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Genetics and biochemistry of yeast multidrug resistance

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1. Introduction

The development of resistance to a wide range of unrelated cytotoxic compounds is a common phenomenon observed in various organisms throughout the evolutionary scale.

Multidrug resistance is a major concern in medical and agricultural developments. In medicine, the emergence of resistance to multiple drugs commonly used in therapy is a major obstacle in the treatment of several tumours as well as of diverse diseases such as malaria, tuberculosis and various bacterial and fungal infections which often complicate major debilitating syndromes like AIDS. In agriculture, the control of resistance of plant pathogens towards natural plant defence toxins and towards common fungicides, as well as the development of parasite-toxins resistant crops, are of major economic importance.

In yeast, studies on multidrug resistance received a recent impetus, not only because of the involvement of some yeast species in pathogenicity for men and plants, but also because yeast is an universal, easy-to-manipulate model system for the study of higher eukaryotic cells. In a previous review [4] we have extensively treated the history of the studies on yeast pleiotropic (or multi) drug resistance (PDR) during the last two decades. A considerable amount of new information has since expanded our view on the molecular genetics of the yeast PDR system. It is the aim of this mini-review to update briefly our current knowledge on genetic networks, and biochemical mechanisms, underlying yeast multidrug resistance.

2. Determinants of yeast multidrug resistance

In *Saccharomyces cerevisiae*, *Schizosaccharomzces pombe* and *Candida albicans*, the known genetic determinants associated with multidrug resistance are at present no fewer than fifteen. The encoded gene products identified so far can be arranged in three major classes: membrane proteins belonging either to the ABC (ATP-Binding-Cassette) or to the MFS (Major Facilitators Superfamily) transport superfamilies and factors for transcription regulation.

2.1. Transporters of the 'ABC' type

The ATP-binding-cassette (ABC) [27] or traffic ATPases [2] superfamily comprises a large number of membrane proteins, conserved from bacteria to man. These proteins, or protein complexes, share a common architecture consisting of four domains, or subunits, two hydrophobic comprising each six transmembrane spans (TM), and two hydrophilic including a conserved cassette of about 200 amino acids with ATP-binding motifs (NBF, nucleotide binding fold). Eukaryotic members of the ABC superfamily display either a duplicated structure, consisting of four alternating domains, or a 'single half' structure, consisting of only one single transmembrane domain fused to a nucleotide binding fold; these 'half-sized' ABC proteins are thought to function upon dimerization. Some ABC proteins are unique in their mechanism since they seem to function both as an ATPase driven pump and as an ATP-dependent ion channel (review, Ref. [27]). Mammalian proteins such as the plasma membrane P-glycoprotein, responsible for multidrug resistance of cancer cells (review, Ref. [32]), and the transmembrane conductance regulator factor CFTR, a chloride channel responsible for cystic fibrosis (review, Ref. [18]),

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belong to the four-domains ABC class; others such as the peroxisomal proteins ADL, determinant of adrenoleukodystrophy [47], and pMP70 [33], determinant of Zellweger syndrome [21], as well as the endoplasmic reticulum proteins TAP, responsible for antigens transport [46], are 'half sized' members of the ABC superfamily.

The first ABC-type protein reported from the yeast *Saccharomyces cerevisiae* was the *STE6* gene product, a plasma membrane pump responsible for the secretion of the mating pheromone **a** [44,39]. The STE6 protein is composed of two homologous halves, each with a transmembrane region followed by the ABC domain, similar to the mammalian P-glycoprotein. The analysis of *STE6* led to the first elucidation of the physiological transported substrate of an eukaryote ABC transporter. *STE6* was not found to confer, upon amplification, resistance to multiple drugs, including cycloheximide, oligomycin, chloramphenicol, benomyl, nocodazole, doxorubicin and trichodermin [44]; however, increased resistance to valinomycin, a potassium ionophore collapsing membrane potential, was reported to be associated to overexpression of *STE6* [39]. Interestingly, mammalian *mdr* genes, encoding P-glycoproteins, were shown to complement yeast *ste6* null mutants and thus to be able to replace the STE6 function of secreting the pheromone **a** in yeast [38,56]. Moreover, the mouse MDR3 P-glycoprotein was shown to function as a fully active drug transporter in yeast [57], underscoring the value of the yeast system for the study of the mechanism of action of P glycoproteins in general.

The first reported ABC transporter gene with an established role in yeast multidrug resistance is *SNQ2* [25,61]. In multicopy the *SNQ2* gene confers resistance to mutagens such as 4-nitroquinoline *N*-oxide (NQO) and triaziquone as well as to sulfomethuron methyl, an inhibitor of the acetolactate synthetase, and to phenantroline. Null *SNQ2* mutants are viable but hypersensitive to NQO [25]. *SNQ2* encodes a putative 169 kDa four-domain ABC protein, similar to the Brown pigment-transport protein of *Drosophila* [61]. The SNQ2 protein displays also a duplicated structure, but, at difference with STE6 and like PDR5, the hydrophylic domains are located at the N-terminal side of the transmembrane spans. The presence of a putative proteolysis stimulating PEST region was observed in the center of SNQ2, raising the (very hypothetical) possibility of a cleavage of the protein in two halves, each constituting a functional subunit [61].

The best characterized yeast pleiotropic drug resistance ABC transporter is the product of the *PDR5* gene. *PDR5* was isolated through its property of conferring, upon amplification, resistance to cycloheximide and sulfomethuron methyl [42]. Disruption of *PDR5* is not lethal but correlated to hypersensitivity to various drugs such as cycloheximide, sulfomethuron methyl and also to the mitochondrial inhibitors chloramphenicol, lincomycin, erythromycin and antimycin [42,45]. *PDR5* was found to encode a duplicated ABC protein, consisting of the repeated alternance of two hydrophilic domains, with an ATP binding cassette, and two hydrophobic domains, with six transmembrane spans, each highly similar to the *SNQ2* gene product [5]. The *PDR5* gene transcript was found to be overexpressed in mutants of another pleiotropic drug resistance locus, *PDR1*, encoding a putative transcription regulator [45,5]. Subsequently, the *PDR5* mRNA was found to be increased also in mutants of other *PDR* loci [17], such as *PDR3*, encoding a transcription regulator homologous to PDR1, as well as *PDR7* and *PDR9*, described further below. The PDR5 protein was found to be overexpressed in the plasma membranes of *pdr1* [5,14] and *pdr3* mutants [14]. These data support the hypothesis that the PDR1 and PDR3 factors would transcriptionally control the expression of the *PDR5* gene, the product of which would be responsible for the active pumping of drugs, and possibly other physiological substrates, out of the cell. The *pdr1* and *pdr3*, mutations, found to confer drug resistance by hyperactivation of the expression of the multidrug pump PDR5, represent the first reported identification of primary genetic sites of lesions leading to overexpression of multidrug resistance pumps.

The *PDR5* gene has been independently reisolated, and renamed *STS1* and *YDR1* respectively, by resistance to either sporidesmin, a mycotoxin pathogenic for men and ruminants [6], or to cerulenin and cycloheximide [28]. The *PDR5(STS1)* gene transcript was reported to be reduced in α-factor arrested cells indicating that the transcription of *PDR5* might be under hormonal control. More specifically, *PDR5* was suggested to be under negative control of the α-factor, in contrast to *STE6* which is positively regulated by the same pheromone α [6]. The transcription of *PDR5(YDR1)*, as well as of its homologue *SNQ2*, was also shown to be induced by stress conditions, such as heat-shock and the presence of drugs, both relevant or irrelevant to the resistance caused by amplification of the *PDR5* gene [28]. A possible role for the *PDR5(YDR1)* gene product was proposed to be the externalization of cytotoxic compounds or metabolites which accumulate during growth and especially in response to environmental stress [28]. Distinct cross-resistance patterns were observed for *PDR5(STS1, YDR1)* and its close homologue *SNQ2*: the overexpression of *PDR5* being specifically correlated to resistance to sporidesmin, cycloheximide [6] cerulenin and compactin [28], while the overexpression of *SNQ2* was associated to resistance to NQO and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidin [25,6,28]. Nevertheless, further analysis revealed that *PDR5(YDR1)* and *SNQ2* share

some overlapping specificities, for staurosporine and fluphenazine [28]. It was also shown that the *STE6* gene could not, despite its structural homology, replace the drug resistance related function of *PDR5(YDR1)* and *SNQ2*; vice versa, *PDR5* and *SNQ2* could not overcome the mating defect due to *STE6* disruption [28].

The PDR5 protein was solubilized and partially purified from the plasma membranes of the naturally PDR5-overexpressing *pdr1* mutants [14]. PDR5 was shown to hydrolyse nucleoside diphosphates and triphosphates. This activity was sensitive to vanadate, oligomycin and various hydrophobic compounds, similar to the features described for the mammalian multidrug resistance P-glycoprotein [14]. The PDR5 protein was also shown to pump Rhodamine 6G in isolated plasma membrane preparations (Kolaczkowski et al., unpublished observations). The subcellular localization of PDR5 in both plasma membranes [5,14,28] and internal membranes, mainly ER and Golgi [6], has been proposed. More refined procedures such as immuno electromicroscopy should provide more conclusive data.

Very recently, the existence of a new *PDR5* homologue, localized on the same right arm of chromosome XV as *PDR5*, has been revealed (Wolfe, K., personal communication). Interestingly, two drug resistance loci, *PDR2* (pleitropic drug resistance) and *SMR3* (resistance to sulfomethuron methyl), had been previously mapped at 5.8 cM and 10.9 cM from PDR5, respectively, on chromosome XV [42].

A homologue of *S. cerevisiae PDR5*, designated *CDR1*, has been very recently isolated from *Candida albicans* and shown to be involved in multidrug resistance (Prasad et al., unpublished data; EMBL accession X77589).

A new yeast four-domain ABC protein, YCF1, which displays a striking homology to the human cystic fibrosis factor CFTR, including the presence of an extensive putative regulatory domain within the hydrophilic domains, has been identified [66]. The *YCF1* gene confers resistance to cadmium when overexpressed and is likely to be transcriptionally controlled by the yeast yAP-1 multidrug resistance regulator [72].

On the first two *S. cerevisiae* chromosomes entirely sequenced in the frame of the European project of systematically sequencing the yeast genome, several new open reading frames encoding ABC proteins have been discovered. On chromosome III, the first 'half-sized' yeast ABC protein has been found to be encoded by the *YCR105* open reading frame and has been designated ADP1 (ATP Dependent Permease) [55]. The ADP1 topology consists of one single ATP-binding cassette followed by one six-transmembrane-helix domain, closely related to the White pigment transporter of *Drosophila*. In addition, ADP1 possesses in its NH_2 terminus a repeated cysteine-rich epidermal growth factor motif, unique among the known ABC proteins. On chromosome XI, two open reading frames, *YKR103* and *YKR104*, separated by a single in-frame stop codon and homologous respectively to the N- and C-terminal parts of the P-glycoprotein, have been uncovered [19]. In addition, the open reading frame *YKL741* [7] was found to encode a 97 kDa homologue of the human ADL protein, responsible for the X-linked disease adrenoleukodystrophy [47], and of the rat peroxisomal membrane protein PMP70, involved in peroxisome biogenesis [33]. These three proteins share significant sequence homology and a common structure, mirroring the one of ADP1 and consisting of one N-terminal hydrophobic domain, with six predicted transmembrane spans, followed by one C-terminal hydrophilic domain with an ATP-binding cassette [7].

Novel ABC homologs encoding genes have been also identified by polymerase chain reaction amplification-based approaches. Two *s*terile *six* *h*omologues, *Ssh1* and *Ssh2*, both residing on chromosome XVI, were isolated and the sequence of their ATP-binding cassette region was reported [37]. The *Ssh1* gene was predicted to encode a non essential 'half size' ABC transporter; *Ssh2* was observed to encode a protein highly similar to the mammalian peroxisomal membrane proteins Pmp70 [37]. Moreover, two *m*ulti*d*rug resistance *l*ike proteins, MDL1 and MDL2, were identified as products of non-essential genes located on chromosomes XII and XVI, respectively [13]. Both encode 'half-sized' ABC proteins, considerably similar to the mammalian MDR and to the antigen transport proteins TAP of the endoplasmic reticulum [13]. The *MDL2* encoded sequence is identical to the ABC domain sequence of Ssh1, suggesting that *Ssh1* and *MDL2* are the same gene. Finally, eight new genes encoding ATP-binding cassette proteins have been independently isolated by PCR amplification of the yeast genomic DNA, one of them, ATM1, being essential and located in mitochondria (Ref. [41] and Leighton and Schatz, personal communication). Further analysis will be required to establish the role of these new *S. cerevisiae* ABC proteins, with respect to their implication in multidrug resistance and related transport processes.

Finally, in the fission yeast *Schizosaccharomyces pombe*, at least two gene products belonging to the ABC superfamily have been described. HMT1 is a 'single half' ABC protein associated with the vacuolar membrane and most similar to the mammalian P-glycoprotein. Overexpression of the *hmt1* gene was correlated to enhanced heavy metal tolerance and increased intracellular accumulation of cadmium. This suggested that HMT1 plays a role in intracellular compartimentalization of heavy metals rather than cellular efflux [53]. The *pmd1*$^+$ gene encodes a 'double half' ABC

protein homologous to MDR and STE6 [49]. Overexpression of *pmd1*$^+$ confers resistance to leptomycin B, cycloheximide and valinomycin; the two latter resistances can be reduced by addition of verapamil, a competitive inhibitor of the mammalian P-glycoprotein. Despite the homology to STE6, pmd1 was not found to be essential for transport of pheromones, since null *pmd1*$^-$ mutants did not show decreased mating abilities [49].

2.2. Transporters of the 'MFS'-type

The Major Facilitators Superfamily comprises over 50 transporters from bacteria and eukaryotes, catalysing the specific uni-, sym- and antiport of sugars, organic acids or drugs. These proteins share a common topology consisting of two-times six transmembrane-helical segments separated by a dispensable central cytoplasmic loop which does not contain the ATP binding cassette [43]. A binary comparison of their sequences [43] has allowed their classification into five distinct families, one of which clusters proteins involved in multidrug resistance, including several bacterial pumps for resistance to various antiseptics like tetracycline [1]. These proteins are also closely related to the mammalian vesicular transporters of the neurotransmitters monoamines, which may also function as multidrug transporters [60]. It has been suggested that all these proteins would function as antiporters exchanging one or more H^+ ions with a substrate molecule [60]. In yeast, at least 12 gene products from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans* and *Candida maltosa* may be classified within the MFS.

The gene *ATR1* was found to confer, when present in multiple copies, resistance to aminotriazole, an inhibitor of the histidine biosynthetic pathway [35]. Overexpression of *ATR1*, and the accompanying aminotriazole resistance, were inversely proportional to the intracellular concentration of aminotriazole. The *ATR1* gene product, a 547 amino acids polypeptide with 12 membrane-spanning regions and a C-terminal weak consensus for an ATP-binding site, was predicted to be a membrane-bound pump for the efflux of aminotriazole and possibly of toxic amino acid precursors [35]. *ATR1* was reported not to be involved in resistance to cycloheximide, chloramphenicol, daunomycin (inhibitors of protein synthesis), oligomycin, antimycin (inhibitors of mitochondrial electron transport), vinblastin (inhibitor of mitosis) triazolealanine, ethionine, canavanine and *p*-fluorophenylalanine (inhibitors of histidine, methionine, arginine and phenylalanine biosynthesis, respectively). However, subsequently reisolated under the name *SNQ1*, the same *ATR1* gene was associated with cross-resistance to 4-nitroquinoline *N*-oxide, a promutagen interacting with DNA, upon intracellular activation [24]. Loss of ATR1 function, by specific deletion of the C-terminal ATP-binding site, caused hypersensitivity to aminotriazole and NQO [35,24]. The transcription of *ATR1* was found to be induced by the presence of aminotriazole, or better by the amino acid starvation conditions caused by aminotriazole, and not by the presence of NQO [35,24].

Three more MFS proteins were found to be encoded on chromosome III of *S. cerevisiae* [23]: YCL 069w and YCR023c showing homology to the bacterial tetracycline resistance gene and YCL070c showing homology to the yeast ATR1 [64]. On chromosome XI, homologues of YCLO69w or YCLO70c were found and designated respectively YKR105c or YKR106w [19]. Further analysis will be required to establish whether these proteins influence resistance to drugs and related transport pathways.

In *Candida* and *Schizosaccharomyces pombe*, three genes were found to encode proteins with structural features typical of the MFS class and functionally involved in drug resistance. The *ORF1* gene from *Candida albicans* encodes a 63 kDa hydrophobic polypeptide involved in resistance to the anti-mitotic drug benomyl and to the dihydrofolate reductase inhibitor methotrexate [20]. Low copies of the *Candida ORF1* were found to be sufficient to confer resistance to benomyl and methotrexate in *S. cerevisiae*; *ORF1* was suggested to be responsible for the intrinsic resistance of *Candida* towards these two drugs. A highly related gene from *Candida maltosa* was reported to confer, when overexpressed, resistance to cycloheximide [58]. In *Schizosaccharomyces pombe*, a homologous gene, *car1*, was isolated by complementation of mutants resistant to amiloride, an inhibitor of various sodium transporters [31]. At difference with the majority of multidrug resistance transporters, the resistance phenotype was associated to disruption of the *car1* gene. Despite the homology to the two *Candida* resistance determinants, *car1* was not found to affect resistance to cycloheximide or methotrexate. The Car1 protein was proposed to carry out the uptake of an unknown substrate, possibly in symport with amiloride [31].

2.3. Transcription regulators

Two pleiotropic drug resistance loci, *PDR1* and *PDR3* were found to encode homologous transcription regulators belonging to the family containing a 'Zinc 2 Cysteine 6' co-ordination complex in the DNA binding domain [3,15]. Mutations of the *PDR1* locus were associated to resistance to more than 20 structurally unrelated inhibitors of both cytoplasmic and mitochondrial functions (review, Ref. [4]). The sequence of eight *pdr1* mutant alleles responsible for increased multidrug resistance has been recently established ([9] and Carvajal, unpublished data). Two mutations of the *PDR3* gene

were found to confer resistance to mucidin, an antifungal antibiotic inhibiting electron transfer, chloramphenicol and cycloheximide [65]. Amplification of the *PDR3* gene was correlated to increased resistance to cycloheximide and oligomycin [36,16]. The *PDR3* locus was recently described to be allelic to another pleiotropic drug resistance locus [36], previously denominated *PDR4*, whose mutation (originally *nra5*, renamed *pdr3–3*) was associated with resistance to cycloheximide, chloramphenicol, tetracycline and the vacuolar dye Neutral red (Ref. [54] and review, Ref. [4]). A fourth mutant allele of the (previous *PDR4*) *PDR3* gene was isolated as suppressor of the cycloheximide-hypersensitivity phenotype due to disruption of *PDR1* [17]. Functional cross-complementation of *pdr1* or *pdr3* mutants with the *PDR3* or *PDR1* gene respectively indicated functional overlapping between the two genes (Cybularz and Balzi, unpublished observations). The *PDR1* and *PDR3* gene products were found to regulate the expression of the *PDR5* gene, encoding an ABC-type drug efflux pump. Genetic interactions between *PDR1* and *PDR5* indicated that expression of the *PDR1*-dependent resistance to cycloheximide and chloramphenicol (but not to oligomycin, lincomycin and erythromycin) requires a functional *PDR5* gene [45]. Mutants of both *PDR1* and *PDR3* were reported to overexpress the *PDR5* mRNA [5,45] and encoded protein [5, 14]. *pdr1* mutants were also observed to abnormally express the mRNA of *STE6* [5] and the gp125 glycoprotein (Decottignies, Fey and Mose-Larsen, unpublished observations) anchored into the yeast plasma membranes [50,69]. Replacement of a mutant *pdr1* allele with a null *pdr1* allele, and double disruptions of *PDR1* and *PDR3* were found to decrease markedly the *PDR5* transcription levels [45,36]. Disruption of *PDR1* led to hypersensitivity to cycloheximide and oligomycin [3,16,36]. Isogenic disruptions of *PDR3* had no detectable effect on cycloheximide tolerance [16,36] but somehow increased sensitivity to oligomycin [16]. Double disruptions of both *PDR1* and *PDR3* had a dramatic effect of increased sensitivity to both cycloheximide and oligomycin compared to single disruptants [16,36]. The cycloheximide hypersensitivity of the double *PDR1 / PDR3* disruptant was similar to the phenotype observed for a *PDR5* disruptant. In contrast, hypersensitivity to oligomycin was not correlated to disruption of *PDR5*. Increase of the *PDR3* gene copies in a *PDR5*-deleted strain led to increased oligomycin resistance, but failed to correct the hypersensitivity to cycloheximide resulting from loss of *PDR5* [36]. The present interpretation of these data is that PDR1 and PDR3 would control, with some overlapping function, the resistance to cycloheximide which depends of the PDR5 pump, and the resistance to oligomycin through interactions with one or several other gene products regulated by PDR1 and/or PDR3.

The *PDR1* gene product was shown to modulate also the intracellular availability of small hormone molecules like estradiol [22]. The indication that estrogen molecules are also substrates for the yeast PDR pathway provided a first direct link between multidrug resistance and hormone tolerance, underscoring the value of studies of yeast *PDR* genes for the understanding of human multidrug resistance [22].

The *PDR3* gene product was shown to interact directly with the *PDR5* promoter in vivo by transactivation assays with a reporter *lacZ* gene under the control of the *PDR5* promoter. In this system, the overexpression of *PDR3* was correlated to increased *PDR5*-driven β-galactosidase activity [36]. The PDR3 protein, produced in bacteria, was found to bind to the *PDR5* promoter in vitro, on at least one site containing rotationally symmetric CCG nucleotide triplets, similar to the binding site for GAL4 but with a different spacing between two triplets [36]. The PDR1 protein was found to bind on the same PDR3-binding site(s) on the *PDR5* promoter (Moye-Rowley and Mahé, personal communication).

The PDR3 protein was shown to display a transcription activation function and was functionally dissected

Footnotes to Table 1

Proteins structurally homologous to multidrug resistance determinants but whose involvement in resistance to multiple drugs has not been established yet are also mentioned. Drugs are abreviated as follows: acri, acriflavine; atr, aminotriazole; amy, antimycin; aml, amyloride; ben, benomyl; bor, borrelidin; caf, caffeine; car, carbomycin; cer, cerulenin; chl, chloramphenicol; cyh, cycloheximide; cmc, carbonylcyanide-*m*-chlorophenylhydrazone; com, compactin; dac, dibenzyldimethylammonium chloride; dqc, dequalinium chloride; ery, erythromycin; ebr, ethidium bromide; flu, fluphenazine; gen, gentamycin; hygB, hygromycin B; lep, leptomycin B; lyn, lyncomycin; myc, myconazole; nin, 1-nitroso-2-naphtol; neo, neomycin; NMNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidin; NQO, 4 nitroquinoline *N*-oxide; ner, Neutral red; met, methotrexate; oli, oligomycin; par, paromomycin; phe, 1–10-phenanthroline, rut, rutamycin; smm, sulfomethuron methyl; sta, staurosporin; tet, tetracycline; teb, triethyltin bromide; tba, tetra-*N*-butylammonium bromide; tio, thiolutin; tpb, triphenylmethylphosphonium bromide; tre, trenimon; tri, triazquione; val, valinomycin. Other abreviations are: NBF, nucleotide binding fold; TM, transmembrane region. References given refer to the isolation and/or sequencing of the relevant gene.

[a] Wolfe, K., personal communication. Considering the high homology of this open reading frame to the *PDR5* gene, the designation *PDR10* is proposed.

[b] Prasad et al., unpublished data.

[c] Leighton, J. and Schatz, G., unpublished data.

[d] Golin, J. and Lambert, L., personal communication.

using LexA-PDR3 fusions. Two activation domains were localized near the N-terminal DNA-binding domain and at the carboxy-terminus, respectively [16]. The importance of the C-terminal region for transcription activation was confirmed by the evidence that loss of function resulting from the deletion of this domain could be corrected by replacement with the strong activation domain of the viral protein VP16 [36].

Next to *PDR1* and *PDR3*, two new pleiotropic drug resistance loci, designated *PDR7* and *PDR9*, were found to control the transcription of the *PDR5* gene [17]. Mutant alleles of *PDR7* and *PDR9*, both located on chromosome II, were isolated as spontaneous suppressors of the hypersensitive *PDR1* deletion. These suppressors restore resistance to cycloheximide and sulfomethuron methyl and overproduce *PDR5* mRNA.

Table 1
List of yeast gene products involved in multidrug resistance

Species	Name(s)	CHR	Structure	Toxic compounds	Features	Ref.
1. ABC MEMBRANE PROTEINS						
(a) Duplicated						
S. cerevisiae	PDR5/STS1/ /YDR1	XV	(NBF-TM)2	cyh, smm, chl, ery, lyn, amy, spo cer, com, sta, flu	homolog of SNQ2	5,6,28
S. cerevisiae	SNQ2		(NBF-TM)2	NQO, tri, smm, phen, NMNG, sta, flu	homolog of PDR5	25,61
S. cerevisiae	STE6	XI	(TM-NBF)2	val	export of a factor	39,44
S. cerevisiae	YCF1		(TM-NBF)2	Cd	homolog of CFTR	66,72
S. cerevisiae	PDR10	XV	(NBF-TM)2		homolog of PDR5	a
P. pombe	pmd1		(TM-NBF)2	lep, cyh, val	homolog of MDR, STE6	49
C. albicans	CDR1	III	(NBF-TM)2	cyh, chl, nys, myc, oli	homolog of PDR5	b
(b) Half-sized						
S. cerevisiae	ADP1	III	NFB-TM		homolog of White	55
S. cerevisiae	YKL741	XI	TM-NBF		homolog of ADL, Pmp70	7
S. cerevisiae	MDL1	XII	TM-NBF		homolog of TAP	13
S. cerevisiae	MDL2	XVI	TM-NBF		probably identical to Ssh1	13
S. cerevisiae	Ssh1	XVI	TM-NBF		probably identical to MDL2	37
S. cerevisiae	Ssh2	XVI	TM-NBF		homolog of ADL, Pmp70	37
S. cerevisiae	ATM1		TM-NBF		mitochondrial	41, c
S. pombe	HMT1		TM-NBF	Cd	vacuolar	53
2. MFS MEMBRANE PROTEINS						
S. cerevisiae	ATR1/SNQ1			atr, NQO		35,24
S. cerevisiae	YCL069w	III			homolog of TetR	51,23,64
S. cerevisiae	YCL023c	III			homolog of TetR	51,23,64
S. cerevisiae	YCL070c	III			homolog of ATR1	51,23,64
S. cerevisiae	YKR105c	XI			homolog YCL069w, TetR	19
S. cerevisiae	YKR106w	XI			homolog YCL070c, ATR1	19
C. albicans	ORF1			ben, met		20
C. maltosa	CYHR			cyh		58
S. pombe	car1			aml		31
3. TRANSCRIPTION REGULATORS						
S. cerevisiae	PDR1	VII	Zn2Cys6	cyh, chl, oli, ven, nys, ebr, ner, rho, bor, muc, tet, smm, car, dqc, acri, amy, cmc, cer, dac, gen, rut, par, neo, teb, tba, tio, tpb	homolog of PDR3	3
S. cerevisiae	PDR3	II	Zn2Cys6	muc, chl, cyh, oli, tet, ner	homolog of PDR1	15,16,36
S. cerevisiae	yAP1/PDR4/ SNQ3/PAR1		bZip	Cd, Zn, cyh, smm, NQO, NMNG, tre, phe, nin, H202	homolog of AP-1	48,42,26,62
S. cerevisiae	CAD1/YAP2		bZip	Cd, Zn, phe	homolog of AP-1	73,8
S. pombe	pap1		bZip	sta	homolog of Ap-1	67
S. cerevisiae	PDR7	II	?	cyh, smm		17
S. cerevisiae	PDR9	II	?	cyh, smm		17
S. cerevisiae	RPD1			cyh		70
S. cerevisiae	RPD3			cyh		71
4. OTHERS						
S. cerevisiae	YGL022	VII	membrane	cyh, smm	homolog of erg24	10
S. cerevisiae	PDR6	VII	soluble	cyh, bor, hygB		11
S. cerevisiae	PDR8	XII	soluble	oli, smm	identical to PEP5	d
S. pombe	sts1		membrane	cyh, sta, caf, chl, divalent cation	homolog of YGL022	63

The recessive *pdr7–1* and semidominant *pdr9–1* resistance alleles were shown to specifically interact with the *PDR5* promoter in vivo, by increasing the expression of a *lacZ* reporter gene under the *PDR5* promoter. Although the nucleotide sequence of these genes remains to be identified, *PDR7* and *PDR9* were proposed to encode regulatory functions, possibly positive for *PDR9* and negative for *PDR7*, controlling the expression of *PDR5* without need for interference with the *PDR1* gene product [17].

Another family of transcription regulators implicated in drug resistance is represented by proteins related to the mammalian proto-oncoprotein c-Jun. These proteins share similar DNA-binding domains and leucine zipper dimerization domains and recognize a common DNA element denominated ARE (AP1 Response Element). Next to different Jun proteins, this family includes the mammalian transcription factor complex AP-1 and the three yeast regulators GCN4, yAP1 and CAD1/YAP2. Interestingly, the mammalian c-Jun protein controls the expression of glutathione *S*-transferase [51] and possibly of P-glycoprotein genes [29], both involved in multidrug resistance; AP-1 binding sites are also present in the promoter of the CFTR gene [12]. The *S. cerevisiae YAP1* gene encodes a 90 kDa polypeptide with a leucine zipper motif, sharing homology, at the level of the DNA-binding domain, to the GCN4 and AP-1 regulators [48]. *YAP1* has been independently reisolated by three different searches of genes conferring drug resistance upon amplification. Under the names of *PDR4*[42], *SNQ3* [26] and *PAR1* [62], *YAP1* has been shown to confer, when overexpressed, resistance to cycloheximide, sulfomethuron methyl [42], NQO, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, Trenimon [26], to the iron chelators 1,10-phenanthroline and 1-nitroso-2-naphthol [62,8] and to heavy metals like cadmium and zinc [73]. Resistance to oligomycin, antimycin and nystatin was unaffected by *YAP1(PDR4)* overexpression [42]. Disruption of *YAP1* was associated with multiple drug hypersensitivity phenotypes [26] as well as with sensitivity to hydrogen peroxide and chemicals which generate superoxide anion radicals [62]. The specific activities of enzymes involved in oxygen detoxification were found to be decreased in *yap1* deletion mutants and increased in *YAP1* overexpressing strains. The *YAP1* gene product was suggested to be involved in gene regulation of oxygen detoxification enzymes, consistent with the finding that H_2O_2 promotes DNA-binding of human c-Jun [62]. *YAP1* dependent resistance to oxidative stress by peroxide radicals was shown to be mediated by activation of the *TRX2* gene, encoding a thioredoxin [40]. The cadmium tolerance controlled by *YAP1* was suggested to be mediated by transcriptional activation of a target gene, *YCF1*, encoding the yeast ABC homolog of the human CFTR factor [72]. On the other hand, the cadmium resistance associated to *YAP1* was not found to be correlated with the *ZRC1* gene product [73], a membrane protein conferring resistance to heavy metals upon overexpression [34]. Interactions between *YAP1* and the *PDR1 / PDR3 / PDR5* network have also been investigated [17]. *YAP1* mediated cycloheximide resistance was found not to depend on the *PDR5* gene product, since multiple copies of *YAP1* confer cycloheximide resistance even in a *PDR5* deleted background. Moreover, multicopy *YAP1* transformants fail to overproduce *PDR5* mRNA [17]. These findings indicated that at least two networks of genes, with different regulations, mediate cycloheximide resistance: the PDR5 pump regulated by at least PDR1 and PDR3, and the yet unidentified target controlled by yAP1 [17].

A homolog of *YAP1*, also involved in multidrug resistance, has been independently isolated and designated *CAD1* [73] or *YAP2* [8], respectively. *CAD1 / YAP2* provides resistance to cadmium, iron chelators and zinc, when present in multiple copies [73,8]. Cross-resistance to cycloheximide and sulfomethuron methyl, but at lower doses than those tolerated by *YAP1* overexpressing strains, were also reported [73]. Although *CAD1 / YAP2* and *YAP1* are closely related, in the structure of their DNA-binding domain and in their capacity of binding the same DNA consensus, disruption of *YAP1* results in hypersensitivity to cadmium, while disruption of *CAD1 / YAP2* does not [73]. In addition, the *yap2* null mutant showed an increased thermotolerance under iron/zinc starvation conditions caused by 1,10-phenanthroline [8]. However, *YAP1* and *YAP2* disruptants have additive effects on Zn^{2+} sensitivity, indicating cooperative action on a common target (Rodrigues-Pousada, personal communication). An homologue of *YAP1* has been also identified in the fission yeast *S. pombe* as product of the *pap1*$^+$ gene, which confers resistance to staurosporine, inhibitor of protein kinases, when overexpressed [67]. The pap1-dependent transcription regulation was found to undergo negative control by an essential nuclear protein, crm1, playing an important role in maintenance of chromosome architecture [68].

Finally, we wish to mention one more type of transcription regulatory factors somehow implicated in resistance to drugs: the *RPD1* [70] and *RPD3* [71] encoded proteins. These non-essential polypeptides of respectively 175 and 45 kDa do not contain evident DNA-binding domains, but do comprise acidic regions reminiscent of transcription activation domains as well as helix-loop-helix like secondary structures possibly involved in protein-protein interactions. Spontaneous mutations and deletions of *RPD1* or *RPD3* display a pleiotropic phenotype including hypersensitivity to cycloheximide, next to mating defects, inability to sporulate, derepression and increased secretion of acid phosphatase and increased potassium uptake. *RPD1*

and *RPD3* were shown to influence, both positively and negatively, the transcription of several, apparently unrelated, genes including *STE6*, and were proposed to be global regulators required for maximal transcriptional states of various target genes [70,71].

2.4. Other multidrug resistance proteins

Some yeast multidrug resistance genes, such as *PDR2* (review, Ref. [4]) *PDR7* and *PDR9* [17], still await a precise identification of their nucleotide sequences. Others have been found to code for proteins not typically belonging to the above described classes.

The *YGL022* gene of S. cerevisiae was shown to encode a putative 56 kDa protein predicted to be membrane buried [10]. Disruption of *YGL022* was correlated to increased sensitivity to cycloheximide and sufomethuron methyl [59]. A highly related protein from *S. pombe*, product of the gene *sts1*$^+$, was reported to influence sensitivity to different drugs such as staurosporine, caffeine, chloramphenicol and cycloheximide [63]. Disruption of the *sts1*$^+$ gene was not only associated to supersensitivity to these drugs, but also to sensitivity to divalent cations, to detergents and to stress conditions such as low temperature and osmotic pressure [63]. However, multiple copies of *sts1*$^+$ did not confer resistance to staurosporine. Interestingly, in a *sts1*$^-$ deleted background, multiple copies of the *S. pombe* gene encoding a regulator homologous to AP-1 were no more able to confer staurosporine resistance [63], suggesting some dependence of the AP-1 regulatory function on a functional *sts1*$^+$ gene product. The *sts1*$^+$ and *YGL022* encoded proteins share homology, both at the levels of primary sequence and hydrophobicity profile, with animal nuclear membrane proteins such as the laminB receptor [63]. In addition, sequence similarities to the ergosterol biosynthesis enzyme, sterol C-14 reductase encoded by the *S. cerevisiae* gene *erg24*, are also observed.

The *PDR6* gene, located in the proximity of *PDR1* on chromosome VII, encodes a non-essential 124 kDa polypeptide with no membrane-buried segments, no obvious functional consensus motif and no significant homology to known proteins [11]. *PDR6* was reported to somehow interact with the *PDR1* gene, since multiple copies of *PDR6* restore sensitivity to cycloheximide, borrelidin and hygromycin B in *pdr1* mutants [11].

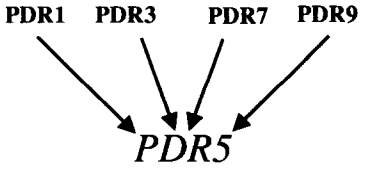

Fig. 1. Multiple regulation of PDR5. One multidrug resistance conferring membrane protein gene, *PDR5*, is transcriptionally controlled by several regulators, PDR1, PDR3, PDR7 and PDR9.

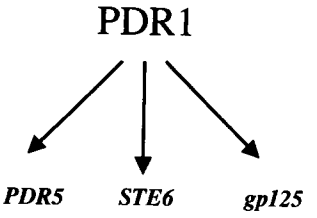

Fig. 2. Multiple regulation by PDR1. One multidrug resistance related transcription factor, PDR1, controls the expression of several target genes encoding plasma membrane proteins.

The *PDR8* gene, isolated as suppressor of oligomycin and sulfomethuron methyl resistance of *pdr1* mutants (Golin et al., unpublished data) has been found to correspond to the *PEP5* gene, whose product is necessary for vacuolar biogenesis (Lambert, L., personal communication).

Conclusions and perspectives

The number of yeast gene products found to be involved in multidrug resistance is rapidly increasing (Table 1). In April 1994 we were able to identify no fewer than 16 different genes involved in drug resistance in *Saccharomyces cerevisiae* and 8 genes in *Schizosaccharomyces pombe* or *Candida albicans*. The global picture resulting from the analysis of the features and interrelations of the yeast PDR determinants points essentially to functions in membrane transport, mainly in cellular efflux but also in organelle (mitochondria, vacuoles) influx. Active drug transport is mediated by at least two different major classes of membrane proteins, ABC and MFS. The energy for transport is probably provided by the hydrolysis of ATP in ABC proteins and by the facilitated diffusion of H^+ in MFS proteins.

The membrane proteins responsible for the active transport of drugs are under the control of complex and multiple transcription pathways. At least two independent regulatory networks, represented by the transcription factors PDR and yAP, respectively, have been identified so far. The PDR network provides an example of complex transcription regulation underlying multidrug resistance. For instance the transporter *PDR5* gene is the common target of several different transcription regulators such as PDR1, PDR3, PDR7 and PDR9 (Fig. 1). On the other hand, a given regulator, like PDR1, controls the expression of several target genes encoding distinct membrane proteins such as PDR5, STE6 and gp125 (Fig. 2). These recent studies fully support our original hypothesis on the pleiotropic function of PDR1 (Fig. 1 in Ref. [4]). The unravelling of these regulatory circuits might provide a model for

the uncovering of similar regulatory pathways controlling in mammals the expression of genes like *mdr*, whose disregulation is likely to be at the basis of multidrug resistance and tumorigenesis in general.

Another key question which remains to be adressed is the elucidation of the physiological functions of the wide and rapidly expanding classes of yeast membrane proteins involved in multidrug resistance. The role of this multitude of membrane proteins is likely to encompass the problem of cellular detoxification in yeast, which is a near virgin scientific field up to now, as well as that of the transport of other physiological substrates poorly considered so far, such as peptides and small hydrophobic proteins.

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