Proc. Indian natn. Sci. Acad. **B49** No 5 pp. 398–402 (1983)

Membrane-Associated Proteins Involvement in Resistance Development to Carboxin in *Saccharomyces cerevisiae*

P GUNASEKARAN and P TAURO*
Department of Microbiology, Madurai Kamaraj University, Madurai 625021

(Received 9 August 1982; after revision 4 April 1983)

Resistance to carboxin in *Saccharomyces cerevisiae* is dependant on protein synthesis and the specific proteins involved in restricting the entry of carboxin into the cell are associated with the cytoplasmic membrane.

Key Words: Carboxin, Yeast membrane, Resistance development

Introduction

Carboxin(2, 3-dihydro-5-carboxyanilido-6-methyl 1, 4-oxathiin, vitavax), a systemic fungistat, effective against a wide range of pathogenic fungi and the yeast, *Saccharomyces cerevisiae* (Edgington & Barron 1967, Gunasekaran 1980). In this yeast growth-inhibition by carboxin is transient and resumption of growth occurs within a short period depending on the fungistat concentration in the medium. The growth-inhibition is due to the suppression of protein synthesis as growth-resumption occurs after cellular alteration, which restricts the entry of the fungistat into the cells (Gunasekaran & Tauro 1982). The present study was undertaken to study the mechanism of growth-resumption of yeast in the presence of carboxin

Materials and Methods

A respiratory-deficient strain of haploid yeast, *S. cerevisiae* 9B (α, ρ^-), was isolated in this laboratory (Tauro, unpublished data) and maintained on YEPD agar slants (yeast extract 0.5%, peptone 1%, dextrose 2% and agar 2%) by serial transfers.

For growth measurement, cells from a 12 hr old slant culture were transferred to 50 ml of synthetic medium (Rose & Nickerson 1956) in a 250 ml Erlenmeyer flask and kept agitated on an incubator (30°C)—shaker (230 rpm). 5 ml samples were withdrawn at intervals, and growth measured as optical density (D_o) at 620 nm using Bausch & Lomb spectronic colorimeter.

Determination of RNA and protein syntheses both in the presence or absence of cycloheximide (10 μg/ml), was effected

*Department of Microbiology, Haryana Agricultural University, Hissar 125004

by methods described (Gunasekaran & Tauro 1982). Plasma membrane was isolated from the protoplasts as described by Longley et al. (1968) and Ferenczy and Maraz (1977) and the membrane-bound proteins were separated on a Sephadex G-100 column (1.5 × 100 cm) and eluted with phosphate buffer (*p*H 7.2; 0.01M) containing 1% sodium *dedocyl* Sulphate (SDS). Fractions of 4 ml were collected using an automatic fraction collector and the radioactivity in each sample was estimated with a Beckman scintillation counter (Hash 1972).

Results and Discussion

Gunasekaran and Tauro (1982) reported a decrease in the rate of entry of the carboxin into the cells after growth resumption. Perhaps, in the presence of carboxin the cells undergo a structural alteration and thereby restrict the entry of carboxin into the cells. Although there are no such reports in fungi, in *Staphylococcus aureus*, one of the mechanism of resistance development to tetracycline has been reported to be due to alteration in the cytoplasmic membrane (Sompolinsky et al. 1970, Levy & Mc Murrey 1978). If growth-resumption in the presence of carboxin is mediated by specific proteins which alter membrane properties, then the inhibition of complete protein synthesis during the early-stages of exposure to carboxin should proportionately affect the length of recovery period. To test this the cells were exposed to cycloheximide in a medium containing carboxin, and the resulting lag period before growth resumption in the presence of carboxin was examined (figure 1). Cycloheximide (10 μg/ml) completely inhibits protein synthesis while RNA synthesis is not affected for 60 min.

Cells exposed to 100 μg/ml carboxin show only a delay of about 60–70 min before growth resumption, while cells exposed to 10 μg/ml cycloheximide and then washed, show a lag of about 30–40 min. Cells treated with cycloheximide at T_0, T_{30} or T_{60} for 30 min in carboxin medium show varying period of lag in the subsequent transfer to carboxin medium (figure 1). The decreases in the length of the lag period with time of addition of cycloheximide suggest that the proteins necessary for restricting the entry of carboxin is induced by carboxin within the first 30–60 min of exposure to carboxin.

Fractionation of Plasma Membrane Proteins

To determine if the proteins involved in allowing growth in the presence of carboxin are membrane-associated, plasma membranes isolated from the cells exposed to carboxin and ^{14}C-threonine for 2 hr were fractionated on a Sephadex G-100 column. Similarly, plasma membrane-proteins from untreated cells were also labelled and fractionated. The isotope distribution profile in the various fractions of the two membrane samples is shown in figure 2. The spectra are almost identical except for two distinct peaks (A and B) seen in the carboxin-exposed membrane fraction. However, the level of certain membrane-proteins (fractions 50-64, 66-80 and 112-128) is altered in the carboxin-exposed membrane fractions. It, therefore, appears that altered level of membrane-proteins and the new proteins synthesized in the presence of carboxin are involved in allowing growth resumption.

Conclusions

In the yeast *S. cerevisiae*, carboxin inhibits growth by partial inhibition of protein

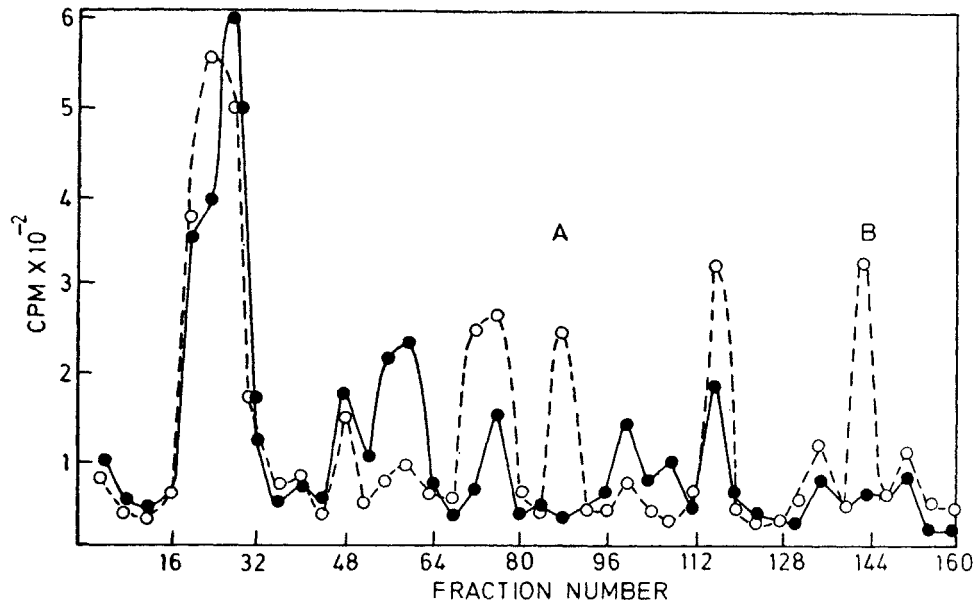

Figure 1 Effect of cycloheximide on resistance development in yeast. **1**, Control; **2**, Cycloheximide(10 μg/ml)—treated cells in carboxin free medium; **3**, Cycloheximide (100 μg/ml)—untreated cells in carboxin medium; **4**, Cycloheximide treatment at T_{60}; **5**, Cycloheximide treatment at T_{30}; **6**, Cycloheximide treatment at T_0; Inset: Effect of cycloheximide on protein and RNA syntheses
A, Protein synthesis; *B*, RNA synthesis.
●—● Control; 0—0 Cycloheximide (10 μg/ml)

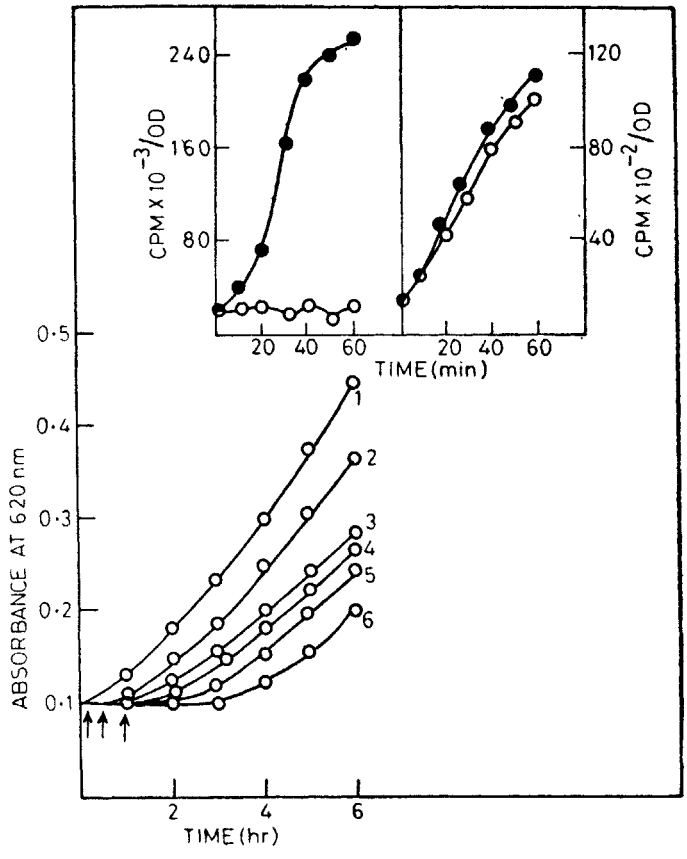

Figure 2 Plasma membrane protein profile:
●—● Control; 0—0 Carboxin treated

synthesis but during this period RNA synthesis remains normal (Gunasekaran & Tauro 1982). It appears that during exposure to carboxin, specific proteins are synthesized which are responsible for restricting the entry of the fungistat into the cells. Resistance development may also involve altered level of certain membrane proteins as shown by the present results. Consequently, total inhibition of protein synthesis during exposure to carboxin extends the length of recovery period. Preliminary studies on the analysis of cytoplasmic membrane-proteins indicate that cells exposed to carboxin possess altered levels of certain membrane proteins and at least two new proteins which are not found in unexposed-cells. It is likely, therefore, that these proteins bring about a structural alteration of the cytoplasmic membrane and thereby restrict the transfer of the fungistat into the cells.

Acknowledgements

The financial assistance to one of the authors (PG) from the Council of Scientific and Industrial Research, during this period of investigation is gratefully acknowledged.

References

Edgington L V and Barron G L 1967 Fungitoxic spectrum of oxathun compounds; *Phytopathol.* **57** 1256

Ferenczy L and Maraz A 1977 Transfer of mitochondria by protoplast fusion in *Saccharomyces cerevisiae*; *Nature* **268** 524

Gunasekaran P 1980 Mechanism of resistance development to fungitoxicants in fungi; Ph.D Thesis, Haryana Agricultural University, Hissar

—— and Tauro P 1982 Mechanism of action of carboxin and development of resistance in yeast; *J. Biosci.* **4** 219

Hash J H 1972 Liquid scintillation counting in Microbiology; in *Methods in Microbiology* Vol 6B p 109 eds J R Norris and D W Ribbon (New York: Academic Press)

Levy S B and McMurrey L 1978 Plasmid-determined resistance involves new transport system for tetracline; *Nature* **276** 90

Longley R P, Rose R H and Knight B A 1968 Composition of protoplast membrane from *Saccharomyces cerevisiae*; *Biochem. J.* **180** 401

Rose A H and Nickerson W J 1956 Selection of nicotinic acid by biotin-dependent yeast; *J. Bacteriol.* **72** 324

Sompolinsky D, Krawitz T, Zaidenzaig Y and Abramova 1970 Inducible resistance to tetracycline in *Staphylococcus aureus*; *J. Gen. Microbiol.* **62** 341