Cloning by Gene Amplification of Two Loci Conferring Multiple Drug Resistance in Saccharomyces

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ABSTRACT

Yeast DNA fragments that confer multiple drug resistance when amplified were isolated. Cells containing a yeast genomic library cloned in the high copy autonomously replicating vector, YEp24, were plated on medium containing cycloheximide. Five out of 100 cycloheximide-resistant colonies were cross-resistant to the unrelated inhibitor, sulfometuron methyl, due to a plasmid-borne resistance determinant. The plasmids isolated from these resistant clones contained two nonoverlapping regions in the yeast genome now designated PDR4 and PDR3 (for pleiotropic drug resistant). PDR4 was mapped to chromosome XIII, 31.5 cM from LYS7 and 9 cM from the centromere. PDR5 was mapped to chromosome XV between ADE2 and H153. Genetic analysis demonstrated that at least three tightly linked genes (PDR5, PDR2 and SMR3) that mediate resistance to inhibitors are located in this region. Insertion mutations in the either PDR4 or PDR3 genes are not lethal, but the insertion in PDR5 results in a drug-hypersensitive phenotype.

In mammalian cells, amplification of specific sequences in no fewer than three genes can result in resistance to a large number of structurally and mechanistically unrelated inhibitors (Howell et al. 1984; Roninson et al. 1984; Varshavsky 1984; Karner et al. 1985; Richert et al. 1985; Stark 1986). For example, in Chinese hamster cell lines, amplification is associated with resistance to Adriamycin and Colcemid. When the duplicated regions are subsequently lost by culturing in media without inhibitor, there is loss of resistance. Often, the multiple drug resistance phenotype is associated with an increase in the amount of a high molecular weight plasma membrane glycoprotein. Recent studies also suggest that the heightened resistance is due to an ATP dependent transport system which actively pumps inhibitors out of the cell (Skorsgaard 1978; Fojo et al. 1985; Gros, Croop and Housman 1986; Willingham et al. 1987).

Multiple drug resistance is not restricted to mammalian cells. It has been suggested that similar genes exist in the malaria parasite resulting in resistance to many of the common antimalarial agents (Krogstad et al. 1987). There is also striking homology between some of the mammalian mdr genes and those involved with energy mediated bacterial transport such as the malk locus of Escherichia coli (Ferro-Luzzi Ames 1986 for review). Furthermore, several such loci have been identified in yeast. The PDR1 locus has been the subject of considerable genetic analysis (Rank and Bech-Hansen 1973; Saunders and Rank 1982). Mutations at this locus can be dominant or recessive, and are sometimes unstable or suppressible. In addition, alterations in the PDR1 locus result in resistance to no fewer than fifteen different inhibitors. Mutations at a second site (Falco et al. 1984), initially designated SMR3, but now called PDR2, produce resistance to at least three different compounds, cycloheximide, oligomycin and sulfometuron methyl. Yet a third gene (PDR3) has been located on chromosome II (Subik, Ulaszewski and Goffeau 1986). Recently, McGarth and Varshavsky (1989) demonstrated that the yeast gene, STE6, shows striking homology to the mammalian gene that codes for the P glycoprotein. Interestingly enough, STE6 does not appear to mediate multiple drug resistance, but mediates mating factor transport.

The PDR1 locus has recently been cloned and sequenced (Balzi et al. 1987). It specifies a 3192 nucleotide open reading frame. The deduced amino acid sequence contains zinc fingers suggesting that the encoded peptide might bind to DNA.

In this report, we describe the isolation of two yeast genes designated PDR4 and PDR5, that lead to multiple drug resistance when carried on a high copy plasmid. Disruption of either gene is not lethal; thus, these loci are not essential to the cell. However, a Tn5 insertion mutation in PDR5 results in a hypersensitive
phenotype. PDR5 is tightly linked to two other genes identified by drug-resistance mutations.

MATERIALS AND METHODS

Strains, plasmids and media: The strains and plasmids used in this study are listed in Table 1. The recipes for media and the conditions for culturing yeast cells are described elsewhere (FALCO and DUMAS 1985). Strains containing plasmids were maintained under selective conditions described elsewhere (FALCO and DUMAS 1985). Strains containing plasmids were maintained under selective conditions described elsewhere (FALCO and DUMAS 1985). Strains containing plasmids were maintained under selective conditions described elsewhere (FALCO and DUMAS 1985). Strains containing plasmids were maintained under selective conditions described elsewhere (FALCO and DUMAS 1985). Strains containing plasmids were maintained under selective conditions described elsewhere (FALCO and DUMAS 1985). Strains containing plasmids were maintained under selective conditions described elsewhere (FALCO and DUMAS 1985).

DNA preparation: The methods for the preparation of yeast chromosomal DNA and plasmid DNA were described previously (GOLIN, FALCO and MARCOLSKE 1986). Strains containing plasmids were maintained under selective conditions described elsewhere (FALCO and DUMAS 1985).

Transformation: Transformation of yeast with plasmid DNA was done according to the procedure of HINNE, HICKS and FINK (1978). Bacterial transformation was performed using the calcium chloride procedure as described by MANIATIS, FRITSCH and SAMBROOK (1982). Restriction enzyme analysis was carried out as described by ROTHSTEIN (1985). Colony hybridization was performed as described by ROTHSTEIN (1985).

Inhibitors: Cycloheximide, antimycin, oligomycin, and nystatin were purchased from Sigma (St. Louis, Missouri). Sulfometuron methyl was obtained from the Agricultural Chemical Department of E. I. duPont de Nemours (Wilmington, Delaware). Inhibitors were dissolved in dimethyl sulfoxide.

Quantitative measurement of drug resistance: To determine the effect of a particular inhibitor, 2-ml cultures of the strains to be studied were grown overnight to saturation. A volume of 0.2 ml of cells was suspended in 3 ml of 1% agar and spread on the appropriate medium. The values reported represent the average of two or more samples.

Genetic analysis: Random spore and tetrad analysis were carried out as described by SHERMAN, FINK and LAWRENCE (1974).

Construction of chromosomal insertion mutations: To determine whether the PDR4 or PDR5 genes are essential for growth, chromosomal insertion mutations were created using the Tn5 bearing plasmids described in the results. A disruption in the PDR5 gene was constructed as follows. The plasmid carrying the Tn5-5 insertion in pDR3.3 (Figure 1) was linearized with SmaI and used to transform a Ura- diploid strain (DBY 703 X RW 2802). This diploid lacks the 2-μm circle plasmid and therefore cannot support autonomous replication of the plasmid. Integrative transformants were obtained in which one chromosome contained the wild type PDR5 gene and the Tn5 insertion mutation separated by vector DNA. The other chromosome had a single wild type gene. Recombinants which resulted from intrachro-
mosomal exchange between the direct repeats (and loss of one copy) were obtained by replica plating several thousand colonies to uracil emission medium and identifying Ura− segregants. The recombinants that were heterozygous for the Tn5 insertion were identified by Southern hybridization. The interruption of PDR4 was carried out in analogous fashion, however the Tn5-F mutation was first subcloned into a YIp5 vector (BOTSTEIN et al. 1979). Following transformation of a ura3 diploid, Ura− segregants were selected on 5-fluoro-orotic acid as described by BOEKE, LACROUTE and FINK (1984). Tn5 insertion heterozygotes were identified by SOUTHERN (1975) hybridization and sporulated for subsequent analysis.

RESULTS

Cloning multidrug resistance genes by DNA amplification: The strategy used to clone multidrug resistance loci was similar to that initially described by RINE et al. (1983). They showed that when cells are transformed with a high copy number plasmid containing the gene coding for yeast UDP-N-acetylglucosamine-1-phosphate transferase, resistance to tunicamycin is conferred. A similar approach was used by FALCO and DUMAS (1985) to clone the yeast ILV2 gene. Overproduction of acetylactate synthase, the product of the locus, results in resistance to the herbicide sulfometuron methyl, an inhibitor of this enzyme.

To identify loci that confer multidrug resistance as a result of gene amplification, we screened a library that was generated by insertion of Sau3A partially digested yeast genomic DNA into the BamHI site of the high copy number, extrachromosomally replicating plasmid, YE24 (BOTSTEIN et al. 1979). Yeast cells (strain FY158) containing this clone bank, selected as Ura− transformants, were plated on cycloheximide medium. Resistant colonies appeared after 48 hr. One hundred were then screened for resistance to sulfometuron methyl. Six cross-resistant colonies were found.

To determine whether the cross resistance was plasmid mediated, the six strains were subcultured in nonselective (YEPD) medium. Under such conditions, the plasmid is lost from some cells in the culture and, after many generations, Ura− colonies derived from such cells were identified. In five of the six cases, all Ura− segregants were also sensitive to both cycloheximide and sulfometuron methyl. One strain remained drug resistant and was not analyzed further.

Structural analysis of the cloned DNA fragments: DNA was prepared from each of the five yeast clones and used to transform E. coli. Restriction enzyme analysis was performed on plasmid DNA extracted from each of the five E. coli transformants to determine whether the clones contained different DNA fragments. The patterns produced from two clones, pDR3.3 and pDR3.2, were related, but unlike those from pDR3.1, pDR3.2 and pDR3.4. The latter group gave identical profiles with all enzymes used. Since yeast cells containing the YEp24 clone bank were grown in culture for many generations prior to plating on cycloheximide medium, the latter group of three almost certainly represents the repeated selection of the same plasmid insertion.

The plasmids pDR3.1 and pDR3.3 were chosen for further study since they carried two unrelated DNA fragments. Reintroduction of these plasmids into strain FY138 resulted in cycloheximide and sulfometuron methyl resistance, confirming the presence of resistance determinants on the plasmids. Restriction enzyme maps of pDR3.1 and pDR3.3 are shown in Figure 1. The sizes of the DNA fragments cloned in the YEp24 vector were 8.0 and 14.7 kb pairs, respectively. Southern blot hybridization indicated that each of these fragments was present once in the yeast genome (not shown).

Tn5 insertion mutagenesis, as described previously by VAN DYK, FALCO and LA ROSA (1986), was used to localize the regions of the clones essential for conferring resistance. The plasmids pDR3.3 and pDR3.1 were put into an E. coli strain (HB101::Tn5) containing Tn5 inserted in the bacterial genome, and independent, highly neomycin-resistant colonies, resulting from transposition of the Tn5 element from the chromosome to the high copy plasmid, were selected. Restriction enzyme analysis of the DNA from these plasmids allowed determination of the site of each Tn5 insertion. Each of the Tn5 mutants was put into yeast strain FY138 by transformation to evaluate the
effect of the insertion. The Tn5 insertions defined a 0.7-kb region essential for the high copy-mediated resistance of pDR3.1 and an approximately 3-kb region in pDR3.3 (Figure 1). These values represent a minimum estimate of the critical region. The results of the Tn5 analysis were further verified in the case of pDR3.3 by constructing a 6.5-kb KpnI deletion in pDR3.3 (named pGL706, see Figure 1). When present in high copy, pGL706 confers drug resistance at levels identical to pDR3.3. pGL706 was used in most of the subsequent experiments. The pleiotropic drug resistance genes of plasmids pDR3.1 and pDR3.3 have been named PDR4 and PDR5, respectively.

Phenotypic characterization of PDR4 and PDR5 genes: The drug resistance phenotypes of strain FY138 bearing plasmids pDR3.1 (PDR4), pDR3.3 (PDR5) or YEp24 are compared in Table 2. Five structurally and functionally unrelated inhibitors were tested: cycloheximide, sulfometuron methyl, nystatin, antimycin and oligomycin. The plasmids pDR3.1 and pDR3.3 conferred resistance to cycloheximide and sulfometuron methyl, as expected since these were used to select clones, but not to any of the other inhibitors. An ANOVA statistical test on the data was ambiguous with regard to whether pDR3.1 increased resistance to sulfometuron methyl. It may be that our choice of dose was not sensitive enough since the resistance phenotype is easily detectable by replica plating to medium containing 1 pg/ml of this inhibitor. To determine if the multidrug resistance phenotype associated with the cloned PDR5 gene requires amplification, pGL706 was introduced into FY138 cells in both circular and linear form. When introduced in circular form, this plasmid replicates autonomously and in high copy number. Linearizing the plasmid with SacI prior to transformation resulted in integration of the plasmid into the chromosome by homologous recombination. Integrative transformants (low copy) were distinguished from the extrachromosomal variety by the markedly increased stability of the Ura" phenotype and confirmed by Southern hybridization. While transformants containing the high copy number autonomously replicating plasmid were resistant to both cycloheximide and sulfometuron methyl, integrative transformants were sensitive (Table 3).

The PDR4 gene was tested for its high and low copy phenotype in a slightly different manner. The high copy effect was monitored in strain DBY947 by transforming it with the original plasmid pDR3.1. To obtain integrative (low copy) transformants, the PDR4 gene was subcloned into YIp5 (Botstein et al. 1979) to produce the plasmid pBT208. Since this plasmid contains no yeast origin of replication, transformants are obtained only when the plasmid is integrated into the chromosome by homologous recombination. The cycloheximide resistance phenotypes of these transformants are also found in Table 3. The integrative transformants, which bears PDR4 in two copies, is slightly more resistant than the untransformed control, while the presence of the gene in high copy number leads to a considerably more resistant phenotype. Thus, as in the case of PDR5, it is increased copy number of PDR4, rather than allelic variation in the cloned DNA that is responsible for drug resistance.

To determine whether the PDR4 and PDR5 genes are essential for growth, chromosomal insertion mutations were created as described in MATERIALS AND METHODS. Each of the two insertion heterozygotes was sporulated and twenty tetrads were dissected to determine whether a lethal mutation was segregating. Dip-

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**TABLE 2**

Zones of inhibition (cm) produced by various inhibitors

<table>
<thead>
<tr>
<th>Strain</th>
<th>Antimycin</th>
<th>Oligomycin</th>
<th>Nystatin</th>
<th>Cycloheximide</th>
<th>Sulfometuron-methyl</th>
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<tbody>
<tr>
<td>FY138 + YEp24</td>
<td>3.3</td>
<td>1.7</td>
<td>3.3</td>
<td>5.7</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>3.3</td>
<td>1.4</td>
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<td></td>
</tr>
<tr>
<td>Average</td>
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<td>1.5</td>
<td>3.3</td>
<td>5.6</td>
<td>6.3</td>
</tr>
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<tr>
<td>Average</td>
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<td>1.5</td>
<td>3.3</td>
<td>3.4</td>
<td>6.0</td>
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<tr>
<td>Average</td>
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<td>1.5</td>
<td>3.2</td>
<td>4.7</td>
<td>5.2</td>
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</tbody>
</table>

Samples of 10 mg/ml of each inhibitor were dissolved in dimethyl sulfoxide and 100 ng were applied to a 0.6-cm disc.
TABLE 3
Phenotypes of integrative transformants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Zones of inhibition (cm)</th>
</tr>
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<tr>
<td></td>
<td>Cyclheximide (50 ng)</td>
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<tr>
<td>FY138 (untransformed)</td>
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<tr>
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</tr>
<tr>
<td>FY138 + pGL706 (integar #8)</td>
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</tr>
<tr>
<td>FY138 + pGL706 (autonomous high copy)</td>
<td>3.3</td>
</tr>
<tr>
<td>DBY947 + YEpl2</td>
<td>4.4</td>
</tr>
<tr>
<td>DBY947 + pBT208 (integar #1)</td>
<td>4.1</td>
</tr>
<tr>
<td>DBY947 + pDR3.1 (autonomous high copy)</td>
<td>3.3</td>
</tr>
</tbody>
</table>

loids bearing either of the insertion mutations exhibited high spore viability with no indication of a 2:2 segregation pattern characteristic of a lethal mutation. Furthermore, in each case, Southern blot hybridization indicated that spores containing the integrated Tn5 element were viable (not shown). This evidence strongly suggests that these genes are not essential. However, the possibility that the insertion mutations do not completely inactivate the genes cannot be ruled out at this point.

The cycloheximide drug resistance phenotypes of segregants bearing a Tn5 insertion mutation were compared to wild-type controls. Replacement of the wild type PDR4 gene with the Tn5-F disruption did not result in any phenotypic change (data not shown). In contrast, however, disruption of PDR5 produced a semidominant hypersensitive phenotype. This is shown in Figure 2. Cells containing the insertion mutation failed to grow after 72-96 hr on media containing low doses (0.5 µg/ml) of cycloheximide that are normally not inhibitory.

The segregation of the drug hypersensitivity phenotype was monitored by tetrad analysis of a heterozygous diploid. Fifty-two tetrads were replicated to a series of genetically marked mapping stocks (Klapolz and Easton-Esposito 1982). Because the integrated plasmid still retains a copy of the 2-µm circle inverted repeat sequences, the chromosome containing the insertion is subject to heightened mitotic recombination and or chromosome loss. As a result, recessive markers originally present in heterozygous configuration are rendered homozygous. The change in phenotype for genes on a particular chromosome indicates the presence of the 2-µm plasmid in that homolog. The results of this analysis indicated that the PDR4 gene present in pDR3.1 mapped to chromosome XIII while the PDR5 gene present in pGL706 was located on chromosome XV between ADE2 and HIS3.

Tetrad analysis was carried out to confirm the chromosomal assignment of PDR4 and to more precisely determine the map position with respect to the LYS7 locus. To do this, PDR4 inserted in the integrating plasmid YEp5 (pBT208) was used to transform DBY947 (LYS7, ura3) to Ura+. The resulting transformant (JC 447) was mated to FY201 (lys7, ura3), the heterozygous diploid was sporulated, and asci were dissected. The results of the tetrad analysis are found in Table 4. PDR4 is located 31.5 cM from the LYS7 locus and 9 cM from the centromere, indicating that it is proximal to LYS7, or on the other arm. No previously identified drug resistance mutation has been mapped to this region.

**Figure 2.**—Hypersensitivity of Tn5 insertion mutations in PDR5. The photograph shows the results of replica plating strains grown on YPD medium containing 0.5 µg/ml cycloheximide. The plates were photographed after 72 hr. Top: wild-type (sensitive) strain. Middle: Tn5 hypersensitive mutation. Bottom: heterozygous diploid. All strains grow vigorously on nutrient medium (left). The sensitive strain grew on cycloheximide media, while the Tn5 insertion mutation renders a cell unable to grow at this low dose. The heterozygous diploid exhibits an intermediate phenotype.
The location of the integrated pGL706 plasmid raised the possibility that PDR5 was allelic to SMR3, alleles of which are known to confer multiple drug resistance (Falco et al. 1986). The SMR3 locus was defined previously by several independently isolated sulfometuron methyl resistant mutants which were shown to map on chromosome XV between ADE2 and HIS3. Complementation tests revealed two complementation groups indicating the presence of two tightly linked genes or alternatively, intragenic complementation (Falco and Dumas 1985). Three mutations in the first complementation group (represented by smr3-37) lead to resistance to sulfometuron methyl only. Four mutations in the second group (represented by smr3-33) cause a multidrug resistance phenotype (Falco et al. 1986). The genetic relationships between the mutations, smr3-33, smr3-37 and SMR3-73 (a dominant multidrug resistance mutation mapping in this region), and the PDR5 gene cloned on pDR3.3 were determined by complementation tests and tetrad analysis.

The first experiment explored the genetic linkage between mutations assigned to the two complementation groups by crossing the smr3-33 and smr3-37 mutants. If the two mutants were separated by exchange, tetratype and nonparental ditype asci would have one and two sensitive spores, respectively. The data, found in Table 5 show a map distance of 6.4 cM indicating that these mutations are in two different genes. The dominant multidrug resistant mutation SMR3-73 was tightly linked to the recessive multidrug resistance mutation smr3-33, suggesting that these mutations are allelic. Thus, by the criteria of complementation and crossing over, two genes are defined by the mutation smr3-37 and the pair smr3-33 and SMR3-73. The first retains the name SMR3, and the second has been designated PDR2 (as previously suggested by Balzi et al. 1987). Thus, the smr3-33 and SMR3-73 alleles have been renamed pdr2-33 and PDR2-73.

To determine whether the multidrug resistance gene cloned in pDR3.3 was allelic to either of the two genes described above, two approaches were taken. In the first experiment, the pDR3.3 subclone, pGL706, was integrated into ura3, pdr2-33 (previously smr3-33) and ura3, smr3-37 mutant strains. Such transformants remained drug resistant, indicating that the DNA fragment on pGL706 could not complement either of these recessive mutants. In the second experiment, the URA3 on the plasmid served as a marker to allow pGL706 (and thus the drug resistance gene that served as its site of integration) to be followed in subsequent crosses, while the sulfometuron methyl resistance was used to monitor either of the two mutant alleles. If the plasmid-borne multidrug resistance gene was not allelic to either smr3-37 or pdr2-33, sulfometuron resistant, Ura- and sulfometuron methyl sensitive, Ura+ recombinants would be found. Such recombinants were observed in both crosses (Table 5) indicating that the plasmid-borne PDR5 gene was distinct from each of these loci. PDR3 was located 5.8 cM from pdr2-33 and 10.9 cM from smr3-37.

In a third experiment, the cycloheximide hypersensitivity of the Tn5 insertion mutation was used as a genetic marker. A haploid containing the Tn5 insertion (JG334) was mated to a strain bearing the pdr2-33 mutation (JG277). Tetrads were screened for cycloheximide hypersensitivity and sulfometuron methyl resistance. The two phenotypes were found to be separable by recombination. In thirty tetrads analyzed, there were three tetratype asci indicating a distance of about 5 cM, which is consistent with that determined using the URA3 gene in pGL706 as a marker (Table 5).

In summary, the genetic experiments described above define three loci by the criteria of complementation and crossing over: a gene represented by smr3-37, a second gene represented by pdr2-33 (and PDR2-73) and a third gene, PDR5, carried on pGL706.

**DISCUSSION**

In this report, we describe the cloning and initial characterization of two yeast genes that result in a
multidrug resistant phenotype when they are present as part of an autonomously replicating high copy plasmid. Both genes, designated PDR4 and PDR5, are present in single copy in the yeast genome of wild-type sensitive cells. PDR4 is located on chromosome XIII, 31.5 cm from LYS7 and 9 cm from the centromere. PDR3 is on chromosome XV between ADE2 and HIS3; it is tightly linked to, but separable from, two other drug resistance genes, SMR3 and PDR2. Inactivation of either the PDR4 or PDR5 sites by insertion mutation is not lethal to the cell. However, a marked drug hypersensitivity is observed for the latter. Mild hypersensitivity was also obtained when deletions of the PDR1 locus were analyzed (BALZI et al. 1987).

The clustering of loci involved in drug resistance on chromosome XV was unexpected. The genetic mapping of the PDR5 gene to chromosome XV between ADE2 and HIS3 initially suggested that the cloned gene was the wild type allele of the previously identified drug resistance gene SMR3 (FALCO and DUMAS 1985). The simplest interpretation of the genetic experiments described in this report is that there are, in fact, three different genes located between ADE2 and HIS3 involved in the cellular response to environmental chemicals. Other explanations, for example a single complex gene with large introns, are possible. Such regions have been found in animal cells, but they are unprecedented in yeast. In addition, the restriction map of Tn5 insertion mutations that inactivate the PDR5 high copy resistance gene suggests that a single continuous 3-kb region is necessary and sufficient for function. Furthermore, trans-splicing or intragenic complementation would be required to explain the complementation results. Sequencing of PDR5 should further aid in determining the exact organization of this region as well as the possible relation to mammalian drug resistance genes.

It is interesting to note that mammalian multiple drug resistance genes are also clustered (STARK 1986). Furthermore, the PDR1 locus of yeast is complex and may be composed of several linked genes. Mutations that map to this region on chromosome VII show varying linkage to other markers (MORTIMER and SCHILD 1980), wide differences in drug resistance phenotype (SAUNDERS and RANK 1982) and complementation (FALCO and DUMAS 1985). In addition to the PDR1 gene, another gene, PMA1, which encodes a plasma membrane ATPase, has been cloned and mapped to this region (SERRANO, KIELLAND-BRANDT and FINK 1986). Mutations in PMA1 also lead to a multidrug resistant phenotype (ULASZEWSKI, GRENSON and GOFFEAU 1983). Thus, at least two genes, wherein mutations leading to a multidrug resistance phenotype have been found, are tightly linked on chromosome VII. A similar situation also exists with regard to the PDR3 locus on chromosome II (SUBIK, ULASZEWSKI and GOFFEAU 1986). The nucleotide sequences of the two genes do not show any obvious similarity, nor do the deduced amino acid sequences of the encoded polypeptides suggest any functional relatedness. Possible interactions between various PDR genes are being explored by making combinations of mutant alleles and by searching for suppressor mutations.

Amplification of the PDR4 or PDR5 genes imparts resistance to cycloheximide and sulfometuron methyl, agents that inhibit protein synthesis and isoleucine and valine production, respectively. The chemical structures of these two compounds do not show obvious similarities, other than the presence of an imide group. A multiple drug resistance phenotype to unrelated inhibitors could be due to at least two different mechanisms already documented in the literature. Resistance could be brought about by changes in the membrane structure so that inhibitors are excluded. For example, various Pseudomonad mutants (HANCOCK 1984) with altered membrane lipid composition have altered resistance. Alternatively, mammalian

### TABLE 5

<table>
<thead>
<tr>
<th>Cross</th>
<th>Pertinent genotype</th>
<th>Tetrads scored</th>
<th>P</th>
<th>NPD</th>
<th>TT</th>
<th>Map distance</th>
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<td>JG277 × JG272</td>
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<td>0</td>
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<tr>
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</table>

The pdr2-33 and PDR2-73 mutations were originally designated as smr2-33 and SMR2-73.
multidrug resistant lines have a more efficient, energy-dependent export of inhibitors (SKOSSGAARD 1978; FOJO et al. 1985). At present no biochemical experiments have been done to reveal which of these, if either, is the mechanism operating in yeast. The association of a resistance phenotype with amplification of the PDR5 gene and hypersensitivity with disruption of the gene is consistent with it encoding a "pump" which exports the inhibitors. Alternatively, the PDR5 gene product might be a positive activator of the pump.

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LITERATURE CITED


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