IN VIVO DIFFERENTIATION OF YEAST CYTOPLASMIC AND MITOCHONDRIAL PROTEIN SYNTHESIS WITH ANTIBIOTICS.

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Received July 27, 1966

We have previously shown that chloramphenicol completely inhibits the formation of cytochromes a, a_3 during active growth of Saccharomyces cerevisiae (Huang et al., 1966) and that the formation of a number of other membrane bound mitochondrial enzymes, notably cytochromes b, c_1 and succinate dehydrogenase are markedly inhibited (Linnane et al., 1966). These results have been correlated with the finding that chloramphenicol inhibits amino acid incorporation by isolated yeast mitochondria (Wintersberger 1965, Linnane et al., 1966) but does not affect amino acid incorporation by cytoplasmic ribosomal systems from yeast (So and Davie 1963). We have suggested that in yeast cells and probably in cells of higher organisms that there are two distinct protein synthesizing systems, one localized in the mitochondria and sensitive to chloramphenicol, the other, the cytoplasmic ribosomal system and insensitive to chloramphenicol (Linnane et al., 1966). As bacterial and yeast mitochondrial protein synthesizing systems are similar in their response to chloramphenicol, we therefore tested some other antibiotics, known to inhibit bacterial protein synthesis (Wolfe and Hahn 1965, Vazquez 1966), for their effects on mitochondrial protein synthesis in S.cerevisiae.

The present communication reports that chloramphenicol, tetracycline, oxytetracycline, erythromycin, carbomycin, spira-

mycin, oleandomycin and lincomycin cause a total or partial inhibition of cytochrome *a*, a_3 formation in growing *S.cerevisiae*. In this respect the response of the mitochondrial protein synthesizing system to these antibiotics further demonstrates its similarity to bacterial protein synthesizing systems.

RESULTS

The results presented in Table I describe the effect of the antibiotics on the growth and cytochrome *a*, a_3 content of the yeast; the changes in the amount of *a* cytochromes formed may be equated with an effect on mitochondrial protein synthesis. Chloramphenicol, tetracycline, oxytetracycline, erythromycin and carbomycin all caused a complete inhibition of cytochrome *a*, a_3 formation; at concentrations of the drugs below those listed in Table I only a partial inhibition of cytochrome *a*, a_3 synthesis was obtained. With spiramycin, oleandomycin and lincomycin only a partial inhibition of cytochrome *a*, a_3 synthesis was obtained with the highest concentration of the drug employed. This partial inhibition could perhaps be due to a limited penetration of the antibiotic into the cell or to a lower activity of the antibiotic in inhibiting mitochondrial protein synthesis.

It was also found that in the absence of cytochrome *a*, a_3 the final cell yield of the yeast was influenced by the amount of fermentable substrate in the medium. Thus with cells grown in the presence of chloramphenicol or similar antibiotics, in a medium containing 1% glucose (Wallace & Linnane 1964) the final cell yield was less than that obtained in the absence of antibiotic. However, if excess fermentable substrate was available (a 5% glucose medium) then the growth of the organism, even in the presence of the highest levels of antibiotics shown in Table

TABLE I

THE GROWTH AND CYTOCHROME a, a_3 CONTENT OF S. CEREVISIAE IN THE PRESENCE OF ANTIBIOTICS.

Antibiotic	Concentration of antibiotic (μg/ml.medium)	Cell Yield (mg.dry wt.cells/ ml. medium)	Cytochromes a, a_3.
NONE	-	3.3	+
Chloramphenicol	500	2.0	—
	4000	1.5	—
Tetracycline hydrochloride	250	1.7	—
	2000	1.4	—
Oxytetracycline hydrochloride	250	1.7	—
	2000	1.4	—
Erythromycin glucoheptonate	250	1.8	—
	20000	1.2	—
Carbomycin base	500	3.2	±
	2000	1.5	—
Spiramycin base	500	3.3	+
	2000	2.3	±
Oleandomycin phosphate	2000	3.1	+
	10000	2.3	±
Lincomycin hydrochloride	500	2.8	+
	2000	2.7	±
Cycloheximide	0.1	Growth rate halved	+
	1.0	No growth	+

The yeast was grown aerobically in a 1% glucose Difco yeast extract-salts medium (Wallace & Linnane 1964) for 16 hours at 28°C, in the presence of the antibiotics as indicated. The cells were harvested, washed and aliquots taken for dry weight and absorption spectrum determinations as described previously (Huang et al., 1966). The occurrence of cytochromes a, a_3 in the yeast is indicated by: + normal, ± partial inhibition of cytochrome a, a_3 formation, — total absence of cytochrome a, a_3.

I was the same as the control, although cytochrome a, a_3 formation was still inhibited (Linnane et al., 1966). These results

have been interpreted to indicate that the cytoplasmic ribosomal protein synthesizing system of the cell is unaffected by high levels of the antibiotics but in the absence of a functional electron transport chain the organism is unable to aerobically utilize the products of fermentation. The growth of yeast is therefore reduced in medium containing limiting amounts of fermentable substrate.

In contrast to the antibiotics discussed above, cycloheximide, does not affect the growth of bacteria or inhibit bacterial cell free protein synthesis, but it does inhibit the growth of some yeasts (Whiffen 1948) and cells from higher organisms (Bennett *et al.*, 1964). Further, cycloheximide has been shown to inhibit protein synthesis by cytoplasmic ribosomes from yeast (Siegel and Sisler 1965) and more highly evolved organisms (Ennis and Lubin 1964). It was therefore examined for its effect on the formation of cytochromes *a*, a_3 by *S.cerevisiae* to try to determine whether the formation of these mitochondrial enzymes was carried out by the cytoplasmic ribosomal protein synthesizing system.

At a cycloheximide concentration of 0.1 μg/ml the growth rate of the yeast was approximately half that of the control. Under these conditions the formation of cytochrome *a*, a_3 was not preferentially affected. This result strongly suggests that the loss of cytochrome *a*, a_3 from the absorption spectrum of the yeast in the presence of chloramphenicol and other antibiotics is not due to a non specific response of the yeast to stress conditions.

DISCUSSION

It appears, on the basis of sensitivity to a number of antibiotics that *S.cerevisiae* has two protein synthesizing

systems. One, the cytoplasmic ribosomal system sensitive to cycloheximide and largely unaffected by the inhibitors of bacterial protein synthesis; the other, the mitochondrial system previously shown to be inhibited both *in vivo* and *in vitro* by chloramphenicol (Linnane *et al.*, 1966) and herein demonstrated to be inhibited *in vivo* by tetracycline, oxytetracycline, erythromycin, carbomycin, spiramycin, oleandomycin and lincomycin but not by cycloheximide.

It is of interest that chloramphenicol has been found to inhibit the synthesis of chlorophyll in heterotrophically grown *Euglena gracilis* (Smillie *et al.*, 1963) and this observation has recently been extended in this laboratory to include tetracycline and erythromycin as inhibitors of chlorophyll synthesis in this organism (Linnane unpublished expts). Furthermore green plants have been found to contain two distinct types of ribosomes characterized by their sedimentation values. Thus chloroplasts from spinach leaves (Lyttleton 1962) and tobacco leaves (Boardman *et al.*, 1965) contain 70S type ribosomes similar to those found in bacteria, while the cytoplasm of these plants contain the 80S type ribosomes generally associated with protein synthesis in organisms other than bacteria and blue green algae.

We suggest, based on this knowledge and the results with the various antibiotics described above, that protein synthesis in yeast mitochondria is probably mediated by ribosomes resembling the 70S type from bacteria and chloroplasts. It also seems likely that some specific characteristics of protein synthesizing systems are proscribed by their ribosomal size type.

ACKNOWLEDGEMENTS

This work was supported by the National Health and Medical Research Council of Australia and the U.S. Public Health Service

Grant GM 10496-04. We also wish to thank Parke Davis & Co. for their generous support of the project.

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