THE INTRACELLULAR SITE OF FORMATION OF THE MITOCHONDRIAL PROTEIN SYNTHETIC SYSTEM.

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Certain antibacterial antibiotics such as chloramphenicol or erythromycin inhibit the formation *in vivo* by growing yeast cells of the membrane bound cytochromes ($\underline{a}$ + $\underline{a}_3$), $\underline{b}$ and $\underline{c}_1$ (Clark-Walker and Linnane, 1966). This observation has led to the suggestion that the mitochondrion itself is probably responsible for the synthesis of these components. However, recent studies of *in vitro* amino acid incorporation by mitochondria indicate that the label becomes associated largely with uncharacterized structural proteins; unequivocal labelling of the cytochromes has not been observed, suggesting that perhaps the latter may be synthesised by a non-mitochondrial system (Kadenbach, 1967; Hansby *et al.*, 1969). The question of the identity of the proteins synthesised by the mitochondrion is of considerable interest as it appears that, quantitatively, not more than 10-15% of the organelle is synthesised by itself (Hansby *et al.*, 1969) and consequently much of the protein must be synthesised in the cytoplasm prior to incorporation into the mitochondrion (Work *et al.*, 1968; Beattie, 1968; Kadenbach, 1967).

In bacteria it has been shown that the mutations leading to resistance to both streptomycin and erythromycin result from changes in single ribosomal proteins (Tanaka, 1968; Kremble and Apirion, 1968). A similar mutation in *Saccharomyces cerevisiae* in which the mitochondrial protein synthesising system becomes resistant to erythromycin (Linnane *et al.*, 1968a) has been shown to be cytoplasmically determined by a one-step mutation (Linnane *et al.*, 1968b; Thomas and Wilkie, 1968; Linnane, 1968) and by analogy it has been suggested that the change in sensitivity is due to a change in the mitochondrial

ribosome similar to that occurring in the bacterial system (Linnane et al., 1968a). At the present state of knowledge it is implicit that the cytoplasmic determinant for erythromycin resistance is localised in the mitochondrial DNA, and thus it would seem that at least one of the proteins of the mitochondrial ribosome is coded by mitochondrial DNA. It has also recently been demonstrated that mitochondrial RNA preparations hybridize with mitochondrial DNA (Wintersberger and Viehhauser, 1968; Suyama and Eyer, 1968). Consideration of the coding capacity of the mitochondrial DNA, however, suggests that its information content may be insufficient for the mitochondrial RNA, plus the total complement of mitochondrial ribosomal proteins, although the above genetic evidence strongly supports the view that at least one of the proteins is coded by mitochondrial DNA. The present communication reports experiments which strongly suggest that not only the mitochondrial ribosomal proteins but indeed the total proteins of the mitochondrial protein synthesising system are formed on the cytoplasmic ribosomes. Küntzel (1969) has also recently reported in vivo pulse labelling experiments in the presence of cycloheximide or chloramphenicol which he interprets to indicate that the total proteins of the mitochondrial ribosome are synthesised by the cytoplasmic protein synthesising system.

RESULTS AND DISCUSSION

The antibiotics chloramphenicol, lincomycin and erythromycin have clearly been shown to inhibit yeast mitochondrial protein synthesis both in vivo and in vitro (Clark-Walker and Linnane, 1966; 1967; Lamb et al., 1968; Huang et al., 1966). We have therefore reasoned that by growing cells in the presence of these antibiotics we would inhibit the mitochondrial protein synthesising system and that any proteins that remained in the mitochondria would have been formed by a non-mitochondrial protein synthesising system.

Cells were grown through 5 generations on 2% glucose medium (Clark-Walker and Linnane, 1967) containing 4 mg/ml chloramphenicol or 1.5 mg/ml lincomycin; or on 2% glucose medium with no antibiotic. The cells grown in the presence of chloramphenicol were completely lacking cytochromes ($a + a_3$), b and c_1, but those grown in the presence of lincomycin still possessed

some cytochrome b. The mitochondria isolated from such cells still retained the capacity to incorporate amino acids into protein (Table 1). The mitochondria as initially isolated showed only limited capacity for amino acid incorporation but on washing the preparation, presumably removing any loosely bound or occluded antibiotic, their activity increased. In the representative experiment shown in Table 1 the activity of mitochondria prepared from cells grown in the presence of chloramphenicol increased through 10, 34 to 50 μμmoles amino acid incorporated per mg mitochondrial protein/20 min, the latter value corresponding to about 65% of the activity of mitochondria prepared from normal cells.

The influence of oxygen on the development of mitochondrial protein synthesis was investigated by isolating mitochondrial profiles from yeast cells grown anaerobically on 5% galactose medium supplemented with Tween 80 and ergosterol (Wallace et al., 1968). As expected, the mitochondrial profiles prepared from the anaerobically grown cells contained no cytochromes ($a + a_3$), b, c_1 or c. However, they retained a capacity to incorporate amino acids into protein (Table 1), albeit at a somewhat lower level than the organelles isolated from aerobic cells, and they had an amino acid incorporating activity with characteristics similar to those from cells grown without antibiotic, judged by the sensitivities of the preparations to the several antibiotics investigated. Again similar to the aerobically cultured cells, when the cells were grown anaerobically in the presence of chloramphenicol or erythromycin the isolated mitochondria were still found to retain the capacity for amino acid incorporation into protein (Table 1).

These results are interpreted to indicate that the total proteins making up the mitochondrial protein synthesising system are formed independently of the protein synthesising system of the mitochondria and that oxygen is not obligatory for their formation. More specifically, the proteins of the mitochondrial ribosome, which genetic evidence indicates are coded for by mitochondrial DNA, are formed by a non-mitochondrial system. It would also follow that as a functional mitochondrial ribosome is formed, the mitochondrial polymerase for RNA also is synthesised by a non-mitochondrial system and so also must the

TABLE 1

IN VITRO INCORPORATION OF ^{14}C-LEUCINE INTO PROTEIN BY MITOCHONDRIA ISOLATED FROM S. CEREVISIAE

Antibiotic Addition to Growth Medium		Incorporating Activity: μμmoles ^{14}C-leucine/mg protein/20 mins.		
		Number of Washes to Remove Antibiotic		
		0	1	2
Aerobic Growth				
	None	75	-	-
4 mg/ml	Chloramphenicol	10	34	50
1.5 mg/ml	Lincomycin	-	-	40
Anaerobic Growth				
	None	18	-	17
2 mg/ml	Chloramphenicol	-	-	12
0.5 mg/ml	Erythromycin	-	-	10

Cells of a haploid strain of Saccharomyces cerevisiae were grown aerobically on 2% glucose medium, with or without antibiotics (Clark-Walker and Linnane, 1967). Anaerobic growth of a locally isolated diploid strain took place on 5% galactose medium supplemented with Tween 80 and ergosterol, with or without antibiotic as described by Wallace et al. (1968). Mitochondria were prepared and assayed for protein synthetic activity as described by Lamb et al. (1968). Cells grown in the presence of the antibiotics were incubated with chloramphenicol (2 mg/ml) or erythromycin (1 mg/ml) present during snail gut digestion to prevent any induction of mitochondrial enzymes during this period. The assay system contained no more than 500-1000 bacteria per ml.

activation enzymes and t-RNA polymerisation factors be formed by a non-mitochondrial protein synthesising system. The question of the site of synthesis of the nucleic acids cannot be unequivocally answered by the present experiments. However, it has been shown that in vitro mitochondria can incorporate nucleotides into high molecular weight DNA (Karol and Simpson, 1968) and RNA (South and Mahler, 1968). Thus it would appear likely that

mitochondria synthesise their own high molecular weight RNA and DNA.

The experiments reported in this communication confirm the conclusion drawn by Küntzel (1969) that the proteins of the mitochondrial ribosome are synthesised by the cytoplasmic protein synthesising system, and extend the observation to include all the other proteins required for the activity of the mitochondrial protein synthesising system.

In view of the genetic data, it is proposed, in contrast with Küntzel's suggestion, that at least some of the proteins of the mitochondrial ribosome are coded by mitochondrial DNA. This leads to the conclusion that m-RNA transcribed from mitochondrial DNA is translated in the cytoplasm and that the resulting proteins are then incorporated into the mitochondrion.

REFERENCES

Beattie, D.S., J. Biol. Chem. 243 (1968) 4027.
Clark-Walker, G.D. and Linnane, A.W., J. Cell Biol. 34 (1967) 1.
Hansby, J., Kellerman, G.M., Lamb, A.J. and Linnane, A.W., J. Cell Biol. Submitted.
Kadenbach, B., Biochim. et Biophys. Acta 134 (1967) 430.
Karol, M.H. and Simpson, M.V., Science 162 (1968) 470.
Kremble, J. and Apirion, D., J. Mol. Biol. 33 (1968) 363.
Küntzel, H., Nature 222 (1969) 142.
Lamb, A.J., Clark-Walker, G.D. and Linnane, A.W., Biochim. et Biophys. Acta 161 (1968) 415.
Linnane, A.W. 1968. In Biochemical Aspects of the Biogenesis of Mitochondria. E.C. Slater, J.M. Tager, S. Papa and E. Quagliariello, editors. Adriatica Editrice. Bari, Italy. 333.
Linnane, A.W., Lamb, A.J., Christodoulou, C. and Lukins, H.B., Proc. Nat. Acad. Sci. 59 (1968)a 1288.
Linnane, A.W., Saunders, G.W., Gingold, E.B. and Lukins, H.B., Proc. Nat. Acad. Sci. 59 (1968)b 903.
South, D.J. and Mahler, H.R., Nature 218 (1968) 1226.
Suyama, Y. and Eyer, J., J. Biol. Chem. 243 (1968) 320.
Tanaka, K., Teraska, H., Temaki, M., Otaka, E. and Osawa, S., Science 162 (1968) 576.
Huang, M., Biggs, D.R., Clark-Walker, G.D. and Linnane, A.W., Biochim. et Biophys. Acta 114 (1966) 434.
Thomas, D.Y. and Wilkie, D., Genet. Res. Camb. 11 (1968) 33.
Wallace, G.J., Huang, M. and Linnane, A.W., J. Cell Biol. 37 (1968) 207.
Wintersberger, E. and Viehhauser, G., Nature 220 (1968) 699.
Work, T.S., Coote, L.J. and Ashwell, M., Fed. Proc. 27 (1968) 1174.