Effect of growth factor deficiency on nystatin sensitivity in *Saccharomyces cerevisiae*

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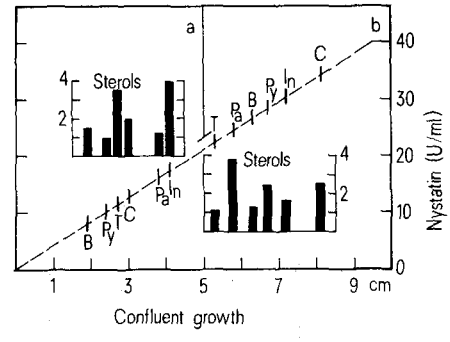
Summary. The nystatin sensitivity, as well as the levels of total sterols and individual phospholipids of *Saccharomyces cerevisiae* are influenced by variations in the supply of growth factors.

Antifungal polyene antibiotics exert their cytotoxic effect by interaction with the sterol constituents of the cytoplasmic membrane of sensitive cells[1,2]. This fact can be connected with observations that the sterol content, quantitatively and qualitatively, varies with the degree of antibiotic resistance[3,4], usually developed by the reculturing of fungi on media containing successively higher concentrations of polyene antibiotics. We observed that a growth factor deficiency stimulates changes in nystatin sensitivity of cells, and we tried to correlate these changes with changes in the content of sterols and of individual phospholipids.

Materials and methods. Nystatin (mycostatin, 4690 U/mg) and growth factors were purchased from Calbiochem, ergosterol from Sigma Chemical Co., St Louis, Miss., USA and individual phospholipid standards from Serva Feinchem., Heidelberg, Germany. *Saccharomyces cerevisiae* strain U 92 was obtained from the culture collection of the Prague Institute of Chemical Technology. The organism was grown in Olson-Johnson synthetic medium[5] with 10 µg biotin; 500 µg thiamine; 50 µg inositol; 5 mg calcium pantothenate; 1 mg pyridoxine per litre (optimum concentrations). Glucose was supplied at 10 g/l. Deficient media (liquid or solidified) were obtained by total omission of biotin or thiamine, by a reduction in the concentration of inositol or pyridoxine to 20%, or by reducing the concentration of pantothenate to 40% (80% or 60% deficiency). The media were solidified with 2% Oxoid agar No. 3. Cultivation in liquid media were carried out under intensive aeration in 100 ml medium at 28 °C. Deficient cultures were inoculated with a 10 ml culture pregrown for about 20 h in the corresponding deficient medium. Gradient plates[6] were prepared in squares (95 × 50 mm) using 25 ml solidified medium per layer. The required amount of antibiotic was dissolved in 2% dimethylformamide, which at this concentration showed no effect on the colony forming ability of deficient or supplemented cells. The gradient plates were inoculated with 0.2 ml culture grown in the absence of nystatin. The minimum inhibitory concentration (MIC) of nystatin was taken as the limit of confluent growth on gradient plates after 40 h of incubation at 28 °C. The total sterol content was determined according to Longley et al.[7] using mild acid pre-treatment[8] of cells and anhydrous ergosterol for calibration. Individual phospholipids were separated and determined by 2-dimensional TLC[9] combined with phosphorus estimation[10]. All determinations were executed in duplicate at the time of inoculation of the gradient plates. Cells were harvested for analysis in the middles of the exponential and stationary phases of individual growth curves.

Results and discussion. Growth factor deficiency may result in changed, mostly enhanced susceptibility of yeast cells to nystatin (figure). The changes are dependent on the growth phase of the cultures. No uniform sterol content - nystatin sensitivity pattern was observed. These results are in agreement with the suggestion that membrane sensitivity to polyene antibiotics is determined by the overall membrane organisation, including the changes in structure of membrane components[4], rather than by the level of a single membrane component[11,12].

In view of our results one may assume that some variations in growth factor supply can stimulate the development of certain membrane alterations. In this connection, the content of individual phospholipids in deficient cells has been



Nystatin sensitivity of *Saccharomyces cerevisiae* cells. The gradient plates were inoculated with cells from the stationary (a) or exponential (b) growth phase taken from nutrient sufficient of deficient media. (– – – – –), linear 0-40 U nystatin gradient; MICs (– + + + + –) in the case of cell populations deficient in biotin (B), thiamine (T), inositol (In), pantothenate (Pa), pyridoxine (Py), and supplemented cells (C). The effect of individual deficiencies on sterol content (figures inserted) is expressed as % of dry cell weight. Values are the means of duplicate measurements.

Effect of growth factor deficiency on the content of individual phospholipids in *Saccharomyces cerevisiae* cells grown on glucose

Phospholipids*	Growth phase	Control	Deficiency (%)				
			Biotin	Thiamine	Pantothenate	Inositol	Pyridoxine
Phosphatidyl	Exponential	18.4	23.0	17.8	17.5	18.9	17.5
choline	Stationary	24.2	33.8	36.5	33.3	35.8	30.2
Phosphatidyl	Exponential	44.5	34.7	33.8	32.9	32.4	35.6
ethanolamine	Stationary	36.7	30.0	28.2	31.1	25.6	31.0
Phosphatidyl	Exponential	25.2	25.0	27.9	27.7	27.6	30.2
inositol	Stationary	28.4	22.1	34.3	22.4	22.3	28.4
Phosphatidyl	Exponential	10.6	10.5	12.5	11.5	11.6	12.1
serine	Stationary	13.4	13.3	3.4	12.4	12.4	14.7

* The contents of individual phospholipids are expressed as lipid phosphorus (µmoles/g dry cell weight). Values are the mean of duplicate measurements.

determined and compared with that of control cells. As can be seen from the table, the respective deficiencies, in stationary cells, stimulate an increase in the content of phosphatidyl choline and a decrease in the content of phosphatidyl ethanolamine. The changes registered in the content of phosphatidyl inositol and phosphatidyl serine in stationary cells are dependent on the nature of the deficiency. A uniform decrease in the content of phosphatidyl ethanolamine was also registered in deficient exponential cells. On the other hand, in these cells the content of phosphatidyl choline, phosphatidyl inositol and phosphatidyl serine is unchanged or only slightly increased.

Considering the role of sterols or phospholipids in the microarchitecture of biological membranes we can assume that both components play a role in membrane susceptibility to polyene antibiotics especially, if polyene sensitivity is dependent on the degree of membrane fluidity. In this connection, our results suffer from the shortcoming that the lipids of both classes are extracted from the whole, unfractionated cells and that changes in the composition of the cytoplasmic membrane only may be masked. Similarly, it should be pointed out that nystatin sensitivity may be determined in part by binding factors in the cell wall[13], and that our analyses would not reveal changes in cell wall organization. Nevertheless, our results suggest that growth factor deficiencies allow us to manipulate the content and composition of membrane components and nystatin sensitivity of cells. This approach could be used for further studies of the mode of action of polyene antibiotics at the molecular level.

1 J.M. Hamilton-Miller, Bact. Rev. *37*, 166 (1973).
2 A.W. Norman, A.M. Spielvogel and R.G. Wong, Adv. Lipid Res. *14*, 127 (1976).
3 M. Fryberg, A.C. Oehlschlager and A.M. Unrau, Archs Biochem. Biophys. *173*, 171 (1976).
4 A.M. Pierce, H.D. Pierce, Jr, A.M. Unrau and A.C. Oehlschlager, Can. J. Biochem. *56*, 135 (1978).
5 B.H. Olson and M.J. Johnson, J. Bact. *57*, 235 (1949).
6 V. Bryson and W. Szybalski, Science *116*, 45 (1952).
7 R.P. Longley, A.H. Rose and B.A. Knights, Biochem. J. *108*, 401 (1968).
8 R.A. Gonzales and L.W. Parks, Biochim. biophys. Acta *489*, 507 (1977).
9 A. Čejková and V. Jirků, Folia microbiol. *23*, 372 (1978).
10 C.W.F. McClare, Analyt. Biochem. *39*, 527 (1970).
11 C.C. HsuChen and D.S. Feingold, Nature, Lond. *251*, 656 (1974).
12 C.C. HsuChen and D.S. Feingold, Biochem. biophys. Res. Commun. *51*, 972 (1973).
13 S.M. Hammond and B.N. Kliger, Antimicrob. Agents Chemother. *9*, 561 (1976).

Encapsulation of *Psorospermium haeckeli* by the haemocytes of *Astacus leptodactylus*

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Summary. Psorospermium haeckeli, a parasite of freshwater crayfish, has sometimes been observed encapsulated within the tissues of *Astacus leptodactylus*. This observation does not agree with earlier reports. The lack of tolerance is discussed in relation to variations affecting either the parasite or its host.

In arthropod species, penetration into the internal medium by either living or inert extraneous bodies generally induces a defense reaction of the host with an encapsulation of these foreign 'organisms'. A progressive accumulation of blood cells tightly grouped around the organism is involved; this is a common defence reaction in invertebrates[1,2]. Such encapsulation effectively prevents the development of parasites such as trematodes, cestodes, nematodes, Hymenoptera and tachinids, as well as pathogenic fungi[3,4]. However, a parasite which is sometimes present in large numbers within the tissues of different crayfish species has always been found unencapsulated, and seems to be tolerated by its host. It was first described by Haeckel[5], and then defined as *Psorospermium haeckeli* by Hilgendorf[6]. This parasite has been observed in several *Astacus* species[5,8-11] and in *Cambarus affinis*[12-14]. It is poorly known and its taxonomic status has not been clearly determined, although it is in fact considered to be a sporozoan protozoon. It invades various tissues[10,11] but, until now, no evident cellular reaction of the crayfish has been reported and the direct damage to the crayfish tissues is unknown[15]. Since 1976 we have periodically found an analogous parasite within the tissues of some groups of *Astacus leptodactylus*, but it sometimes appears to be encapsulated. It has been identified, with the assistance of M. Vey, as belonging to the species *Psorospermium haeckeli*. However it is able to induce an encapsulation reaction by the host. Thus, it seems that the reaction of the crayfish may show different modalities after aggression by this pathogen.

Materials and methods. Adult crayfish *Astacus leptodactylus* originating from Central Europe were obtained from local fish merchants. 25 to 30 groups of crayfish, each with 20-30 animals, were all examined; a total of over 500 subjects. Pieces of several tissues, as well as hemolymph withdrawn on anticoagulant (10% aqueous sodium citrate = 1:9), were collected and examined through an optical microscope to look for parasites. Histochemical tests with PAS hematin were performed after fixing in formalin.

Results. Every year the parasites were found only in certain groups of crayfish, mainly during the spring and sometimes early in the autumn. The rate of infestation of each type and each animal was not precisely recorded since the presence of this parasite was a common feature. All these groups of crayfish were infected and in 2 of them, cellular reactions of encapsulation were observed. Both encapsulated or unencapsulated parasites invaded the same tissues: they were located at the base of the antenna and the antennula, within the ventral sinus, the sub-epidermal tissues, and in fact everywhere within the adipocyte rich organs. The vitality of the crayfish, as well as their outer appearance, seemed to be unchanged. Parasites with 2 different shapes were observed; the majority were ovoid but a few were round. The organisms had a very thick outer shell, which contained spherical bodies of different sizes