Diazaborine Resistance in the Yeast Saccharomyces cerevisiae Reveals a Link between YAP1 and the Pleiotropic Drug Resistance Genes PDR1 and PDR3*

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We have investigated the mechanisms underlying resistance to the drug diazaborine in Saccharomyces cerevisiae. We used UV mutagenesis to generate resistant mutants, which were divided into three different complementation groups. The resistant phenotype in these groups was found to be caused by allelic forms of the genes AFG2, PDR1, and PDR3. The AFG2 gene encodes an AAA (ATPases associated with a variety of cellular activities) protein of unknown function, while PDR1 and PDR3 encode two transcriptional regulatory proteins involved in pleiotropic drug resistance development. The isolated PDR1–12 and PDR3–33 alleles carry mutations that lead to a L1044Q and a Y276H exchange, respectively. In addition, we report that overexpression of Yap1p, the yeast homologue of the transcription factor AP1, results in a diazaborine-resistant phenotype. The YAP1-mediated diazaborine resistance is dependent on the presence of functional PDR1 and PDR3 genes, although PDR3 had a more pronounced effect. These results provide the first evidence for a functional link between the Yap1p-dependent stress response pathway and Pdr1p/Pdr3p-dependent development of pleiotropic drug resistance.

One of several possibilities by which cells exert resistance to inhibitory substances is the exclusion of these toxic compounds by membrane-located efflux pumps. In eukaryotic cells, certain pumps may be able to transport unspecifically a variety of unrelated drugs, resulting in resistance to more than one inhibitor, a phenomenon called pleiotropic drug resistance (1). The genes involved in this process are referred to as pleiotropic drug resistance (PDR) genes. In general, pleiotropic drug resistance involves the overexpression of membrane pumps from the ABC (ATP-binding cassette) or MFS (major facilitators superfamily) type, all of which transport toxic compounds out of the cell or into the vacuole, thus preventing inactivation of the drug target (1–3). Well known examples for such pumps in Saccharomyces cerevisiae are Pdr5p, Snq2p, and Yor1p (4–7).

Each of these transporters manages the export of several unrelated compounds. To date, the best characterized ABC protein involved in pleiotropic drug resistance is Pdr5p. This 175-kDa protein of the ABC transporter family contains a duplicated six-membrane span domain with a repeated ATP-binding motif (4, 5). Pdr5p displays ATPase activity and confers upon overexpression resistance to cycloheximide, sulfonylurea, cadmium, zinc, and reactive oxygen species (1). Yap1p-mediated cycloheximide resistance is independent of PDR5 overexpression and is thought to involve an as yet unidentified transporter. Yap1p is a transcription activator belonging to the bZIP type family (8).

The PDR1 and PDR3 genes have also been found to mediate resistance to a wide variety of compounds, since mutations in the respective genes lead to resistance to many structurally unrelated drugs (1). PDR1 and PDR3 code for homologous transcriptional regulators of the Zn(2)Cys(6) family, and both gene products were demonstrated to interact with the PDR5, SNQ2, and YOR1 promoters where they exert transcription activator functions (5, 7, 9, 10, 13). Gain-of-function alleles of both PDR1 and PDR3 or overexpression of the wild type genes was reported to cause overexpression of the Pdr5p and Snq2p proteins, explaining the correlation of these regulatory proteins and resistance (11–13). PDR1-dependent resistance to cycloheximide and chloramphenicol requires a functional PDR5 gene (14). In addition to PDR5, SNQ2, and YOR1, Pdr1p appears to regulate several other genes, some of which are involved in pleiotropic drug resistance development (1).

In this paper, we report the results from our studies on diazaborine resistance in S. cerevisiae. Diazaborines are heterocyclic boron-containing compounds that exhibit strong antibacterial activity (15, 16). These drugs cause inhibition of fatty acid and phospholipid biosyntheses in Gram-negative bacteria, like Escherichia coli or Salmonella typhimurium (17). The target for diazaborine in E. coli and S. typhimurium was identified as the enoyl-(acyl carrier protein) reductase (FabI), catalyzing a key regulatory step in the elongation cycle of fatty acid biosynthesis (18). Diazaborine was found to bind to the purified

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FabI protein only in the presence of NAD(H), thereby inhibiting its essential enzymatic function. Diazaborine-resistant mutants were isolated and characterized from E. coli and S. typhimurium and found to contain allelic forms of the fabI gene (19).

We demonstrate here that a eukaryotic organism, the yeast S. cerevisiae, is also sensitive to diazaborine. To study the mechanism of action of diazaborine in yeast, we used UV mutagenesis to generate drug-resistant mutants. Three classes of resistant mutants were obtained. We show that allelic forms of the genes AFG2, PDR1, and PDR3 cause resistance and that overexpression of YAP1 also leads to diazaborine resistance in yeast. YAPI-mediated resistance is dependent on functional PDR1 and PDR3 genes, demonstrating for the first time a functional interaction between YAPI-mediated gene regulation and the yeast PDR network.

EXPERIMENTAL PROCEDURES

Yeast Strains and Culture Conditions—All S. cerevisiae strains used in this study are listed in Table I. An isogenic set of wild type and PDR3-2 strains was obtained by back-crossing a PDR3-2 strain (kindly provided by J. Subik) several times into the W303–1A background to generate the strain YYM14-A4 (MATa leu2–3,112 his3–11,15 can1 ura3–52 ade2–1 his3 his3 ura3 leu2 ade2) and its isogenic PDR3 segregant YYM14-03 (MATa PDR3-2 ura3 leu2 his3 his3 ura3 leu2 ade2). Synthetic medium (SD), supplemented with appropriate nutrients for maintenance of plasmids, or rich medium (YPD), were prepared exactly as described by Sherman et al. (20). Yeast were grown routinely at 30°C. Yeast transformations were carried out by the method of Ho et al. (21).

UV Mutagenesis and Classification of the Resistant Mutants—UV mutagenesis of wild type strain A2 was performed according to the protocol of Jardrosits et al. (22). It allowed the isolation of 17 stable mutants with diazaborine-resistant phenotypes. To test if resistance is dominant or recessive, we crossed the mutants DM1 to DM17, all of them MATa, with the diazaborine-sensitive strain L1544 (MATa lys9). All diploids from these crosses were diazaborine-resistant, indicating that the mutations are dominant. The diploid strains were sporulated and subjected to tetrad analysis. From every cross, at least eight complete tetrads were examined. All of them were found to segregate 2:2 for diazaborine resistance and diazaborine sensitivity, demonstrating that the mutations reside at a single genetic locus. From one of the mutants we cloned an allelic form of AFG2 (DRG1–1).2 DRG1–1 was introduced into the wild type strain A2, resulting in strain ADR6. To determine the number of complementation groups, strain ADR6 was crossed with the diazaborine-sensitive strain L1544 (MATa lys9). After sporulation and tetrad analysis, we isolated a haploid strain, AL1 (MATa lys9, DRG1–1). With this strain, we backcrossed all of the remaining original 16 mutants. After sporulation and tetrad analysis, the segregation pattern of the diazaborine-resistant phenotype was studied. At least six complete tetrads from each cross were checked. Only resistant spores were obtained in 10 cases, indicating that all 10 mutants fall into the same complementation group as ADR6, i.e. drg1. To examine if the remaining six mutants form a homogeneous complementation group, we crossed one of these mutants (DM6) with strain L1544 (MATa lys9), sporulated the diploid, and isolated resistant haploid MATa cells. One was selected to be used for a further cross. Each of the remaining five mutants of the second recombination group was crossed with this strain and subjected to tetrad dissection. At least eight complete tetrads were examined. In three cases, only resistant spores could be found, indicating that the cells harbor mutations in the same locus. A similar analysis revealed that the remaining two mutants belong to a third recombination group. These recombination groups were designated drg2 and drg3, respectively.

Construction of Genomic Libraries—The genomic libraries of yeast strains DM6, DM12, DM6 (yap1), and DM12 (yap1) were constructed in BamHI-digested plasmid YEp351 by using SacI-digested genomic DNA with a size of fragments in the range of 5–10 kb. After transformation into E. coli, in all cases, at least 4.5 × 106 transformants were obtained. Transformants from each library were pooled, and a plasmid preparation was performed.

Genomic Screening, DNA Manipulations, and Sequencing—Yeast strain A2 was transformed with a 2-μm YEp351-based genomic library from DM6 or DM12, respectively, to leucin prototrophy and diazaborine resistance (50 μg/ml) under double selection conditions. In each case about 100,000 transformants were screened. Eleven transformants from the DM6 and 17 from the DM12-derived library were obtained. Plasmid loss and retransformation of plasmids into the sensitive strain A2 confirmed plasmid-dependent resistance. For further subcloning procedures, plasmid pFW100 derived from the DM6 library was selected. By fragment exclusion experiments, a minimal 2.8-kb pet/drl subfragment was identified as the cause of diazaborine resistance. The plasmid harboring this fragment was designated plasmid pFW100. The presence of a common consensus fragment in all of the isolated plasmids from either the DM6 or the DM12 library was verified by Southern blot analysis with a digoxigenin-labeled 1.8-kb pet/drl fragment. Furthermore, a 4.3-kb pet/drl fragment from plasmid pFW100 was cloned into pBlueScript, resulting in plasmid pBSF00. From this plasmid, a 1-kb Rpm1 and a 0.6-kb KpnI/BamHI fragment were again cloned into pBlueScript, and both ends of the inserts were sequenced using T3 and T7 primers by the Sanger dideoxy chain termination method (25). Searches

### Table I

| Strain | Genotype | Source |
|--------|----------|--------|
| A2     | MATa leu2–2,112 his3–11,15 can1 | Vivian L. MacKay Ref. 44 |
| L1544  | MATa lys9 | This study |
| ADR6   | MATa leu2–2,112 his3–11,15 can1 DRG1–1 | This study |
| AL1    | MATa lys9 DRG1–1 | This study |
| DM6    | MATa leu2–2,112 his3–11,15 can1 DRG2–1 pet | This study |
| DM12   | MATa leu2–2,112 his3–11,15 can1 DRG3–1 pet | This study |
| FW52   | MATa leu2–2,112 his3–11,15 can1 YAPI1–HIS3 | This study |
| DM6 (yap1) | MATa leu2–2,112 his3–11,15 can1 DSERG1–2 pet yap1:HIS3 | This study |
| DM12 (yap1) | MATa leu2–2,112 his3–11,15 can1 DSERG1–3 pet yap1:HIS3 | This study |
| KPH1   | MATa leu2–2,112 his3–11,15 can1 PDR1–12:HIS3 pet | This study |
| KPH2   | MATa leu2–2,112 his3–11,15 can1 PDR3–33:HIS3 pet | This study |
| YYM14–03 | MATa PDR3 trp1 his3 ura3 leu2 ade2 | This study |
| YYMIA-A4 (PDR3–2) | MATa PDR3–2 trp1 his3 ura3 leu2 ade2 | This study |
| YALA-B1 | MATa ura3–52 leu2–3,112 his3–11,15 trp1–1 PDR1 | Ref. 13 |
| YALA-G4 (PDR1–3) | MATa ura3–52 leu2–3,112 his3–11,15 trp1–1 PDR1–3 | Ref. 13 |
| FY1679–28C | MATa ura3–52 leu2Δ1 his3Δ200 trp1Δ63 | Refs. 13 and 28 |
| FY1679–28C (Δpdr1) | MATa ura3–52 leu2Δ1 his3Δ200 trp1Δ63 Δpdr3::TRP1 | Ref. 28 |
| FY1679–28C (Δpdr3) | MATa ura3–52 leu2Δ1 his3Δ200 trp1Δ63 Δpdr3::HIS3 | Ref. 28 |
| W303–1A | MATa ura3–1 leu2–3 his3–11,15 trp1–1 ade2–1 can1–100 | Ref. 44 |
| YPH51 | MATa ura3–52 leu2Δ1 his3Δ200 trp1Δ63 lys2–801mb ade2–101w | Ref. 29 |
| YKDR13 (Δpdr5) | MATa ura3–52 leu2Δ1 his3Δ200 trp1Δ63 lys2–801mb ade2–101w Δpdr5::TRP1 | Ref. 4 |
| YYM4 (Δpdr5 Δsnq2) | MATa ura3–52 leu2Δ1 his3Δ200 trp1Δ63 lys2–801mb ade2–101w Δpdr5::TRP1 Δsnq2::hisG | Ref. 29 |

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for homology, using the FASTA algorithm (24), were performed on this sequence and showed the presence of gene YAP1.

Genomic libraries of DM6 (yap1) and DM12 (yap1) were used to transform the diazaborine-sensitive strain S. cerevisiae A2. Growth of the transformants on synthetic media lacking leucine yielded at least 4.5 × 10⁶ colonies for each library. After double selection on plates lacking leucine but containing 15 μg/ml diazaborine for the DM6 (yap1) library, or 10 μg/ml diazaborine for the DM12 (yap1) library, 13 diazaborine-resistant transformants were obtained in the first instance, and 11 in the second. Upon transformation into sensitive yeast A2, all plasmids from these strains were able to generate resistant phenotypes. The flanking regions of the inserts of the plasmids obtained from the DM6 (yap1) library were sequenced with forward and reverse primers for pBlueScript. Sequence comparison revealed that both plasmids contained the PDR1 gene. A digoxigenin-labeled 1-kb PstI fragment of PDR1 was used to perform Southern blot analysis of the remaining 11 plasmids, which showed a common sequence in all 13 plasmids. The insert of the smallest plasmid, pKP100, was sequenced entirely. Similarly, sequencing of the flanking regions of the inserts of three plasmids derived from the DM12 (yap1) library showed that all plasmids contained the PDR3 gene. A common sequence in all 11 plasmids was confirmed by Southern blot analysis using a digoxigenin-labeled 1.4-kb NcoI fragment of PDR3. The smallest plasmid obtained from the DM12 (yap1) library, pKP300, was further reduced in size by using the singular SalI site. The insert of the resulting plasmid pKP310 was completely sequenced.

Gene Disruption Mutations—The starting material for construction of the YAP1 gene, marked with a HIS3 cassette, was plasmid pBS300. A 1.75-kb HIS3 BamHI cassette was cloned into the singular SplI site, and a linear EcoRI/XbaI fragment was used to transform strain A2, resulting in strain FW52. Appropriate recombinants were identified by Southern blotting (26). Furthermore, this fragment was cloned into YEp351 and transformed into A2 to exclude any side effects due to the insertion of the HIS3 cassette.

Gene disruptions were constructed using the one-step gene disruption method of Rothstein (26). Disruption was performed as above except that the HIS3 cassette was inserted into a singular Hip1 site.

The resulting strains were designated DM6 (yap1) and DM12 (yap1). To allow selection after the allele exchange experiment of gene PDR1–12, a 1.75-kb HIS3 cassette was introduced into the downstream ApaI site on plasmid pKP100, giving plasmid pKP120, which still mediated resistance. Strain KPHJ1 was obtained by transformation of the linear 6.1-kb SacI/SalI fragment from plasmid pKP120 into the diazaborine-sensitive strain A2. Transformants were tested for correct chromosomal integration by polymerase chain reaction.

The allele exchange of PDR3–33 was performed after introducing a 1.75-kb HIS3 cassette into the downstream BsuIII site of plasmid pKP300, resulting in plasmid pKP320, which still conferred resistance. The linear 6.4-kb SacI/SalI fragment of pKP320 was transformed into the diazaborine-sensitive strain A2. The strain obtained was histidine-prototrophic and diazaborine-resistant and was designated KPHJ2. Correct integration of the construct was confirmed by polymerase chain reaction and Southern hybridization.

Measurement of Growth Inhibition by Diazaborine—The effects of diazaborine on different yeast strains were quantitated by growing them overnight in YPD medium or, when plasmid-carrying strains were used, in minimal medium lacking leucine. The cell suspension was diluted to an A₆₀₀ of 0.1. From this cell suspension, 100 μl were used to inoculate 2 ml of fresh YPD medium containing different concentrations of diazaborine. After 24 h, the A₆₀₀ was measured spectrophotometrically to quantitate the effects of diazaborine on each strain. Each experiment was repeated at least three times.

Yeast Cell Extracts and Immunoblotting—Exponentially growing cells were harvested and washed once with water to prepare cell extracts as described before (27). Briefly, after pelleting the cells, one A₆₀₀ equivalent of cells was treated for 10 min on ice with 150 μl of 1.85 M NaOH containing 7.5% mercaptoethanol. 150 μl of 50% trichloroacetic acid were added, and the samples were incubated for an additional 10 min on ice. Precipitates were collected by centrifugation at 5000 rpm for 5 min and washed once with ice-cold 2% trichloroacetic acid. The sample was then resuspended in SDS-PAGE sample buffer (40 mM Tris-HCl, pH 6.8, 8 M urea, 5% SDS, 0.1 mM EDTA, 1% mercaptoethanol, 0.01% bromphenol blue) and neutralized by the addition of 1/10 volume of 1 M Tris base. After heating at 37 °C for 15 min, insoluble material was removed by a 10-min high speed centrifugation in an Eppendorf microcentrifuge. Approximately 0.5 A₆₀₀ equivalent or 10 μl of each sample was fractionated by SDS-polyacrylamide gel electrophoresis on a 7.5% polyacrylamide gel and analyzed by immunoblotting. The poly-
troduced it into the isogenic background of the wild type strain A2 via homologous recombination. The resulting strain FW52 showed no drug resistance. We conclude from this experiment that resistance due to YAP1 is the result of overexpression of the wild type gene and not of a mutation.

Inactivation of YAP1 Has No Influence on the Resistance of Strains DM6 and DM12—Since we were able to clone only the YAP1 gene from the libraries prepared from strains DM6 and DM12, we decided to inactivate YAP1 in these two mutants. We were particularly interested to see whether elimination of YAP1 would influence the resistance phenotype of these two strains. YAP1 gene disruptions were made in both strains, DM6 and DM12. The resulting yap1 mutant strains were subsequently tested for their ability to grow in the presence of diazaborine. We could not detect any changes in drug resistance in yap1-disrupted strains of either DM6 or DM12 background. However, they showed hypersensitivity to hydrogen peroxide, a characteristic property of yap1 disruptants (30).

Allelic forms of PDR1 and PDR3 Cause Diazaborine Resistance—Since gene YAP1 was obliterating the search for genes causing resistance in strains DM6 and DM12, we prepared genomic libraries from the disrupted strains DM6 (yap1) and DM12 (yap1), respectively. These genomic libraries were screened for plasmids that conferred diazaborine resistance to the sensitive strain A2. A total of 13 diazaborine-resistant transformants were obtained from the DM6 (yap1)-derived and 11 from the DM12 (yap1)-derived library. Upon retransformation into sensitive yeast, all plasmids were able to mediate drug resistance. For still unknown reasons, these strains showed a somewhat retarded growth on complete medium (Fig. 1).

Southern blotting and restriction analysis demonstrated that all 13 plasmids derived from the DM6 (yap1) library contained fragments sharing a common sequence (data not shown). The recombinant plasmid with the smallest insert of 4.3 kb from the DM6 (yap1) library was designated pKP100 (Fig. 2) and subjected to DNA sequencing. Comparison with the Saccharomyces Genome Database revealed that this fragment was identical to a sequence of the S. cerevisiae chromosome VII containing gene PDR1, except for a single nucleotide exchange. This T to A transversion changes codon 1044 (CTA  CA), resulting in a L1044Q exchange in the amino acid sequence of Pdr1p. In addition, we sequenced the wild type PDR1 gene from our parent strain A2, verifying the L1044Q mutation in the new PDR1 gain-of-function allele, which we designated PDR1–12. This result suggested that PDR1–12 might be responsible for diazaborine resistance. To provide conclusive proof, an allelic exchange experiment was performed, replacing the wild type PDR1 gene in strain A2 with the mutated PDR1–12 allele to generate strain KPHJ1. Strain KPHJ1 was diazaborine-resistant, showing that the mutation was indeed responsible for the resistance phenotype.

All 11 plasmids isolated from the DM12 (yap1) library contained fragments with a common DNA sequence different from the PDR1-specific fragments. The plasmid from this library carrying the smallest insert of 5.1 kb was designated pKP300. Shortening this fragment to 4.6 kb using the single SalI site in the insert yielded plasmid pKP310, which still conferred diazaborine resistance. The insert of pKP310 contained only one complete open reading frame, which was identified as the PDR3 gene (Fig. 2). A deletion affecting the PDR3 gene up to the SpH1 site resulted in complete loss of diazaborine resistance. Hence, we conclude that resistance is due to PDR3 expression. The nucleotide sequence of the pKP310 insert was determined, revealing three differences to the published PDR3 sequence in the Saccharomyces Genome Database. One mutation was a T to C transition (TAT  CAT) changing amino acid
To diazaborine. The minimum inhibitory concentration of this strain FY1679–28C (PDR1–3) was more resistant than the strain FY1679–28C (PDR1–12), which resembles the situation found with our trations of diazaborine than the previously described multiple drug-resistant PDR1–3 allele, YYMIA-A4 and YALA-G4, respectively. The isogenic wild type strains A2, YYM14-A4, and YALA-B1 served as controls.

We also studied the effects of the drug on mutants carrying the isolated PDR3–3 alleles were subsequently tested for overexpression of Pdr5p and Snq2p. As shown in Fig. 3, the PDR1–12 allele leads to similar levels of Pdr5p and Snq2p as compared with PDR1–3. The PDR3–3 allele leads to even higher levels of these two pumps than the previously described multiple drug-resistant PDR3–2 allele.

Effect of Diazaborine on the Growth of Other Described PDR Mutants—To investigate if other known PDR gain-of-function alleles can also confer diazaborine resistance and to test if strains deleted in the PDR1 and PDR3 genes are supersensitive to the drug, strains YALA-G4 (PDR1–3), YYMIA-A4 (PDR3–2), and FY1679–28C (Δpdr1 Δpdr3) were tested for their ability to grow in the presence of diazaborine. As shown in Fig. 4, the strains YALA-G4 and YYMIA-A4 are resistant to diazaborine but exhibited differences in the level of resistance i.e. the PDR1–3 strain tolerates the presence of higher concentrations of diazaborine than the PDR1–2 strain. This closely resembles the situation found with our PDR1–12 allele, which was more resistant than the PDR3–3 allele (data not shown).

Strain FY1679–28C (Δpdr1 Δpdr3) proved to be hypersensitive to diazaborine. The minimum inhibitory concentration of this strain was 15 μg/ml, while that of the wild type strain FY1679–28C was 30 μg/ml.

Within the last few years, the antibacterial drug diazaborine has gained considerable attention due to its unique mode of action (31) and because it inhibits the Gram-negative homologue of protein InhA, which is involved in isoniazide resistance of clinical isolates of Mycobacterium tuberculosis (32). Although serious side effects are known, diazaborine could serve as a serious side effects are known, diazaborine could serve as a

![Fig. 3. Strains carrying the diazaborine-resistant PDR1–12 and the PDR3–33 allele overexpress Pdr5p and Snq2p. Crude extracts from the S. cerevisiae strains KPHJ2 (PDR3–33) and KPHJ1 (PDR1–12) were blotted and probed with polyclonal anti-Pdr5p or anti-Snq2p antibody as described under “Experimental Procedures.” The level of Pdr5p and Snq2p expression was compared with that of strains carrying the previously described PDR3–2 and PDR1–3 allele, YYM14-A4 and YALA-G4, respectively. The isogenic wild type strains A2, YYM14-A4, and YALA-B1 served as controls.](image)

![Fig. 4. Effect of diazaborine on the growth of various known PDR alleles. Cultures of the strains YALA-G4 (PDR1–3), YALA-B1 (PDR1), YYMIA-A4 (PDR3–2), YYMIA-A4 (PDR3), FY1679–28C (Δpdr1 Δpdr3), and FY1679–28C were grown overnight in YPD medium and diluted to an A600 of 0.1. From this cell suspension, 100 μl were used to inoculate 2 ml of YPD medium containing 0–80 μg/ml diazaborine. After 24 h of incubation, the A600 was measured spectrophotometrically and plotted against the diazaborine concentration.](image)

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**DISCUSSION**

Within the last few years, the antibacterial drug diazaborine has gained considerable attention due to its unique mode of action (31) and because it inhibits the Gram-negative homologue of protein InhA, which is involved in isoniazide resistance of clinical isolates of Mycobacterium tuberculosis (32). Although serious side effects are known, diazaborine could serve as a...
The mechanism of resistance specified by no AAA protein has been associated with resistance to growth inhibition. The enzyme Afg2p belongs to the family of AAA proteins (33, 34). Up to now, overproduction of drug efflux pumps (5, 7, 9, 10). In contrast, this enzymatic activity is part of the multifunctional fatty acid synthase that is isolated from the yeast enoyl-(acyl carrier protein) reductase, because the enzyme is part of the multifunctional fatty acid synthase and therefore structurally very different from that in E. coli (35).

Here we describe our results on PDR1 and PDR3 and, for the first time, show a link to the transcription factor Yap1p, which also causes diazaborine resistance when overexpressed. Pdr1p and Pdr3p are highly related regulatory proteins of the Zn(2)Cys(6) family. They act on the same upstream sequences of certain PDR-responsive genes (1) and functionally overlap, at least for the genes of the efflux pumps PDR5, SNQ2, and YOR1 (5, 7, 9, 10, 13). The gain-of-function alleles of PDR1 and PDR3 that we have isolated here contain mutations that lead to a single amino acid change in each of the encoded proteins. The exchange in Pdr1p is located in the C-terminal part of the protein and results in an L1044Q exchange. Interestingly, this region of Pdr1p shows significant homology to the C-terminal portion of Pdr3p and Gal4p (Fig. 7). In Gal4p, this segment is involved in transactivation and interaction with the negative regulator Gal80p (36). Moreover, the transactivation domain of Pdr1p resides within the last 251 amino acids of the protein (37). Martens et al. (37) also demonstrated that the C-terminal region of Pdr1p and Pdr3p interact with the ADA coactivator/repressor complex. This association inhibits the transactivation activity of Pdr1p. The gain-of-function mutation in our PDR1–12 allele could therefore affect either the transactivation itself or the interaction with a negative regulator. In contrast to Pdr1p, the exchange in PDR3–33 leading to diazaborine resistance is located in the middle portion of the protein, a region without any significant homology to Gal4p. This region, however, is conserved in the amino acid sequence of Pdr1p and the putative regulatory protein YER184C, which belongs to the same family of regulatory proteins. This motif may therefore be of functional importance (Fig. 7).

Pdr1p and Pdr3p could regulate another yet unidentified target protein that might be the major resistance determinant. This is confirmed by the fact that a double deletion mutant exhibits very high sensitivity, surpassing each single deletion mutant. Since transcriptional regulation of PDR3 is known to involve both control by Pdr1p, and by Pdr3p itself via an autoregulatory loop (38), it is still an open question whether Pdr1 and Pdr3 act simultaneously on the same or neighboring promoter elements (pleiotropic drug resistance elements) of their target genes or whether there is a sequential gene activation.

Moreover, we have found that overexpression of the wild type YAP1 gene also generates diazaborine resistance. We have also isolated a truncated version of this gene, which was sufficient to confer resistance when overexpressed. This deletion lacks the 212 C-terminal codons of YAP1. Very recently, it was demonstrated that yeast cells relocate Yap1p from the cytoplasm to the nucleus upon exposure to reactive oxygen (39). The information for this regulated process is located within a C-terminal, cysteine-rich, domain of the Yap1p protein. Mutations exchanging these cysteines or a deletion of the whole domain result in nuclear localization of Yap1p. Such truncated Yap1p proteins were shown to have retained their potential to
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1,10-phenanthroline

cycloheximide

Yap1p

Pdr3p

Pdr1p

4-NQO

diazaborine

FIG. 8. Scheme to explain the connection of Yap1p and the PDR network. Yap1-mediated resistance to 1,10-phenanthroline, cycloheximide, and probably cadmium (43) is independent from PDR1 or PDR3. In contrast, Yap1-mediated resistance to diazaborine and 4-NQO is exerted mainly via PDR3. The contribution of PDR1 to diazaborine resistance may be either direct or indirect through PDR3.

Taken together, we describe here for the first time a functional connection of the Yap1p activity with the master regulators of the pleiotropic drug resistance network, namely Pdr1p and Pdr3p.

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