MOLECULAR AND CELLULAR BIOLOGY, Dec. 1995, p. 6875–6883 Vol. 15, No. 12
0270-7306/95/$04.00+0

Expression of an ATP-Binding Cassette Transporter-Encoding Gene (*YOR1*) Is Required for Oligomycin Resistance in *Saccharomyces cerevisiae*

DAVID J. KATZMANN,[1] TIMOTHY C. HALLSTROM,[1] MARLEEN VOET,[2] WILLIAM WYSOCK,[3] JOHN GOLIN,[3] GUIDO VOLCKAERT,[2] AND W. SCOTT MOYE-ROWLEY[1,4]*

Molecular Biology Program[1] and Department of Physiology and Biophysics,[4] University of Iowa, Iowa City, Iowa 52242; Laboratory of Gene Technology, KU Leuven, Leuven, Belgium[2]; and Department of Biology, Catholic University, Washington, D.C.[3]

Received 14 July 1995/Returned for modification 9 August 1995/Accepted 12 September 1995

Semidominant mutations in the *PDR1* or *PDR3* gene lead to elevated resistance to cycloheximide and oligomycin. *PDR1* and *PDR3* have been demonstrated to encode zinc cluster transcription factors. Cycloheximide resistance mediated by *PDR1* and *PDR3* requires the presence of the *PDR5* membrane transporter-encoding gene. However, *PDR5* is not required for oligomycin resistance. Here, we isolated a gene that is necessary for *PDR1*- and *PDR3*-mediated oligomycin resistance. This locus, designated *YOR1*, causes a dramatic elevation in oligomycin resistance when present in multiple copies. A *yor1* strain exhibits oligomycin hypersensitivity relative to an isogenic wild-type strain. In addition, loss of the *YOR1* gene blocks the elevation in oligomycin resistance normally conferred by mutant forms of *PDR1* or *PDR3*. The *YOR1* gene product is predicted to be a member of the ATP-binding cassette transporter family of membrane proteins. Computer alignment indicates that Yor1p shows striking sequence similarity with multidrug resistance-associated protein, *Saccharomyces cerevisiae* Ycf1p, and the cystic fibrosis transmembrane conductance regulator. Use of a *YOR1-lacZ* fusion gene indicates that *YOR1* expression is responsive to *PDR1* and *PDR3*. While *PDR5* expression is strictly dependent on the presence of *PDR1* or *PDR3*, control of *YOR1* expression has a significant *PDR1*/*PDR3*-independent component. Taken together, these data indicate that *YOR1* provides the link between transcriptional regulation by *PDR1* and *PDR3* and oligomycin resistance of yeast cells.

ATP-binding cassette (ABC) transporters are involved in a wide range of energy-dependent transport events across cell membranes (22). These proteins have a bipartite structure, consisting of a set of membrane-spanning domains and a characteristic nucleotide-binding domain (NBD). The NBD regions of ABC transporters are the most highly conserved domains of this group of proteins and are believed to couple ATP hydrolysis to a transport event, in many instances (16). The NBDs of ABC transporters show sequence similarity across the entire approximately 200-amino-acid stretch of polypeptide that constitutes the NBD (25). These NBDs contain Walker A and B motifs that are found to be associated with many other nucleotide-binding proteins (48). However, the sequence similarity of the NBDs of ABC transporter proteins extends across the entire NBD and is not constrained to the two short Walker elements.

Although there is a great deal of sequence similarity in ABC transporter NBDs, the number of amino acids between the functional motifs is quite variable. This spacing consideration has allowed two distinct subclasses of ABC transporters to be recognized (10). We will refer to these two subclasses as class 1, represented by human Mdr1p, and class 2, represented by human cystic fibrosis transmembrane conductance regulator (CFTR). Class 1 has many more members and contains the majority of known ABC transporters. Additionally, class 1 proteins contain an additional segment of amino acids (13 more in the comparison of Mdr1p with CFTR [10]) that is absent from class 2 proteins. The spacing between the Walker A and B domains is important, as the most prevalent allele giving rise to cystic fibrosis is an in-frame deletion of a phenylalanine codon lying between the Walker A and B domains of NBD1 in CFTR (47).

In tumor cells, overexpression of ABC transporter genes leads to the simultaneous acquisition of resistance to several different cytotoxic agents (17). Two different ABC transporter-encoding loci in human cells have been commonly found to elicit this multidrug resistance when overproduced, *MDR1* (19, 40) and *MRP1* (10). In animal cells, overexpression of these genes in multidrug-resistant cells is most often elicited by amplification of their respective structural genes (39).

Saccharomyces cerevisiae also contains loci that have the potential to give rise to a multidrug-resistant phenotype. In *S. cerevisiae*, these genes are referred to as pleiotropic drug resistance (PDR) genes. A number of loci involved in yeast PDR have been identified, and information is now available concerning how their respective gene products interact (5). An ABC transporter-encoding locus, *PDR5*, that is a critical determinant for several resistances, including cycloheximide, sulfometuron methyl, and chloramphenicol, has been characterized (6, 7, 23). *PDR5* is under the transcriptional control of two homologous zinc finger transcription factors, Pdr1p (3) and Pdr3p (13, 27), as well as the products of the regulatory genes *PDR7* and *PDR9* (15). Dominant mutations in the *PDR1* and *PDR3* structural genes lead to overexpression of *PDR5* and high-level drug resistance to cycloheximide and oligomycin (27, 34). However, only the *PDR1*/*PDR3*-mediated cycloheximide resistance requires the *PDR5* gene to be present. These data led to the prediction that another *PDR1*/*PDR3* target gene was responsible for oligomycin resistance.

Here, we isolated a gene that is a strong candidate for the additional *PDR1*/*PDR3* target gene conferring oligomycin re-

* Corresponding author. Phone: (319) 335-7874. Fax: (319) 335-7330. Electronic mail address: moyerowl@blue.weeg.uiowa.edu.

sistance. This locus has been designated *YOR1* (yeast oligomycin resistance 1) and found to encode an ABC transporter protein. *YOR1* is regulated by *PDR1*/*PDR3* but, most important, is required for the oligomycin resistance that is normally mediated by these transcription factors. Unlike *PDR5*, *YOR1* gene expression has a significant *PDR1*/*PDR3*-independent component. Yor1p is a novel member of the class 2 group of ABC transporter proteins, with unique spacing in its NBD1. In a separate study, a genetic approach has also identified *YOR1* as an important oligomycin resistance locus (51).

MATERIALS AND METHODS

Yeast strains and media. Yeast transformations were performed by either the alkali cation technique of Ito et al. (26) or a high-efficiency method (18). Yeast strains were propagated on SD medium (43) containing supplements appropriate for growth of auxotrophic strains. Selection for oligomycin resistance was accomplished by adding oligomycin (35) to YPGE medium. Oligomycin (5 mg/ml) was dissolved in 95% ethanol. Relative resistance levels were assessed by streaking colonies on plates containing different drug concentrations, a spot test assay (50), or a zone-of-inhibition test (31). The yeast strains used in this study are SEY6210 (*MAT*α *leu2-3,112 ura3-52 his3-*Δ*200 trp1-*Δ*901 lys2-801 suc2-*Δ*9* Mel$^-$), PB2 (SEY6210 *pdr3-*Δ*1::hisG*), PB3 (SEY6210 *pdr1-*Δ*2::hisG*), PB4 (*MAT*α *leu2-3,112 ura3-52 his3-*Δ*200 trp1-*Δ*901 lys2-801 suc2-*Δ*9* Mel$^-$ *pdr1-*Δ*2::hisG pdr3-*Δ*1::hisG*), and DKY7 (*MAT*α *leu2-3,112 ura3-52 his3-*Δ*200 trp1-*Δ*901 lys2-801 suc2-*Δ*9* Mel$^-$ *yor1-1::hisG*). The *pdr* and *yor1* mutant strains were all derived from SEY6210 by one-step gene disruption.

Plasmids. The *YOR1-lacZ* fusion plasmid was constructed by inserting a 1.5-kb *Eco*RI-*Eco*RV fragment containing the *YOR1* promoter and N-terminal coding sequence into *Eco*RI- and *Sma*I-cut pSEYC102. A *Bam*HI-*Sac*II fragment containing 3 kb of the *YOR1* coding sequence was inserted into pBluescriptKSII$^+$ to form pSM102. The plasmids pDOC10-2, pDOC12, and pDOC65-2 contain various segments of yeast genomic DNA cloned as *Sau*3A fragments into the *Bam*HI site of YEp24.

Gene disruption. A gene disruption of *YOR1* was produced by inserting a *Bam*HI-*Bgl*II fragment from pNKY51 (1) into the *Bgl*II site of pSM102. The resulting construct was designated pDK30 and contained a 4.3-kb insert of *hisG-URA3-hisG* at codon 316 in *YOR1*. The pDK30 plasmid was cut with *Bam*HI and *Sac*II prior to transformation of SEY6210 cells. *URA3* transformants were selected and purified. Chromosomal DNA was prepared (24) from each transformant, and the presence of the correct integration event was verified by Southern blotting. The *URA3* gene was removed through use of 5-fluoro-orotic acid as described before (8). A representative *yor1* disruptant designated DKY7 was used in these studies.

RNA methods. Total RNA was extracted by a rapid method (42). Northern (RNA) blotting was performed as described before (41). Antisense *YOR1* and *RPC40* probes were prepared by T7 polymerase transcription of appropriate subclones of each gene. The *YOR1* subclone was a pBluescriptKSII$^+$ derivative containing *YOR1* sequences from an *Eco*RI site to the unique *Eco*RV site in the coding sequence. The 5′ end of *RPC40* was subcloned as an *Eco*RI-*Cla*I fragment in pBluescriptKSII$^+$. RNase mapping was performed essentially as described by the manufacturer of the reagents used (Ambion). Equal amounts of RNA were used in both the RNase protection and Northern analyses. Verification that the spectrophotometric quantitation of the RNA was accurate came from visual inspection of the level of the rRNA subunits (data not shown). Protected RNA species were separated by urea-polyacrylamide gel electrophoresis (PAGE) and visualized by autoradiography.

Immunological methods. An anti-Yor1p antiserum was produced by immunizing rabbits with a glutathione *S*-transferase (GST)-Yor1p fusion protein (21). This fusion protein was produced by preparing a PCR fragment of *YOR1* that encoded the extreme carboxy-terminal 80 amino acids. This PCR fragment was cloned as a *Sal*I-*Hin*dIII fragment into pGEX-KG (20) and sequenced to ensure that no errors had occurred during PCR amplification. The resulting rabbit anti-Yor1p antiserum was used to analyze Western immunoblots of yeast protein extracts. The location of the antigen-antibody complex was detected by use of a donkey anti-rabbit immunoglobulin-horseradish peroxidase secondary antibody and ECL reagents (Amersham).

Protein extraction and analysis. Cells were grown in minimal medium to an optical density at 600 nm of 0.7 to 1.0, harvested by centrifugation, resuspended in sorbitol buffer (0.3 M sorbitol, 0.1 M NaCl, 5 mM $MgCl_2$, 10 mM Tris [pH 7.4], with protease inhibitors), broken with glass beads, and microcentrifuged at 2,000 rpm for 5 min at 4°C to remove debris. The protein concentration was determined (32), and equal amounts of protein were solubilized with Laemmli sodium dodecyl sulfate (SDS) sample buffer (28). Immunoreactive protein species were detected by standard Western blotting (46) with the anti-Yor1p antiserum.

RESULTS

Identification of oligomycin resistance-conferring loci. Dominant mutations in the *PDR1* or *PDR3* gene were found to lead to large increases in cycloheximide and oligomycin resistance (reviewed in references 4 and 44 for *PDR1* and *PDR3*, respectively). Genetic and biochemical studies indicated that the cycloheximide resistance conferred by *PDR1* and *PDR3* occurred through activation of *PDR5* gene transcription (27, 34). However, the oligomycin resistance component of the *PDR1*/*PDR3* drug resistance phenotype was clearly *PDR5* independent. In order to identify the gene(s) required for *PDR1*/*PDR3*-mediated oligomycin resistance, we set out to clone genes that affected this phenotype.

A YEp24-based yeast genomic library was transformed into a wild-type yeast strain, and ~24,000 individual *URA3* transformants were recovered. These transformants were then replica plated to YPGE medium containing various concentrations of oligomycin. A total of 101 colonies were found to have elevated resistance to oligomycin relative to the YEp24 vector. Plasmid DNA was recovered from these transformants by transformation into *Escherichia coli*. Restriction analysis indicated that 23 different classes of plasmids had been isolated. Retransformation of representative plasmid isolates into the original wild-type strain determined that three different classes of plasmids reproducibly led to elevated levels of oligomycin resistance. These plasmids were designated pDOC10-2, pDOC12, and pDOC65-2.

Each of the three different classes of plasmids was then transformed into a strain lacking both *PDR1* and *PDR3*. The oligomycin resistance conferred by pDOC65-2 was unaffected by the loss of both the *PDR1* and *PDR3* genes (Fig. 1). However, no oligomycin resistance was seen if either pDOC10-2 or pDOC12 was introduced into a Δ*pdr1,pdr3* strain (data not shown). A high-copy plasmid containing the *PDR5* gene was assayed as a control for a *PDR1*/*PDR3*-dependent gene. Cycloheximide resistance was elevated by increasing the copy number of *PDR5*, but only in the wild-type background. These data indicated that the oligomycin resistance locus in pDOC65-2 was still able to confer a strong resistance phenotype in the absence of *PDR1*/*PDR3* gene function. In comparison, cycloheximide resistance provided by *PDR5* required the presence of *PDR1* and/or *PDR3*.

The approximate origin of the genomic DNA carried in each recombinant plasmid was determined by hybridization to separated yeast chromosomes and a collection of λ phage and cosmid clones carrying known segments of the yeast genome (37). The DNA from pDOC10-2 came from chromosome VIII, near *CUP1*. The genomic insert in pDOC12 was derived from chromosome XII, near *SKI2* and *PEP3*, while the insert in pDOC65-2 originated on chromosome VII, close to the right telomere. We focused our analysis on the insert in pDOC65-2 for two reasons. First, this plasmid conferred the highest level of oligomycin resistance of the three isolates (data not shown). Additionally, the gene carried on this plasmid is required for *PDR1*/*PDR3*-mediated oligomycin resistance (see below).

Sequence of *YOR1*. In order to localize the oligomycin resistance gene present in pDOC65-2, two different derivatives were prepared and phenotypically tested (Fig. 1). A 6-kb *Sac*I-*Sac*II fragment was subcloned into pRS426, and this construct was designated pSM100. This chimera was transformed into wild-type cells and found to no longer elevate oligomycin resistance. The unique *Xho*I restriction site present in pDOC65-2 was filled in with the Klenow fragment, and the resulting plasmid was designated pSM99. This plasmid was still able to confer high-level oligomycin resistance. These findings sug-

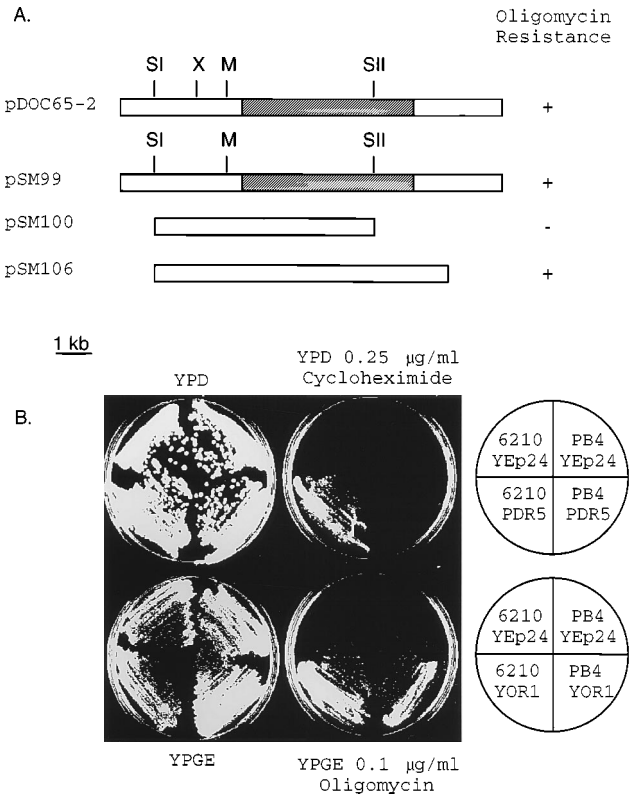

FIG. 1. Subcloning and phenotype analysis of *YOR1*. (A) The yeast genomic segments present in several *YOR1* subclones are shown. Oligomycin resistance represents the ability of a high-copy plasmid carrying the indicated genomic fragment to elevate oligomycin resistance in an otherwise wild-type strain. The location of the *YOR1* coding sequence is indicated by the hatched box. All cloned fragments were present in either the 2μm *URA3* vector YEp24 (pDOC65-2 and pSM99) or pRS426 (pSM100 and pSM106). Relevant restriction sites are indicated: SI, *Sac*I; X, *Xho*I; M, *Mlu*I; SII, *Sac*II. The bar at the bottom of the figure denotes the scale in kilobase pairs of DNA. (B) Yeast transformants carrying high-copy plasmids were tested for their relative ability to grow on cycloheximide and oligomycin by streaking on the indicated media. All plasmids carried the 2μm origin and the *URA3* gene for selection. YEp24 was used as a control plasmid lacking an additional yeast gene. *PDR5* indicates the presence of plasmid pDR3.3 (31), containing the *PDR5* structural gene. *YOR1* denotes transformants containing pSM106, which carries the *YOR1* gene. SEY6210 has wild-type alleles of relevant *PDR* genes, while PB4 lacks both the *PDR1* and *PDR3* loci (27).

gested that the oligomycin resistance determinant was likely to span the *Sac*II site but did not require an intact *Xho*I site for function.

With this functional information, DNA sequence analysis was initiated from the *Sac*I and *Sac*II restriction sites. The DNA sequence around the *Sac*II restriction site was found to lie within a gene that was sequenced by G. Volckaert and colleagues in the frame of the effort to sequence the entire yeast genome (47a). We determined that this gene had the potential to encode a protein that was predicted to be a member of the ABC transporter family of membrane proteins (see below). We designated this open reading frame *YOR1* (yeast oligomycin resistance). To confirm that *YOR1* was the oligomycin resistance gene present in pDOC65-2, 1 kb of DNA extending from the internal *Sac*II site to a *Sna*BI site downstream was inserted into pSM100 to form pSM106. Insertion of this additional 1 kb of DNA was predicted to complete the *YOR1* open reading frame. Consistent with this prediction, pSM106 was able to confer high-level oligomycin resistance. Gene disruption of the *YOR1* locus also led to extreme oligomycin sensitivity (see below), further supporting the role of this locus in oligomycin resistance.

The DNA sequence of the *YOR1* open reading frame predicted a protein product of 1,477 amino acids, with a molecular mass of 166 kDa. Hydropathy analysis suggested the presence of multiple membrane-spanning domains, consistent with the idea that Yor1p is likely to be an integral membrane protein. Analysis of the 5′ noncoding sequence of the *YOR1* gene indicated the presence of a likely binding site for Pdr1p and/or Pdr3p upstream of the *YOR1* ATG. The sequence TTCCGTG GAA is located between −402 and −393 (relative to the translation start). This sequence is identical to an element in the *PDR5* promoter, and we have recently found that both Pdr1p and Pdr3p will bind to this *YOR1* element in vitro (20a). This element and its relatives will be referred to as the Pdr1p/Pdr3p response element (PDRE). We are currently analyzing the function of the *YOR1* promoter to confirm that this element is responsible for Pdr1p/Pdr3p control of *YOR1* expression.

Homology of Yor1p to ABC transporters. A BLAST search (2) of the GenBank database with the predicted amino acid sequence of Yor1p indicated the presence of strong sequence similarity with ABC transporter proteins. The two most closely related sequences were the mammalian multidrug resistance-associated protein Mrp1p (10), with 33% identity, and yeast Ycf1p (45), with 32% identity. The sequence identities of these proteins extended through the entire length of the polypeptide chains, suggesting that these factors are likely to have similar overall structures. Yor1p also had 28% sequence identity with the cystic fibrosis transmembrane conductance regulator (CFTR) (38). Alignment of these four ABC transporter protein sequences is shown in Fig. 2.

While many ABC transporters exhibit strong sequence similarity, a unique feature shared by Mrp1p, CFTR, and Ycf1p is the spacing between the functional motifs present in NBD1. Two of these functional motifs are found associated with many nucleotide-binding proteins and are designated Walker A and Walker B (48). The third motif, unique to ABC transporter proteins, is known as the LSGGQ sequence (25). These three elements are arrayed (from the N to the C terminus along the NBD chain) as Walker A-LSGGQ-Walker B. Comparison of the distance between these motifs in Ycf1p, Mrp1p, and CFTR showed that the spacing of these elements was identical in these three proteins (45). However, in the case of Yor1p, a single-amino-acid gap had to be introduced to maintain the sequence similarity. Other than this one-amino-acid gap, the rest of Yor1p NBD1 showed high sequence identity with Ycf1p, Mrp1p, and CFTR. This one-amino-acid gap is striking in light of the mutational data available for CFTR and Ycf1p (see Discussion).

Genetics of *YOR1* and *PDR1*. *YOR1* was isolated by virtue of its ability to confer oligomycin resistance when present at a high gene dosage. We constructed an isogenic pair of *YOR1*/*yor1* strains to determine if the single-copy *YOR1* gene also had an effect on oligomycin resistance. A *YOR1* gene disruption allele was prepared by inserting a *hisG-URA3-hisG* DNA fragment into the *Bgl*II site spanning *YOR1* codons 315 to 317. This in vitro-generated allele was introduced into the chromosomal *YOR1* gene by transformation. The resulting *yor1* allele is an insertion mutation.

Yeast strains containing the *yor1-1*::*hisG* allele were found to be hypersensitive to low concentrations of oligomycin (Fig. 3). Strains with a wild-type *YOR1* locus were able to tolerate oligomycin at up to 0.3 μg/ml, while isogenic *yor1-1*::*hisG* strains were unable to form colonies at 0.1 μg/ml. This result is consistent with the notion that a normal physiological role of Yor1p is to provide oligomycin resistance to the cell.

```
Mrp    .......MAL RGFCSADGSD PLWDWNVTWN TSNPDFTKCF QNTVLVWVPC
Ycf1p  MAGNLVSWAC KLCRSPEGFG PISFYG.... ....DFTQCF IDGVILNLSA
Yor1p  .......... .......... .......... .......... ..........
Cftrp  .......... .......... .......... .......... ..........

Mrp    FYLWACFPFY FLYLSRHDRG YIQMTPLNKT KTALGFLLWI VCWADLFYSF
Ycf1p  IFMITFGIRD LVNLCKKKHS GIKYRRNWII VSRMALVLLE IAFVSLASLN
Yor1p  .......... .......... .......... .......... ..........
Cftrp  .......... .......... .......... .......... ..........

Mrp    WERSRGIFLA PVFLVSPTLL GITTLLATFL IQLE.RRKGV QSSGIMLTFW
Ycf1p  ISKEEAENFT IVSQYASTML SLFVALALHW IEYD.R..SV VANTVLLFYW
Yor1p  .......... .........M TITVGDAVSE TELENKSQNV VLSPKASASS
Cftrp  .......... .......... .......... .......... ..........

Mrp    LVALVCALAI LRSKIMTALK EDAQ..VDLF RDITFYVYFS LLLIQ....L
Ycf1p  LFETFGNFAK L...INILIR HTYE..GIWY SGQTGFILTL FQVITCASIL
Yor1p  DISTDVDKDT SSSWDDKSLL PTGEYIVDRN KPQTYLNSDD IEKVTESDIF
Cftrp  .......... .......... .......... .......... ..........

Mrp    ....VLSCFS DRSPL....F SETIHDPNPC PESSASFLSR ITFWWITGLI
Ycf1p  ....LLEALP KKPLMPHQHI HQTLTRRKPN PYDSANIFSR ITFSWMSGLM
Yor1p  PQKRLFSFLH SKKI...PEV PQTDDERKIY PLFHTNIISN MFFWWVLPIL
Cftrp  .......... .......... ......MQRS PLEKASVVSK LFFSWTRPIL

Mrp    VRGYRQPLEG SDLWSLNKED TSEQVVPVLV KNWKKECAKT RKQPVKVVYS
Ycf1p  KTGYEKYLVE ADLYKLPRNF SSEELSQKLE KNWENE.... ..........
Yor1p  RVGYKRTIQP NDLFKMDPRM SIETLYDDFE KNMIYYFEKT RKKYRK....
Cftrp  RKGYRQRLEL SDIYQIPSVD SADNLSEKLE REWDRE.... ..........

Mrp    SKDPAQPKES SKVDANEEVE ALIVKSPQKE WNPSLFKVLY KTFGPYFLMS
Ycf1p  .......... .......... ......LKQK SNPSLSWAIC RTFGSKMLLA
Yor1p  .......... ....RHPEAT EEEVMENAKL PKHTVLRALL FTFKKQYFMS
Cftrp  .......... .......... ......LASK KNPKLINALR RCFFWRFMFY

Mrp    FFFKAIHDLM MFSGPQILKL LIKFVND... .......... .......TKA
Ycf1p  AFFKAIHDVL AFTQPQLLRI LIKFVTDYNS ERQDDHSSLQ GFENNHPQKL
Yor1p  IVFAILANCT SGFNPMITKR LIEFVEE... .......... ...KAIFHSM
Cftrp  GIFLYLGEVT KAVQPLLLGR IIASYDPDN. .......... ........KE

Mrp    PDWQGYFYTV LLFVTACLQT LVLHQYFHIC FVSGMRIKTA VIGAVYRKAL
Ycf1p  PIVRGFLIAF AMFLVGFTQT SVLHQYFLNV FNTGMYIKSA LTALIYQKSL
Yor1p  HVNKGIGYAI GACLMMFVNG LTFNHFFHTS QLTGVQAKSI LTKAAMKKMF
Cftrp  ERSIAIYLGI GLCLLFIVRT LLLHPAIFGL HHIGMQMRIA MFSLIYKKTL

Mrp    VITNSARKSS TVGEIVNLMS VDAQRFMDLA TYINMIWSAP LQVILALYLL
Ycf1p  VLSNEASGLS STGDIVNLMS VDVQKLQDLT QWLNLIWSGP FQIIICLYSL
Yor1p  NASNYARHCF PNGKVTSFVT TDLARIEPAL SFQPFLAGFP AILAICIVLL
Cftrp  KLSSRVLDKI SIGQLVSLLS NNLNKFDEGL ALAHFVWIAP LQVALLMGLI

Mrp    WLNLGPSVLA GVAVMVLMVP VNAVMAMKTK TYQVAHMKSK DNRIKLMNEI
Ycf1p  YKLLGNSMWV GVIILVIMMP LNSFLMRIQK KLQKSQMKYK DERTRVISEI
Yor1p  IVNLGPIALV GIGIFFGGFF ISLFAFKLIL GFRIAANIFT DARVTMMREV
Cftrp  WELLQASAFC GLGFLIVLAL FQAGLGRMMM KYRDQRAGKI SERLVITSEM

Mrp    LNGIKVLKLY AWELAFKDKV LAIRQE...E LKVLKKSAYL SAVGTFTWVC
Ycf1p  LNNIKSLKLY AWEKPYREKL EEVRNNK..E LKNLTKLGCY MAVTSFQFNI
Yor1p  LNNIKMIKYY TWEDAYEKNI QDIRTKEISK VRKMQLSRNF LIAMAMSLPS
Cftrp  IENIQSVKAY CWEEAMEKMI ENLRQT...E LKLTRKAAYV RYFNSSAFFF

Mrp    TPFLVALCTF AVYVTIDENN ILDAQTAFVS LALFNILRFP LNI.LPMVIS
Ycf1p  VPFLVSCCTF AVFV.YTEDR ALTTDLVFPA LTLFNLLSFP LMI.IPMVLN
Yor1p  IASLVTF..L AMYKVNKGGR ..QPGNIFAS LSLFQVLSLQ MFF.LPIAIG
Cftrp  SGFFVVFLSV LPYALIKG.. .IILRKIFTT ISFCIVLRMA VTRQFPWAVQ

Mrp    SIVQASVSLK RLRIFLSHEE LEP..... .......... ..........
Ycf1p  SFIEASVSIG RLFTFFTNEE LQP..... .......... ..........
Yor1p  TGIDMIIGLG RLQSLLEAPE DDPNQMIEMK PSPGFDPKLA LKMTHCSFEW
Cftrp  TWYDSLGAIN KIQDFLQKQE YKTLE..... ........YN LTTTEVVMEN

Mrp    ......DSIE RRP.VKDGGG TNSITVRNAT FTWARSD..P P.........
Ycf1p  ......DSVQ RLPKVKNIGD VAINIGDDAT FLWQRKPEYK V.........
Yor1p  EDYELNDAIE EAKGEAKDEG KKNKKKRKDT WGKPSASTNK AKRLDNMLKD
Cftrp  VTAFWEEGFG ELFEKAKQNN NNRKTSNGDD SLFFSN.... ..........

Mrp    .......... ...TLNGITF SIPEGALVAV VGQVGCGKLS LLSALLAEMD
Ycf1p  .......... ...ALKNINF QAKKGNLTCI VGKVGSGKTA LLSCMLGDLF
Yor1p  RDGPEDLEKT SFRGFKDLNF DIKKGEFIMI TGPIGTGKSS LLNAMAGSMR
Cftrp  ......FSLL GTPVLKDINF KIERGQLLAV AGSTGAGKTS LLMMIMGELE
                                        Walker A

Mrp    KVEGHVAIKG SVAYVPQQAW IQNDSLRENI LFGCQLEEPY YRSVIQACAL
Ycf1p  RVKGFATVHG SVAYVSQVPW IMNGTVKENI LFGHRYDAEF YEKTIKACAL
Yor1p  KTDGKVEVNG DL.LMCGYPW IQNASVRDNI IFGSPFNKEK YDEVVRVCSL
Cftrp  PSEGKIKHSG RISFCSQFSW IMPGTIKENI IFGVSYDEYR YRSVIKACQL

Mrp    LPDLEILPSG DRTEIGEKGV NLSGGQKQRV SLARAVYSNA DIYLFDDPLS
Ycf1p  TIDLAILMDG DKTLVGEKGI SLSGGQKARL SLARAVYARA DTYLLDDPLA
Yor1p  KADLDILPAG DMTEIGERGI TLSGGQKARI NLARSVYKKK DIYLFDDVLS
Cftrp  EEDISKFAEK DNIVLGEGGI TLSGGQRARI SLARAVYKDA DLYLLDSPFG
                                 Linker                Walker B

Mrp    AVDAHVGKHI FENVIGPKGM LKNKTRILVT HSMSYLPQVD VIIVMSGGKI
Ycf1p  AVDEHVARHL IEHVLGPNGL LHTKTKVLAT NKVSALSIAD SIALLDNGEI
Yor1p  AVDSRVGKHI MDECLT..GM LANKTRILAT HQLSLIERAS RVIVLGTDGQ
Cftrp  YLDVLTEKEI FESCVCK..L MANKTRILVT SKMEHLKKAD KILILNEGSS

Mrp    SEMGSYQELL .ARDGAFAEF L......... .......... ..........
Ycf1p  TQQGTYDEIT KDADSPLWKL L......... .......... ..........
Yor1p  VDIGTVDEL. KARNQTLINL L......... .......... ..........
Cftrp  YFYGTFSELQ NLQPDFSSKL MGCDSFDQFS AERRNSILTE TLHRFSLEGD

Mrp    .......... RTYASTEQEQ DAEENGVTGV SGPGKEAKQM ENGMLVTDSA
Ycf1p  .......... NNYGK..... ..KNNGKSNE FGDSSESSVR ESSIPV.EGE
Yor1p  .......... QFSSQNSEKE DEEQEAVVAG .......... ELGQLKYESE
Cftrp  APVSWTETKK QSFKQTGEFG EKRKNSILNP INSIRKFSIV QKTPLQMNGI

Mrp    GKQLQR.... .......... .......... .......... .....QLSSS
Ycf1p  LEQLQK.... .......... .......... .......... .....LNDLD
Yor1p  VKELTE.... .......... .......... .......... .....LKKKA
Cftrp  EEDSDEPLER RLSLVPDSEQ GEAILPRISV ISTGPTLQAR RRQSVLNLMT

Mrp    SSYSGDISRH HNSTAELQKA EAKKEETWK. .......... ...LMEADKA
Ycf1p  FGNSDAISLR RASDATLGSI DFGDDE..N. .......... ...IAKREHR
Yor1p  TEMSQ..... ...TANSGKI VADGHTSSK. .......... .......EER
Cftrp  HSVNQGQNIH RKTTASTRKV SLAPQANLTE LDIYSRRLSQ ETGLEISEEI

Mrp    QTGQVKLSVY WD........ ....YMKA.. ...IGLFISF LSIFLFMCNH
Ycf1p  EQGKVKWNIY LE........ ....YAKACN PKSVCVFILF IVISMFLS..
Yor1p  AVNSISLKIY RE........ ....YIKAAV GKWGFIALPL YAI.LVVGTT
Cftrp  NEEDLKECLF DDMESIPAVT TWNTYLRYIT VHKSLIFVLI WCLVIFLAEV

Mrp    VSALASNYWL .......... SLWTDDPIVN GTQEHTKVRL SVYGALGISQ
Ycf1p  ...VMGNVWL .......... KHWSEVNSRY GSNPNAARYL AIYFALGIGS
Yor1p  FCSLFSSVWL .......... SYWTENKFK. ..NRPPSFYM GLYSFFVFA.
Cftrp  AASLVVLWLL GNTPLQDKGN STHSRNNSYA VIITSTSSYY VFYIYVGVAD

Mrp    GIAVFGYSMA V.SI.GGILA SRCLHVDLLH SILRSPMSFF ERTPSGNLVN
Ycf1p  ALATLIQTIV L.WVFCTIHA SKYLHNLMTN SVLRAPMTFF ETTPIGRILN
Yor1p  AFIFMNGQFT I.LCAMGIMA SKWLNLRAVK RILHTPMSYI DTTPLGRILN
Cftrp  TLLAMGFFRG LPLVHTLITV SKILHHKMLH SVLQAPMSTL NTLKAGGILN

Mrp    RFSKELDTVD SMIPEVIKMF MGSLFNVIGA CIVILLATPI AAIIIPPLGL
Ycf1p  RFSNDIYKVD ALLGRTFSQF FVNAVKVTFT ITVICATTWQ FIFIIIPLSV
Yor1p  RFTKDTDSLD NELTESLRLM TSQFANIVGV CVMCIVYLPW FAIAIPFLLV
Cftrp  RFSKDIAILD DLLPLTIFDF IQLLLIVIGA IAVVAVLQPY IFVATVPVIV

Mrp    IYFFVQRFYV ASSRQLKRLE SVSRSPVYSH FNETLLGVSV IRAFEEQERF
Ycf1p  FYIYYQQYYL RTSRELRRLD SITRSPIYSH FQETLGGLAT VRGYSQQKRF
Yor1p  IFVLIADHYQ SSGREIKRLE AVQRSFVYNN LNEVLGGMDT IKAYRSQERF
Cftrp  AFIMLRAYFL QTSQQLKQLE SEGRSPIFTH LVTSLKGLWT LRAFGRQPYF

Mrp    IHQSDLKVDE NQKAYYPSIV ANRWLAVRLE CVGNCIVLFA ALFAV..ISR
Ycf1p  SHINQCRIDN NMSAFYPSIN ANRWLAYRLE LIGSIIILGA ATLSVFRLKQ
Yor1p  LAKSDFLINK MNEAGYLVVV LQRWVGIFLD MVAIAFALII TLLCVTR..A
Cftrp  ETLFHKALNL HTANWFLYLS TLRWFQMRIE MIFVIFFIAV TFISILTTGE

Mrp    HSLSAGLVGL SVSYSLQVTT YLNWLVRMSS EMETNIVAVE RLKEY.....
Ycf1p  GTLTAGMVGL SLSYALQITQ TLNWIVRMTV EVETNIVSVE RIKEY.....
Yor1p  FPISAASVGV LLTYVLQLPG LLNTILRAMT QTENDMNSAE RLVTY.....
Cftrp  ...GEGRVGI ILTLAMNIMS TLQWAVNSSI DVDSLMRSVS RVFKFIDMPT

Mrp    .......... ..S.ETEKEA PWQIQETRPP SSWPQVGRVE FRNYCLRYRE
Ycf1p  .......... ..A.DLKSEA PLIVEGHRPP KEWPSQGDIK FNNYSTRYRP
Yor1p  .......... ..ATELPLEA SYRKPEMTPP ESWPSMGEII FENVDFAYRP
Cftrp  EGKPTKSTKP YKNGQLSKVM IIENSHVKKD DIWPSGGQMT VKDLTAKYTE

Mrp    DLDFVLRHIN VTINGGEKVG IVGRTGAGKS SLTLGLFRIN ESAEGEIIID
Ycf1p  ELDLVLKHIN IHIKPNEKVG IVGRTGAGKS SLTLALFRMI EASEGNIVID
Yor1p  GLPIVLKNLN LNIKSGEKIG ICGRTGAGKS TIMSALYRLN ELTAGKILID
Cftrp  GGNAILENIS FSISPGQRVG LLGRTGSGKS TLLSAFLRLL N.TEGEIQID
                                        Walker A

Mrp    GINIAKIGLH DLRFKITIIP QDPVLFSGSL RMNLDPFSQY SDEEVWTSL.
Ycf1p  NIAINEIGLY DLRHKLSIIP QDSQVFEGTV RENIDPINQY TDEAIWRAL.
Yor1p  NVDISQLGLF DLRRKLAIIP QDPVLFRGTI RKNLDPFNER TDDELWDALV
Cftrp  GVSWDSITLQ QWRKAFGVIP QKVFIFSGTF RKNLDPYEQW SDQEIWKV..
```

```
Mrp     .....ELAHL KDFVSALP.D K......... .LDHECAEGG ENLSVGQRQL
Ycf1p   .....ELSHL KEHVLSMSND G......... .LDAQLTEGG GNLSVGQRQL
Yor1p   RGGAIAKDDL PEVKLQKPDE NGTHGKMHKF HLDQAVEEEG SNFSLGERQL
Cftrp   ....ADEVGL RSVIEQFPGK .......... .LDFVLVDGG CVLSHGHKQL
                                                    Linker

Mrp     VCLARALLRK TKILVLDEAT AAVDLETDDL IQSTIRTQFE DCTVLTIAHR
Ycf1p   LCLARAMLVP SKILVLDEAT AAVDVETDKV VQETIRTAFK DRTILTIAHR
Yor1p   LALTRALVRQ SKILILDEAT SSVDYETDGK IQTRIVEEFG DCTILCIAHR
Cftrp   MCLARSVLSK AKILLLDEPS AHLDPVTYQI IRRTLKQAFA DCTVILCEHR
                    Walker B

Mrp     LNTIMDYTRV IVLDKGEIQE YGAPSDLL.Q QRGLFYSMAK DAGLV.....
Ycf1p   LNTIMDSDRI IVLDNGKVAE FDSPGQLLSD NKSLFYSLCM EAGLVNENZ.
Yor1p   LKTIVNYDRI LVLEKGEVAE FDTPWTLFSQ EDSIFRSMCS RSGIVENDFE
Cftrp   IEAMLECQQF LVIEENKVRQ YDSIQKLL.N ERSLFRQAIS PSDRVKLFPH

Ycf1p   .......... .......... ........
Yor1p   NRS....... .......... ........
Cftrp   RNSSKCKSKP QIAALKEETE EEVQDTRL
```

FIG. 2. Homology between Mrp, Ycf1p, Yor1p, and CFTR. A computer-generated alignment between the protein sequences of Mrp, Ycf1p, Yor1p, and CFTR (Cftrp) is shown. This alignment was produced by using the Genetics Computer Group package and the subroutine Pileup (14). Amino acids are listed in the one-letter code, and dots indicate positions where gaps were introduced to maintain the alignment. The predicted transmembrane domains in the CFTR are underlined, while the functional motifs in each NBD are indicated (Walker A, LSGGQ [Linker], and Walker B).

Previously, we demonstrated that production of a PDR3-VP16 fusion protein led to acquisition of high-level oligomycin resistance (27). This high-level oligomycin resistance was not dependent on the presence of the *PDR5* gene. Others have found that dominant alleles of *PDR1* also elicit a strong oligomycin resistance phenotype (3). We used strains containing the *yor1-1*::*hisG* mutation to determine if oligomycin resistance produced by *PDR1* and/or *PDR3* required the presence of *YOR1*.

Introduction of a *CEN* plasmid carrying the semidominant drug resistance *PDR1-6* allele into a *YOR1* strain (SEY6210) produced a strain capable of growing more rapidly than an isogenic wild-type strain with 0.3 μg of oligomycin per ml (Fig. 3). However, when the same plasmid was transformed into a strain (DKY7) lacking the *YOR1* gene, the resulting strain was only able to tolerate 0.1 μg/ml. Note that the *yor1* strain was able to grow on medium containing 0.1 μg of oligomycin per ml only if the *PDR1-6* allele or the plasmid expressing the PDR3-VP16 fusion protein was present. This result indicates that even in a *yor1* background, Pdr1p and Pdr3p have an additional target gene that can be activated to elevate oligomycin resistance. Similarly, the presence of the *yor1-1*::*hisG* allele blocked the ability of the PDR3-VP16 fusion protein to elevate oligomycin resistance. However, loss of *YOR1* from the cell had no effect on the ability of either the *PDR1-6* allele or the PDR3-VP16 fusion protein to confer cycloheximide resistance. These data are consistent with the belief that *YOR1* is a major determinant of oligomycin resistance in *S. cerevisiae.*

A more quantitative comparison of the relative levels of oligomycin resistance in several different strains was carried out with a zone-of-inhibition assay. Wild-type (SEY6210) cells exhibited a zone of inhibition of 1.3 ± 0.1 cm around a filter disk containing 50 μg of oligomycin. The size of the zone of inhibited growth increased to 2.3 ± 0.14 cm when the isogenic *yor1-1*::*hisG*-containing strain was tested. A strain lacking both the *PDR1* and *PDR3* genes had a zone of inhibition of 1.7 ± 0.14 cm. Thus, loss of the *YOR1* gene had a dramatic effect on oligomycin tolerance, nearly doubling the size of the zone of inhibition, while removal of *PDR1* and *PDR3* caused a less severe degree of inhibition by oligomycin.

Analysis of *YOR1* mRNA. Dominant mutations in the *PDR1* gene confer elevated resistance to oligomycin and cycloheximide (3). The elevated resistance to cycloheximide comes about through overproduction of the *PDR5* gene product (34). To determine if *YOR1* mRNA levels correlated with the oligomycin hyperresistance phenotype of a semidominant *PDR1-6* mutant, we performed Northern blot analysis. RNA was isolated from a wild-type strain carrying a *CEN* plasmid containing the *PDR1-6* gene or the vector plasmid alone. These RNA samples were electrophoresed in a denaturing agarose gel, transferred to a nylon membrane, and probed with an antisense *YOR1* riboprobe. The resulting autoradiogram (Fig. 4) indicated that there was a large elevation in *YOR1* steady-state mRNA levels in response to the presence of the *PDR1-6* allele relative to the wild-type *PDR1* gene. The estimated size of the *YOR1* transcript was consistent with size of the coding sequence required to encode this relatively large protein.

The start site of *YOR1* gene transcription was identified by RNase protection. An antisense *YOR1* RNA probe was prepared by in vitro transcription, annealed to aliquots of RNA prepared from wild-type cells or cells containing the *PDR1-6* allele, and digested with RNase. The resulting duplex RNA was electrophoresed next to size markers, and the protected RNA species was detected by autoradiography (Fig. 4). An antisense probe was also prepared for the *RPC40* gene, a locus not known to be under the control of Pdr1p or Pdr3p (33).

The *YOR1* antisense probe gave rise to a protected RNA of 400 nucleotides. This protected fragment was more abundant in the RNA prepared from the strain containing the *PDR1-6* allele than in RNA isolated from the wild-type strain. The size of the protected fragment indicated that the 5′ end of the *YOR1* mRNA is located approximately 200 nucleotides upstream of the deduced *YOR1* ATG. There are no other ATG elements in this 200-nucleotide stretch of *YOR1* sequence. The levels of the control RNA from the *RPC40* gene did not change. These data indicate that the steady-state levels of *YOR1* mRNA responded to the type of *PDR1* allele present in the cell. Additionally, this finding of the location of the *YOR1* mRNA start point supports the suggestion that the PDRE could serve as an upstream activation element for this gene.

Control of *YOR1* by *PDR1*. To facilitate measurement of *YOR1* gene expression, a *YOR1-lacZ* fusion gene was constructed. The fusion gene that was constructed contained 1,250 bp of 5′ noncoding sequence and 213 bp from the *YOR1* open reading frame fused to *E. coli lacZ*. The resulting chimera was transformed into several yeast strains with different genetic backgrounds in order to assess the response of the *YOR1-lacZ*

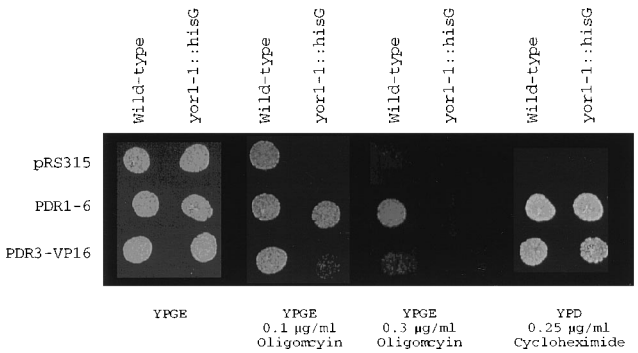

FIG. 3. *YOR1* required for normal oligomycin tolerance. Yeast strains containing (*YOR1*) or lacking (*yor1-1*::*hisG*) a normal copy of the *YOR1* gene were transformed with the plasmids indicated on the left-hand side of the figure. Vector refers to the presence of the low-copy plasmid pRS315, while *PDR1-6* denotes transformants carrying the *PDR1-6* allele cloned in pRS315. PDR3-VP16 corresponds to a high-copy plasmid expressing a PDR3-VP16 fusion protein that elevates oligomycin resistance in wild-type and Δ*pdr5* cells (27). The relative resistance of each transformant was analyzed by a spot test assay (50).

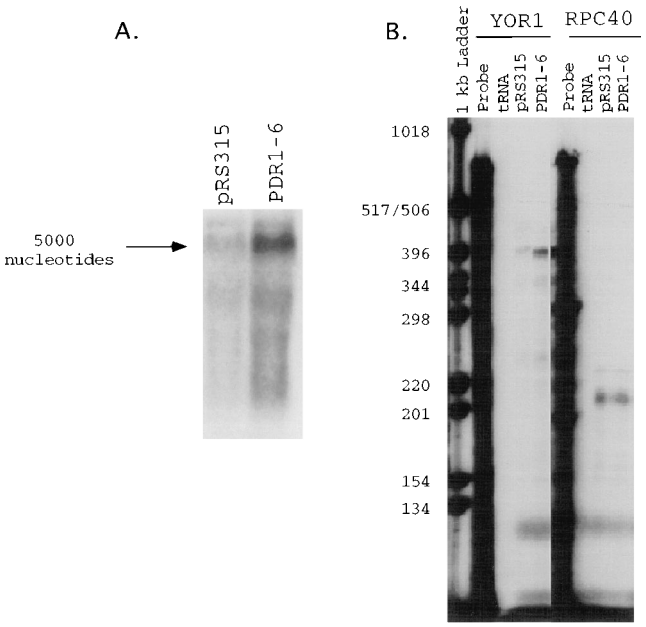

FIG. 4. Identification of *YOR1* mRNA species. (A) Northern blot. Total RNA was prepared from a wild-type yeast strain (SEY6210) transformed with a low-copy plasmid carrying the *PDR1-6* allele (PDR1-6) or the low-copy vector alone (pRS315). Equal amounts of RNA were electrophoresed through a formaldehyde-agarose gel and transferred to a nylon membrane. *YOR1* mRNA was detected by probing the membrane with an antisense riboprobe. The estimated size of the *YOR1* transcript is shown on the left-hand side of the figure. (B) RNase mapping of the *YOR1* transcription start site. Antisense RNA probes were prepared against *YOR1* or *RPC40* by in vitro transcription in the presence of [α-^{32}P]UTP. The presence of each of these probes is indicated at the top of the figure. Probe denotes the untreated probe, while tRNA indicates that the probe was annealed to tRNA and then treated with RNase. RNAs from wild-type cells carrying a low-copy vector (pRS315) or cells carrying pRS315 expressing the *PDR1-6* allele were also annealed to the probe and digested with RNase. After digestion, the samples were electrophoresed through a 5% urea–polyacrylamide gel in parallel with an aliquot of the 1-kb ladder (Bethesda Research Laboratories) that was ^{32}P labeled. The sizes of the DNA fragments are shown on the left (in nucleotides).

fusion gene to changes in the type of *PDR1* or *PDR3* allele present.

Introduction of the *YOR1-lacZ* fusion gene into cells with a wild-type set of *PDR* loci led to the production of 16 U of β-galactosidase activity per optical density unit (Table 1). The presence of this reporter plasmid in an isogenic *pdr1* strain

TABLE 1. Expression of a *YOR1-lacZ* fusion gene exhibits a major *PDR1*/*PDR3*-independent component[a]

Strain	β-Galactosidase activity[b] (U/OD unit)	
	YOR1-lacZ	*PDR5-lacZ*
Wild type	16	30
Δ*pdr1*	7	13
Δ*pdr3*	9	15
Δ*pdr1,3*	5	0.5
PDR1-6[c]	66	344

[a] An isogenic set of strains with the indicated *PDR* genotypes were transformed with plasmids containing either a *YOR1-lacZ* (pSM109) or a *PDR5-lacZ* (pKV2) fusion gene.

[b] β-Galactosidase activities are the average of at least two determinations and have an error of <20%. Enzyme activities were determined as described before (27). OD, optical density.

[c] This genetic background consists of a wild-type strain (SEY6210) transformed with a low-copy plasmid carrying the *PDR1-6* allele.

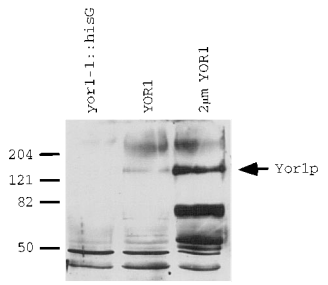

FIG. 5. Identification of Yor1p by Western blot analysis. Protein extracts were prepared from yeast strains containing multiple copies (2μm *YOR1*), a single copy (*YOR1*), or no functional copies (*yor1-1*::*hisG*) of the *YOR1* gene. Total protein (100 μg) was electrophoresed for each sample and transferred to nitrocellulose. The location of immunoreactive Yor1p was determined by probing the filter with an anti-Yor1p antiserum. The positions of molecular mass standards are indicated on the left (in kilodaltons). The position of full-length Yor1p is noted on the right-hand side of the figure.

dropped the *YOR1*-dependent enzyme activity to 7 U per optical density unit. Loss of only the *PDR3* gene elicited a decrease to 9 U per optical density unit. Transformation of a cell lacking both *PDR1* and *PDR3* decreased the expression of the *YOR1-lacZ* gene to 5 U per optical density unit. Introduction of the *PDR1-6* allele into a cell containing the *YOR1-lacZ* fusion plasmid led to a nearly fivefold increase in β-galactosidase activity. This increase in *YOR1*-dependent β-galactosidase activity is consistent with the increase in *YOR1* mRNA seen in a *PDR1-6* background (Fig. 4). The decrease in *YOR1*-dependent β-galactosidase activity upon loss of *PDR1* and/or *PDR3* from the cell provides further support for the notion that normal expression of *YOR1* is dependent on the presence of *PDR1* and *PDR3* in the cell.

For comparison, a *PDR5-lacZ* fusion was assayed as a control for the effect of the various *PDR* mutations on gene expression. As seen previously (27), the expression of *PDR5*-dependent β-galactosidase was only modestly affected by the loss of either *PDR1* or *PDR3* individually. However, when both of these loci were removed from the cell, the expression of the *PDR5-lacZ* fusion gene was reduced to 2% of the level seen in wild-type cells. Thus, while both *YOR1* and *PDR5* are regulated by *PDR1* and *PDR3*, *YOR1* expression is much less dependent on the function of these two transcription factors than is *PDR5*.

Immunological detection of Yor1p. Both the Northern blot and *lacZ* fusion data are consistent with the belief that overproduction of Yor1p leads to increased oligomycin resistance. To confirm this suggestion, we generated an antiserum directed against Yor1p. This antiserum was used in Western blot analysis of protein extracts from yeast strains with various gene dosages of *YOR1*. Detergent-soluble protein extracts were prepared from strains containing the *yor1-1*::*hisG* allele, the wild-type *YOR1* gene, or a high-copy plasmid containing *YOR1*. These extracts were then electrophoresed through SDS-PAGE, transferred to a nitrocellulose membrane, and probed with the anti-Yor1p antiserum (Fig. 5).

A 163-kDa species was detected in a strain containing either the high-copy *YOR1* plasmid or the normal chromosomal *YOR1* gene by the anti-Yor1p antiserum. This polypeptide was not found in an extract from cells lacking a functional copy of the *YOR1* gene. The observed molecular mass of 163 kDa agrees well with the expected size predicted from the *YOR1* DNA sequence (166 kDa). We conclude that this 163-kDa protein is the product of the *YOR1* gene. The relative oligomycin resistances exhibited by these three strains correlate well

with the relative expression levels of Yor1p in each genetic background. We believe that the other protein species appearing on this blot correspond to aggregation or breakdown products of full-length Yor1p. These data suggest that Yor1p directly functions to detoxify oligomycin, although other possibilities, such as Yor1p's regulating the function of the direct mediator of oligomycin resistance, cannot be excluded at this time.

DISCUSSION

The identification of *YOR1* as a target gene important for *PDR1*/*PDR3*-mediated oligomycin resistance provides a key piece of information in understanding the network of pleiotropic drug resistance genes in *S. cerevisiae*. This analysis of *YOR1* confirms the previous suggestion that the effects of *PDR1* and *PDR3* on cycloheximide and oligomycin resistance come about through control of expression of different loci. It is interesting that all known *PDR1*/*PDR3* target genes encode either ABC transporter proteins or the PDR3 protein itself. In addition to *YOR1*, genes that have been demonstrated to fall under *PDR1* and *PDR3* control include *PDR5* (27), *SNQ2* (11), and the *PDR3* gene (12).

The degree of *PDR1*/*PDR3* dependence is quite different for the coregulated *PDR5* and *YOR1* genes. Loss of the *PDR1* and *PDR3* genes severely reduces *PDR5* expression, while in this same genetic background, *YOR1*-dependent expression remains relatively high. It was anticipated that *YOR1* expression would show a strong *PDR1*/*PDR3* dependence, as does *PDR5*, since Δ*pdr1* and Δ*pdr1,3* mutants are extremely oligomycin sensitive (13, 27). Thus, it was surprising to find that, unlike *PDR5*, *YOR1* has a major *PDR1*/*PDR3*-independent component for its expression (Table 1). How can the oligomycin sensitivity of a Δ*pdr1,3* strain be rationalized in light of the relatively minor effect of this genetic background on *YOR1* expression? This apparent paradox can be explained by the ability of *PDR1*/*PDR3* to activate the expression of at least one other gene involved in oligomycin resistance. The requirement for this other gene can be suppressed by overproduction of Yor1p from a 2μm plasmid. This finding is supported by the observation that a 2μm plasmid carrying *YOR1* still confers strong oligomycin resistance even in a Δ*pdr1,3* background (Fig. 1). It is possible that the other two genes identified in this screen represent other targets important for *PDR1*/*PDR3*-mediated oligomycin resistance.

PDR5 and *YOR1* are coregulated drug resistance genes, through the common presence of PDREs in their promoter regions, but exhibit clear differences in their transcriptional control. This suggests that other *PDR1*/*PDR3*-regulated genes will also show distinct patterns of regulation and rely on these transcription factors to various degrees. Identification of the protein(s) and site(s) mediating the *PDR1*/*PDR3*-independent expression of *YOR1* will provide important new information concerning the network of factors that regulate drug resistance genes.

ABC transporters constitute a large and constantly growing class of membrane proteins. However, the ABC transporter proteins with which Yor1p has the greatest sequence identity represent only a small subgroup of this family of proteins. These molecules are grouped based on the strong conservation of both sequence and spacing in their NBD1 regions. The finding of the one-amino-acid gap in the Yor1p NBD1 is striking in light of mutational data available concerning single NBD1 amino acid deletions in CFTR (reviewed in reference 49) and Ycf1p (45, 49a). Loss of the phenylalanine codon at position 508 in CFTR or 713 in Ycf1p causes these proteins to lose their ability to function normally. A second CFTR mutation, ΔI507, also elicits a loss-of-function phenotype (9). These

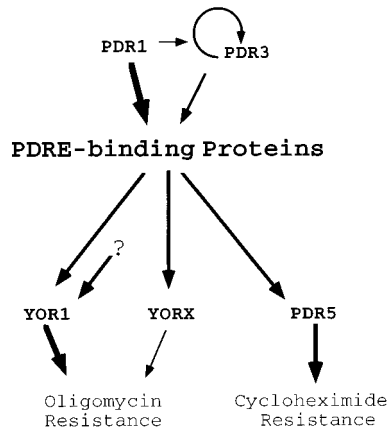

FIG. 6. Model for *PDR* gene interactions. A scheme describing the functional interactions of the known *PDR* genes involved in cycloheximide and oligomycin resistance is shown. Arrows indicate positive interactions. The large arrow drawn from *PDR1* indicates the greater contribution of this gene to the pool of PDRE-binding proteins than that of *PDR3*. The three PDREs present in *PDR5* (8a) and the one identified PDRE in *YOR1* are indicated by the arrows. The ? refers to the other protein(s) involved in transcriptional control of *YOR1*.

results indicate that the number of amino acids present in NBD1 is not totally flexible, at least in the region close to the important phenylalanine. Other than this alteration in spacing, the rest of NBD1 in Yor1p is very similar to the analogous regions of CFTR, Mrp, and Ycf1p. It will be interesting to investigate the consequence of a ΔF670 mutation in Yor1p as well as to vary the spacing elsewhere in NBD1 of this ABC transporter protein.

While the homology of Yor1p with other ABC transporter proteins suggests a possible mode of action of this protein, no details are yet available concerning the biochemical activity of Yor1p. The two proteins that show the highest degree of sequence similarity with Yor1p are Mrp1p and Ycf1p. These two proteins have recently been demonstrated to act as glutathione conjugate transporters (29, 35a). Since transport of glutathione conjugates is a key step in Mrp-mediated detoxification (29), perhaps Yor1p pumps an oligomycin-glutathione conjugate as a step in the neutralization of this agent in *S. cerevisiae*. The availability of mutant strains lacking or overproducing Yor1p will allow this hypothesis to be tested.

Along with the biochemical activity of Yor1p, a key issue to be resolved is the location of this protein. Subcellular localization will be a defining property in proposing models for how Yor1p might function in oligomycin resistance. Pdr5p is a plasma membrane protein that mediates the efflux of target drugs out of the cell (30). Hmt1p, a vacuolar ABC transporter from *Schizosaccharomyces pombe*, has recently been shown to confer cadmium resistance through pumping of phytochelatin-cadmium complexes into the vacuole (36). Since the target of oligomycin is the mitochondrially located F_1 ATPase protein (35), Yor1p may function by removing oligomycin from the cell or possibly by sequestering the drug so that it cannot reach the mitochondria. The anti-Yor1p antiserum that we have developed will allow the determination of where Yor1p acts in the cell.

We have examined a large number of other drugs and found no major effect of Yor1p on resistance to these other inhibitors (50a). The possibility that yeast cells possess a dedicated oligomycin detoxification system seems unlikely, since oligomycin is only toxic when cells are utilizing nonfermentable carbon sources like glycerol or ethanol. It is expected that other drugs to which Yor1p also mediates resistance will be found.

A model to explain the various interactions of genes involved in oligomycin and cycloheximide resistance is shown in Fig. 6. The contribution of the *PDR1* gene to the pool of PDRE-binding proteins has a greater functional consequence that of *PDR3*. Strains lacking the *PDR1* locus exhibit a profound reduction in cycloheximide and oligomycin resistance, while loss of *PDR3* has a negligible effect on these resistance phenotypes (27). Pdr1p and Pdr3p then act on several sites in the *PDR5* promoter and at least one in the 5′ noncoding sequence of *YOR1*. Dominant mutations in either *PDR1* or *PDR3* confer cycloheximide tolerance through transactivation of *PDR5* gene expression, while the oligomycin resistance of these lesions is produced through stimulation of *YOR1* transcription. Expression of *PDR5* is strongly dependent on the presence of either *PDR1* or *PDR3*, while *YOR1* transcription does not exhibit such a strong requirement for these regulatory proteins.

Our finding that the *PDR1-6* allele can partially suppress the strong oligomycin-hypersensitive phenotype of the *yor1-1*::*hisG* mutation indicates the presence of at least one additional gene that can contribute to oligomycin resistance (Fig. 3). This locus provides a minor component of cellular resistance to oligomycin but is still responsive to the *PDR1-6* allele. This suggests that an additional target gene that is under control of Pdr1p (and likely Pdr3p) that can provide oligomycin resistance in a Yor1p-independent fashion exists. Possible candidates for this unknown locus or loci are the genes carried in the other two plasmids recovered in our high-copy plasmid library screen. This suggestion is currently under investigation.

ACKNOWLEDGMENTS

This work was supported in part by NIH grant GM49825 to W.S.M., NATO grant CRG940493 to E. Balzi and W.S.M., NSF grant MCB 9307962 to J.G., and by the EU-Biotech Program, the Belgian Office for Science Policy, and the K.U. Leuven Research Fund to G.V. W.S.M. is an Established Investigator of the American Heart Association.

We thank A. Goffeau, E. Balzi, D. Ortiz, and I. Arias for useful discussions and communication of unpublished results. Thanks to E. Carvajal for providing the pRS315-PDR1 clones used in these experiments. The assistance of John Wemmie and Cynthia Leveille in the production of the anti-Yor1p antiserum was invaluable.

REFERENCES

1. **Alani, E., L. Cao, and N. Kleckner.** 1987. A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. Genetics **116:**541–545.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Balzi, E., W. Chen, S. Ulaszewski, E. Capieaux, and A. Goffeau.** 1987. The multidrug resistance gene *PDR1* from *Saccharomyces cerevisiae*. J. Biol. Chem. **262:**16871–16879.
4. **Balzi, E., and A. Goffeau.** 1991. Multiple or pleiotropic drug resistance in yeast. Biochim. Biophys. Acta **1073:**241–252.
5. **Balzi, E., and A. Goffeau.** 1995. Yeast multidrug resistance: the *PDR* network. J. Bioenerg. Biomembr. **27:**71–76.
6. **Balzi, E., M. Wang, S. Leterme, L. Van Dyck, and A. Goffeau.** 1994. *PDR5*: a novel yeast multidrug resistance transporter controlled by the transcription regulator *PDR1*. J. Biol. Chem. **269:**2206–2214.
7. **Bissinger, P. H., and K. Kuchler.** 1994. Molecular cloning and expression of the *S. cerevisiae STS1* gene product. J. Biol. Chem. **269:**4180–4186.
8. **Boeke, J. D., F. Lacroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine 5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:**345–346.

8a. **Carvajal, E., et al.** Unpublished data.

9. **Cheng, S. H., R. J. Gregory, J. Marshall, S. Paul, D. W. Souza, G. A. White, C. R. O'Riordan, and A. E. Smith.** 1990. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell **63:**827–834.
10. **Cole, S. P. C., G. Bhardwaj, J. H. Gerlach, J. E. Mackie, C. E. Grant, K. C. Almquist, A. J. Stewart, E. U. Kurz, A. M. V. Duncan, and R. G. Deely.** 1992. Overexpression of a transporter gene in multidrug-resistant human lung cancer cell line. Science **258:**1650–1654.
11. **Decottignies, A., L. Lambert, P. Catty, H. Degand, E. A. Epping, W. S. Moye-Rowley, E. Balzi, and A. Goffeau.** 1995. Identification and characterization of *SNQ2*, a new multidrug ABC transporter of the yeast plasma membrane. J. Biol. Chem. **270:**18150–18157.
12. **Delahodde, A., T. Delaveau, and C. Jacq.** 1995. Positive autoregulation of the yeast transcription factor Pdr3p, involved in the control of the drug resistance phenomenon. Mol. Cell. Biol. **15:**4043–4051.
13. **Delaveau, T., A. Delahodde, E. Carvajal, J. Subik, and C. Jacq.** 1994. *PDR3*, a new yeast regulatory gene, is homologous to *PDR1* and controls the multidrug resistance phenomenon. Mol. Gen. Genet. **244:**501–511.
14. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
15. **Dexter, D., W. S. Moye-Rowley, A.-L. Wu, and J. Golin.** 1994. Mutations in the yeast *PDR3*, *PDR4*, *PDR7* and *PDR9* pleiotropic drug resistance loci affect the transcript level of an ATP binding cassette transporter encoding gene, *PDR5*. Genetics **136:**505–515.
16. **Doige, C. A., and G. F.-L. Ames.** 1993. ATP-dependent transport systems in bacteria and humans: relevance to cystic fibrosis and multidrug resistance. Annu. Rev. Microbiol. **47:**291–319.
17. **Endicott, J. A., and V. Ling.** 1989. The biochemistry of P-glycoprotein-mediated multidrug resistance. Annu. Rev. Biochem. **58:**137–171.
18. **Gietz, D., A. St. Jean, R. A. Woods, and R. H. Schiestl.** 1992. Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. **20:**1425.
19. **Gros, P., J. Croop, and D. Housman.** 1986. Mammalian multidrug resistance gene: complete cDNA sequence indicates strong homology to bacterial transport proteins. Cell **47:**371–380.
20. **Guan, K.-L., and J. E. Dixon.** 1991. Eukaryotic proteins expressed in *Escherichia coli*: an improved thrombin cleavage and purification procedure of fusion proteins with glutathione S-transferase. Anal. Biochem. **192:**262–267.

20a. **Hallstrom, T.** Unpublished data.

21. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Higgins, C. F.** 1992. ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. **8:**67–113.
23. **Hirata, D., K. Yano, K. Miyahara, and T. Miyakawa.** 1994. *Saccharomyces cerevisiae YDR1*, which encodes a member of the ATP-binding cassette (ABC) superfamily, is required for multidrug resistance. Curr. Genet. **26:**285–294.
24. **Hoffman, C. S., and R. Winston.** 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of E. coli. Gene **57:**267–272.
25. **Hyde, S. C., P. Emsley, M. J. Hartshorn, M. M. Mimmack, U. Gileadi, S. R. Pearce, M. P. Gallagher, D. R. Gill, R. E. Hubbard, and C. F. Higgins.** 1990. Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. Nature (London) **346:**362–365.
26. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:**163–168.
27. **Katzmann, D. J., P. E. Burnett, J. Golin, Y. Mahe, and W. S. Moye-Rowley.** 1994. Transcriptional control of the yeast *PDR5* gene by the *PDR3* gene product. Mol. Cell. Biol. **14:**4653–4661.
28. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
29. **Leier, I., G. Jedlitschky, U. Buchholz, S. P. C. Cole, R. G. Deeley, and D. Keppler.** 1994. The *MRP* gene encodes an ATP-dependent export pump for leukotriene C_4 and structurally related conjugates. J. Biol. Chem. **45:**27807–27810.
30. **Leonard, P. J., P. K. Rathod, and J. Golin.** 1994. Loss-of-function mutation in the yeast multiple drug resistance gene *PDR5* causes a reduction in chloramphenicol efflux. Antimicrob. Agents Chemother. **38:**2492–2494.
31. **Leppert, G., R. McDevitt, S. C. Falco, T. K. Van Dyk, M. B. Ficke, and J. Golin.** 1990. Cloning by gene amplification of two loci conferring multiple drug resistance in *Saccharomyces*. Genetics **125:**13–20.
32. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
33. **Mann, C., J.-M. Buhler, I. Treich, and A. Sentenac.** 1987. *RPC40*, a unique gene for a subunit shared between yeast RNA polymerases A and C. Cell **48:**627–637.
34. **Meyers, S., W. Schauer, E. Balzi, M. Wagner, A. Goffeau, and J. Golin.** 1992. Interaction of the yeast pleiotropic drug resistance genes *PDR1* and *PDR5*. Curr. Genet. **21:**431–436.
35. **Ooi, B. G., C. E. Novitski, and P. Nagley.** 1985. DNA sequence analysis of the *oli1* gene reveals amino acid changes in mitochondrial ATPase subunit 9 from oligomycin-resistant mutants of *Saccharomyces cerevisiae*. Eur. J. Biochem. **152:**709–714.

35a. **Ortiz, D., and I. Arias.** Personal communication.

36. **Ortiz, D. F., T. Ruscitti, K. F. McCue, and D. W. Ow.** 1995. Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. J. Biol. Chem. **270:**4721–4728.
37. **Riles, L., J. E. Dutchik, A. Baktha, B. K. McCauley, E. C. Thayer, M. P. Leckie, V. V. Braden, J. E. Depke, and M. V. Olson.** 1993. Physical maps of

the six smallest chromosomes of *S. cerevisiae* at a resolution of 2.6 kb. Genetics **134:**81–150.

38. **Riordan, J., J. M. Rommens, B.-S. Kerem, N. Alon, R. Rozmahel, Z. Grzelczack, J. Zielenski, W. Lok, N. Plavsic, J.-L. Chou, M. L. Drumm, M. C. Iannuzzi, F. S. Collins, and L.-C. Tsui.** 1989. Identification of the cystic fibrosis gene: cloning and characterization of the cDNA. Science **245:**1066–1073.
39. **Riordan, J. R., K. Deuchars, N. Kartner, N. Alon, J. Trent, and V. Ling.** 1985. Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. Nature (London) **316:**817–819.
40. **Roninson, I. B., J. E. Chin, K. Choi, P. Gros, D. E. Housman, A. Fojo, D. W. Shen, M. M. Gottesman, and I. Pastan.** 1986. Isolation of human *MDR* DNA sequences amplified in multidrug resistant KB carcinoma cells. Proc. Natl. Acad. Sci. USA **83:**4538–4542.
41. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
42. **Schmitt, M. E., T. A. Brown, and B. L. Trumpower.** 1990. A simple and rapid method for preparation of RNA from *S. cerevisiae*. Nucleic Acids Res. **18:**3091–3092.
43. **Sherman, F., G. Fink, and J. Hicks.** 1979. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. **Subik, J., S. Ulaszewski, and A. Goffeau.** 1986. Genetic mapping of nuclear mucidin resistance mutations in *S. cerevisiae*. Curr. Genet. **10:**665–670.
45. **Szczypka, M. S., J. A. Wemmie, W. S. Moye-Rowley, and D. J. Thiele.** 1994. A yeast metal resistance protein similar to human CFTR and multidrug resistance-associated protein. J. Biol. Chem. **269:**22853–22857.
46. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
47. **Tsui, L.-C.** 1992. The spectrum of cystic fibrosis mutations. Trends Genet. **8:**392–398.

47a. **Volckaert, G., M. Voet, and P. Verhasselt.** Unpublished data.

48. **Walker, J. E., M. Saraste, M. J. Runswick, and N. Gay.** 1982. Distantly related sequences in the alpha and beta subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. **8:**945–951.
49. **Welsh, M. J., and A. E. Smith.** 1993. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell **73:**1251–1254.

49a. **Wemmie, J., et al.** Submitted for publication.

50. **Wu, A., J. A. Wemmie, N. P. Edgington, M. Goebl, J. L. Guevara, and W. S. Moye-Rowley.** 1993. Yeast bZIP proteins mediate pleiotropic drug and metal resistance. J. Biol. Chem. **268:**18850–18858.

50a. **Wysock, W., and J. Golin.** Unpublished data.

51. **Wysock, W., J. Shallom, D. Katzmann, Sr., S. Cronin, T. Schmitt, W. S. Moye-Rowley, and J. Golin.** Genetic identification of drug resistance-conferring target genes of the yeast Pdr1p protein. Submitted for publication.