Functional Expression of the Multidrug Resistance-associated Protein in the Yeast Saccharomyces cerevisiae*

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The multidrug resistance-associated protein (MRP) is a member of the ATP binding cassette superfamily of transporters which includes the mammalian P-glycoproteins (P-gp) family. In order to facilitate the biochemical and genetic analyses of MRP, we have expressed human MRP in the yeast Saccharomyces cerevisiae and have compared its functional properties to those of the mouse Mdr3 P-gp isoform. Expression of both MRP and Mdr3 in the anthracycline hypersensitive mutant VASY2563 restored cellular resistance to Adriamycin in this mutant. MRP and Mdr3 expression produced pleiotropic effects on drug resistance in this mutant, as corresponding VASY2563 transformants also acquired resistance to the anti-fungal agent FK506 equivalent to the K⁺/H⁺ ionophore valinomycin. The appearance of increased cellular resistance to the toxic effects of Adriamycin (ADM) in MRP and Mdr3 transformants coincided with a reduced intracellular concentration of [14C]ADM in spheroplasts prepared from these transformants. Moreover, MRP and Mdr3, but not the control plasmids, could mediate a time-dependent decrease in cell-associated [14C]ADM indicating the presence of an active ADM transport mechanism in MRP and Mdr3 transformants. Finally, human MRP expression in the yeast S. cerevisiae was found to complement the biological activity of the yeast periplasmic permease Ste6 and, therefore, restored mating in a sterlne colonies. These findings suggest that despite some structural homology, MRP and P-gp share both functional aspects, since both can mediate transport of chemotherapeutic drug molecules as GSH conjugates in yeast.

MRP, multidrug resistance-associated protein; P-gp, P-glycoproteins; VAL, valinomycin; LA, lactate.
chloride channel in which mutations cause cystic fibrosis in humans (18), the TAP1 and TAP2 peptide pumps participating in antigen presentation in lymphocytes (22), and the family of mammalian P-gps (1, 2). A number of eukaryotic proteins of this group, including P-gps (2) and CFTR (18), appear to be formed by two relatively similar halves each encoding six putative transmembrane domains and one nucleotide binding site (17).

We have previously used the yeast S. cerevisiae as to characterize the mechanism of action of the three murine P-gp isoforms (23–27). The Mdr1 and Mdr3 P-gp isoforms expressed in yeast bind drug analogs and confer cellular resistance to known MDR drugs (e.g., the anti-fungal macrolide peptide FK506) by reducing their intracellular accumulation (23, 24). The structural similarity between P-gps and the endogenous yeast Ste6 peptide pheromone transporter translates into functional homology, since the mouse Mdr3 P-gp isoform can complement the biological activity of Ste6 and partially restore mating in a sterile ste6 null mutant (23). Studies of P-gp expressed in the membrane of secretory vesicles from the yeast mutant sec4-4 have shown that P-gp uses direct ATP hydrolysis to transport drug molecules across the membrane, this being independent of an intact membrane electrochemical gradient (Δν, ΔpH) (26). Finally, using the same expression system, we have been able to show that the normal physiological role of the liver-specific P-gp isoform Mdr2 is an ATP-dependent phosphatidylcholine flippase that translocates lipid molecules from the inner to the outer leaflet of the membrane lipid bilayer (27).

The exact mechanism by which MRP confers multidrug resistance and the normal physiological function of this protein in a system that would permit genetic analyses, we have expressed in a number of mutant strains of yeast. We have observed that phenotypic characteristics similar to those observed in the mouse P-gp isoform, with respect to cytotoxic drugs, accumulation, and complementation of a null mutation in the Ste6 gene.

**EXPERIMENTAL PROCEDURES**

**Materials—**Zymolyase-100T was purchased from ICN Biomedicals; Amano Protease inhibitors were obtained from Amersham Corp. Protease inhibitors, dihydroorbidin, bacitracin, and concanavalin A were purchased from Sigma Chemical Company. 

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**Yeast Strains, Plasmids, Transformation, and Culture Conditions—**The S. cerevisiae strain J PY201 (MATa, ste6-4, and VASY2563 (MATa, his4-15, lys9, ura3-52, erg5, leu2-2his G, rad2::LEU2) were used throughout this study. For high level protein expression the plasmid vector pVT101-U (pVT) was used to transform both strains (28). A full-length cDNA for the human MRP gene (9) was introduced in the pVT1 site of pVT101-U as a SacI/Xhol fragment repaired with T4 DNA polymerase, using standard cloning techniques, to create plasmid pVT-MRP. The pVT-mdr35 and pVT-mdr3F constructs containing wild type and mutant mdr3 cDNA inserts, respectively, have been described elsewhere (23). The MRP-GFP fusion protein was constructed by introducing the green fluorescent protein (GFP) (29) cDNA (Columbia Immuno-Engineering Enterprise, New York, NY) as a full-length fragment (repaired with T4 DNA polymerase) into the unique Nde I site of the MRP cDNA at position 4780, which is positioned 9 nucleotides upstream of the stop codon. DNA transformation of J PY201 cells was performed by the lithium acetate method of Ito et al. (30). The transformation of the VASY2563 strain required the following modifications (31) of the standard protocol (30). Cultures were grown to mid-logarithmic phase in YPD (1% yeast extract, 2% Bactopeptide, 2% glucose), diluted to A600 = 0.5, and the cells were further incubated for 3 h at 30 °C with constant shaking. Aliquots (250 ml) were harvested, washed once in lithium acetate buffer (LIAT buffer: 100 mM lithium acetate, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and resuspended in a final volume of 1 ml of LIAT buffer. In a 1.5-ml Microfuge tube, 10–20 μg of plasmid DNA of the various constructs in 10 μl of LIAT buffer was mixed with 40 μg (in 10 μl) of freshly prepared total yeast RNA (32) and 150 μg of the yeast cell suspension. After incubation for 2 h at 30 °C, the mixture was suspended in 0.7 ml of 36% polyethylene glycol, 100 μg of 10 mM Tris-HCl, pH 8.0, and incubated for an additional 2 h at 30 °C. The samples were then heat-shocked for 15 min at 42 °C, the cells pelleted, washed once in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and plated on synthetic medium lacking uracil (SD-Ura). Five to ten transformants were picked, pooled, grown as a mass culture in SD-Ura medium, followed by freezing at −80 °C in SD-Ura medium supplemented with 30% glycerol. All subsequent cultures were always started from these stock samples and grown either in SD-Ura or YGC medium (0.75% yeast nitrogen base without amino acids, 0.35% bacto-casamino acids, 2% glucose) as indicated.

**Quantitative Mass Mating Assay—**Quantitative mass mating was performed as described previously (23), but with the following minor modifications. Briefly, J PY201 transformants were grown at 30 °C in YPG medium to mid-logarithmic cultures (A600 = 1.5–2) and diluted with the same medium to A600 = 0.6. After an additional growth period of 3 h at 30 °C, 0.75-ml aliquots were mixed with 0.25 ml of a mid-logarithmic phase culture (A600 = 1.5) of the DC17 MATa tester strain. One-third of each suspension was then concentrated onto a glass fiber filter (Whatman, GF/C filters), transferred to a YPD plate, and incubated at 30 °C for 2 h to allow mating. The cells were then removed from the filters by vigorous agitation in MM medium containing 0.68% yeast nitrogen base without amino acids and 2% glucose (2 ml, in a 6-ml push-cap tube). Equal aliquots were plated in parallel on SD-Ura plates (number of total haploid J PY201 cells) and on MM plates (number of J PY201 diploid cells), and the ratio of the two numbers defined the mating frequency.

**Analytical Methods—**Immunofluorescence analysis of yeast transformants expressing either the MRP or the Mdr3 proteins was performed according to a standard protocol (32). The anti-MRP antiserum MRP-2 (15), and the anti-P-gp antiserum E54 (33), were used for the detection of the two proteins.

**RESULTS**

The aim of this study was to use yeast as an experimental system to express and functionally characterize the MRP.
clonal or polyclonal antibodies (15) failed to detect the presence of MRP by immunoblotting and immunoprecipitation (23, 24), similar activity of yeast cells transformed with pVT plasmids carrying full-length cDNAs for wild type mdr3, wild type MRP, or the MRP-GFP chimera to the anti-fungal macrolactone FK506 (at 50 μg/ml), a known P-gp substrate (24). In these experiments, we included as an additional control a mutant mdr3 cDNA carrying a single Ser to Thr substitution at position 939 (Mdr3F) which shows decreased physiological activity in mammalian and yeast cells. We then determined if MRP expression in yeast JPY201 cells could alter sensitivity to cytotoxic drugs. We tested the sensitivity of yeast cells transformed with pVT plasmids carrying full-length cDNAs for wild type mdr3, wild type MRP, or the MRP-GFP chimera to the anti-fungal macrolactone FK506 (at 50 μg/ml) by growth inhibition assay in liquid culture the capacity of MRP to confer resistance to anthracyclines such as ADM. In addition, this strain carries a null allelic at the ERG6 locus with pleiotropic effects of P-gp resistance to ADM (and other MDR drugs), precluding the analysis of MRP or Mdr3 activity against ADM in these strains (data not shown). To circumvent this limitation, we took advantage of the unique phenotype of the mutant S. cerevisiae strain VASY2563 (MATa, his4–15, lys9, ura3–52, erg6Δ, leu2::his G, rad52::LEU2). This strain carries a null allele at the ERG6 locus with pleiotropic effects of membrane permeability and fluidity (31), including hyper-sensitivity to anthracyclines such as ADM. In addition, this strain carries a mutation at the rad52 locus, which is phenotypically expressed as a defect in DNA repair. We tested in a growth inhibition assay in liquid culture the capacity of MRP and wild type Mdr3 (Mdr3S) to increase cellular resistance to ADM in this strain. Control, MRP-, Mdr3S-, and Mdr3F-expressing cells were incubated for 48 h with ADM (1, 2, and 3 μg/ml). After 48 h of incubation, cell densities at 24 h similar to those observed in the presence of drug. Likewise, yeast expression plasmids pVT containing full-length cDNA for mouse wild type MRP and mouse wild type Mdr3 were transformed into S. cerevisiae (34). Control experiments in Fig. 1B (left panel) show only a small growth retardation (lag phase), but reaching cellular densities at 24 h similar to those of the MRPositive cells. Likewise, Mdr3S, and Mdr3F substrates strongly affected, reaching cellular densities at 24 h similar to those of the MRPositive cells. Likewise, Mdr3S, and Mdr3F strains were exposed to the anti-Pgp polyclonal antiserum ES4 (32) at a 1:100 dilution, whereas Mdr3S cells (panel 2) were incubated for 24 h in a 1:2000 dilution in all experiments except for control, MRP-, and Mdr3S-expressing cells. MRP protein expression was monitored by immunofluorescence on permeabilized spheroplasts using a polyclonal antiserum raised against a MRP peptide (15) at a 1:100 dilution, whereas Mdr3S cells (panel 2) were incubated for 24 h in a 1:250 dilution. Rhodamine-conjugated goat anti-rabbit IgG was used at a 1:2000 dilution in all experiments except for control, MRP-, and Mdr3S-expressing cells. MRP protein expression was monitored by immunofluorescence using either a specific antiserum or by expression of a fusion construct containing the C-terminal 84 amino acids of MRP fused to the C-terminus of GFP. A major goal of our study was first, to determine if MRP can be expressed in yeast JPY201 cells. To this end, we included as an additional control a mutant mdr3 cDNA carrying a single Ser to Thr substitution at position 939 (Mdr3F) which shows decreased physiological activity in mammalian and yeast cells. We then determined if MRP expression in yeast JPY201 cells could alter sensitivity to cytotoxic drugs. We tested the sensitivity of yeast cells transformed with pVT plasmids carrying full-length cDNAs for wild type mdr3, wild type MRP, or the MRP-GFP chimera to the anti-fungal macrolactone FK506 (at 50 μg/ml), a known P-gp substrate (24). In these experiments, we included as an additional control a mutant mdr3 cDNA carrying a single Ser to Thr substitution at position 939 (Mdr3F) which shows decreased physiological activity in mammalian and yeast cells. Yeast expression plasmids pVT containing full-length cDNA for human MRP and mouse wild type Mdr3 were transformed into S. cerevisiae strain JPY201(MATa, ste6Δ::his3). MRP protein expression was monitored by immunofluorescence on permeabilized spheroplasts using a polyclonal antiserum raised against a MRP peptide (15). As shown in Fig. 1A (panel 1), a strong fluorescent signal was detected in JPY201 cells transformed with the pVT-MRP plasmid, which was absent in control cells transformed with pVT (panel 2). Likewise, a strong fluorescent signal was produced by the polyclonal anti-P-gp antiserum ES4 in JPY201 cells transformed with the pVT-mdr3 plasmid (panel 3). While in Mdr3 cells, the fluorescent signal had a ring-like pattern, seemingly associated with cell membrane expression of the protein, the anti-MRP antibody generated a strong additional punctuate fluorescence in the cytoplasm, suggesting the presence of MRP protein associated with intracellular organelles. Although expression of wild type and mutant Mdr3 proteins in yeast plasma membranes can be readily demonstrated by immunoblotting and immunoprecipitation (23, 24), similar attempts using a number of well characterized anti-MRP monoclonal or polyclonal antibodies (15) failed to detect the presence of full-length MRP polypeptides (data not shown). These results suggest a high turnover and short half-life of mammalian MRP in yeast cells, resulting in low steady state levels of intact protein. To determine if full-length MRP was indeed synthesized in our yeast strain, we introduced a DNA fragment coding for the naturally fluorescent GFP (29) in-frame at the carboxyl terminus of MRP and determined if a full-length fluorescent MRP-GFP protein could be synthesized in yeast. As shown in panel 4, expression of this MRP-GFP construct in JPY201 cells resulted in the production of a naturally fluorescent polypeptide (ring-like signal), which was also recognized by the anti-MRP antiserum (panel 5). These results suggest that a full-length recombinant MRP-GFP protein was indeed synthesized in these yeast cells.

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Functional expression of MRP in S. cerevisiae
ADM (at all concentrations tested), FK506 and VAL inhibited growth of control pVT and Mdr3F transformants. By contrast, Mdr3S- and MRP-expressing cells were resistant to all concentrations of ADM; growth inhibition observed in control wells free of drugs. The means and standard deviations of triplicate measurements of three independent experiments are shown.

Growth inhibition of VASY2563 transformants was rapid and followed identical kinetics in the three groups (Fig. 4). ADM inhibition in control, MRP, and Mdr3S spheroplasts was rapid and reflected cellular resistance of the MRP and Mdr3S VASY2563 transformants. Culture aliquots were removed after 45 min of incubation. By contrast, the rate of ADM accumulation continued at the same rate in control pVT spheroplasts to reach a maximum of approximately 200 pmol/10^7 cells after 45 min of incubation. By contrast, the rate of ADM accumulation in both MRP and Mdr3S spheroplasts started to decrease significantly after the initial 15-min incubation period, resulting in a lower level of steady state ADM accumulation in these cells compared with pVT controls at 45 min (110–125 pmol/10^7 cells; Fig. 4). These results suggest that Mdr3S and MRP are functionally expressed in yeast and may mediate cellular resistance and reduced ADM accumulation through an active drug transport process.

We next used [14C]ADM to test the possibility that cellular resistance to this drug in MRP and Mdr3S VASY2563 transformants may be due to a reduced intracellular accumulation. The low specific activity of radiolabeled ADM, together with the high nonspecific binding of this drug to intact yeast cells and spheroplasts required to determine first optimal experimental conditions for ADM accumulation in control yeast cells. Spheroplasts from J PY201 and VASY2563 cells grown in either YPD or YCG medium were found to accumulate [14C]ADM poorly, with total cellular radioactivity reaching only a 2–3-fold increase after a 4-h incubation period (data not shown). J PY201 spheroplasts derived from ADM-resistant strains of J PY201 sphero- plasts required to determine first optimal experimental conditions for ADM accumulation in control yeast cells. Spheroplasts were diluted with total cell-associated radioactivity reaching only a 2–3-fold increase after a 4-h incubation period (data not shown). J PY201 spheroplasts derived from ADM-resistant strains of J PY201 sphero- plasts required to determine first optimal experimental conditions for ADM accumulation in control yeast cells. Spheroplasts were diluted

![Graph](https://example.com/graph.png)

**Fig. 2.** Growth inhibition of control and MRP- and Mdr-expressing VASY2563 cells by structurally unrelated cytotoxic drugs. Growth inhibition of VASY2563 cells transformed with either control plasmid pVT (open bars), pVT-MRP (filled bars), pVT-mdr3S (hatched bars), or pVT-mdr3F (stippled bars) by FK506, VAL, or ADM was determined in liquid cultures as described under “Experimental Procedures.” Cell growth after 48-h incubation in drug-containing medium was quantitated by optical density and is expressed as a percent

![Diagram](https://example.com/diagram.png)

**Fig. 3.** Drug resistance characteristics of control and MRP- and Mdr-expressing VASY2563 cells. VASY2563 transformants were grown and diluted as described for the growth inhibition assay under “Experimental Procedures.” After a 4-h incubation with FK506, VAL, and ADM, 20-μl aliquots were spotted on YPD plates (free of drugs) and grown for 72 h at 30 °C. In addition, 100-μl aliquots (in triplicates) were plated on 100-μl YPD-plates to determine relative growth efficiencies.
The spheroplast suspensions were diluted 20-fold in cold LA medium and placed at 30 °C. At 5-min intervals, aliquots were removed and cell-associated [14C]ADM was determined by separating the spheroplasts from the drug containing medium after centrifugation through an oil cushion as described under "Experimental Procedures." Data represent means of six determinations of two independent experiments.

FIG. 4. ADM accumulation in spheroplasts prepared from control and MRP- and Mdr3S-expressing JPY201 cells. Control (I) and MRP (II) and Mdr3S-expressing (III) cells were grown in LA medium to midlogarithmic cultures and converted to spheroplasts. Subsequently, ADM accumulation into spheroplasts from control and MRP- and Mdr3S-expressing (II) cells was initiated and monitored as described in the legend to Fig. 4. BSA (1 mg/ml) was added to the reaction mixture (arrow) at 25 min. Data represent means of six determinations of two independent experiments.

FIG. 5. ADM release from spheroplasts prepared from control and MRP- and Mdr3S-expressing JPY201 cells. A, emission spectra of ADM (1 μM) in LA medium in the absence (O) and presence (●) of BSA (1 mg/ml). B, ADM accumulation into spheroplasts from control (I) and MRP (II) and Mdr3S-expressing (III) cells was initiated and monitored as described in the legend to Fig. 4. BSA (1 mg/ml) was added to the reaction mixture (arrow) at 25 min. Data represent means of six determinations of two independent experiments.

FIG. 6. Complementation of the ste6 mutation by MRP and Mdr3S. JPY201 cells transformed with either control pVT plasmid or with the same plasmid carrying cDNAs for mdr3S, Ste6, or MRP, were allowed to mate in selective medium monitored as described under "Experimental Procedures." A depicts the growth of diploid cells on selective medium after 48-h incubation at 30 °C. B, data from Figs. 4 and 5B show the relative rate of ADM accumulation in steady state levels obtained from control pVT transformants (Fig. 5B). These results suggest that MRP shares some of the functional characteristics of Mdr3S in yeast. The presence of extracellular MRP causes a small decrease in the rate of drug accumulation into the total amount of free cell-associated ADM available for uptake, and 2) possibly forming complexes with ADM molecules extruded from preloaded cells.

The addition of BSA to MRP and Mdr3S yeast cells resulted in a small decrease of the rate of drug accumulation, with a 5–10% reduction in the steady state level of ADM (compare Figs. 4 and 5B). The addition of BSA to MRP and Mdr3S spheroplasts caused a small reduction in the rate of ADM accumulation in these cells, which was followed 10 min later by an actual decrease in the total amount of spheroplasts-associated radioactivity. This decrease resulted in a reduction of total ADM accumulation of 20–25% from peak levels attained in the presence of BSA (Fig. 5B, compare 45- and 90-min time points) and 30–35% from peak levels reached in these cells in the presence of Ste6 (Fig. 5B). The addition of BSA to MRP and Mdr3S spheroplasts after the initial 25-min exposure to ADM caused a small decrease of the rate of drug accumulation, with a 5–10% reduction in the steady state level of ADM (compare Figs. 4 and 5B). The addition of BSA to MRP and Mdr3S spheroplasts caused a small reduction in the rate of ADM accumulation in these cells, which was followed 10 min later by an actual decrease in the total amount of spheroplasts-associated radioactivity. This decrease resulted in a reduction of total ADM accumulation of 20–25% from peak levels attained in the presence of BSA (Fig. 5B, compare 45- and 90-min time points) and 30–35% from peak levels reached in these cells in the presence of Ste6 (Fig. 5B).
DISCUSSION

MRP was originally identified in the P-gp-negative drug-resistant small-cell lung carcinoma cell line H69AR, in which the gene is both amplified and overexpressed (3, 4). The specific drug resistance profile of this cell line together with that characteristic of MRP transfectants (10) indicate that MRP overexpression also confers cellular resistance to a large group of structurally distinct drugs such as vincristine, VP-16, daunorubicin, and calcitriol, all of which are known MDR drugs and P-gp substrates. Also analogous to P-gp isoforms, MRP seems to be expressed at the cell surface where it functions as an active, ATP-dependent drug transporter (10, 11, 15), although it may also be active in subcellular membranous compartments. Distinguishing features of MRP, however, are that it can confer resistance to heavy metal oxanions (10) and can efficiently transport anionic amphiphiles such as cyssteyn leukotrienes and glutathione S-conjugates (12, 14). This suggests that MRP may have a broader substrate specificity than P-gp, being able to recognize and transport anionic (leukotrienes) and possibly conjugates or metabolites of cationic amphiphiles (MDR drugs). Although attractive, this model is difficult to reconcile with the observations that 1) MRP overexpression does not confer cellular resistance to cisplatinum (9), a drug detoxified after modification via glutathione conjugation, and 2) ADM, a drug to which MRP confers resistance to (9), is metabolized to a C-13-OH, and is not known to be biotransformed to conjugates (35). In the present study, we have used yeast as a heterologous expression system to initiate the characterization of the mechanism of MRP action.

Immunofluorescence was used to monitor MRP expression in yeast. Anti-MRP antibodies transformants a fluorescent signal within the pattern typical of cell membrane. In a strong cytoplasmic punctate pattern that a portion of the overexpressed MRP with intracellular membrane-enriched fraction. In blotting, we were unable to weight compatible with the predicted sequence analysis (in the yeast MRP at 95–100-kDa polypeptides with anti-MRP antibodies in cell fraction analysis, used that the majority of the MRP was either truncated or rapidly degraded during the isolation procedure. Degradation or aberrant processing of heterologous membrane proteins by the yeast machinery is linked to the absence of important signals missing from the primary amino acid sequence of the protein (for sorting, targeting) and/or to very active proteolysis in these cells. Finally, the standard plasma membrane isolation protocol used here includes as a first step, the conversion of intact cells to spheroplasts by enzymatic digestion of the cell wall with Zymolyase. This enzymatic step is critical and can be significant cause of degradation, as not only exogenous but also endogenous yeast membrane proteins (such as the PMA1 H^+ATPase) may be very sensitive to proteases frequently contaminating the commercial preparations of this enzyme. To unequivocally demonstrate that MRP was indeed produced in yeast as a full-length protein, we constructed a chimeric MRP cDNA encoding at its 3' end the GFP protein (29). The staining pattern of the expressed MRP-GFP fusion protein detected by fluorescence analysis suggested that the MRP-GFP chimera was associated with the plasma membrane. In addition, we observed that both MRP- and MRP-GFP-expressing yeast cells showed cellular resistance toward the P-gp substrate and anti-fungal drug FK506, but could also restore mating in an otherwise sterile ste6 mutant. These results strongly suggest that at least a fraction of the MRP protein in yeast is intact, expressed properly folded at the plasma membrane, and functional.

Since ADM is the drug that had been used in the initial selection of the H69AR cell line, we focused on this cytotoxic drug as a tool to further characterize MRP in yeast. The use of ADM as a cytotoxic drug and a pharmacological transport substrate turned out to be problematic in yeast, for various reasons. First, most common laboratory strains of S. cerevisiae are resistant to extremely high concentrations of this drug. Second, ADM is photolabile and quite unstable at room temperature, as ADM solutions of less than 5 μM are known to lose 95% of their activity in less than 1 h at 20°C (36). Third, ADM binds nonspecifically to plastic and other surfaces and can also form aggregates with itself or other molecules at concentrations higher than 1 μM (36). Despite these limitations, we found that MRP expression in the mutant S. cerevisiae strain VASY2563, which is hypersensitive to anthracyclines and other lipophilic compounds, dramatically increased resistance to ADM. We found that MRP expression in these cells also conferred cellular resistance to two P-gp substrates FK506 and to ADM (7, 10) and other drugs (24). Overall, MRP behaved in a manner very similar to P-gp and conferred cellular resistance to structurally unrelated drugs.

We found that increased resistance to ADM in Mdr3 transformants is associated with overexpression of the MDR3/GMRP, and increased drug accumulation (Fig. 5B). MRP transfectants behaved in a manner very similar to P-gp and conferred cellular resistance to two P-gp substrates (24). Overall, MRP behaved in a manner very similar to P-gp and conferred cellular resistance to structurally unrelated drugs.

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fact that one of these three cytotoxic drugs is known to act at the level of the cell membrane without binding to an intracellular target clearly suggests that MRP and P-gp can recognize and act on intact drug molecules that are not otherwise modified. The data presented here, together with the previously demonstrated ability of MRP to transport leukotrienes and glutathione S-conjugates (12–14), are in agreement with the proposal that MRP may be capable of acting on both anionic and cationic amphiphiles. Therefore, although both proteins may act according to a common mechanistic basis, their substrate specificity, although overlapping, is clearly distinct. We have demonstrated that MRP can complement a null mutation at the Ste6 locus and restore a mating factor transport and mating in an otherwise sterile yeast mutant (Fig. 6). These experiments suggest that structural and/or functional determinants required for the transport of this pheromone by MRP, Mdr3, and Ste6 proteins have been evolutionarily conserved in these three very distant members of the ABC superfamily. The nature of these common determinants is unknown and can only be speculated upon. The a mating pheromone is a dodecapeptide, which is modified on its terminal cysteine by methylation and by the addition of a farnesyl lipid moiety, and both modifications of the peptide backbone are absolutely required for export. Since we have previously established that one of the members of the mouse P-gp family (the liver-specific Mdr2 isoform) is a lipid flippase capable of actively translocating phosphatidylcholine molecules from the inner to the outer leaflet of the cell membrane (27), it is tempting to speculate that an aspect of lipid transport by Mdr2 may be complemented and be important for the translocation of this pheromone by MRP, Mdr3, and Ste6. Such a proposition is highly speculative, such a proposition having been experimentally tested in yeast.

The yeast proved to be an extremely useful system for the study of membrane transporters such as these. In yeast, the protein in yeast will permit the genetic analyses of the mechanism of transport of secretory vesicles from secretory vesicles of transport can be exploited as specific inhibitors or with the useful yeast genetic background.

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Functional Expression of the Multidrug Resistance-associated Protein in the Yeast _Saccharomyces cerevisiae_

Stephan Ruetz, Martine Brault, Christina Kast, Charles Hemenway, Joseph Heitman, Caroline E. Grant, Susan P. C. Cole, Roger G. Deeley and Philippe Gros

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Additions and Corrections

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Functional expression of the multidrug resistance-associated protein in the yeast *Saccharomyces cerevisiae*.

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We have been unable to reproduce complementation of the yeast *Saccharomyces cerevisiae ste6* mutant by the human *MRP* gene in 200 new yeast transformants tested. Additional studies suggest that the original *MRP*-associated mating activity was caused by contaminating *STE6* sequences that were detected by Southern blotting in frozen vials of *MRP* transformants from that period. We were unable to reproduce high levels of Adriamycin and valinomycin resistance in MRP transformants of yeast strain VASY. Additional experiments suggest that MRP-mediated high level resistance to these two drugs was associated with contamination of yeast cultures with a multidrug-resistant microbial contaminant. Finally, we have been able to reproducibly observe MRP-mediated drug resistance to FK506, but only in the VASY strain (*rad52, erg6*) and not in the JPY201 strain. Thus, we are retracting the above manuscript (with the exception of MRP-mediated FK506 resistance). Please do not hesitate to contact P. Gros (gros@med.mcgill.ca) should you need additional information or wish to discuss further any aspect of this work.

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Identification of the *cis*-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors.

Hiderou Yoshida, Kyosuke Haze, Hideki Yanagi, Takashi Yura, and Kazutoshi Mori

Page 33744, Fig. 2, A and B: The locations of the first C residue of CCAAT of human GRP94 ERSE3 and murine ERp72 ERSE1 and ERSE2 should be −195, −179, and −194, respectively. We thank Dr. Amy S. Lee for pointing out these errors.

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