Pdr5p is the yeast *Saccharomyces cerevisiae* ATP-binding cassette transporter conferring resistance to several unrelated drugs. Its high overproduction in Pdr1p transcription factor mutants allows us to study the molecular mechanism of multidrug transport and substrate specificity. We have developed new *in vitro* and *in vivo* assays of Pdr5p-mediated drug transport. We show that in spite of little sequence homology, and inverted topology in respect to that of mammalian P-glycoproteins, Pdr5p shares with them common substrates. Pdr5p extrudes rhodamines 6G and 123, from intact yeast cells in an energy-dependent manner. Plasma membrane preparations from a Pdr5p-overproducing yeast cells exhibit ATP hydrolysis-dependent, osmotically sensitive rhodamine 6G fluorescence quenching. The quenching is competitively inhibited by micromolar concentrations of many anticancer drugs, such as vinblastine, vincristine, taxol, and verapamil, and of ionophoric peptides as well as steroids. In contrast, other anticancer drugs, like colchicine and some multidrug resistance modifiers, such as quinidine, exert noncompetitive inhibition. Our experimental system opens new possibilities for the analysis of structure-function relationship of multidrug transporter substrates and inhibitors.

The growth of yeast cell shows little sensitivity to most of the anticancer agents (1), and it is not known whether any of the yeast ABC1 proteins exhibits the broad, yet limited multidrug resistance to anticancer agents (1), and it is not known whether any of the yeast ABC1 proteins exhibits the broad, yet limited multidrug resistance to anticancer agents. Overproduction of Pdr5p confers resistance to cycloheximide and several unrelated compounds, but resistance to anticancer drugs or ionophores was not tested so far (10, 11, 16, 17).

In this study, we show that Pdr5p shares with mammalian P-glycoproteins a series of common substrates and inhibitors, which were kinetically characterized in a *new in vitro* Pdr5p drug transport assay.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Rhodamine 6G and sodium orthovanadate were from British Drug Chemicals, oligomycin from Janssen Chimica, 2 deoxy-<i>D</i>-glucose, progesterone, vinblastine, vincristine, taxol, tamoxifen, verapamil, puromycin, nifedipine, colchicine, yohimbine, quinidine, β-estradiol, gramicidin D, nigericin, monensin, ATP disodium salt (A-3377), GTP, UTP, and sodium deoxycholate were from Sigma, ATP•<i>S</i> tetraphosphate, CTP, ATP, chloramphenicol, Triton X-100, and n-octyl glucoside were from Boehringer Mannheim, valinomycin, FCCP, and CCCP were from Fluka, S13 was a kind gift from Dr. P. Haman (Monsanto Co., St. Louis, MO), SF6847 was from Sumitomo Chemical Co. Ltd. (Osaka, Japan). Deoxycorticosterone was kindly provided by Dr. T. Wright. All other reagents were of analytical grade.

**YEAST STRAINS**—The following *S. cerevisiae* strains were used in this study: US50-18C (MAT<i>A</i>, PDR 1-3, ura 3, his-1) (18), US50-D5 (US50-18C disrupted by pdr5-Δ2:URA3) (19), RW2892 (MAT<i>A</i>, leu2, met5, ura3-52, mak17, KRE1), JG436 (RW2892 bearing PDR5::Tn5 insertion) (17), JG365-5C (MAT<i>A</i>, pdr1-3, PDR5::Tn5, met5, ura3) (17) FY1679-28C (MAT<i>A</i>, ura3-52, trp1Δ63, leu2Δ1, his3Δ200, GAL2+) (C. Fairhead), FY1679-1D (MAT<i>A</i>, ura3-52, trp1Δ63, his3Δ200, GAL2+) (C. Fairhead), FYMKU-1/U1 (FY1679-28C disrupted by pdr5-Δ1::hisG) (this study), FYMRK-2/1 (diploid from cross between FYMKU-1/U1 and FY1679-1D (this study), FYMKU-1/U1 (FY1679-28C disrupted by pdr5-Δ1::hisG) (this study), FY1679-28C and FY1679-1D are segregants from a cross between FY23 and FY73 (20). The *PDR5* gene disruption was performed as described previously (7) except that the disrupted allele was verified by polymerase chain reaction amplification using the *PDR5* wild type (*G1ATACGCGGACGCAACGAGGAA*, *GGCAATCCATTGGGGGGAAGGTAGC*) and deleted allele (*AAGCAGGCGGCAGAAGAAGTAACA*) specific primers used together in one reaction. The monogenic character of the disruption was verified by tetrad analysis. The *URA3* gene was removed by 5-fluoro-otic acid treatment (21) of appropriate transformants.

This paper is available online at http://www-jbc.stanford.edu/jbc/
**Plasmids**—The KpnI fragment was removed from the previously described Yep24-based pDR5-3 plasmid (16), yielding pMKR3-3. The KpnI-BamHI fragment containing the entire PDR5 gene and its promoter was obtained by partial digestion of pMKR3-3 and cloned into the KpnI-BamHI sites in the polynuclein of pRS315 vector (22) partially digested with KpnI and BamHI, yielding pMK315-5. Yeast transformation was carried by the lithium acetate procedure (23).

**In Vivo Drug Sensitivity Assays**—Cells grown on YPG medium, pH 6.8, were poured in top agar (seeded with 10⁵ cells/ml) onto YPG plates digested with BamHI and KpnI moter was obtained by partial digestion of pMKR3-3 and cloned into the BamHI site of pRS424 (23). The resulting supernatants were centrifuged for 40 min at 15000 × g. Pellets were resuspended in 10 mM Tris-acetic acid, pH 7.5, 1 mM EDTA, and mitochondrial membranes were removed by acetic acid precipitation at pH 5.2 and pelleting by 2.5-min centrifugation at 15000 × g. The resulting supernatants were centrifuged for 40 min at 20,200 × g. Pellets were resuspended in 10 mM Tris-acetic acid, pH 7.5, 1 mM EDTA, and mitochondrial membranes were removed by acetic acid precipitation at pH 5.2 and pelleting by 2.5-min centrifugation at 7000 × g, as described before (25). The resulting supernatants were centrifuged for 36 min at 27,000 × g. Each pellet, highly enriched in plasma membranes, was resuspended in 50 mM Hepes-NaOH, pH 7.0, 15000 × g, and stored at −70 °C. The quality of the membranes was determined by 7% SDS-polyacrylamide gel electrophoresis, according to Laemmli (26), and by ATPase activity measurements, as described by Dufour et al. (25).

**Fluorescence Measurements in Plasma Membrane Fractions**—Membranes (30 μg of protein) were resuspended in 2 ml of 50 mM Hepes-NaOH, pH 7.0, 5 mM MgCl₂, 20 to 600 mM rhodamine 6G or 123, at 35 °C. The reaction was started by addition of 5 mM ATP-NaOH, pH 7.0. Other parameters for fluorescence measurements were as described above for whole cells. Rhodamine 6G was used for inhibition kinetics measurements. Inhibitors were added as ethanol solutions. The ethanol concentration never exceeded 1%, and this solvent did not cause any measurable disturbances. The kinetic analysis of rhodamine 6G fluorescence quenching inhibitors was performed using the GraFit program (27), assuming the model developed for P-glycoproteins by Michelson and Slate (28). Trypsin digestion of plasma membrane fractions was carried out for 30 min in 50 mM Hepes-NaOH, pH 7.5, with 250 μg/ml trypsin (1 μg of trypsin/mg protein).

**Determination of Intracellular ATP Levels**—The CLS ATP bioluminescence assay (Boehringer Mannheim) was used to measure the ATP content in cell extracts, prepared according to the LKB bioluminescence assay instructions. Briefly, cell suspensions (prepared as described above) were diluted 50 times in boiling 0.1 M Tris-EDTA buffer (pH 8.0), and heated for 1 min. The resulting cell extracts were used for determination of ATP content in the integrating photometer 3000 of the SAI Technology Co. Ability of Pdr5p to mediate resistance to growth inhibition by the fluorescent dyes, anticancer drugs, ionophores, and steroids. Plates containing YPG, pH 6.8, were seeded with cells of US50-18C or US50-D5 strains (except for deoxycorticosterone where FY1679-28C and FYMK-1/1 were used, and monensin where JG365-5C transformed either with Yep24 or pMKR3-3 were compared on minimal YNB medium), were made and analyzed as described under “Experimental Procedures.”

**RESULTS**

**In Vivo Drug Sensitivity Assays**—We have compared drug sensitivity of the pdr 1-3, mutant US50-18C, overproducing Pdr5p and the otherwise isogenic PDR5-deleted US50-D5 strains by agar diffusion assay. The former strain proved more resistant to growth inhibition by the fluorescent dyes rhodamine 6G, rhodamine 123, daunorubicin, and doxorubicin (Fig. 1). These dyes are well-known substrates of mammalian P-glycoproteins, the latter two used in chemotherapy. We further found that Pdr5p confers resistance to growth inhibition by the anticancer drug tamoxifen and also by the MDR modifier trifluoperazine (Fig. 1). Other anticancer drugs, such as vinblastine, vincristine, taxol, verapamil, colchicine, MDR modifiers, such as puromycin, nifedipine, and also gramicidin D were not toxic to our yeast strains under the conditions used. The effect of oligomycin and of the protonophores FCCP and CCCP was not appreciably modified by Pdr5p. In contrast, the Ca²⁺ ionophore A23187, and the ionophoric peptides monensin and nigericin were less toxic in the Pdr5p-overproducing strain than in the PDR5-deleted strain (Fig. 1). Deletion of PDR5 increases also sensitivity to progesterone and deoxycorticosterone in contrast to β-estradiol which was not toxic (Fig. 1). The case of valinomycin is more curious. Initially, identical growth inhibition zones were observed for the PDR5-overexpressing and -deleted strains. Upon further incubation, however, the inhibition zone disappeared in the case of the PDR5-deleted strain, but remained unchanged in the case of the overexpressing strain (Fig. 1).

That the phenotypes are related to Pdr5p was confirmed by complementation of PDR5 deletion in the FYMK-1/1 strain.
The excitation and emission spectra of rhodamine 6G (Fig. 2B) after incubation of Pdr5p-expressing cells with glucose, as compared to controls where either Pdr5p or glucose was absent (Fig. 2B). The excitation and emission spectra of rhodamine 6G with PDR5 borne on a centromeric plasmid and by cosegregation of rhodamine 6G hypersensitivity with PDR5 deletion (data not shown).

**Extrusion of Rhodamine from Whole Cells**—Rhodamines 6G and 123 are taken up by yeast cells and are commonly used to stain mitochondria (30). We found the dyes to accumulate much more abundantly in the strain with the disrupted PDR5 gene, as evidenced by fluorescence microscopy, by visual observations of red colonies on plates containing rhodamine 6G, and by the red color of sedimented cell pellets after addition of rhodamine to liquid cultures (data not shown). Prolonged incubation of the cells in the presence of 2-deoxy-D-glucose (a glycolysis inhibitor) and rhodamine 6G (a mitochondrial ATP-synthase inhibitor) resulted in marked depletion of the intracellular ATP pool from 10 to 1 nmol of ATP/mg of cell proteins and in an almost equal loading of PDR5-overexpressing and PDR5-deleted cells with the dye, as compared to equal amounts of preloaded cells produced fluorescence signals of similar intensity (Fig. 2A). The survival rate was similar for both strains after this incubation, as observed by vital staining and cell plating. The active Pdr5p-mediated extrusion of rhodamine 6G from intact cells was measured indirectly by monitoring glucose-stimulated fluorescence quenching in de-energized, dye-preloaded cells (Fig. 2A) and by directly measuring the fluorescence of extruded rhodamine in the assay buffer (Fig. 2B). Addition of the energy source (glucose) caused the intracellular ATP level to rapidly increase up to 8 nmol of ATP/mg of protein in both strains. This was accompanied by rapid dequenching of rhodamine 6G fluorescence in the case of the Pdr5p-expressing cells but not in the case of the otherwise isogenic PDR5 disruptant (Fig. 2A). A parallel increase in rhodamine 6G fluorescence was observed in the external buffer after incubation of Pdr5p-expressing cells with glucose, as compared to controls where either Pdr5p or glucose was absent (Fig. 2B). The excitation and emission spectra of rhodamine 6G observed before cell loading and after extrusion were indistinguishable (data not shown). FY1679-28C was also found to extrude rhodamine 6G at a higher rate than FYMK-1/1 (data not shown). Pdr5p-producing cells were found to extrude rhodamine 123 at a higher rate than PDR5 disruptants (data not shown). These results, together with the in vivo drug sensitivity results, identify Pdr5p as an efficient cell detoxification system, which can be inhibited by depletion of the intracellular ATP pool.

**Quenching of Rhodamine 6G Fluorescence by Pdr5p-containing Plasma Membrane Preparations**—Further characterization of Pdr5p activity was done with a plasma membrane-enriched fraction containing the transporter in high amount, as shown in Fig. 3. Rapid quenching of rhodamine 6G fluorescence by Pdr5p-containing membranes occurred in the presence of Mg2+ and ATP. This quenching was not observed when membranes from the isogenic PDR5 disruptant were used (Fig. 4), although they appeared to be of the same quality, as revealed by similar SDS-polyacrylamide gel electrophoresis profiles (Fig. 3) and similar H+ -ATPase activity (data not shown). The reaction leading to rhodamine 6G fluorescence quenching was inhibited by nanomolar concentrations of oligomycin and micromolar concentrations of vanadate, these being known inhibitors of Pdr5p ATPase activity (6). The reaction was also inhibited by a wide variety of mammalian MDR P-glycoprotein substrates, including the anticancer drugs vinblastine, vincristine, verapamil, and taxol at micromolar concentrations. When these inhibitors were added after rhodamine 6G quenching had reached a steady state, dequenching of fluorescence was observed as shown in Fig. 4 for oligomycin. Oligomycin at this concentration did not affect the rhodamine 6G fluorescence in the absence of Pdr5p. The rate of dequenching was proportional to the inhibitor concentration. When the inhibitor was added together with ATP before the start of the reaction, quenching occurred at a decreased rate, inversely proportional to the inhibitor concentration, as shown for tamoxifen in Fig. 5. The nonhydrolyzable ATP analogue ATP-S (31) could not substitute for ATP and was inhibitory in the presence of ATP, suggesting that hydrolysis of ATP is required to energize the reaction. Furthermore, rhodamine 6G fluorescence quenching was fully relieved by addition of detergent at low concentrations, such as 1.45 mM octyl glucoside, 0.1 mM sodium deoxycholate, or 0.005% Triton X-100. This shows that the dye was neither degraded nor modified. The process responsible for fluorescence quenching was not inhibited by 10 mM sodium azide, 100 mM potassium nitrate, or by protonophores such as 10 μM CCCP, 10 μM FCCP, 1 μM S13, 1 μM SF6847, added either before addition of ATP or after the steady state was reached.
reached. Quenching of rhodamine 6G fluorescence was completely abolished by a 30-min preincubation of the membranes with trypsin. Rhodamine 6G and rhodamine 123 had similar responses (data not shown).

Characterization of Rhodamine 6G Fluorescence Quenching Reaction—Modification of extravascular osmolarity was achieved by preincubation of membranes with different amounts of sucrose in the assay buffer for 60 min at room temperature as described previously by Horio et al. (32). Then the assay was performed at the same concentration of sucrose as that of preincubated membrane suspension. Fig. 6A shows that the Pdr5p-mediated, MgATP-dependent rhodamine 6G quenching reaction is osmotically sensitive. Its optimal temperature is 35 °C (Fig. 6B). The rhodamine 6G quenching reaction shows narrow optimum at pH 7 (Fig. 6C) and shows a clear preference for MgATP as energy source (Fig. 6D) with no activity observed without magnesium and with MgADP.

Characterization of Rhodamine 6G Fluorescence Quenching Inhibitors—The rate of rhodamine 6G fluorescence quenching in Pdr5p-enriched plasma membrane fractions reached a plateau value at rhodamine 6G concentrations above 400 nM (Fig. 7). Michaelis-Menten kinetics revealed an apparent \( K_m \) of Pdr5p for rhodamine 6G of 144 nm. Each tested inhibitor of rhodamine 6G fluorescence quenching was kinetically characterized. Some inhibitors displayed competitive, others noncompetitive inhibition (Fig. 7, A and B). These inhibitors did not affect the rhodamine 6G fluorescence in the absence of Pdr5p. For each tested inhibitor, we determined the \( I_{50} \) concentration at the rhodamine 6G concentration of 146 nm, as shown for tamoxifen in Fig. 5. The results are summarized in Table I.

DISCUSSION

Using our newly developed assays based on in vitro rhodamine 6G fluorescence measurements and in vivo drug sensitivity, we show that in spite of little sequence homology to the mammalian P-glycoproteins and inverted topology, the yeast...
abolished by protease treatment. This, together with our
from the
molecular weight of the mixture components.

...nescence in the presence of Mg$^{2+}$, taking the average
molecuar weight of the mixture components.

Others

Oligomycin 65 ng/ml$^b$ Competitive
Chloramphenicol 394 μM Competitive
Vanadate 3.3 μM Noncompetitive

$^a$ This concentration corresponds to ~146 nM, taking the average molecular weight of the mixture components.
$^b$ This concentration corresponds to ~82 nM, taking the average molecular weight of the mixture components.

multidrug transporter Pdr5p shares with them common sub-
strates and inhibitors. The in vitro assay allowed us to kineti-
cally characterize 22 inhibitors of the Pdr5p-mediated fluo-
rescence quenching of rhodamine 6G.

We first identified, on the basis of in vivo toxicity assays
performed on PDR5-deleted and -expressing strains, toxic com-
 pounds likely to be transported by Pdr5p such as the fluores-
cent dyes rhodamine 6G and rhodamine 123, the anticancer
drugs daunorubicin, doxorubicin, tamoxifen, and ATP. Similar
preparations to quench rhodamine 6G or 123 fluorescence

The observed quenching cannot be due to chemical modifi-
cation of the fluorescent dye: addition of detergents completely
restores the initial fluorescence intensity, and the rhodamine
6G excitation and emission spectra recorded before and after
extraction from whole cells, or before and after fluorescence
quenching by membrane preparations, are indistinguishable
(data not shown). The possible involvement in rhodamine 6G
fluorescence quenching of proton motive force or of one of its
components is unlikely, as shown by the lack of inhibition by
protonophores, sodium azide (an inhibitor of mitochondrial
ATPase), and by nitrate (an inhibitor of vacuolar ATPase and a
membrane-permeating, Δp-collapsing anion). Likewise, drug
transport by the mouse MDR1 and MDR3 P-glycoproteins
expressed in yeast secretory vesicles has been found not to be
influenced by the proton motive force (35).

Several lines of evidence suggest that MDR transporters can
recognize their substrates in the cytoplasmic leaflet of the
bilayer (36) and may function as flippases (37). The ability of
isolated membrane fractions to quench rhodamine 6G or 123
fluorescence provided they contain Pdr5p might be explained
by modification of the distribution of the dye within the lipid
bilayer although accumulation by inside-out plasma membrane
vesicles cannot be excluded. This latter possibility would for
example be consistent with the osmosensitivity of our assay
and with the fact that rhodamine 6G forms at higher concen-
trations nonfluorescent dimers and trimers, which quench
the monomer fluorescence (38). We do not wish to take a position
between these two possibilities.

Interestingly, the low $I_{50}$ values recorded for vanadate and
oligomycin in our in vitro rhodamine 6G fluorescence quench-
ing assay are strikingly similar to those observed for inhibition
of Pdr5p nucleoside triphosphatase activity (6). Micromolar
concentrations of vanadate can also effectively inhibit the
ATPase activity of mammalian P-glycoproteins (39). As previ-
ously reported (6, 19), we have confirmed that in our samples
the plasma membrane bound Pdr5p hydrolyses UTP, CTP,
GTP, and ITP at rates comparable with ATP with a broad pH
optimum. However, the in vitro rhodamine transport has a
narrow pH optimum and is energized by ATP. The difference in
the pH sensitivity between ATPase activity and rhodamine 6G
fluorescence quenching might be connected with the protona-
tion state of the dye. The difference in nucleoside triphosphatase
specificity, however, sheds new light on the poorly understood
mechanism of coupling ATP hydrolysis to drug transport by
ABC transporters, since it indicates that although UTP and
other triphosphonucleosides can be effectively hydrolyzed by
Pdr5p, only the interaction of the nucleotide binding site with
ATP produces proper energy transduction required for drug
translocation.

The kinetic characterization of Pdr5p rhodamine 6G quench-
ing inhibitors is consistent with the in vitro drug sensitivity
assay results, showing that compounds whose toxicity is re-
duced by Pdr5p are competitive inhibitors of rhodamine trans-
port including chloramphenicol, previously reported to be sub-
strate of Pdr5p (40). There is one exception, however, Pdr5p
clearly mediates trifluoperazine resistance which appears to be
a noncompetitive inhibitor of rhodamine 6G transport. It is
possible that trifluoperazine is a transported substrate recog-
nized at a site different from that of rhodamine 6G. Indeed,
alterations of drug resistance profiles, particularly segregation
of vinblastine resistance from daunomycin and colchicine
resistance, have been observed in mutant P-glycoproteins (41–
43). This, together with the observations of Tamai and Safa
(44) that azidopine noncompetitively interacts with vinblastine

### Yeast Multidrug Resistance Transporter

#### Table I

<table>
<thead>
<tr>
<th>Inhibitor type</th>
<th>$I_{50}$ concentration</th>
<th>Type of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticancer drugs and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chemosensitizers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>0.6 μM</td>
<td>Competitive</td>
</tr>
<tr>
<td>Taxol</td>
<td>0.7 μM</td>
<td>Competitive</td>
</tr>
<tr>
<td>Vinristine</td>
<td>1 μM</td>
<td>Competitive</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>1.4 μM</td>
<td>Competitive</td>
</tr>
<tr>
<td>Verapamil</td>
<td>3.4 μM</td>
<td>Competitive</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>18 μM</td>
<td>Competitive</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>1.2 μM</td>
<td>Noncompetitive</td>
</tr>
<tr>
<td>Quinidine</td>
<td>2.5 μM</td>
<td>Noncompetitive</td>
</tr>
<tr>
<td>Puroymycin</td>
<td>8.7 μM</td>
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</tr>
<tr>
<td>Yohimbine</td>
<td>36 μM</td>
<td>Noncompetitive</td>
</tr>
<tr>
<td>Colchicine</td>
<td>79 μM</td>
<td>Noncompetitive</td>
</tr>
</tbody>
</table>

Inhibitors

Valinomycin 15 nm Competitive
Gramicidin D 0.3 μg/ml$^a$ Competitive
Nigericin 0.6 μM Competitive
Monensin 1.1 μM Competitive
Ionophore A23187 4.1 μM Competitive
Steroid hormones

Progestosterone 5.5 μM Competitive
β-Estradiol 8.3 μM Competitive
Deoxycorticosterone 8.9 μM Competitive

Others

Oligomycin 65 ng/ml$^b$ Competitive
Chloramphenicol 394 μM Competitive
Vanadate 3.3 μM Noncompetitive

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*D This concentration corresponds to ~146 nM, taking the average molecular weight of the mixture components.

* This concentration corresponds to ~82 nM, taking the average molecular weight of the mixture components.
and cyclosporin A binding to P-glycoprotein and observations of Ferry and co-workers (45, 46), that the vinca alkaloid binding site is allosterically coupled to binding sites for dihydropyridines and taxanes, might reflect the presence of different drug-binding sites on P-glycoproteins for different classes of transported substrates. In this perspective it is interesting to note that colchicine noncompetitively inhibits Pdr5p-mediated rhodamine 6G fluorescence quenching in yeast plasma membranes whereas the vinca alkaloids vinblastine and vincristine were competitive inhibitors. Alternatively, in vitro trifluoperazine might bind to the ATP binding site of Pdr5p, as is the case for sarcoplasmic calcium ATPase (47). In vivo, such an interaction could be prevented by direct extrusion of the compound from the membrane, before it reaches the nucleotide binding sites.

Another important aspect of our work is that Pdr5p mediates resistance to steroid hormones such as progesterone and deoxycorticosterone. The hypersensitivity to both compounds after deletion of PDR5 was observed in several strains bearing either the PDR1 wild type or pdr1-3 mutant allele. Although sensitivity to progesterone and deoxycorticosterone is influenced by the genetic background, the resistance was restored by transformation of PDR5 deleted strain by PDR5 on centromeric plasmid (data not shown). This and the fact that both hormones are potent competitive inhibitors of Pdr5p-mediated rhodamine 6G transport shows their direct interaction with Pdr5p and strongly suggests that they are indeed transported substrates of the pump. This adds new aspects to the observations made by Kralli et al. (12) that Pdr5p does not modify the accumulation in intact cells of progesterone and deoxycorticosterone, but affects accumulation of dexamethasone and triamcinolone acetonide. This conclusion was based on indirect measurements of hormone-inducible reporter gene activity, which was expressed differently in PDR5 wild type and a pdr5 mutant. Our data support the view that they used a mutant of which was expressed differently in either the

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Anticancer Drugs, Ionophoric Peptides, and Steroids as Substrates of the Yeast Multidrug Transporter Pdr5p
Marcin Kolaczkowski, van der Rest Michel, Anna Cybularz-Kolaczkowska, Jean-Philippe Soumilion, Wil N. Konings and Goffeau André

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