expression of *PDR5* and *PDR15* and increases the PDR in a manner dependent on *RPD3* and *PDR3* in ρ^0 cells.

2. Materials and Methods

2.1. Yeast Strains, Plasmids, and Media

The FY1679-28C (*MAT*a, *ura3-52*, *leu2-D1*, *trp1-D63*, *his3-D200*, *GAL2*+) strain was used as the wild-type strain [45–49]. To construct its derivatives with single or double gene deletions, the open reading frames of *UME6*, *PDR3*, or *RPD3* were replaced with *KanMX* or *bleMX6* gene cassettes by PCR-mediated one-step gene disruption in the FY1679-28C background [45,46,49]. The ρ^0 derivatives of the strains described above were obtained by plating the cells once on YPD agar plates (2% glucose, 1% yeast extract, 2% Bactopeptone, and 2% agar) containing 40 µg/mL of ethidium bromide [50]. The yeast strains used in this work are listed in Table 1.

Table 1. Yeast strains used.

Yeast Strain	Genotype	Mitochondrial Genotype	Source or Reference
FY1679-28C	*MATa ura3-52 leu2-Δ1 trp1-Δ63 his3-Δ200 GAL2+*	ρ^0	[47]
ume6Δ	*MATa ume6Δ::bleMX6 ura3-52 leu2-Δ1 trp1-Δ63 his3-Δ200 GAL2+*	ρ^0	[45]
ume6rpd3Δ	*MAT ume6Δ::bleMX6 rpd3Δ::kanMX ura3-52 leu2-Δ1 trp1-Δ63 his3-Δ200 GAL2+*	ρ^0	This study
ume6pdr3Δ	*MAT ume6Δ::bleMX6 pdr3Δ::kanMX ura3-52 leu2-Δ1 trp1-Δ63 his3-Δ200 GAL2+*	ρ^0	This study

The centromeric plasmid pRS313 (cen, *HIS3*) was purchased from the National Bio-Resource Project, Japan [51]. *UME6* was PCR-amplified from genomic DNA using PrimeSTAR GXL Polymerase (TaKaRa) and the following primers homologous at −405 and +3011: *UME6* forward, 5′-GGCGTCGACTATACAAAAGCGACACGTCGTCTGA-3′ (the underline is the *Sal*I recognition sequence) and *UME6* reverse, 5′-CCGCCCGGGCAATGAATTTTGCAAATCCAACGTG-3′ (the underline is the *Xma*I recognition sequence). The resulting PCR product and the pRS313 plasmid were digested with *Xma*I and *Sal*I. The digested PCR product was cloned and inserted into the linearised pRS313 plasmid using DNA Ligation Kit Mighty Mix (TaKaRa Bio Inc., Shiga, Japan). The resulting plasmid was termed pRS313-*UME6*.

Yeast cells were grown in YPD medium (2% glucose, 1% yeast extract, 2% Bactopeptone) at 30 °C.

2.2. Spot Dilution Assay

The relative resistance of each yeast strain to fluconazole or cycloheximide was estimated with a spot dilution assay using YPD media [45,46,49]. The ρ^0 cells from each yeast strain were aerobically grown to the logarithmic phase (at an OD_{600} of 0.6–0.9) at 30 °C in YPD media in triplicate. Five microlitre aliquots of 10-fold serial dilutions of cultures containing the same number of cells were spotted on YPD plates with or without 10 µg/mL of fluconazole (Nacalai Tesque, Kyoto, Japan) (or 0.3 µg/mL of cycloheximide (Wako Pure Chemicals, Osaka, Japan)) and incubated at 30 °C for 7 days.

2.3. RNA Extraction from ρ^0 Cells of Each Yeast Strain Grown to the Logarithmic Growth Phase

ρ^0 cells from each yeast strain were grown to an OD_{600} of 7–9 in YPD media in duplicate. The cultures were diluted to an OD_{600} of 0.1 and grown to an OD_{600} of 0.4–0.8 [45,46,49]. The cultures were recovered, and the cells in the cultures were pelleted, washed, frozen at −80 °C, and used for total RNA extraction [45,46,49]. Total RNA was isolated from the yeast cells using a NucleoSpin RNA kit (TaKaRa Bio Inc., Shiga, Japan) according to the manufacturer's protocol.

2.4. Real-Time RT–PCR

Reverse transcription of total RNA was performed using FastGene Scriptase II cDNA 5× ReadyMix (NIPPON Genetics, Tokyo, Japan) and oligo dT primers (TaKaRa Bio Inc.,

Shiga, Japan). SYBR Green qRT–PCR for cDNA from the individual duplicate samples was performed using TB Green Premix Ex Taq II (TaKaRa Bio Inc., Shiga, Japan) in a Step One Real-time PCR system (Applied Biosystems, Foster City, CA, USA) [52]. A minus reverse transcriptase control was used as the negative control. Serial dilutions of control cDNA were prepared to produce a standard curve for each primer pair. The primers used for qRT–PCR are listed in Table S1. The mRNA levels of each target gene were measured by qRT–PCR and normalised to those of the housekeeping gene *ACT1*, which was used as an endogenous control. The normalised mRNA levels are shown relative to samples from the wild-type strains, which were set to 1.

2.5. Statistical Analysis

An unpaired Student's *t*-test was used for statistical analysis. Results with $p < 0.05$ and $p < 0.01$ were considered statistically significant.

3. Results

3.1. UME6 and RPD3 Are Required for the Upregulation of the Steady-State mRNA Levels of SNQ2, PDR15, and PDR5 in ρ^0 Cells

We previously reported that activated *PDR5* transcriptional expression by retrograde signalling was significantly reduced in ρ^0 cells of the *rpd3*Δ and *ume6*Δ strains. Thus, we investigated the mRNA levels of the major ABC transporter genes *PDR15*, *PDR10*, *SNQ2*, and *YOR1*, including *PDR5*, in ρ^0 cells of the wild-type, *ume6*Δ, and *rpd3*Δ strains by qRT–PCR. qRT–PCR revealed that the *YOR1*, *SNQ2*, *PDR15*, *PDR10*, and *PDR5* mRNA levels were statistically significantly lower in the *ume6*Δ strain than in the wild-type strain ($p < 0.05$) (Figure 1). In contrast, the expression of *SNQ2*, *PDR15*, and *PDR5*, but not that of *YOR1* or *PDR10*, was statistically significantly lower in the *rpd3*Δ strain than in the wild-type strain ($p < 0.05$) (Figure 2). In both the *ume6*Δ and *rpd3*Δ mutants, although the *SNQ2* mRNA levels were relatively mildly reduced, the mRNA levels of *SNQ2*, *PDR15*, and *PDR5* were reduced compared with those in the wild-type strain (Figures 1 and 2). These results suggest that *SNQ2*, *PDR15*, and *PDR5* are upregulated by Ume6 via Rpd3 in ρ^0 cells.

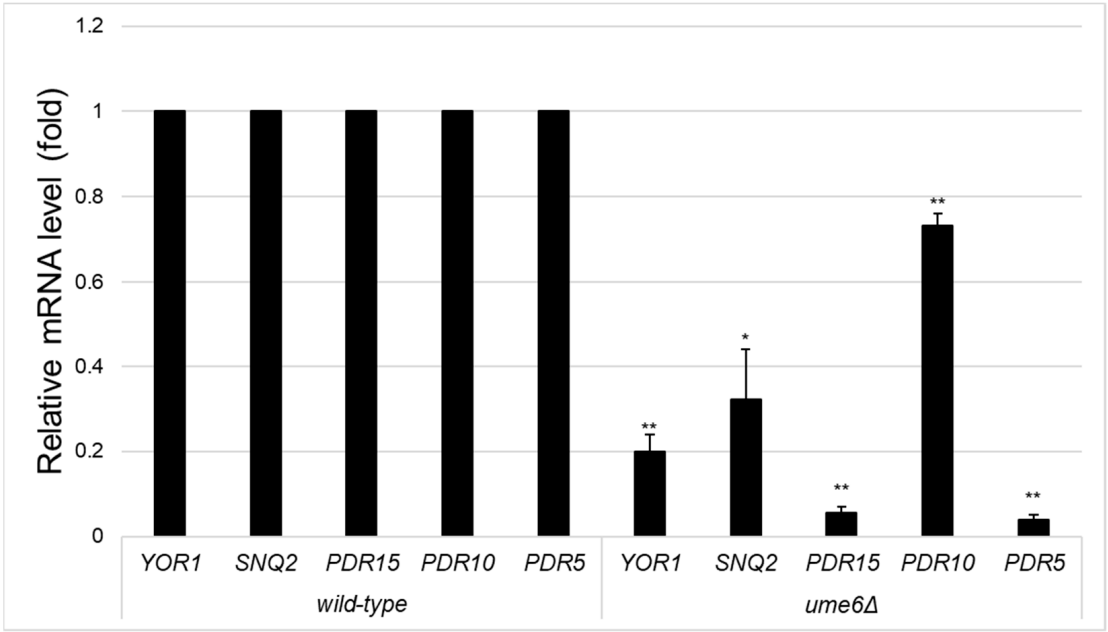

Figure 1. Transcription levels of ABC transporters in ρ^0 cells of the wild-type and *rpd3*Δ strains in the logarithmic growth phase. Relative *YOR1*, *SNQ2*, *PDR15*, *PDR10*, and *PDR5* mRNA levels in ρ^0 cells of the wild-type and *ume6*Δ strains in the logarithmic growth phase were determined by qRT–PCR. One asterisk (*) or two asterisks (**) indicate *p* values less than 0.05 or 0.01, respectively.

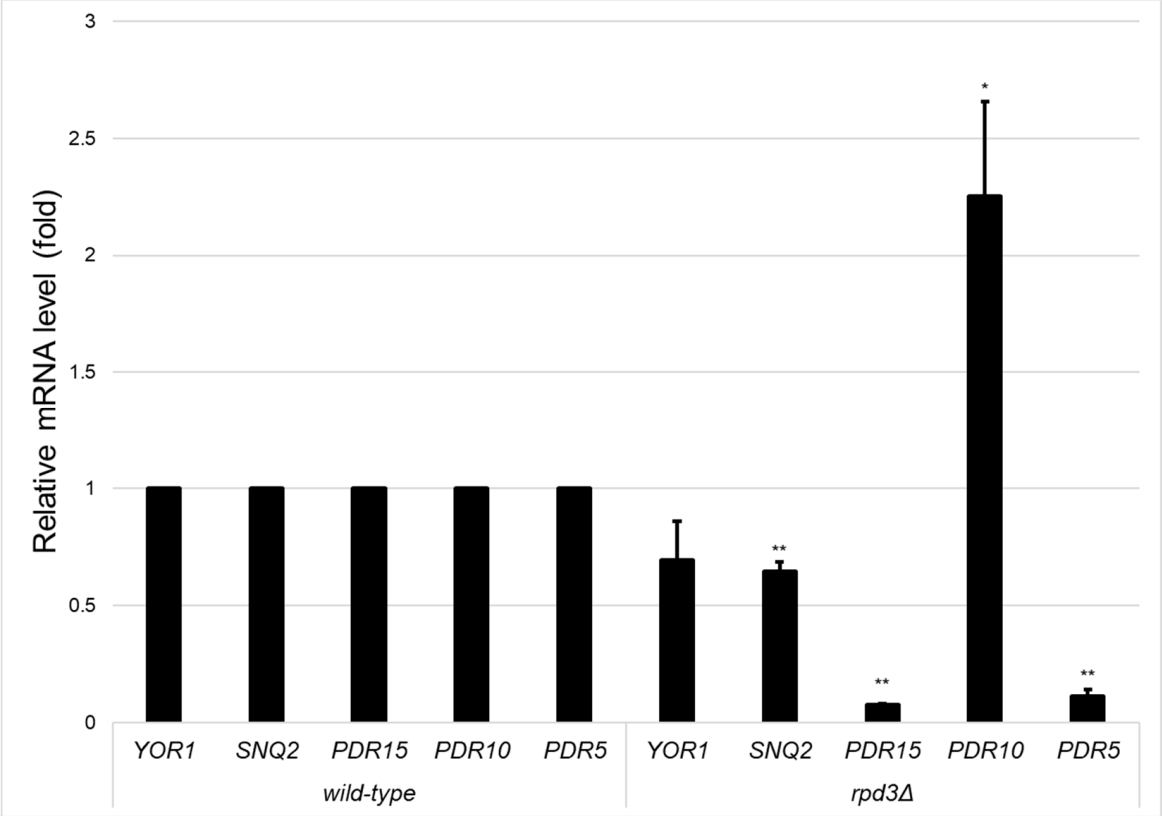

Figure 2. Transcription levels of ABC transporters in ρ^0 cells of the wild-type and *rpd3*Δ strains in the logarithmic growth phase. The relative transcription levels of *YOR1*, *SNQ2*, *PDR15*, *PDR10*, and *PDR5* were determined in ρ^0 cells of the wild-type and *rpd3*Δ strains in the logarithmic growth phase by qRT–PCR. One asterisk (*) or two asterisks (**) indicate *p* values less than 0.05 or 0.01, respectively.

*3.2. RPD3 and PDR3 Are Required for the Partial Rescue of the Reduction in PDR15 and PDR5 Expression in the ume6*Δ *Strain by Exogeneous UME6 Expression*

To validate whether reduced mRNA levels of *PDR15* and *PDR5* in the *ume6*Δ mutant result from the deletion of *UME6*, we investigated whether decreased mRNA levels of *PDR15* and *PDR5* in the *ume6*Δ mutant are complemented with exogeneous expression of *UME6*. Although *PDR15* and *PDR5* mRNA levels were statistically significantly lower in *ume6*Δ pRS313 than in the wild-type strain ($p < 0.01$), the reductions in basal *PDR15* and *PDR5* mRNA levels in *ume6*Δ pRS313 were incompletely restored to wild-type pRS313 levels in *ume6*Δ pRS313–*UME6* ($p < 0.01$) (Figure 3). The incomplete rescue by introduction of pRS313–*UME6* into the *ume6*Δ mutant could result from nonphysiological levels of *UME6* expression, because qRT–PCR revealed that *UME6* is overexpressed at high levels—at least sevenfold—in *ume6*Δ pRS313–*UME6* compared with wild-type pRS313. Regardless, this restoration of *PDR15* and *PDR5* mRNA levels suggests that the deletion of *UME6* is a cause of reduced mRNA levels of *PDR15* and *PDR5* in the *ume6*Δ mutant.

Next, we investigated whether *RPD3 and PDR3* are required for the partial rescue of reduced mRNA levels of *PDR15* and *PDR5* in the *ume6*Δ mutant by exogenous expression of *UME6*. Although there was no significant difference in the mRNA levels of *PDR15* and *PDR5* between *ume6*Δ pRS313 and *ume6*Δ*rpd3*Δ pRS313 ($p > 0.05$), no upregulation of *PDR15* and *PDR5* mRNA levels was detected in *ume6*Δ*rpd3*Δ pRS313–*UME6* compared to *ume6*Δ*rpd3*Δ pRS313 ($p > 0.05$) (Figure 3). Similarly, there was no significant difference in the mRNA levels of *PDR15* and *PDR5* between *ume6*Δ pRS313 and *ume6*Δ*pdr3*Δ pRS313 ($p > 0.05$), while the mRNA levels of *PDR15* and *PDR5* in *ume6*Δ*pdr3*Δ pRS313–*UME6* were not statistically significantly higher than those in *ume6*Δ*pdr3*Δ pRS313 ($p > 0.05$) (Figure 3). These results indicate that *RPD3 and PDR3* are required for incomplete rescue

of the reduction in *PDR15* and *PDR5* mRNA levels in the *ume6*Δ mutant by exogenous expression of *UME6*.

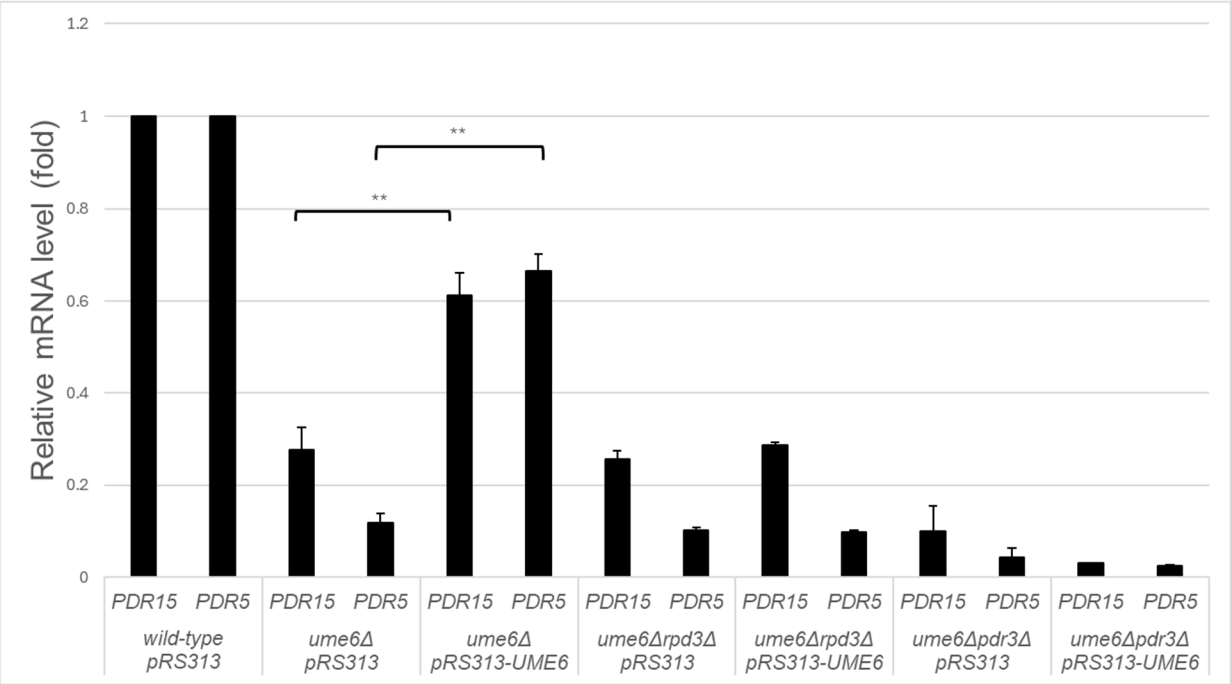

Figure 3. *RPD3* and *PDR3* are required for partial complementation of the reduction in *PDR15* and *PDR5* mRNA levels caused by exogenous *UME6* expression in ρ^0 cells of the *ume6*Δ mutant. Relative *PDR15* and *PDR5* mRNA levels in the logarithmic growth phase were determined in ρ^0 cells of each strain by qRT–PCR. The wild-type strain transformed with an empty pRS313 plasmid is termed wild-type pRS313. The *ume6*Δ mutants transformed with an empty pRS313 plasmid or pRS313-*UME6* are designated *ume6*Δ pRS313 or *ume6*Δ pRS313-*UME6*, respectively. The *ume6*Δ*rpd3*Δ mutants transformed with an empty pRS313 plasmid or pRS313-*UME6* are designated *ume6*Δ*rpd3*Δ pRS313 or *ume6*Δ*rpd3*Δ pRS313-*UME6*, respectively. The *ume6*Δ*pdr3*Δ mutants transformed with an empty pRS313 plasmid or pRS313-*UME6* are termed *ume6*Δ*pdr3*Δ pRS313 or *ume6*Δ*pdr3*Δ pRS313-*UME6*, respectively. two asterisks (**) indicate *p* values less than 0.01.

*3.3. RPD3 and PDR3 Are Required for Incomplete Restoration of Susceptibility to Fluconazole and Cycloheximide in the ume6*Δ *Strain via Exogeneous UME6 Expression*

To examine whether exogeneous *UME6* expression from a plasmid with a low copy number can rescue the susceptibility of *ume6*Δ mutant ρ^0 cells to the PDR substrates fluconazole and cycloheximide, a spot dilution assay was carried out. We found that *ume6*Δ pRS313 displays greater susceptibility to fluconazole and cycloheximide than does wild-type pRS313 (Figure 4). In contrast, exogeneous *UME6* expression in *ume6*Δ pRS313–*UME6* incompletely rescued the susceptibility to fluconazole and cycloheximide in *ume6*Δ pRS313 (Figure 4). These results can be explained by the partial rescue of *PDR15* and *PDR5* reduction by exogenous *UME6* expression in *ume6*Δ pRS313–*UME6*, as shown in Figure 3. This partial restoration of susceptibility to fluconazole and cycloheximide also suggests that the deletion of *UME6* is a cause of high susceptibility to fluconazole and cycloheximide in the *ume6*Δ mutant.

We next examined whether *RPD3* and *PDR3* are required for incomplete restoration of high susceptibility to fluconazole and cycloheximide in the *ume6*Δ mutant by exogenous *UME6* expression. In contrast to the case of *ume6*Δ pRS313 and *ume6*Δ pRS313–*UME6*, high susceptibility to fluconazole and cycloheximide in *ume6*Δ*rpd3*Δ pRS313 and *ume6*Δ*pdr3*Δ pRS313 was not complemented with exogenous *UME6* expression in *ume6*Δ*rpd3*Δ pRS313–*UME6* and *ume6*Δ*pdr3*Δ pRS313–*UME6* (Figure 4). These results suggest that Ume6-mediated activation of PDR in ρ^0 cells is dependent on *RPD3* and *PDR3*.

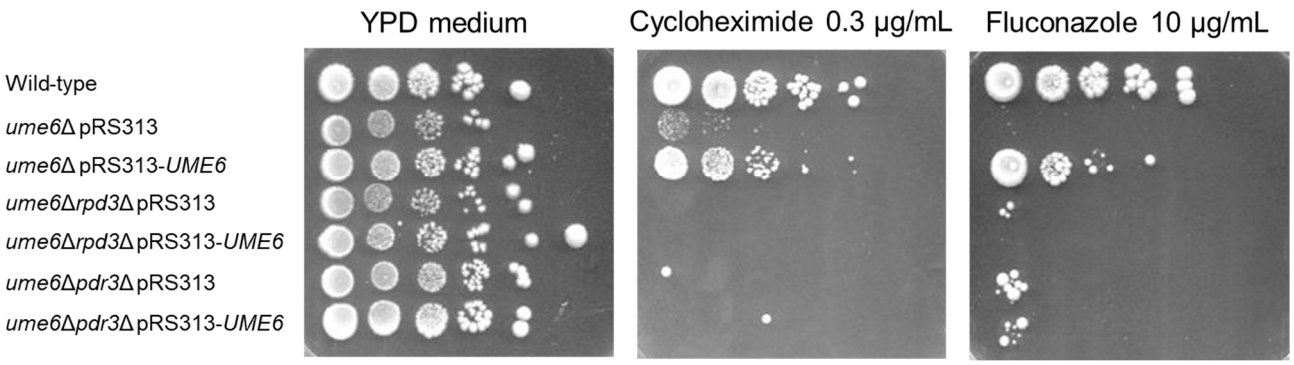

Figure 4. *RPD3* and *PDR3* are required for partial complementation of sensitivity to fluconazole and cycloheximide by exogenous *UME6* expression in ρ^0 cells of the *ume6*Δ mutant. Fluconazole or cycloheximide resistance in the ρ^0 cells of the wild-type, *ume6*Δ, *ume6*Δ*rpd3*Δ, and *ume6*Δ*pdr3*Δ strains with an empty pRS313 plasmid or a pRS313-*UME6* plasmid was determined by the spot dilution assay.

4. Discussion

In this report, we showed that *UME6* and *RPD3* are required for upregulating the steady-state mRNA expression of *SNQ2*, *PDR15*, and *PDR5* in *S. cerevisiae* ρ^0 cells. Although *SNQ2*, *PDR15*, *PDR5*, and *PDR10* are activated by Pdr3 in ρ^0 cells [50], the mRNA levels of *SNQ2*, *PDR15*, and *PDR5* but not that of *PDR10* were increased by Ume6 and Rpd3 in ρ^0 cells (Figures 1 and 2). Thus, Ume6 and Rpd3 may bind to the promoter regions of *SNQ2*, *PDR15*, and *PDR5* but not to the promoter region of *PDR10* and increase the transcription of *SNQ2*, *PDR15*, and *PDR5* via Pdr3 in ρ^0 cells. Although Ume6 is bound to the promoters of *PDR5*, *PDR10,* and *YOR1* but not to that of *SNQ2* or *PDR15* in *S. cerevisiae* ρ^+ cells, it is unknown whether Ume6 is localised at the *SNQ2*, *PDR15*, and *PDR5* promoters in ρ^0 cells [53,54]. Alternatively, the upregulation of *SNQ2*, *PDR15*, and *PDR5* mRNA expression mediated by Rpd3 and Ume6 in ρ^0 cells may be indirectly caused by changes in the expression of other genes. In addition, the expression of *PDR10* was significantly lower in the *ume6*Δ strain but significantly greater in the *rpd3*Δ strain than in the wild-type strain (Figures 1 and 2). This may be because Rpd3 deacetylates proteins involved in the regulation of *PDR10* independent of Ume6.

In an example similar to the transcriptional regulation of *SNQ2*, *PDR15*, and *PDR5* by Ume6 and Rpd3 in ρ^0 cells, the histone chaperone Rtt106 specifically localises to the *SNQ2*, *PDR15*, and *PDR5* promoters in a manner dependent on Pdr3 but not Pdr1 in *S. cerevisiae* ρ^+ cells [54]. The histone chaperone Rtt106 is also essential for Pdr3-mediated basal expression of *SNQ2*, *PDR15*, and *PDR5* in ρ^+ cells [54]. In addition, *PDR3* carrying the gain-of-function allele *pdr3-7* in ρ^+ cells has fewer target genes than Pdr3 activated by retrograde signalling in ρ^0 cells [50]. These findings suggest that other transcription factors act in concert with Pdr3 in ρ^0 cells. Therefore, the colocalisation of cooperative factors such as Ume6 and Rpd3 with Pdr3 at promoters may be required for the activation of target genes by Pdr3 in ρ^0 cells.

We also revealed that Ume6 activates *PDR15* and *PDR5* transcription and PDR via *RPD3* and *PDR3* in ρ^0 cells. Although it is currently unknown whether the histone deacetylase activity of Rpd3 is required for the activation of *PDR15* and *PDR5* transcription and PDR by retrograde signalling in ρ^0 cells, histone deacetylation by Rpd3 can lead to the transcriptional activation of DNA damage-inducible and osmoresponsive genes [43,55]. Therefore, the histone deacetylase activity of Rpd3 recruited to the *PDR15* and *PDR5* promoters by Ume6 may activate the transcription of *PDR15* and *PDR5.* Moreover, in an example similar to the dependency of *UME6* on *RPD3* and *PDR3* for the activation of *PDR15* and *PDR5* transcription and PDR in ρ^0 cells, *PDR1* is required for the activation of *PDR5* and *YOR1* transcription by the C-terminal region of Zuo1 (Zuo1C) in ρ^+ cells [56]. Zuo1, a ribosome-associated J protein, can positively regulate the transcription of *PDR5*

and *YOR1*, increasing the activity of the transcription factor Pdr1 when it is not bound to ribosomes [56]. Exogenous expression of Zuo1C increases the expression of reporter genes driven by the *PDR5* or *YOR1* promoter (*PDR5*–lacZ and *YOR1*–lacZ) in the order of 10- and 4-fold, respectively, in both wild-type cells and *pdr3*Δ cells [56]. In contrast, no activation of *PDR5*–lacZ or *YOR1*–lacZ was observed in *pdr1*Δ cells expressing Zuo1C [56]. In addition, similar levels of drug resistance were observed in wild-type and *pdr3*Δ cells expressing Zuo1C, whereas no drug resistance in the presence of the drug was observed in *pdr1*Δ cells [56]. Thus, Prunuske et al. concluded that Zuo1-mediated activation of *PDR5*, *YOR1*, and PDR is dependent on Pdr1 [56]. In addition, Hallstrom et al. investigated the dependency of *PDR13* on *PDR1* for *PDR13*-mediated induction of cycloheximide resistance in ρ^+ cells using a spot assay [57]. Hallstrom et al. concluded that *PDR1* is required for the induction of cycloheximide resistance by *PDR13* in ρ^+ cells [57]. These reports support the relevance of our approach in examining the dependency of *UME6* on *RPD3* and *PDR3* for the activation of *PDR15* and *PDR5* transcription and PDR.

In addition, how Ume6 and Rpd3 are involved in the activation of *PDR15* and *PDR5* transcription and PDR by Pdr3 in ρ^0 cells is unknown. It has been reported that the Rpd3 complex is required for the normal function of the transcriptional activator Upc2 and its stable binding to the promoter of the anaerobic gene *DAN1* [44]. Therefore, Ume6 and Rpd3 may also localise to the *PDR15* and *PDR5* promoters in ρ^0 cells and facilitate the normal function of Pdr3 and its stable binding to these promoter regions (Figure 5).

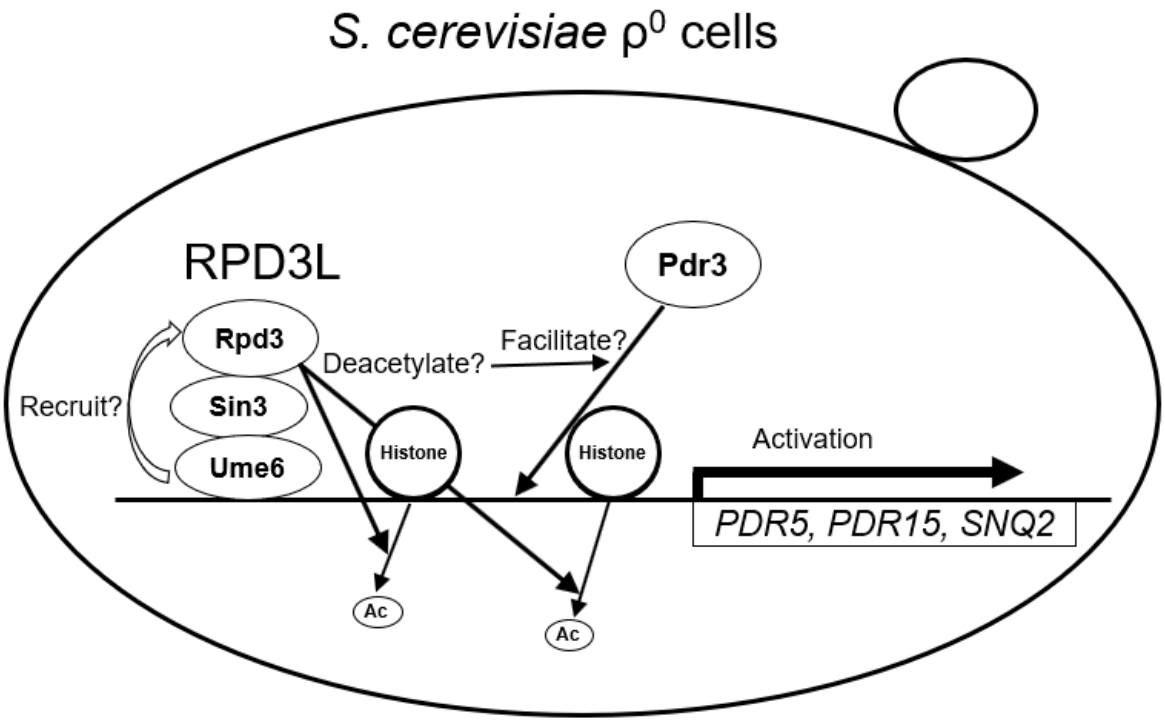

Figure 5. Model of the correlation among Ume6, Rpd3, and Pdr3 and the activation of *SNQ2*, *PDR15*, and *PDR5* in ρ^0 cells. In this model, Ume6 recruits Rpd3 to the *SNQ2*, *PDR15*, and *PDR5* promoter regions, and the histone deacetylase activity of Rpd3 facilitates the binding of Pdr3 to these promoter regions.

Currently, the relationships among the molecules required for the activation of ABC transporter genes in ρ^0 cells are unknown. We revealed that *UME6* activates basal *PDR15* and *PDR5* transcription and PDR in a manner dependent on *RPD3* and *PDR3* in ρ^0 cells (Figures 3 and 4). In contrast, we previously showed that *UME6* suppresses basal *PDR5* expression and PDR in ρ^+ cells [46]. Med12 in the L-Mediator complex also contributes to the induction of *PDR5* expression in ρ^0 cells but not in ρ^+ cells. The transcriptional mediator complex serves as the interface between gene-specific transcription factors and

the RNA polymerase II machinery [58]. The L-Mediator complex in *S. cerevisiae* contains the core mediator complex and the Cdk8 subcomplex. The Cdk8 subcomplex is composed of Med12/Srb8, Med13/Srb9, the cyclin-dependent kinase Cdk8/Srb10, and cyclin C/Srb11 [59]. Pdr1 and Pdr3 can bind to the KIX domain of a mediator subunit called Med15/Gal11 of the L-Mediator complex [32]. Loss of Med12 from the Cdk8 complex completely suppresses the induction of *PDR5* expression in ρ^0 cells but not in ρ^+ cells [29]. In addition, Lge1 is required for proper *PDR5* induction in ρ^0 cells but not in ρ^+ cells, independent of its role in histone H2B ubiquitination [31]. These results indicate a difference in the regulatory machinery of *PDR5* transcription between ρ^+ and ρ^0 cells. Thus, the identification of all molecules specifically required for the activation of ABC transporter genes and PDR in $\rho^{0/-}$ cells is needed to reveal the relationships among these molecules.

C. albicans and *C. glabrata* are the two most common yeast pathogens in humans [60,61]. *S. cerevisiae* is phylogenetically closer to *C. glabrata* than to *C. albicans* [62]. Loss of the mitochondrial genome also leads to increased PDR in *C. glabrata* [63]. However, *C. albicans* cannot survive the loss of mitochondrial DNA and therefore is petite negative [63]. The molecules involved in PDR pathway activation in *S. cerevisiae* ρ^0 cells are parallel to those in *C. glabrata* ρ^0 cells. For example, the Pdr3 homologue CgPdr1 in *C. glabrata*, upon compromise of mitochondrial function, upregulates the expression of *CDR1* and *CDR2*, the homologues of *S. cerevisiae PDR5* [64]. In addition, in *C. glabrata* ρ^+ cells, the loss of the Rpd3 orthologue CgRpd3 increases susceptibility to caspofungin at high concentrations [65]. However, no mechanistic explanation—for example, gene targets or changes in histone modifications—has been provided for the caspofungin hypersensitive phenotype. Thus, the Ume6 orthologue Zcf11 in *C. glabrata* may also be responsible for multidrug resistance via transcriptional regulation of efflux genes. Therefore, identifying specific inhibitors of Zcf11 may lead to the development of drugs with activity against the multidrug-resistant pathogen *C. glabrata*.

5. Conclusions

We previously showed that *RPD3* and *UME6* are required for the activation of *PDR5* and PDR in ρ^0 cells. This study investigated the dependence of Ume6 on Rpd3 and Pdr3 in basal transcription of the ABC transporters, including *PDR5*, and PDR in *S. cerevisiae* ρ^0 cells. Using a real-time PCR, *RPD3* and *UME6* were responsible for the activated basal expression of the ABC transporter-encoding genes *SNQ2*, *PDR15*, and *PDR5* in *S. cerevisiae* ρ^0 cells. Furthermore, Ume6 increased the basal expression of *PDR5* and *PDR15*, and induced PDR in a manner dependent on *RPD3* and *PDR3* in ρ^0 cells. This work may contribute to an elucidation of the relationships between molecules required for the activation of the ABC transporter genes in $\rho^{0/-}$ cells.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/microbiolres15020048/s1: Table S1: Primers used for qRT–PCR.

Author Contributions: M.F., M.O. and Y.Y. performed experiments. M.F. and Y.Y. analysed data. Y.Y. made contributions to the conception, design, and drafting of the manuscript. All authors have read and agreed to the published version of the manuscript.

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