Multiple Drug Resistance in the Fission Yeast *Schizosaccharomyces pombe*: Correlation Between Drug and Amino Acid Uptake and Membrane ATPase Activities

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Summary. *Cyh3* and *cyh4*, multiple drug resistant strains of *Schizosaccharomyces pombe*, show a much reduced uptake of trichodermin, chloramphenicol, cycloheximide, L-lysine, glycine, L-threonine, L-glutamine, L-arginine and L-glutamic acid when compared to wild type. The plasma membrane and mitochondrial ATPase activities of these mutants are also greatly reduced. Since the uptake of such compounds is likely to be driven by a proton electrochemical gradient set up by the membrane ATPase it is suggested that the primary effect of these mutations is at the level of the membrane ATPase. Another drug resistant strain, *cyh1*, which is resistant only to high levels of cycloheximide, shows increased uptake of trichodermin, L-lysine, glycine, L-threonine, L-glutamine when compared to wild type. The plasma membrane and mitochondrial ATPases of *cyh1* are considerably greater than those of wild type. It has been shown previously that *cyh1* possesses an altered 60S ribosomal subunit protein when compared to wild type and this makes it resistant to cycloheximide. There is no obvious explanation as to how this change could lead to the alterations in drug and amino acid uptake and in ATPase activities observed.

**Key words:** Fission yeast – Membrane ATPase – Amino acid uptake – Drug resistance

Introduction

The proposal of a general membrane permeability barrier as the basis of the multiple drug resistance observed in some yeast, bacterial and cancer cells is one that has had a lot of support throughout the years. Generally, such proposals are made, when in vitro studies of the mutant antibiotic target sites show them to be as sensitive as those of wild type and when the antibiotics concerned are chemically, structurally and functionally so diverse that enzymatic inactivation seems improbable. In bacteria however, all evidence indicates that multiple drug resistance is mediated by plasmids (R-factors) carrying separate genes coding for each of the specific inactivating enzymes responsible for resistance to the different antibiotics (Watanabe 1963; Falkow 1975, Koch 1981). In yeast and cancer cells, no evidence exists for multiple drug resistance via plasmid encoded inactivating enzymes and the general membrane permeability barrier hypothesis is still an attractive one.

However, the experimental evidence for the existence of such permeability barriers is limited and the mechanism of how such a barrier could be produced remains obscure. Studies on the oligomycin resistant mutants of *Saccharomyces cerevisiae oli PR1* and *oli PR2* (alleles at the same locus) which were cross resistant to several inhibitors of mitochondrial electron transport, to chloramphenicol and to cycloheximide, showed them to have a much reduced accumulation (about 1%) of chloramphenicol when compared to wild type (Rank et al. 1975 and 1977). It was proposed that the pleiotropic phenotype could be explained by the modification in expression of a single nuclear gene that affects the permeability of the plasma membrane and which resulted in some physiological alterations in both the plasma and mitochondrial membranes (Rank et al. 1977). The ATPase specific activities of both plasma and mitochondrial membranes isolated from the mutant strain *oli PR1*–2 were approximately 20% lower than those of wild type.

Considerable attention has been focused on the transport of amino acids into fungi because it offers both a
genetical and physiological way of dissecting the transport processes themselves and their regulation. A common strategy used by the fungi is one of a limited number of permeases which transport families of structurally related amino acids. In *Neurospora crassa*, three constitutive amino acid transport systems have been detected. The neutral system, which transports neutral, aliphatic and aromatic amino acids; the basic system which is specific for cationic amino acids and a general system with affinity for all classes of amino acids (DeBusk and DeBusk 1980). The yeast *Saccharomyces cerevisiae* has also been shown to have a number of specific amino acid permeases and in addition a general amino acid permease (Grenson and Hennaut 1971). Studies on the regulation of such permeases have been aided by the availability of mutants lacking one or more of these functional transport systems. Transport of amino acids in *Neurospora crassa* and *Saccharomyces cerevisiae* has been shown to be under genetic control regulated by both substrate inhibition and ammonium repression (DeBusk and DeBusk 1980; Grenson and Hennaut 1971). From the results of these studies on transport mutants, it was suggested that the different amino acid transport systems showed a “specific factor” or one or more “common elements” for their functioning and it was tentatively proposed that the process of energy coupling was a likely candidate.

The existence of a proton-pumping ATPase in the plasma membrane of yeast cells has been postulated in order to explain acidification of the external medium by proton ejection, membrane potentials and the observed co-transport of nutrients with protons (Serrano 1978). In at least four species of fungi, *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Rhodotorula gracilis* and *Neurospora crassa*, there is evidence for electronegic proton extrusion driven by the plasma membrane ATPase where the resulting proton electrochemical gradient functions as a driving force in the uptake of sugars, amino acids and probably ions (Goffeau and Slayman 1981; Eddy 1978 and 1982; Borst-Pauwels 1981). It was for this reason that the ATPase activities and properties of membrane preparations from wild type and antibiotic resistant mutants were examined and compared.

Yeast plasma membranes are lipoprotein structures which have been shown to be organized and to function in ways consistent with the fluid mosaic model of membranes (Singer and Nicolson 1972; Singer 1974). In view of their lipoprotein composition and the consequent protein-protein, protein-lipid and lipid-lipid interactions which occur therein, the importance of such co-operative effects on membrane functions might be reflected in the complex pleiotropic effects that different mutations in such systems may cause. A general membrane permeability barrier might arise from an alteration of some factor which is an integral part or common feature of many transport processes. The composition and structure of the plasma membrane and energy interactions involved in transport, are two likely candidates. In this paper we report experiments which were designed to demonstrate the connection between general amino acid transport processes and energy utilisation in the yeast plasma membrane.

We have already shown (Johnston and Coddington 1982) that *cyh3* and *cyh4*, multiple drug resistant mutant strains of the fission yeast *Schizosaccharomyces pombe* have a greatly reduced uptake of the actively transported amino acid L-tyrosine when compared to wild type. A permeability barrier which confers resistance to a number of unrelated antibiotics is more likely to be a general or non-specific one which could affect the transport of nutrients, metabolites and ions into the cell as well as that of the antibiotics concerned. The mutant strains *cyh3* and *cyh4* do in fact have slower growth rates than wild type, which would be consistent with this proposal. Hence, as a further test the uptake of various [*14C*]-labelled antibiotics and amino acids by each of the drug resistant strains *cyh1*, *cyh3* and *cyh4* was measured and compared with that of wild type. *Cyh1* is of interest in that it is a cycloheximide resistant strain which has an altered target site (the 60S ribosomal subunit) and does not show the multiple drug resistance phenotype (Berry et al. 1978). Hence, it would be expected to resemble wild type in its uptake properties.

### Experimental

**Materials.** [*14C*] Acetic anhydride, *L-[U-*14C]* arginine monohydrochloride, *L-[U-*14C]* leucine, *L-[U-*14C]* threonine, *L-[U-*14C]* glutamine, *L-[1,*14C]* glutamic acid and [*14C*] glycine were obtained from the Radiochemical Centre, Amersham. [*14C*] Trichodermin was prepared from [*14C*] acetic anhydride and trichodermin (kindly supplied by Dr. S. Godtfredsen, Leo Pharmaceuticals, Ballerup, Denmark) by the method of Barbacid and Vazquez (1974). All other reagents were of AR standard and obtained from one of the following: British Drug Houses, Poole, Dorset, UK; Fisons Scientific Apparatus, Loughborough, Leics, UK; and Sigma Chemical Co, Poole, Dorset, UK. Yeast extract and yeast nitrogen base were obtained from Difco Laboratories, West Molesey, Surrey, UK.

**Strains.** The *Schizosaccharomyces pombe* strain 972 h− (wild type) was obtained from Professor Urs Leupold, Institute for General Microbiology, University of Bern, Switzerland. The origin and genetical properties of the mutant strains used, *cyh1-C7, cyh3-K10* and *cyh4-U96* have been described previously (Ibrahim and Coddington 1976).

**Uptake of [*14C*] Labelled Trichodermin and Amino Acids.** The penetration of [*14C*]-labelled trichodermin and amino acids was studied during the exponential phase of growth as determined by absorption methods. Cells were grown to mid log phase in 100 ml batches of liquid complete media (Yeast extract media, YEL) as described previously (Johnston and Coddington 1982). Cells were harvested by centrifugation in an MSE bench centri-