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 (pleiotropic drug resistance) 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 YAP2.

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_{2}C_{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; YDRI, 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_{2}C_{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