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Interactions between the gene products of *pma1* encoding plasma membrane H^+-ATPase, and *pdr1* controlling multiple drug resistance in *Saccharomyces cerevisiae**

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In *Saccharomyces cerevisiae*, the *pma1* mutations controlling the vanadate resistance of the H^+-ATPase activity from the plasma membrane, map on chromosome VII in the vicinity of *pdr1* mutations controlling multiple drug resistance. However, the *pma1-1* mutants exhibit a genotype and a multidrug resistant phenotype quite different from those obtained for *pdr1* mutants. Quantitative modifications of cycloheximide and *N,N'*-(*p*-xylylidene)-bis-aminoguanidine-2HCl resistance are observed in diploids containing the *pma1* and *pdr1* genes in *trans* configuration. Each of the *pdr1* mutations interacts with *pma1* as shown by a decrease in the ATPase activity in *pdr1/pma1* diploids. The *in vitro* resistance of ATPase activity to vanadate is totally or partially suppressed in *pdr1* mutants in haploid double mutants. These results suggest that the expression of *PMA1* might be controlled by the *PDR1* gene product.

Two genetic loci commanding multiple drug resistance of *Saccharomyces cerevisiae* are located near *leu1* on chromosome VII, within a span of 2.3 recombination units [1]. One is *PMA1* encoding the H^+-ATPase from plasma membrane [2] and the other is *PDR1* encoding a regulatory protein [3] controlling the pleiotropic drug resistance phenotype originally reported by Rank & Bech-Hansen [4]. The plasma membrane H^+-ATPase provides energy for the active transport of nutrients and regulates intracellular pH (for the review see Goffeau & Slayman [5] and Serrano [6]).

The first *pma1* mutations affecting the plasma membrane ATPase of *S. cerevisiae* [2] were obtained by selecting mutants resistant to the ATPase inhibitor Dio-9. These mutations modified several catalytic properties of the ATPase activity measured *in vitro*, including vanadate resistance. Sequence analysis and molecular mapping of *pma1* mutant alleles [7] have fully demonstrated that the *pma1* mutations are located in the structural ATPase gene sequenced by Serrano *et al.* [8] and later by Van Dyck *et al.* [9]. There is experimental evidence that the transport of nutrients into the yeasts *Schizosaccharomyces pombe* and *S. cerevisiae* is associated with the function of the plasma membrane ATPase [10 - 13]. There are also indications that, in *pma1* mutants, the transport of inhibitors such as Dio-9, miconazole, ethidium bromide, guanidine derivatives [2, 7, 11, 12] and hygromycin B [14, 15] is modified. The resistance to these compounds did not affect the ATPase activity measured *in vitro* and it results likely from a modification of the membrane potential, as

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shown recently for *pma1* mutants resistant to hygromycin B [15].

Modification of permeability has been proposed to be the reason of multiple resistances to drugs of different structure and targets in mammalian cancer [16 - 19] and yeast cells [11, 20 - 23]. In *S. cerevisiae* the pleiotropic drug resistance mutants *pdr1* have been known for a long time [4, 20, 22, 24 - 35]. According to Saunders & Rank [33] and Balzi *et al.* [3]. The inheritance of multiple drug resistance in a series of *BOR2, cyh3, til1, AMY1, pdr1-1, pdr1-2* and *pdr1-3* mutants can be explained by assuming independent mutations in the same nuclear gene *PDR1*. This gene is centromere linked and maps closely to *leu1* on chromosome VII [1, 33, 36]. The deduced amino-acid sequence of the *PDR1* polypeptide resembles that of several nucleic acid-binding proteins involved in the control of gene expression [3]. Therefore, transcriptional control by the regulatory protein *PDR1* of the several permeability functions responsible for multiple drug resistance in yeast has been suggested. It has been more recently proposed that the *PDR5* gene is a target for regulation by the *PDR1* gene product [37]. In this work, we have investigated the interactions between *pma1* and *pdr1* mutations to assess whether there are functional relations between these two loci commanding two multiple drug resistance phenotypes.

MATERIALS AND METHODS

Strains and genetic methods. The strains used are listed in Table 1. Conventional genetic procedures for yeast crossing, sporulation and tetrad analysis were used. Diploids were isolated with the aid of a micromanipulator De Fonbrune.

Media and growth conditions. The complete medium contained: 1% yeast extract Difco, 2% bactopeptone Difco and 2% glucose (YEPD) or 4% glycerol (YEPG). Minimal medium (SD) contained yeast nitrogen base Difco without amino acids and 2% glucose. In supplemented SD medium, the final concentration of amino acids and adenine sulfate was 20 μg/ml, except for L-tryptophan (10 μg/ml) and L-lysine (40 μg/ml). For plating, the media were solidified with 2% of bacto-agar (Difco).

Drug resistance was evaluated on solid media according to Saunders & Rank [33]. The strains were tested against a wide range of inhibitor concentrations using either YEPG or YEPD media: 0.1, 0.25, 0.5, or 1.0 μg oligomycin/ml; 0.1, 0.5, 1, 2.5, 5 or 10 μg venturicidin/ml; 1 or 4 mg chloramphenicol/ml; 0.1, 0.25, 0.5 or 1 μg cycloheximide/ml; 50 or 100 μg decamethylene-diguanidine; 500, 600, 650 or 750 μg *N,N'*-(*p*-xylylidene)-bis-aminoguanidine-2HCl/ml; 25, 50 or 75 μg ethidium bromide/ml; 50, 100, 150, 200 or 300 μg hygromycin B/ml. In the case of YEPG, the medium was buffered with 0.1 M K_2HPO_4 and adjusted to pH 6.8 prior to autoclaving. All drugs were dissolved in ethanol, except XBAG[1], VAN, EBR, HYG and SYN which were dissolved in water.

Isolation of crude membrane fraction and ATPase assays. The strains were grown in liquid YEPD medium. After 12 h at 30°C the cultures were harvested and yielded 1.1×10^8 cells/ml. The cells were suspended in 2.5 vol. of 250 mM sucrose and 50 mM imidiazole, pH 7.5, and homogenized for 3 min with glass beads in a refrigerated Braun homogenizer. The crude membrane fraction was obtained by differential centrifugation of the subcellular homogenate at 3000 × *g* twice for 10 min, at 4000 × *g* for 10 min and at 15000 × *g* for 40 min. The pellet was suspended in 10 mM Tris/acetate, pH 7.5, 1 mM ATP and 1 mM EDTA. Plasma membrane ATPase activities in the presence or absence of 50 μM vanadate were determined at 35°C, in 1 ml reaction mixtures containing 5 mM ATP, 9 mM $MgCl_2$, 50 mM MES, pH 6.0, 10 mM NaN_3 and 0.5 - 1.5 mg protein membrane fraction. After 8 or 16 min the reaction was stopped by addition of 3 volumes of 1% (w/v) SDS. Inorganic phosphate was measured as described by Pullman & Penefsky [38]. Protein was deter-

[1]Abbreviations: MES, 2-[*N*-morpholino]ethanesulfonic acid; SDS, sodium dodecyl sulfate; OLI, oligomycin was purchased from Sigma; VEN, venturicidin was from Upjohn and Glaxo; EBR, ethidium bromide and CYH, cycloheximide were from Boehringer; CHL, chloramphenicol was from Serva; DMG, decamethylenediguanidine was from Schering; VAN, sodium orthovanadate was from Aldrich; HYB, hygromycin B was from Sigma; XBAG, *N,N'*-(*p*-xylylidene)-bis-aminoguanidine-2HCl was kindly donated by the Department of Drug Synthesis from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland.

Table 1
Strains used

Strain	Ploidy	Genotype	Isogenicity*	Reference
Σ128b	n	α *PMA1*	isogenic	[2]
MG2130	n	α *pma1-1*	isogenic	[2]
MG2132	n	α *pma1-1*	isogenic	[2]
20-584A	n	a *pma1*	isogenic	[2]
20-584B	n	a *pma1*	isogenic	[2]
20-584C	n	α *PMA1-1*	isogenic	[2]
20-584D	n	a *PMA1*	isogenic	[2]
22-295C	n	a *PMA1 ura3*	isogenic	[7]
X2928-3D	n	α *gal1 ade1 leu1 his2 ura3 trp1*	not isogenic	Donner Laboratory Berkeley
X901-35C	n	a *gal2 ade6 leu1 his6 ura1 trp5 lys1 met1 arg4-1 thr1 hom2 aro1C*	not isogenic	Donner Laboratory Berkeley
US7-15D	n	a *leu1 ade6 trp5*	half isogenic	Cross MG2130 × X901-35C
IL125-2b	n	α *his1 [ome$^-$C^{S}E^{S}O^S]*	isogenic only to DR19/T8	[3]
DR19/T8	n	α *pdr1-3 his1 [cir$^+$ome$^-$ rho$^+$]*	isogenic only to IL125-2b	[27]
GR359	n	a *pdr1-1 his6 met8-1 trp1 [ery^R cir$^+$ rho$^+$]*	isogenic only to GR350	[28, 29, 33] ATCC42879
2-20	n	a *pdr1-2 ade2-1 his1-480 lys1 met8-1 SUP4-3 [cir$^+$ psi$^+$ rho$^+$]*	not isogenic	[28, 29, 33] ATCC42880
GR350	n	a *his6 met8-1 trp1 [ery$^+$ cir$^+$ rho$^+$]*	isogenic only to GR359	[28, 29, 33]
US27-74A	n	a *pma1 pdr1-2 lys1 met8-1*	half isogenic	US27 (this paper)
US51-10B	n	α *ura3*	half isogenic	Cross 22-295C × DRI9/T7
US62-24B	n	a *pma1 pdr1-1 ura3 leu1*	half isogenic	Cross US13-25-6B × US53-19A
US6	2n		half isogenic diploid	Cross 20-584A × X2928-3D
US7	2n		half isogenic diploid	Cross MG2130 × X901-35C
US9	2n		half isogenic diploid	Cross 20-584B × DRI9/T8
US11	2n		half isogenic diploid	Cross 20-584C × DRI9/T8
US12	2n		isogenic	Cross 20-584B × 20-584D
US13	2n		near isogenic	Cross MG2130 × US7-15D
US26	2n		half isogenic	Cross 20-584A × GR359
US27	2n		half isogenic	Cross 20-584A × 2-20
US36	2n		half isogenic	Cross US26-70D × Σ1278b
US53	2n		half isogenic	Cross US51-10B × GR359
US82	2n		half isogenic	Cross Σ1278b × GR350
US83	2n		half isogenic	Cross 22-295C × IL125-2b

*Isogenicity related to Σ1278b wild type parental strain.

mined by the method of Lowry *et al.* [39] using bovine serum albumin as a standard.

RESULTS

Drug resistance in *pma1* and *pdr1* strains

Four spontaneous *pma1* mutants were previously selected from the wild-type strain Σ1278b on the basis of their ability to grow on glucose-proline minimal medium containing 10 µg Dio-9/ml or on glucose-ammonia minimal medium containing 100 µg Dio-9/ml [1]. Since Dio-9 is no longer commercially available, the biological activity of the new ATPase inhibitor *N,N'*-(*p*-xylylidene)-bis-aminoguanidine-2HCl (XBAG) was studied [7, 40].

The growth of the parental strain Σ1278b was completely inhibited at XBAG concentrations above 500 µg/ml, whereas all *pma1* strains grew well at 650 µg/ml. This increased resistance *in vivo* to XBAG did not affect ATPase activity in the isolated plasma membranes (not shown). The drug resistance of the haploid strains *pma1* and *pdr1* listed in Table 2 was evaluated at different concentrations of XBAG and 6 other inhibitors. As previously reported by Rank [26], the pleiotropic drug resistant strains GR359 *(pdr1-1)* and 2-20 *(pdr1-2)* showed resistance to OLI, VEN, CHL and CYH, whereas the strain GR350 *(PDR1)* a complete revertant of GR359 was sensitive. However, all these strains were sensitive to 500 µg XBAG/ml. Some differences were observed among the three *pdr1* strains. The *pdr1-2* mutant was less resistant to CHL than *pdr1-1*. The resistance of the *pdr1-3* strain DRI9/T8 isolated by Guerineau *et al.* [27] was generally similar to that of 2-20 *(pdr1-2)*, except that its resistance to CYH was higher and that to OLI and CHL was lower. In addition, slightly higher sensitivity to XBAG and also to DMG and EBR was observed in DRI9/T8.

Compared to their parental strain Σ1278b, the *pma1* strains MG2130, MG2132 and 20-584A showed similar sensitivity to VEN, CHL and CYH and increased sensitivity to OLI and CYH. However, they were resistant to XBAG, EBR, DMG and HYG.

The CYH^R trait is thus a representative marker for the *pdr1* mutants, whereas $XBAG^R$ is a convenient marker for the *pma1* mutants.

Interactions between *pma1* and *pdr1* mutated genes

Table 3 shows that the CYH^R phenotype expressed in *pdr1* haploids is observed at a reduced level in heterozygous *PDR/pdr* diploids. The CYH^R phenotype is thus semi-dominant. Therefore, complementation can not be used for allelism tests between different *pdr1* mutations. The presence of the *pma1-1* gene, when associated to the *pdr1-1*, *pdr1-2* or to *pdr1-3* in diploids, further lowers the CYH resistance. The $XBAG^R$ phenotype of the *pma1* haploid strain is also expressed in *PMA1/pma1* hete-

Table 2

Drug resistance of haploid strains

Growth of different strains was tested on YEPD or YEPG* agar plates containing the inhibitors indicated as described in Materials and Methods

Strain	Genotype	Highest non-inhibitory drug concentration						
		OLI*	VEN*	CHL*	CYH	EBR	DMG	XBAG
		µg/ml	µg/ml	mg/ml	µg/ml	µg/ml	µg/ml	µg/ml
GR350	*PDR1*	< 0.1	0.1	< 1.0	> 0.1	> 75	> 100	500
GR359	*pdr1-1*	> 1.0	> 10	4.0	1.0	> 75	> 100	< 500
2-20	*pdr1-2*	> 1.0	> 10	< 4.0	1.0	> 75	> 100	< 500
IL125-2b	*PDR1*	< 0.05	< 0.1	1.0	0.1	> 100	> 50	500
DRI9/T8	*pdr1-3*	0.25	> 10	4.0	> 1.0	> 50	< 50	< 500
Σ1278b	*PMA1*	< 0.1	0.1	> 1.0	< 0.1	25	> 50	500
MG2130	*pma1*	0.1	0.1	> 1.0	< 0.1	> 75	< 100	700
MG2132	*pma1*	0.1	0.1	> 1.0	< 0.1	> 75	> 100	700
20-584A	*pma1*	0.1	0.1	> 1.0	< 0.1	> 75	> 100	700

Table 3

Drug resistance of different pma1 and pdr1 diploid strains

Three not isogenic wild type *PDR1* strains were used. $PDR1^a$ was from GR350, $PDR1^b$ from Σ1278^b and $PDR1^c$ from IL125-2b.

Strain	Genotype	Highest non-inhibitory drug concentration	
		CYH µg/ml	XBAG µg/ml
US12	*pma1* $PDR1^b$ / *PMA1* $PDR1^b$	< 0.10	600
US26	*pma1* $PDR1^b$ / *PMA1 pdr1-1*	> 0.50	600
US53	*PMA1* $PDR1^b$ / *PMA1 pdr1-1*	< 0.50	< 500
US27	*pma1* $PDR1^b$ / *PMA1 pdr1-2*	0.25	600
US9	*pma1* $PDR1^b$ / *PMA1 pdr1-3*	0.25	600
US11	*PMA1* $PDR1^b$ / *PMA1 pdr1-3*	> 0.50	< 500
US82	*PMA1* $PDR1^b$ / *PMA1* $PDR1^a$	0.10	500
US83	*PMA1* $PDR1^b$ / *PMA1* $PDR1^c$	0.10	500

rozygous diploids (Table 3). In this case also, the level of resistance is intermediate between those of the *PMA1* and *pma1* haploids. The $XBAG^R$ seems thus semidominant as previously reported [2] for the DIO^R phenotype of *pma1*.

As reported in Table 4, the diploids US26 and US27 derived from the cross 20-584A *(pma1-1)* with GR359 *(pdr1-1)*, or 2-20 *(pdr1-2)* gave an overall monogenic 2:2 segregation for CYH^R OLI^R VEN^R and $XBAG^R$ with few 3R:1S or 1R:3S tetrads. From the 242 tetrads screened, only 11 gave a 1R:3S segregation ratio for $XBAG^R$. Four of them, two from each diploid, were further analysed for *in vitro* vanadate resistance of ATPase activity. They showed a 1R:3S cosegregation *in vivo* with $XBAG^R$ and 1R:3S cosegregation *in vitro* with VAN^R, suggesting interaction *in vivo* between *pma1* and *pdr1* double mutants. In all cases the CYH^R, OLI^R and VEN^R spores were $XBAG^S$. It was concluded that in these tetrads, the recombination occurred between *pma1* and *pdr1*, yielding double *pma1 pdr1* mutants. In these haploid double mutants, the $XBAG^R$ phenotype of *pma1* was totally suppressed *in vivo* and *pma1* VAN^R *in vitro* was also totally or partially suppressed by the presence of *pdr1-1* or *pdr1-2* mutations (Fig. 1). The diploid US9 from the cross 20-584B *(pma1)* x DRI9/T8 *(pdr1-3)* also gave a 2:2 segregation for CYH^R and $XBAG^R$ but irregular segregations were slightly more frequent than for US26 and US27. The presence of a tetratype was evidenced by the observed 1R:3S segregation of $XBAG^R$, by analogy to the above mentioned crosses US26, US27 between *pma1* and *pdr1-1*. The same *pma1* allele controlling XBAG resistance exhibited standard Mendelian inheritance in a control cross of US12 and US13.

Plasma membrane H^+-ATPase activity in *pma1* and *pdr1* mutants

In contrast to the wild type and to the *pdr1* mutants, the ATPase activity of the *pma1* mutants was resistant to vanadate (Fig. 1). The progenies of heterozygous cross of *pma1* mutant with wild type (diploid US13 listed in Table 4) exhibited in 52 tetrads a clear 2:2 Mendelian segregation of $XBAG^R$. The *in vivo* $XBAG^R$ and *in vitro* VAN^R traits cosegregated in the 16 tetrads tested [7].

Table 4

Meiotic segregation of XBAG drug resistance

The strains were tested on 650 µg/ml XBAG. All tetrads segregated 2:2 for auxotrophic markers, except in some tetrads issued from the diploid US27 where the amber suppressor *SUP4-3* influenced this segregation. Three not isogenic wild type *PDR1* strains were used. *PDR1*^{a} was from GR350, *PDR1*^{b} from Σ1278b and *PDR1*^{c} was from IL25-2b. The number of tetrads for the given segregations are indicated in brackets. The resistance (R) and sensitivity (S) to OLI, CYH and VEN usually segregated $2^R:2^S$ as excepted for a true heterozygote, without statistically significant deviation.

Diploids	Crosses	Number of tetrads	Segregation for resistance to XBAG (R:S)
US9	*pma1 PDR1*^{b} × *PMA1 pdr1-3*	80	2 : 2 (75) 3 : 1 (3) 1 : 3 (2)
US12	*pma1 PDR1*^{b} × *PMA1 PDR1*^{b}	44	2 : 2 (44)
US13	*pma1 PDR1*^{b} × *PMA1 PDR1*^{a}	52	2 : 2 (51) 3 : 1 (1)
US26	*pma1 PDR1*^{b} × *PMA1 pdr1-1*	140	2 : 2 (136) 1 : 3 (4)
US27	*pma1 PDR1*^{b} × *PMA1 pdr1-2*	102	2 : 2 (94) 3 : 1 (1) 1 : 3 (7)
US83	*PMA1 PDR1*^{b} × *PMA1 PDR1*^{c}	60	0 : 4 (60)

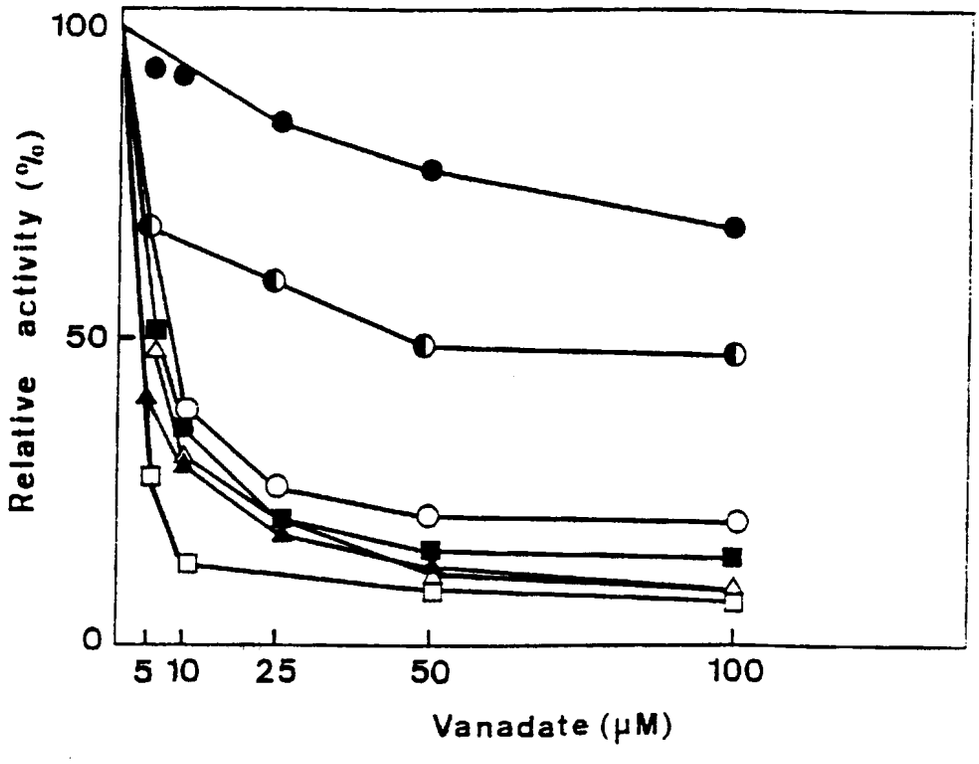

Fig. 1. Effect of vanadate on the ATPase activity in crude membrane fractions of pma1, and pma1 pdr1 mutants.

The strains 20-584A, US62-24B, US27-74A, 2-20, GR359, DRI9/T8 and Σ1278b (Table 1) were used for *pma1-1* (●), *pma1-1 pdr1-1* (◐), *pma1-1 pdr1-2* (○), *pdr1-2* (■), *pdr1-1* (△), *pdr1-3* (▲) and *PMA1 PDR1* (□), respectively. The activity is presented as percentage of the ATPase activity in the absence of vanadate. Specific plasma membrane ATPase activities in the presence or absence of 50 µM vanadate are listed in Table 5. For details see Materials and Methods.

Table 5

Plasma membrane H^+-ATPase activity and vanadate effect in pma1, pdr1 and pma1 pdr1 haploid strains

Strain	Genotype	ATPase specific activity* (μmol Pi $\times$ $min^{-1} \times mg^{-1}$)			
		Vanadate-sensitive		Total	
		exp. 1	exp. 2	exp. 1	exp. 2
Σ1278b	*PMA1 PDR1*	0.57	0.70	0.63	0.77
20-584A	*pma1*	0.07	0.08	0.34	0.36
GR350	*PMA1 PDR1*	0.62	0.65	0.70	0.73
GR359	*pdr1-1*	0.61	0.75	0.70	0.85
2-20	*pdr1-2*	0.46	0.58	0.61	0.80
IL125-2b	*PMA1 PDR1*	0.36	0.47	0.56	0.64
DRI9/T8	*pdr1-3*	0.24	0.25	0.28	0.32
US27-74A	*pma1 pdr1-2*	0.25	0.40	0.32	0.48
US62-24B	*pma1 pdr1-1*	0.09	0.20	0.18	0.38

*ATPase assays (see Materials and Methods) were carried out in the absence (Total) and presence of 50 μM vanadate in crude membrane fractions from two separate cultures (experiment 1 and 2). Vanadate-sensitive is the total ATPase activity minus that observed in the presence of vanadate.

Table 5 shows that in the strains *pdr1-1* and *pdr1-2* the vanadate sensitive ATPase activity was in the same range as that of their isogenic strain GR350.

The vanadate sensitive ATPase activity of *pdr1-3* was slightly lower (0.24 - 0.25 μmol Pi $\times$ $min^{-1} \times mg^{-1}$) than that of the parental strain IL125-2b (0.36 - 0.47 μmol Pi $\times$ $min^{-1} \times mg^{-1}$). However, the low ATPase activity associated with *pdr1-3* is probably due to a mutation in a gene other than *pdr1-3* (not shown).

Table 6 shows that the diploids US26, US9 and US27 containing *pma1* and either *pdr1-1*, *pdr1-2* or *pdr1-3* alleles exhibited lower ATPase activity (0.07 to 0.19 μmol Pi $\times$ $min^{-1} \times mg^{-1}$) than the diploids *pma1/PMA1*, *pdr1-1/PDR1* and *pdr1-3/PDR1* (0.33 to 0.69 μmol Pi $\times$ $min^{-1} \times mg^{-1}$). It thus appears that, when present in *trans* configuration, *pdr1-1*, *pdr1-2* or *pdr1-3* tends to lower the level of vanadate-sensitive ATPase activity of a *pma1* heterozygote, while neither the *pdr1* mutations nor the *pma1* mutation alone markedly affect the ATPase activity in heterozygous diploids.

DISCUSSION

The mutations $oli^{PR}1$-1, $oli^{PR}1$-2 and DRI9/T8 obtained originally as resistant to oligomycin were redesignated by Saunders & Rank [33] as pleiotropic drug resistances *pdr1-1*, *pdr1-2* and *pdr1-3*, respectively. More recently, the hypothesis that the *pdr1-1*, *pdr1-2* and *pdr1-3* mutations occur in the same *PDR1* locus was validated by Balzi *et al.* [3] who cloned and sequenced the *PDR1* gene and proposed that its product acts as a transcription regulator.

The data reported elsewhere by Ułaszewski *et al.* [7] and in the present paper show that the ATPase mutants *pma1* also exhibit multiple drug resistance but have a phenotype quite different from that of *pdr1*. The *pma1* mutants which were originally shown to be resistant to Dio-9, D-lysine (tested in the presence of proline as a nitrogen source) and miconazole are now found to be also resistant to *N,N'*-(*p*-xylylidene)-bis-aminoguanidine-2HCl, ethidium bromide and synthalin. In contrast, the *pdr1* mutants are resistant to oligomycin, venturicidin, chloramphenicol and cycloheximide, and show a slightly enhanced sensitivity to the drugs to which *pma1* is resistant. Furthermore, the *pma1* ATPase is resistant to vanadate *in vitro* while the *pdr1* ATPase is vanadate sensitive.

The presented results suggest that *pdr1* mutations might also exert some control on ATPase activity since all *pdr1* mutations reduce the enzyme activity when combined with *pma1* in diploids in *trans* configuration. Moreover, interactions between *pma1* and *pdr1* mutations are also indicated by interferences in drug re-

Table 6

Plasma membrane H^+-ATPase activity in pma1 and pdr1 diploid strains

Strain	Genotype	ATPase specific activity* (μmol Pi × min^{-1} × mg^{-1})			
		Vanadate-sensitive		Total	
		exp. 1	exp. 2	exp. 1	exp. 2
US53	*PMA1 PDR1*^{b} *PMA1 pdr1-1*	0.56	0.69	0.63	0.79
US6	*pma1 PDR1*^{b} *PMA1 PDR*	0.31	0.35	0.58	0.558
US26	*pma1 PDR1*^{b} *PMA1 pdr1-1*	0.10	0.16	0.18	0.30
US11	*PMA1 PDR1*^{b} *PMA1 pdr1-3*	0.33	0.33	0.37	0.44
US9	*pma1 PDR1*^{b} *PMA1 pdr1-3*	0.11	0.19	0.21	0.23
US27	*pma1 PDR1*^{b} *PMA1 pdr1-2*	0.07	0.09	0.19	0.20
US82	*PMA1 PDR1*^{b} *PMA1 PDR1*^{a}	0.53	0.61	0.59	0.66
US83	*PMA1 PDR1*^{b} *PMA1 PDR1*^{c}	0.41	0.44	0.46	0.49

*Three not isogenic wild type *PDR1* strains were used. *PDR1*^{a} was from GR350, *PDR1*^{b} from Σ1278b and *PDR1*^{c} from IL125-2b. ATPase assays (see Materials and Methods) were carried out in the absence (Total) and presence of 50 μM vanadate in crude membrane fractions from two separate cultures (experiment 1 and 2). Vanadate-sensitive is the total ATPase activity minus that observed in the presence of vanadate.

sistance. In diploids, the combination of *pma1* and *pdr1* alleles decreases the resistance to CYH as compared to the diploid containing only *pdr1*. Furthermore, in the haploid double mutant *pma1 pdr1*, the *in vivo* $XBAG^R$ and *in vitro* VAN^R phenotype of *pma1* are totally or partially suppressed by *pdr1* mutation.

Balzi *et al.* [3] reported that the deduced amino-acid sequence of the *PDR1* polypeptide resembles that of several nucleic acid-binding proteins involved in the control of gene expression in Eucaryotes. The mRNA transcript of the *PDR5* gene is overexpressed in *pdr1-3* mutant [37]. This points to a control by a regulatory protein of several permeability functions responsible for multiple drug resistance in yeast. It has been previously proposed that *PDR1* could be either a positive regulator of drug efflux or a negative regulator of drug influx [35].

The predicted *PDR1* polypeptide may modify the expression of various target genes *PDR4, PDR5, STE6, ATR1* and *ADP1*, mediating multiple drug resistance in yeast [35] including the plasma membrane ATPase gene *PMA1* (this paper). Resistance in yeast *ATR1* and a decreased expression of *PMA1* could in turn modify the cellular uptake of drugs and therefore induce drug resistance *in vivo*. Strong support for this model has been brought previously by Perlin *et al.* [15] who have shown that *pma1* mutants exhibit a reduced membrane potential, and by Capieaux *et al.* [41] who have shown that deletions in the promotor region of *PMA1* produce strains with decreased ATPase activity and concomitant increased resistance to hygromycin B. Finally, we can not exlude the possibility that *pdr1* mutations might also induce either a new plasma membrane [42] or vacuolar ATPase activity [43] interfering for example with the *PMA1* gene product activity in the haploid double mutant *pma1 pdr1*. The nature of the interactions among different determinants of multiple drug resistance in yeast should now be studied at the molecular level.

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