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Biochemistry

Functional complementation of the *ste6* gene of *Saccharomyces cerevisiae* with the *pfmdr1* gene of *Plasmodium falciparum*

(multiple drug resistance)

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ABSTRACT **The *pfmdr1* gene has been associated with a drug-resistant phenotype in *Plasmodium falciparum*, and overexpression of *pfmdr1* has been associated with mefloquine- and halofantrine-resistant parasites, but little is known about the functional role of *pfmdr1* in this process. Here, we demonstrate that the *pfmdr1* gene expressed in a heterologous yeast system functions as a transport molecule and complements a mutation in *ste6*, a gene which encodes a mating pheromone a-factor export molecule. In addition, the *pfmdr1* gene containing two mutations which are associated with naturally occurring chloroquine resistance abolishes this mating phenotype, suggesting that these genetic polymorphisms alter this transport function. Our results support the functional role of *pfmdr1* as a transport molecule in the mediation of drug resistance and provide an assay system to address the nature of this transport function.**

The emergence of drug-resistant organisms that cause human disease has plagued clinical and epidemiological efforts to combat them. Drug-resistant *Plasmodium falciparum*, which causes malaria in humans, emerged over 30 years ago, but increasing levels of resistance to drugs such as chloroquine and mefloquine, as well as cross-resistance to these and other compounds, such as quinine and halofantrine, has made treatment and prophylaxis of malaria increasingly difficult. Neither the targets of these drugs nor the mechanisms of drug resistance are well understood. A candidate gene, the *P. falciparum* multiple drug resistance gene or *pfmdr1*, proposed to encode a transport molecule involved in drug resistance, has been identified (1, 2).

The *pfmdr1* gene is a member of the ATP-binding cassette gene family (3), whose members encode transport molecules, such as the P-glycoproteins, known to efflux drugs and thereby confer drug resistance in mammalian cells (4). It has been hypothesized that the *pfmdr1* gene has a similar role in drug resistance in *P. falciparum* (5). Although the mechanism of drug resistance in *P. falciparum* is not known, it has been demonstrated that chloroquine-resistant parasites accumulate less chloroquine than chloroquine-sensitive parasites (6). It has been suggested that an efflux mechanism is involved in drug resistance (6) and that agents like verapamil which reverse P-glycoprotein-mediated drug efflux in mammalian cells also reverse chloroquine resistance in *P. falciparum* (5). However, more recent studies have demonstrated rapid efflux of chloroquine in both chloroquine-sensitive and chloroquine-resistant parasites (7), suggesting that decreased chloroquine accumulation in resistant parasites may involve mechanisms other than efflux of drug and, furthermore, that reversing agents, such as verapamil, may function by a mechanism other than by inhibition of drug efflux (8). In addition, a proposed role for *pfmdr1* in drug resistance is controversial since other work involving a genetic cross between a chloroquine-sensitive and a chloroquine-resistant strain of *P. falciparum* has dissociated the *pfmdr1* gene from these phenotypic characteristics of efflux and reversal by verapamil (9). Finally, overexpression of *pfmdr1* is not associated with chloroquine-resistant parasites from the wild (2), and parasites selected *in vitro* for increased chloroquine resistance deamplify the *pfmdr1* gene (10) while increasing their sensitivity to mefloquine.

In contrast with chloroquine resistance, there is consistent evidence for amplification of the *pfmdr1* gene in mefloquine-resistant *P. falciparum*, both in field isolates (11) and in laboratory strains subjected to mefloquine pressure (12, 13). In addition, cross-resistance to compounds such as halofantrine (11–13) and quinine (13) has been associated with mefloquine resistance, suggesting that mechanisms involved in mefloquine resistance are distinct from those involved in chloroquine resistance (ref. 14; reviewed in ref. 15).

The goal of the work presented here was to develop a system for functional analysis of the *pfmdr1* gene and its product, the Pgh1 protein (16), and to resolve its role as a transport molecule in drug resistance. In addition, we wanted to develop a screening system to identify drugs which interact with the Pgh1 protein in this system, as well as modulators which may affect these drug interactions.

The *ste6* gene of *Saccharomyces cerevisiae* is also a member of the ATP-binding cassette gene family (17, 18) whose function is defined. The *ste6* gene encodes a membrane protein that exports **a**-factor mating pheromone, and mutants which are deficient in *ste6* expression are sterile and cannot mate (18). Mating is used as a measure of **a**-factor export in a very sensitive complementation assay, where mating frequencies of 0.01% are considered to reflect significant **a**-factor export (19). Other members of the ATP-binding cassette gene family have been shown to complement *ste6*, including the mouse *mdr3* (*mdr1a*) gene (20) and a chimera of the *cftr* gene (21); however, the human *MDR1* gene failed to complement a mutation in *ste6* (22). The demonstration that some ATP-binding cassette gene family members can complement *ste6* function suggests a specific structural requirement for **a**-factor transport. This assay system may therefore be used to address the functional nature of a transport molecule and to characterize the structural components of this molecule which determine or specify this function.

The purpose of these experiments is to develop a functional assay for the *pfmdr1* gene of *P. falciparum* in *S. cerevisiae* and to determine if expression of *pfmdr1* can functionally complement *ste6* and transport mating pheromone **a**-factor. A *pfmdr1* gene containing genetic polymorphisms associated with chloroquine resistance (23) is also expressed in this yeast system to address the effect of these genetic changes on the function of *pfmdr1* in this complementation analysis.

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MATERIALS AND METHODS

Media and Strains. Yeast strains and transformants were grown at 30°C in liquid culture or on solid agar plates containing yeast/peptone/dextrose (YPD) medium or selective synthetic dextrose-uracil (SD-URACIL) medium, and mating assays were performed on minimal synthetic dextrose (SD) agar plates (24). For growth in galactose, dextrose was replaced in the above media at a final concentration of 2%. Yeast strains (25) (the kind gift of Susan Michaelis, Johns Hopkins University, Baltimore) included SM1068 (MATα *lys1*), an α-tester strain for mating, or SM1086 (MATα *sst2-1 rme his6 met1 can1 cyh2*), an α-tester strain for pheromone assays, and SM1563 (MATa *trp1 leu2 ura3 his4 can1 ste6::LEU3*), a yeast strain with a mutation in *ste6*. Yeast transformations were carried out by the lithium acetate method (26).

Constructs. Shuttle plasmids constructed with the pYES-2 vector (Invitrogen) contained either the wild-type *pfmdr1* gene (pYpfmdr1) or a mutant *pfmdr1* gene with a serine to cysteine change at position 1034 and an asparagine to aspartic acid change at position 1042 (pYpfmdr1CD) (2). As a control, the *ste6* gene was also inserted into this vector (pYste6) by using a *Hin*dIII restriction fragment from the pSM580 vector (Susan Michaelis, personal communication).

Northern Analysis. Yeast RNA was isolated from transformants (20-ml cultures) grown to a density of 2×10^8 cells per ml in SD-URA. Cells were centrifuged (Sorvall 6000T; 3500 rpm; 4°C; 10 min), resuspended in 1 ml of extraction buffer (0.1 M Tris·HCl, pH 7.5/0.1 M LiCl/0.1 M EDTA), added to 0.5 g of acid-washed glass beads (425–600 μm in diameter) in 1 ml of phenol/chloroform (50:50), and vortexed repeatedly with equal, intermittent incubations on ice for 5 min. The volume of each phase was increased to 10 ml and extracted twice with an equal volume of phenol/chloroform (50:50). Both aqueous phases were combined and precipitated with ethanol. Northern analysis was performed (14) by using one-tenth of the total yeast RNA for each sample (2×10^8 cells) with hybridization as described (14). The *pfmdr1* probe was a mixture of PCR-amplified DNA from the regions between nt 510 and 830 and 3609 and 4792 (2).

Quantitative Mating Assay and Quantitative Pheromone Assay. Quantitative mating assays were performed by a plate mating procedure as described (25). Mating frequency was calculated as the number of diploids formed relative to the number of input MATa cells. The results that are presented represent three or more separate experiments and are given with the standard deviation. Quantitative pheromone assays were performed as described (25) and are represented as a 1:2 dilution series of concentrated culture supernatant from each of the transformants. Experiments were performed in medium containing either dextrose or galactose. Mating of both the MATa strain SM1058 (25), which is isogenic to SM1563 but contains a wild-type *ste6* gene, and the pYste6 transformant was inhibited in the presence of galactose. Thus, all the data presented here are from cells grown in dextrose-containing media.

Indirect Immunofluorescence Assay of Yeast. Yeast transformants were subjected to an indirect immunofluorescence assay (27) with a polyclonal rabbit anti-Pgh1 antiserum (α-Pgh1) as the primary antibody and a fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulin as the secondary antibody. The α-Pgh1 antiserum was raised in a rabbit immunized with a fusion protein generated from a construct containing the *pfmdr1* gene region from nt position 4158 to 4428 (2) in frame with the β-galactosidase sequences of the pUR vector (28) and expressed in *Escherichia coli*. Yeast transformants were visualized at a magnification of 630 and at a wavelength of 495 nm.

Sequence Comparison. Analysis of sequences for the Pgh1 (2), Mdr3 (29), and STE6 (17, 18) protein coding regions was performed by using the software programs from PG/GENE (IntelliGenetics) and the Genetics Computer Group (University of Wisconsin; ref. 30). Data base searching was performed at the National Center of Biotechnology Information by using the BLAST (31) network services with a gap weight of 3.00 and gap-length weight of 0.10.

RESULTS

Expression of the *pfmdr1* Gene in Yeast. Shuttle plasmids containing either the *pfmdr1* coding region (Fig. 1*A*) or the *ste6* coding region (17, 18) were introduced into SM1563 (25), and independent Ura^+ colonies were chosen for further analysis. The *pfmdr1* gene expressed in these transformants was either the wild-type sequence (pYpfmdr1) or the mutant sequence (pYpfmdr1CD) containing two naturally occurring mutations in *pfmdr1* associated with chloroquine-resistant *P. falciparum* (23). Ura-selected transformants were analyzed by Southern analysis to demonstrate the presence of predicted plasmids (data not shown), and expression of *pfmdr1* in these yeast transformants was examined by Northern analysis (Fig. 1*B*). This analysis demonstrated that the *pfmdr1* probe hybridized with a transcript of ≈4 kb in RNA harvested from yeast transformed with either the wild-type *pfmdr1* gene or the mutant *pfmdr1* gene but not in RNA from yeast transformed with the control plasmid (pY) alone. This transcript size of 4 kb is consistent with the size of the *pfmdr1* coding region, which was put into the shuttle vector for expression in yeast (Fig. 1*A*). The two *pfmdr1* transcripts from *P. falciparum*, which are shown for comparison, have previously been shown to have sizes of 8.5 kb and 7.5 kb (14) since they include sequence information beyond the *pfmdr1* coding region of 4 kb.

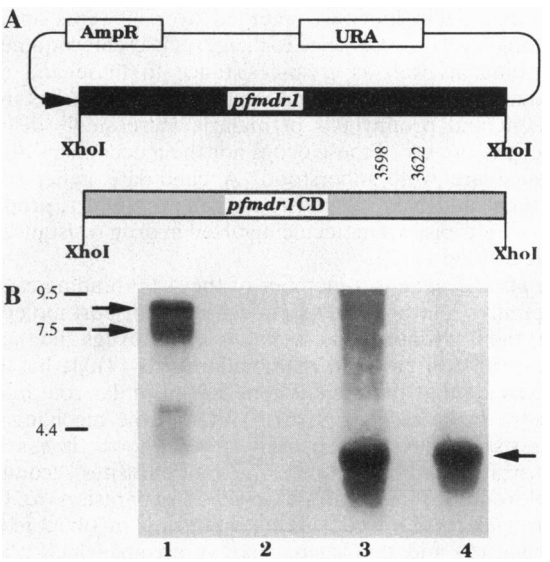

FIG. 1. Construction of expression plasmids and Northern analysis of yeast transformants. (*A*) Shuttle plasmids containing the wild-type *pfmdr1* gene (solid box) or the mutant *pfmdr1* gene (stippled box) containing the C1034 and D1042 mutations were constructed by using the *Xho* I restriction site of the pYES-2 plasmid. The mutations in *pfmdr1*CD are indicated by nt number 3598 (2), resulting in cysteine at position 1034, and by nt number 3622 (2), resulting in an aspartic acid at position 1042 (23). (*B*) Northern analysis of RNA isolated from either the W2mef strain of *P. falciparum* (1), lane 1; or the yeast transformants pY, lane 2; pYpfmdr1, lane 3; or pYpfmdr1CD, lane 4. Size markers for 9.5 kb, 7.5 kb, and 4.4 kb are indicated at the left, along with two arrows next to lane 1 to identify the two *pfmdr1* transcripts of 8.5 kb and 7.5 kb (14) and the single arrow on the right to identify the 4-kb *pfmdr1* transcript in the pYpfmdr1 and pYpfmdr1CD transformants.

To determine if the Pgh1 protein was expressed in yeast transformed with *pfmdr1*, indirect immunofluorescence analysis was performed. Immunofluorescence was detected with α-Pgh1 antiserum in yeast transformed with either the wild-type *pfmdr1* (pYpfmdr1) or the mutant *pfmdr1* (pYpfmdr1CD) gene containing two chloroquine-resistance-associated mutations but not in yeast expressing the control plasmid (pY) only (Fig. 2). The staining pattern in the yeast transformed with either the wild-type or the mutant *pfmdr1* gene was punctate rather than homogeneous, and immunofluorescence was observed as discrete points of signal localized either in the plasma membrane or in internal vesicles (see Fig. 2). These same cells incubated with preimmune rabbit serum as the primary antibody showed no staining (data not shown). These results demonstrated that the yeast transformed with either the wild-type or the mutant *pfmdr1* gene construct express Pgh1 protein and that, as detected at the level of light microscopy, there appeared to be a similar distribution of Pgh1 in each of these transformants.

Phenotypic Analysis of *pfmdr1* Expression. Mating assays were used to test whether *pfmdr1* could complement the mutation in *ste6* when expressed in the Δ*ste6* strain SM1563. Representative plates of these mating assays are shown with the calculated mating frequency in Fig. 3. This analysis demonstrated that the number of diploids formed between the MATα mating strain (25) and the pYpfmdr1 transformant was at a level 1000-fold above background. In a similar experiment using the yeast transformed with either the control plasmid, pY, or the plasmid containing the mutant *pfmdr1* gene, pYpfmdr1CD, only background-level mating was observed. Failure to detect mating by the pYpfmdr1CD transformant is not due to the lack of *pfmdr1* expression since both pYpfmdr1 and pYpfmdr1CD expressed *pfmdr1* similarly (Fig. 2). The level of complementation of the null *ste6* allele by the wild-type *pfmdr1* gene expressed in yeast is similar to the results of Raymond *et al.* (20) for expression of *mdr3* in a similar yeast system, where the *ste6* transformant mated at a frequency of 6.7×10^{-2} and the *mdr3* transformant mated at a frequency of 7.1×10^{-4}.

Quantitative pheromone assays were used to demonstrate the presence of **a**-factor in the culture supernatant of these cells. Results of these experiments (Fig. 4) show that the culture supernatant of pYpfmdr1 contains an **a**-factor titer which inhibited growth of the MATα lawn to a dilution of 1:8, whereas the supernatant from pYste6 transformants inhibited this lawn growth to a dilution of 1:64. Neither pY nor pYpfmdr1CD inhibited the growth of the MATα lawn in this analysis. These data demonstrated that detectable levels of mating factor were present in the supernatant of yeast transformants expressing wild-type *pfmdr1* but not in yeast expressing *pfmdr1* containing the two chloroquine-resistance-associated changes.

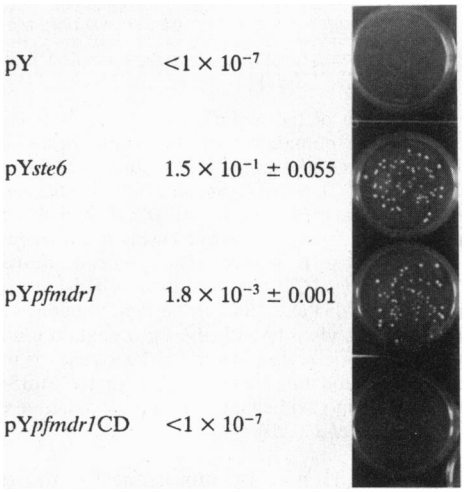

FIG. 3. Mating analysis of yeast transformants. SM1563 yeast transformed with plasmid only (pY), plasmid containing the *ste6* gene (pYste6), plasmid containing the wild-type *pfmdr1* gene (pYpfmdr1), or plasmid containing the mutant *pfmdr1* gene (pYpfmdr1CD) were mixed with MATα cells (SM1068) and grown on minimal medium agar plates to select for diploid yeast. Representative plates are shown with 10^7 transformed MAT**a** (SM1563) cells for each of the transformants except pYste6, which is shown at a 1:10,000 dilution, and pYpfmdr1, which is shown at a 1:100 dilution, to illustrate the similar cell densities observed at these dilutions. Mating frequency was calculated as described in *Material and Methods.*

Location of the Chloroquine Resistance-Associated Mutations. The genetic changes in the *pfmdr1* gene expressed in the pYpfmdr1CD transformant reside in the predicted transmembrane domain 11 of the *pfmdr1* gene, a region which is proposed to form an amphipathic helix (20, 23) and may be involved in substrate recognition and transport by the P-glycoprotein superfamily of transporters. A comparison of these changes in Pgh1 with the STE6 and Mdr3 protein sequences is shown in Fig. 5. The *mdr3* gene which complements ste6 contains a serine residue at position 939, a site proposed to be analogous to the serine residue at position 1034 in *pfmdr1* (20) (Fig. 5), and a change in this serine residue to a phenylalanine residue abolishes the ability of *mdr3* to complement *ste6* (20). These data suggest that the serine at

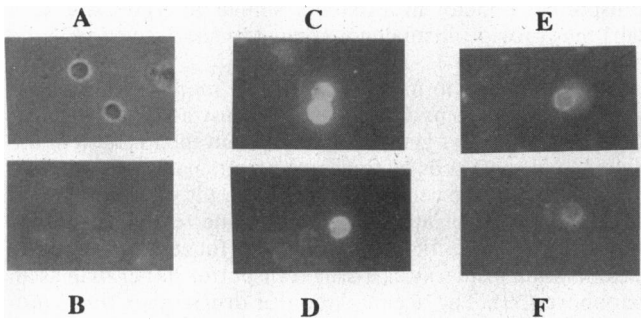

FIG. 2. Identification of Pgh1 in yeast transformants. SM1563 yeast were transformed with plasmid pY only (*A* and *B*), with plasmid pYpfmdr1 containing the wild-type pfmdr1 gene (*C* and *D*), or with plasmid pYpfmdr1CD containing a mutant *pfmdr1* gene (*E* and *F*). Formaldehyde-fixed cells were probed with α-Pgh1 antisera followed by secondary staining with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin and visualized by light microscopy at 495 nm (*B–F*). *A* shows a phase-contrast image of *B*.

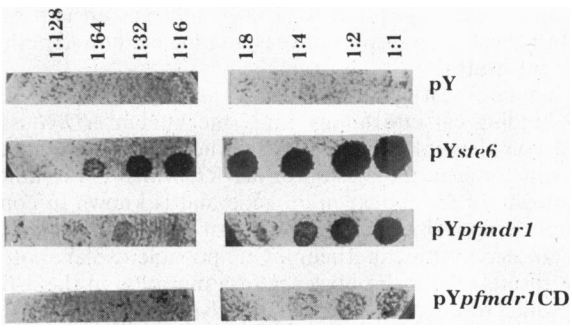

FIG. 4. Pheromone analysis of **a**-factor present in the culture supernatant. SM1563 yeast transformed with plasmid only (pY); plasmid containing the *ste6* gene (pYste6); plasmid containing the wild-type *pfmdr1* gene (pYpfmdr1); or plasmid containing the mutant *pfmdr1* gene (pYpfmdr1CD) were grown as described in *Material and Methods.* A dilution series (1:2) from the fully concentrated culture supernatant (1:1) to a dilution of (1:128) from these transformants was spotted onto a lawn of MATα (SM1086) cells.

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Consensus  1081  KRAIV-G---  SFTQ----FI  -A--Y-FG--  LV-----T--  1120
STE6        940  KRAIATGFGI  SMTNMIVMCI  QAIIYYYGLK  LVMIHEYTSK   979
Mdr3        929  KKAHVFGITF  SFTQAMMYFS  YAACFRFGAY  LVAQQLMTFE   968
Pgh1       1022  RRIIVNAALW  GFSQSAQLFI  NSFAYWFGSF  LIKRGTILVD  1061
CLQR       1022  ********** **C******* D********* **********  1061
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FIG. 5. Comparison of the amino acid sequences from the predicted transmembrane domain 11 of the STE6, Mdr3, and Pgh1 proteins. The alignment of the amino acid sequences from Pgh1 (from aa 1022 to aa 1061; ref. 2), Mdr3 (from aa 929 to aa 968; ref. 29), and STE6 (from aa 940 to aa 979; refs. 17 and 18), as well as a consensus sequence from aa 1081 to aa 1120 derived using BLAST as described in *Materials and Methods*, is shown. The chloroquine-resistance-associated Pgh1 sequence (CLQR) is identical to Pgh1, except for the mutations in position 1034 and 1042 of the Pgh1 sequence (23), with the asterisks indicating identity with the Pgh1 sequence above. The proposed transmembrane region of the Pgh1 sequence is underlined (2), and the serine residue indicated in boldface in the Mdr3 sequence corresponds to the amino acid changed to a phenylalanine residue in the work of Raymond *et al.* (20).

position 1034 of Pgh1 may be important for the transport function of this protein.

DISCUSSION

This work demonstrates that the *pfmdr1* gene expressed in a strain of yeast with a mutation in *ste6* complements STE6 function and transports mating pheromone **a**-factor. In addition, our results suggest that the expression of a *pfmdr1* gene with two naturally occurring polymorphisms associated with chloroquine resistance, C1034 and D1042 (23), abolishes this functional complementation of **a**-factor transport, as determined by mating assays, and that **a**-factor is not present in significant amounts in the supernatant of yeast cultures that express this mutant *pfmdr1* gene. The functional role of the *pfmdr1* gene in the mediation of drug resistance in malaria is not known; however, genetic polymorphisms in *pfmdr1* associated with a chloroquine-resistant phenotype (23) abolish this transport function in yeast, suggesting that these changes may be important for accumulation of drug in the parasite itself.

These genetic changes reside in the predicted transmembrane domain 11 of the *pfmdr1* gene, a region proposed to form an amphipathic helix and which may be involved in substrate recognition and transport by the P-glycoprotein superfamily of transporters (20, 23). In addition, the *mdr3* gene that complements *ste6* contains a serine residue at position 939, a site proposed to be analogous to the serine residue at position 1034 in *pfmdr1* (20). Altering this Ser-939 residue similarly abolishes the ability of *mdr3* to complement *ste6*. This change to a phenylalanine residue at position 939 in *mdr3* has been associated with dramatic changes in the substrate specificity of this gene product in mammalian systems (32–34). These data argue that the serine at position 1034 in *pfmdr1* has an important functional role in transport of yeast mating factor and perhaps other substrates.

A similar yeast system has been used to test if another ATP-binding cassette family gene, the human *MDR1* gene, could complement STE6 function when expressed in yeast deficient for *ste6*. Expression of *MDR1*, which is the human homologue of the mouse *mdr3* gene and is known to confer multiple drug resistance in mammalian systems, did not complement *ste6* in this experiment. One possible explanation for these results (19) is the presence of a mutation in the *MDR1* gene which resulted in a change from glycine to valine at amino acid position 185. This mutation, which resides near transmembrane domain three, phenotypically enhances colchicine selectivity in mammalian cells. These data imply that changes at or near transmembrane regions are important for transport in these ATP-binding cassette gene family members and are consistent with findings in mammalian systems where substrate specificity is often determined by transmembrane domain regions (4).

The observation that residues in transmembrane domain regions determine the specificity and level of resistance to different drugs, as well as the hydrophobic nature of the compounds which the *mdr* genes transport, leads to a current model which views drug transport as a biphasic process involving partitioning into the membrane followed by transport across it (35). This model proposes that substrates which can be transported by the P-glycoprotein molecule partition into the lipid bilayer of the membrane because of their hydrophobic nature, then interact with the P-glycoprotein transporter in the membrane at or near the transmembrane regions.

The genetic changes in the mutant *pfmdr1* gene used in the work presented here occur naturally in chloroquine-resistant parasites (23). The functional relevance of these mutations in *pfmdr1* on chloroquine sensitivity was tested in another system. The *pfmdr1* gene expressed in Chinese hamster ovary cells results in an increased sensitivity of these cells to chloroquine, and these same mutations in *pfmdr1* at positions 1034 and 1042 abolish this increase in chloroquine sensitivity in this system (36). These data support the findings in this work which demonstrate that these genetic polymorphisms identified in a naturally occurring, chloroquine-resistant parasite (23) are associated with altered transport of substrate. This argues that expression of the *pfmdr1* gene may play an important role in the mediation of drug transport in *P. falciparum* itself.

Additional experiments have been done with a similar heterologous yeast system expressing *mdr3* to demonstrate that expression of *mdr3* in this yeast system confers drug resistance to the immunosuppressive and antifungal agent FK520 (37). Furthermore, the *mdr3* gene containing a serine to phenylalanine mutation at position 939 expressed in this yeast system failed to confer this resistance phenotype. This suggests that expression of *pfmdr1* in yeast may be similarly tested for a functional role in drug transport and that the effect of the chloroquine-resistance-associated mutations in *pfmdr1* (23) may be addressed.

We have demonstrated that at the level of light microscopy *pfmdr1* is expressed in yeast in both internal vesicles and the plasma membrane. Although higher resolution of this localization is required, this observation is consistent with the finding that Pgh1 is localized to the digestive vacuole in *P. falciparum* (16). These data may seem to contrast with the finding that *pfmdr1* expression in Δ*ste6* yeast allows **a**-factor to be exported from the cell for mating, but recently it has been demonstrated that STE6 is mainly associated with internal membranes and not the cell surface in yeast, suggesting that STE6 secretes **a**-factor into endocytic vesicles and that the pheromone is released extracellularly through exocytosis (38). We propose that expression of *pfmdr1* in yeast allows for the transport of **a**-factor in a manner similar to STE6, and that Pgh1 may provide an analogous drug transport function in the malaria parasite.

Drug resistance in malaria is possibly mediated by several different mechanisms. This work demonstrates that the malaria parasite *pfmdr1* gene can complement the function of the *ste6* gene in yeast cells by transporting the mating pheromone **a**-factor from these cells but that a naturally occurring mutation in *pfmdr1* associated with chloroquine resistance is inactive. This establishes that Pgh1 is likely to function to alter drug accumulation primarily as a drug transporter rather than as an ionophore (39). The accumulation of drugs other than chloroquine, such as mefloquine and halofantrine, may be influenced differently by *pfmdr1* expression in the parasite, and it is possible that some drugs may be transported by proteins other than Pgh1. Further analysis of the role of Pgh1 as a transport molecule and how expression of *pfmdr1* may mediate a mechanism of drug resistance in malaria needs to be carried out. This yeast expression system can be utilized to identify the characteristics of *pfmdr1* which are important for transport

functions possibly relevant to a mechanism of drug resistance in *P. falciparum.*

We have functionally expressed *pfmdr1* in *S. cerevisiae* and have established that the *pfmdr1* gene product functions as a transporter. In this system, *pfmdr1* transports a small hydrophobic peptide, the yeast mating pheromone **a**-factor, easily assessed by mating assays. Furthermore, this yeast expression system can be easily manipulated to address the characteristics of *pfmdr1* that are important for this transport function and which may be relevant to a mechanism of drug resistance in *P. falciparum.*

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