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Yeast Multidrug Resistance: The PDR Network

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This minireview describes a network of genes involved in multiple drug resistance of the yeast *S. cerevisiae*. The transcription regulators, PDR1, PDR3, PDR7, and PDR9 control the expression of the gene *PDR5*, encoding a membrane protein of the ATP-binding-cassette superfamily and functioning as a drug extrusion pump. Next to *PDR5*, several other target genes, encoding membrane pumps of the ABC type, such as *SNQ2*, *STE6*, *PDR10*, *PDR11*, *YOR1*, but also other membrane-associated (such as *GAS1*, *D4405*) or soluble proteins (such as *G3PD*), involved or not in multidrug resistance, are found to be controlled by PDR1. On another side, the PDR3 regulator participates with its homolog PDR1 to co- and auto-regulation circuits of yeast multidrug resistance.

KEY WORDS: Multidrug resistance; MDR; PDR; ABC membrane proteins; transcription regulation.

INTRODUCTION

Multidrug resistance in the yeast *Saccharomyces cerevisiae* has been described since more than two decades as a generalized resistance of a broad spectrum of functionally and structurally unrelated drugs. The history of the genetic determination of the main *PDR* (*p*leiotropic *d*rug *r*esistance) loci, mediating multidrug resistance, has been reviewed elsewhere (Balzi and Goffeau, 1991). More recent molecular analyses have confirmed the existence of a complex genetic network of no fewer than twenty yeast genes underlying tolerance to cytotoxic compounds and more generally implicated in membrane transport functions (review by Balzi and Goffeau, 1994).

Briefly, most of the (multi)drug resistance identified so far may be classified into three major classes: membrane transport proteins belonging either to the ATP-binding cassette (ABC) superfamily, such as SNQ2, PDR5, and YCF1, or to the major facilitators superfamily (MFS), such as ATR1 and SGE1, and factors for transcription regulation, such as PDR1, PDR3, PDR7, PDR9, YAP1, and YAP 2.

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Various genetic interactions connecting PDR regulators to drug pumps have been uncovered. As a first example, the regulators PDR1, PDR3, PDR7, and PDR9 have been shown to control the transcription of the multidrug pump gene *PDR5*, encoding an ABC type protein. Another example is given by the YAP regulators which are associated to the transcriptional control of the drug resistance genes, such as *YCF1* encoding another ABC pump involved in resistance to cadmium and strongly homologous to the human cystic fibrosis transmembrane conductance regulator (Szczypka *et al.*, 1994; Wemmie *et al.*, 1994). Whether the "PDR" and "YAP" regulatory networks are totally independent or share some yet unknown interactions remains to be established. The specific object of the present minireview is a revision of the rapidly evolving knowledge on the multidrug resistance network controlled by the PDR regulators in yeast.

A FIRST ESTABLISHED INTERACTION IN THE PDR NETWORK: THE REGULATOR PDR1 CONTROLS THE EXPRESSION OF THE DRUG PUMP PDR5

The pleiotropic drug resistance locus *PDR1* was first defined by a series of nuclear mutations, initially

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isolated by selection in the presence of one or two drugs and shown to display cross resistance to a total of nearby 30 different inhibitors affecting unrelated, cytoplasmic or mitochondrial, functions (reviewed in Balzi and Goffeau, 1991). No fewer than twenty independent mutations conferring multidrug resistance have been attributed to the *PDR1* locus [*pdr1-1* to *1-6*, *snr2-1* to *2-7*, *ant1-1*, *AMY1*, *cyh3*, *NRA2*, *till* (references cited in Balzi and Goffeau, 1991), *pdr1-7* (Golin *et al.*, personal communication), and *pdr1-8* (Clavilier, 1976; Gilbert *et al.*, 1993)]. The very high frequency of isolation of alleles of *PDR1* in the course of independent searches for mutations suppressing toxicity suggests that *PDR1* plays a primary role in the multidrug resistance phenotypes of yeast.

The phenotype associated to *pdr1* mutations is pleiotropic and not merely restricted to multidrug resistance. The *pdr1-2* mutant allele was, for example, related to physiological alterations such as respiratory deficiency and inability to grow under adverse conditions, such as elevated pH, temperature, and osmolality (Rank *et al.*, 1976). The *pdr1-8* multidrug resistance allele was shown to modulate the intracellular availability in yeast cells of human hormone molecules, such as estradiol (Gilbert *et al.*, 1993). The complexity of the *pdr1* phenotype was also reflected by genetic data such as the isolation of a large number of *pdr1* revertants suppressing different facets of the original pleiotropic phenotype, and such as the frequent observation of a variety of interactions between genetic factors of both nuclear and cytoplasmic origins (reviewed by Balzi and Goffeau, 1991). These facts suggest that *PDR1* affects a wide range of functions, encompassing resistance to chemical and physical stresses, membrane transport, and organelle functions.

Molecular cloning and characterization of the *PDR1* gene product confirmed the genetic predictions of a complex function. *PDR1* was found to encode a factor for transcription regulation with a Zn_2C_6 binuclear cluster motif as DNA binding domain (Balzi *et al.*, 1987). The hypothesis was thus proposed that the *PDR1* gene product would influence multidrug resistance by regulating the expression of different target genes, encoding proteins mediating transport of drugs and other substrates across different cell membranes (Balzi *et al.*, 1987; Balzi and Goffeau, 1991).

The first target gene shown to undergo transcriptional regulation of PDR1 was *PDR5*. The *PDR5* locus (also denoted: *STS1*, Bissinger and Kuchler, 1994; *YDR1*, Hirata *et al.*, 1994) was isolated by virtue of its ability to confer multidrug resistance as a multicopy plasmid-borne wild type allele (Leppert *et al.*, 1990) and was found to encode a membrane protein belonging to the ATP-binding-cassette superfamily, possibly functioning as a drug-extrusion pump (Balzi *et al.*, 1994). Genetic interactions were shown to take place between the *PDR1* and *PDR5* loci, indicating that the expression of the specific resistances to cycloheximide and chloramphenicol associated to a *pdr1* mutation requires the presence of a functional *PDR5* allele (Meyers *et al.*, 1992). It was also shown that the *PDR5* transcript is increased in multidrug resistant *pdr1* mutants and decreased after disruption of *PDR1* (Meyers *et al.*, 1992). Similarly, the PDR5 protein was found to be overexpressed in the plasma membrane of the same *pdr1* mutants and to disappear after replacement of *pdr* with a null *pdr1* allele (Balzi *et al.*, 1994; Decottignies *et al.*, 1994). *In vitro* evidences for a physical interaction between the PDR1 protein, produced in bacteria as an N-terminal fragment, and the *PDR5* promoter were provided by gel-mobility shift assays (Y. Mahé *et al.*, personal communication). DNAseI protection assays revealed the presence of three PDR1-binding sites in the *PDR5* promoter (490 nt analyzed) and proposed TCCGCGA as a consensus sequence for the binding of PDR1 (Y. Mahé *et al.*, personal communication). This consensus includes two rotationally symmetric CCG triplets as typical traits for the binding of Zn_2C_6 cluster proteins.

The PDR1 regulator has been molecularly and functionally dissected. The six multidrug resistance alleles *pdr1-1,-2,-3,-6,-7,-8*, leading to multidrug resistance through amplification of ABC pumps like PDR5, have been sequenced and five of them have been found to consist each a missense point mutation, located in a total of three clusters located approximately at the positions: 300, 800, 1000) (E. Carvajal *et al.*, 1993). Transactivation experiments with the mutant *pdr1-3*, *pdr1-6*, and *pdr1-8* alleles, representative of each of the three mutation clusters, showed that these alleles increase 10-fold the induction by PDR1 of the *PDR5* promoter fused to a reporter β-galactosidase gene (E. Carvajal *et al.*, personal communication). A transcription activation domain has been identified in the carboxy terminus (approximately 100 amino acids) of the PDR1 protein, by the use of a PDR1::lexA fusion system (A. Cybularz *et al.*, personal communication). The *pdr1-8* drug resistance mutation, contained within this C-terminal domain, increases the activation

capacity of the PDR1 : : lexA fusion protein (A. Cybularz *et al.*, personal communication).

Four *pdr 1* intragenic suppressor mutations of the *pdr1-7* multidrug resistance phenotype were sequenced. Two of them, *pdr7-100* and *pdr7-101*, are point mutations in the DNA binding domain of PDR1, located in the N-extremity of the protein. The PDR1 proteins produced from these revertants lost their capacity to bind the *PDR5* promoter (E. Carvajal *et al.*, personal communication). Two mutations partially suppressing the *pdr1-7* mediated drug resistance (to cycloheximide and sulfometuron methyl) were mapped at the *PDR5* locus and termed *pdr5-1* and *pdr5-2* (J. Golin *et al.*, in preparation). The *pdr5-1* and *pdr5-2* mutant alleles have been sequenced and found to consist of frame-shifts provoking a premature termination (J. Zhao *et al.*, personal communication).

The PDR5 protein has been partially purified and biochemically characterized from the overexpressing *pdr1* mutants (Decottignies *et al.*, 1994). PDR5 was strongly enriched in plasma membrane fractions. After solubilization by N-dodecyl maltoside, the PDR5 protein was separated from the plasma membrane H^+-ATPase activity by glycerol gradient centrifugation and found to be associated to a strong nucleoside triphosphatase activity, with a broad specificity for nucleotides and large pH tolerance. These properties, as well as its sensibility to inhibitors such as vanadate and oligomycin, liken PDR5 to the mammalian multidrug resistance P-glycoprotein (Decottignies *et al.*, 1994). Plasma membrane vesicles from PDR5 overexpressing strains were shown to pump a variety of cytotoxic compounds (Kolaczkowski *et al.*, personal communication). The disruption of the *PDR5* gene was accompanied by a marked reduction in cellular chloramphenicol efflux (Leonard *et al.*, 1994). The null *pdr5* mutants were used to isolate, by heterologous complementation, a homologous gene from the pathogenic yeast *Candida albicans*, *CDR1*, conferring resistance to multiple drugs and antifungals (Prasad *et al.*, 1995). All together the biochemical properties of the yeast PDR5 protein seem similar to those of the mammalian MDR1 protein even though the general topology of the membrane and ATP domains of the two proteins is inverted.

The range of drugs affected by *PDR5* (most typically cycloheximide, chloramphenicol, and sulfometuron methyl) corresponds only partially to the wider range of drugs affected by *PDR1*. For instance, the oligomycin-resistance controlled by PDR1 is not affected by the disruption of *PDR5*. This indicates that other genes controlled by PDR1 must contribute to multidrug resistance. On another side, although disruption of *PDR1* or *PDR5* both leads to cycloheximide sensitivity, the level of hypersensitivity to cycloheximide is much higher in *PDR5* than in *PDR1* deleted strains (Meyers *et al.*, 1992), indicating that other regulators must intervene, next to PDR1, in the control of *PDR5* expression. Both suggestions were validated at the molecular level by the uncovering of novel targets for PDR1 regulation and of other regulators acting on *PDR5*.

MULTIPLE TARGETS FOR PDR1

The yeast ABC membrane proteins SNQ2 and STE6 respectively involved in resistance to multiple drugs and secretion of the yeast mating pheromone **a** are homologs to the PDR5 protein; however, the relative positions of the membrane spanning and ATP-binding domains of PDR5 and SNQ2 are inverted compared to that of STE6 and the mammalian MDR. Both *SNQ2* and *STE6* genes were found to be transcriptionally influenced by PDR1. The mRNA level of *STE6* is increased in *pdr1* mutants, but unaffected after disruption of *PDR1* (Balzi *et al.*, 1994). No typical PDR1-binding consensus is detected in the promoter region of *STE6* (unpublished observation), implying either that other PDR1-binding sites exist or that the effect of the *pdr1* mutations on the *STE6* transcript is indirect. The *SNQ2* gene shares, with *PDR5*, the control of common drug resistances such as toward staurosporine and fluphenazine, but displays some distinct drug specificity, namely toward 4NQO (Hirata *et al.*, 1994). The *pdr1* mutants are cross-resistant to 4NQO, and disruption either of *PDR1* or *SNQ2* in a *pdr1* mutant background increases sensitivity to 4NQO (A. Decottignies *et al.*, personal communication). In the *pdr1* null strains, the level of *SNQ2* gene transcript and protein in the plasma membranes are dramatically decreased (A. Decottignies *et al.*, personal communication). These evidences for control by PDR1 of the expression of *SNQ2* are confirmed by the presence of PDR1-binding sites in the *SNQ2* promoter (A. Decottignies *et al.*, personal communication). The SNQ2 protein has also been solubilized and partially purified from *pdr1* overexpressing mutants deleted of *PDR5*, and associated to a nucleotide triphosphatase activity

distinct from that of PDR5 (A. Decottignies *et al.*, personal communication).

Another property shared by *PDR5* and *SNQ2* is transcriptional induction by the presence of drugs and in response to heat shock, similar to the induction of mammalian multidrug resistance genes by chemical and physical insults (Hirata *et al.*, 1994). Whether the induction of *PDR5* and *SNQ2* in response to environmental stress is mediated by PDR regulatory factors is under investigation.

Another gene encoding a membrane-associated protein controlled by PDR1 was detected on chromosome IV of *S. cerevisiae* by a systematic search of promoters containing PDR1-binding sites (J. L. Jonniaux, personal communication). The transcript of this gene (D4405), encoding a membrane protein not belonging to the ABC superfamily, is remarkably amplified in *pdr1* mutants and depressed after disruption of *PDR1* (L. Lambert, personal communication). Similarly, the presence of potential PDR1-binding sites in the promoter of a new *PDR5*-homologous gene, *PDR10* (K. Wolfe, personal communication), suggests that this gene is likely to undergo regulation by PDR1. Very recently, two new yeast ABC protein encoding genes have been discovered: *YOR1*, on chromosome VII, influences resistance to oligomycin (S. Moye-Rowley and G. Volckaert, personal communication), whereas the other gene (proposed name *PDR11*; accession: Z38113, NCBI gi:558389[11]), on chromosome IX, is a close homologue of *SNQ2*. The expression of both these new ABC genes seem to be regulated by PDR1 (A. Decottignies, personal communication). Numerous other targets of regulation by PDR1 are being uncovered by two-dimensional gel electrophoresis analysis of proteins from the *pdr1–3* mutant and derived *pdr* disrupted strain (S. Fey and P. Mose-Larsen, personal communication). Among these proteins, at least two were identified as being the plasma-membrane anchored 125-kDa glycoprotein encoded by the gene *GAS1* (Nuoffer *et al.*, 1991; Vai *et al.*, 1991), and the glycolytic enzyme glycerol-3-phosphate dehydrogenase (G3PD). These findings, which are under further investigation, open the perspective of a control, by PDR1, over cellular functions not associated to membrane transport and possibly related to mechanisms of reaction to stress. Finally, it has been proposed that a potential target for regulation by PDR1 could be the plasma membrane H^+ATPase gene *PMA1* (Ulaszewski, 1993). While *pma1* mutations are also associated to resistance to drugs, although different to those related to *pdr1* mutations, the association of *pdr1* and *pma1* mutations has been observed to alter both plasma membrane ATPase activity and drug-resistance profiles (Ulaszewski, 1993). It is, however, still not clear whether these interactions are direct or indirect.

MULTIPLE PDR REGULATORS

A second site of mutations conferring multidrug resistance similar to *PDR1* is the *PDR3* locus, localized in proximity of chromosome II centromere (Subik *et al.*, 1986). *PDR3* was recently found to be allelic to a locus previously termed *PDR4* (Preston *et al.*, 1991; Katzman *et al.*, 1994). Taken into account this allelism, four multidrug resistance mutations can be attributed to the *PDR3* locus: *pdr3-1*, *pdr3-2* (Subik *et al.*, 1986), *pdr4-1* (Preston *et al.*, 1991) which has been renamed *pdr3-3* (Katzmann *et al.*, 1994), and *pdr4–2* (Dexter *et al.*, 1994). Some *pdr3-1* mutants were reported to contain additional independent drug-resistance mutations, in particular one specific for cycloheximide resistance and one for oligomycin resistance (Ruttkay-Nedecky *et al.*, 1992; Dexter *et al.*, 1994). This case is typical of a problem frequently occurring during the isolation of drug-resistant mutants: the simultaneous appearance of multiple, unlinked resistance mutations, often with synergistic effects (Rank, 1986; Ruttkay-Nedecky *et al.*, 1992). Similarly, one of the very first multidrug-resistant mutants isolated, DRI9 (Guérineau *et al.*, 1974), turned out to contain two mutations in *PDR1* and *PDR3* respectively. Two separate mutants DRI9/T8 (*pdr1-3*) and DRI9/T7 (*pdr3-2*) have been segregated from the original DRI9 strain.

Sequenced under the name of *YBLO23*, the *PDR3* gene was found to encode a transcription regulator of the Zn_2C_6 cluster family homologous to PDR1 (36% identity over the entire protein length and a highly conserved DNA binding domain) (Delaveau *et al.*, 1991, 1994; Katzmann, 1994). The *PDR3* encoded product was shown to be a transcription regulator, by demonstrating that DNA-bound LexA-PDR3 protein fusions stimulate the expression of a nearby promoter (Delaveau *et al.*, 1994). Two transcription activation domains where identified respectively near the NH-terminal DNA-binding domain and at the carboxy-terminus of PDR3 respectively (Delaveau *et al.*, 1994). The *PDR1* and *PDR3* gene products share not only structural homology but also functional analogies. The *PDR3* wild type gene

was shown to restore wild type drug sensitivity in *pdr3* mutants (Delaveau *et al.*, 1994), similar to the complementation of *pdr1* mutants by the wild type *PDR1* gene (Balzi *et al.*, 1987). In addition, wild type alleles of either *PDR1* or *PDR3* were shown to cross-complement *pdr3* or *pdr1* mutations respectively (A. Cybularz and E. Balzi, personal communication), indicating functional overlappings between the two genes. This was confirmed and elucidated by the finding that the *PDR1* and *PDR3* gene products regulate the expression of at least one common target, the *PDR5* gene.

Indeed, multidrug resistant *pdr3* mutants were reported to overexpress the *PDR5* gene transcript (Dexter *et al.*, 1994) and the PDR5 protein in plasma membranes (Decottignies *et al.*, 1994), just like *pdr1* mutants. Analysis of a series of isogenic mutants lacking either *PDR1*, *PDR3*, *PDR5* or *PDR1* and *PDR3* shows that while disruption of *PDR3* or *PDR1* has no detectable effect on the steady-state level of *PDR5* transcript, simultaneous loss of both *PDR1* and *PDR3* results in a dramatic reduction of *PDR5* mRNA (Katzmann *et al.*, 1994). Whereas single *PDR1* or *PDR3* disruptions are generally associated to hypersensitivity to drugs such as cycloheximide, oligomycin, and chloramphenicol, more pronounced resistance is obtained by disruption of *PDR1* than of *PDR3*, indicating a major role of *PDR1* in the control of multidrug resistance (Delaveau *et al.*, 1994; Katzmann *et al.*, 1994). However, the double disruption of *PDR1* and *PDR3* results in much more drastic hypersensitivity to drugs compared to the single disruptions, indicating that *PDR1* and *PDR3* act in concert in the control of multidrug resistance (Delaveau *et al.*, 1994; Katzmann *et al.*, 1994). An isogenic strain lacking *PDR5* is hypersensitive to cycloheximide like the double *PDR1*, *PDR3* disruptant, indicating that *PDR1*, and *PDR3* share overlapping essential functions for the expression of *PDR5*-mediated resistance of cycloheximide. A high copy number *PDR3* plasmid increases resistance to cycloheximide and oligomycin. This latter trait is, however, independent of the presence of *PDR5*, indicating that PDR3 influences oligomycin resistance like PDR1 and in concert with PDR1, but through another target other than *PDR5* (Katzmann *et al.*, 1994).

In vivo transactivation experiments, using fusions between progressive deletions of the *PDR5* promoter and the β-galactosidase reporter gene, have demonstrated that the *PDR5* promoter responds to induction by PDR3 (Katzmann *et al.*, 1994). The PDR3 protein, produced in bacteria, was shown to bind to at least one site within the responsive region (Katzmann *et al.*, 1994). This PDR3 binding site in the *PDR5* promoter, centered around the motif TCCGCGGA, was shown to bind also PDR1. Subsequently, two to four additional PDR3-binding sites, possibly with different affinities, were identified in an extended *PDR5* 5′-flanking region (Delahodde *et al.*, 1995). Thus, the finding that PDR1 and PDR3 potentially share common binding sites in the *PDR5* promoter validates the observations of overlapping and complementary functions for these two regulators. Addressing the question of whether PDR1 and/or PDR3 bind with similar or different affinities to these sites *in vivo* and unravelling interactions taking place among PDR1, PDR3, and possibly other co-regulatory factors, could shed further light on the respective roles of these two regulators, which are expected to contribute in concert to the fine tuning of drug resistance. In this perspective, a recent study has elegantly demonstrated an autoregulatory process for PDR3 and a hierarchical regulation by PDR1 over the expression of the regulator PDR3 (Delahodde *et al.*, 1995).

Two PDR3/PDR1 binding sites are present and functional in the *PDR3* promoter (Delahodde *et al.*, 1995). Both PDR1 and PDR3 were shown to activate the promoter of *PDR3*, fused to the reporter gene *lacZ*. The transactivation effect of PDR3 depends on the integrity of the two PDR3-binding sites, since mutations in either of the two sites reduce the induction of the *PDR3* promoter. A reduced induction is also observed in a *PDR1*-deleted background,

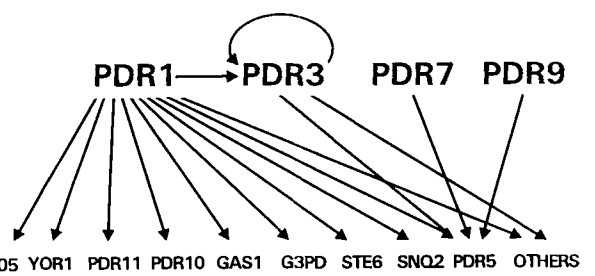

Fig. 1. The yeast pleiotropic drug resistance PDR network. Multiple *S. cerevisiae* multidrug resistance regulators, such as PDR1, PDR3, PDR7, and PDR9 (top row), control the expression of different target genes (bottom row and PDR3), for some of which (PDR5, SNQ2, YOR1) a role in multidrug resistance is established. Arrows indicate regulatory interactions. Direct proofs for regulations have been reported for the targets PDR5, SNQ2, and PDR3; the other suggested interactions are under investigation. References for each determinant are given in the text.

suggesting a cooperation between PDR1 and PDR3 in the activation of the *PDR3* promoter. The two PDR3/PDR1 recognition sequences were shown to bind the PDR3 and PDR1 proteins *in vitro*, with cooperative effects and with a predominant role for the upstream site toward binding of *PDR3* (Delahodde *et al.*, 1995). The autoactivation of *PDR3* was shown to be involved in the process of conferring resistance to cycloheximide. These studies provide a first mechanistic model to explain how the homologous regulators PDR1 and PDR3, which exhibit complementary but still somehow distinct functions, could interact in the fine regulation of expression of the multidrug resistance determinant PDR5. The predominant role of PDR1, next to the necessity for both PDR1 and PDR3 to achieve a most efficient response to drugs, is in agreement with the view of a regulatory circuit where PDR1, capable of controlling PDR3, would be the first element to respond to toxic stress, and PDR3, boosted by autoactivation, would allow a most effective and rapid response (Delahodde *et al.*, 1995).

Next to the exquisite case of fine-tuned regulation elucidated for PDR1 and PDR3, at least two more *PDR* genes have been shown to be involved in the control of *PDR5* gene expression. The *PDR7* and *PDR9* loci were identified by mutations capable of establishing multidrug resistance, through amplification of *PDR5*, even in the absence of *PDR1* (Dexter *et al.*, 1994).

In conclusion, Fig. 1 illustrates our present knowledge of interactions within the network of PDR determinants. Several interactions suggested by this scheme remain to be confirmed. The outlined network is also likely to be incomplete and is expected to rapidly integrate newly discovered targets regulated by PDR1 and PDR3, and possibly new findings on other PDR regulators. The complexity of the PDR network in yeast augurs the existence of system of similar complexity for the control of multiple drug resistance in mammalian cell.

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