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Studies on the Drug Specificity and Novel Functions of the Multidrug Resistance ABC Transporter Protein Pdr5p

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Multidrug resistance ABC proteins are ubiquitous among eukaryotic cells, suggesting that these mechanisms are important for survival of the organisms. The budding yeast *Saccharomyces cerevisiae* was the first eukaryotic organism to have its complete genome sequence determined, revealing 30 distinct genes encoding ABC proteins. Of these, the Pdr5p drug efflux pump is thought to be most important in yeast multidrug resistance for its ability to confer resistance to a wide range of xenobiotic compounds. Analysis of mutant ABC proteins with altered drug specificity is an obvious approach to investigate the function of the transporter protein. In mammalian cell systems, technical difficulties are anticipated in the isolation and analysis of such mutants. The yeast system, in contrast, will allow us detailed functional analysis of the ABC proteins at the molecular level, due to the powerful genetic techniques available with it. We, therefore, investigated the mechanism underlying the drug specificity of the ABC transporter proteins in yeast.

1. The isolation and characterization of the Pdr5p mutants with altered drug specificity

To understand the mechanism by which Pdr5p recognizes and transports hundreds of structurally unrelated compounds, we isolated and analyzed the Pdr5p mutants with altered drug specificity. The p-Bluescript-born *PDR5* gene was mutagenized by inducing random mutagenesis by hydroxylamine treatment. Following transformation with the mutagenized plasmid of the strain in which the chromosomal *PDR5* gene had been deleted, mutants were screened for drug sensitivity using cycloheximide, fluphenazine, cerulenine, tautomycin and staurosporin. The mutants that exhibited the sensitivity to one or more out of five drugs were selected, eliminating the mutants that showed sensitivity to all of the tested drugs. The mutation point of each

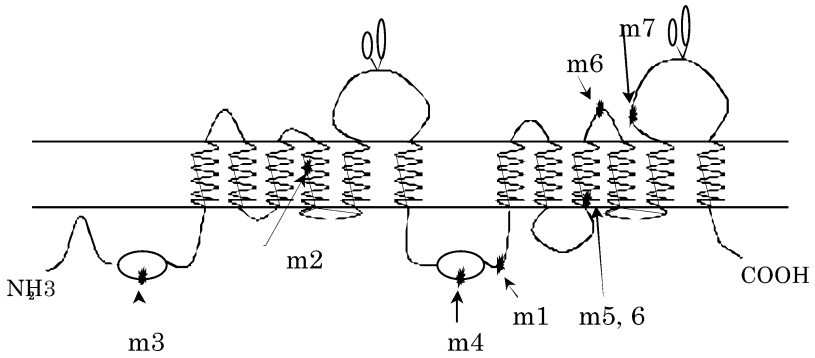

Figure. Mutation points identified in the Pdr5p mutants with altered drug specificity.

Table. Drug phenotypes of various Pdr5p mutants

Mutant	Phenotype of *Δpdr5 Δsnq2* strain carrying indicated *pdr5* mutants					Mutation point
	Cyh	tau	Cer	Tau	Fp	
pdr5-1	S	R	S	R	R	S1048V
pdr5-2	S	R	S	R	R	S648F
pdr5-3	S	R	S	R	R	T257I
pdr5-4	S	R		S	R	G1040D
pdr5-5	S	R	S	R	R	E1289K
pdr5-6	S	R	S	R	S	E1289K Y1311S
pdr5-7	S	R	S	R	R	T1393I

S: sensitive, R: resistant

mutant was determined by DNA sequencing and shown in Figure. Conferred phenotypes of each mutant are summarized in Table. Unexpectedly, however, the mutation points were not confined to the transmembrane domain of Pdr5p, but spread all over the length of the protein, including both cytoplasmic and extracellular domains of Pdr5p. These results suggested that the recognition of the drugs by Pdr5p may be mediated by a mechanism that involves the entire three dimensional structure of Pdr5p.

2. Pdr5p is responsible for the sensitivity to certain drugs

The yeast Pdr5p is important for acquiring the resistance to literary hundreds of xenobiotics. Surprisingly, however, during the course of our study to compare the resistance of wild-type and *Δpdr5* strains to various drugs, we found that the *Δpdr5* strain exhibits increased resistance, while the *PDR5* over-expressing strain exhibits reduced resistance to a few compounds, which included p-chlorophenol, Naban, β-octylglucoside. We are now examining the possibility whether Pdr5p is responsible for the import of these drugs from the medium.

3. The response of Pdr5p to Ca^{2+} signal

By western blot analysis of Pdr5p using antibody against Pdr5p, we found that Pdr5p is rapidly degraded by the activation of the Ca^{2+} signal pathways, which involves calcineurin and Mpk1p MAP kinase cascade. The degradation of Pdr5p was mediated by SCF^{Cdc4}. These results suggested that Pdr5p might be involved in Ca^{2+} transport. In fact, apparent uptake rate of Ca^{2+} from the medium was reduced in the *Δpdr5* strain. We are now developing an efficient drug screening procedure for the inhibitors of Pdr5p on the basis of our previous observation that excessive uptake of Ca^{2+} causes the inhibition of the growth of the yeast cells.