CORRELATION OF MEMBRANE BOUND SUCCINATE DEHYDROGENASE WITH THE OCCURRENCE OF MITOCHONDRIAL PROFILES IN SACCHAROMYCES CEREVISIAE.

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This laboratory has recently described an electron microscope study of the cytological transformations initiated by aeration of non-respiring, anaerobically grown yeast cells (Wallace and Linnane, 1964, Linnane 1965). It was reported that anaerobically grown cells appear to be free from recognizable mitochondrial profiles, the mitochondria characteristic of aerobic cells developing as the aeration proceeds. Some controversy has developed over the claim for an absence of mitochondrial membrane in the anaerobic cells, an electron microscope study by Morpurgo et al (1964) has shown the presence of mitochondria in some cell types grown anaerobically whereas Polakis et al (1964) have confirmed our earlier claim for their absence.

Schatz (1965) has adopted an alternate approach to this question and employed succinate dehydrogenase as a marker for the presence of mitochondria or membrane particles derived from mitochondria in anaerobically grown yeast. He has suggested that succinate dehydrogenase might be regarded as one of the few enzymes characteristically associated with mitochondrial membrane, so that the occurrence of this enzyme in membrane fragments from anaerobic yeast might be taken as presumptive evidence for the presence of mitochondrial membrane in these cells. Schatz has reported the presence of considerable amounts of succinate dehydrogenase in a particle fraction from anaerobically grown yeast and based on the foregoing argument has challenged

the validity of the electron microscope studies purporting to show the absence of mitochondrial membrane.

The respiratory activity of aerobically grown yeast has been known for some time to be under the control of a catabolite repression. Thus cells grown on high concentrations of a rapidly fermentable substrate such as glucose, develop low respiratory activity (Slonimski, 1953) while cells grown on high concentrations of slowly fermentable substrate, such as galactose, develop high respiratory capacity (Strittmatter 1957; Tustanoff & Bartley, 1964). More recently Linnane (1965) has shown that the number as well as the composition of the individual mitochondria within aerobically grown yeast are influenced by the main carbon source of the growth medium. The present communication reports that the occurrence of membrane bound succinate dehydrogenase and mitochondrial profiles in anaerobically grown yeast is determined by the composition of the growth medium.

The results are summarized in Table I. Cells grown aerobically on glucose or galactose media contain numerous mitochondria and considerable amounts of succinate dehydrogenase, the enzyme content of galactose grown cells being some three to four times higher than cells cultured on glucose. The difference in total amount of enzyme in the cells is well correlated with the total number of mitochondria in each cell type. The formation of the mitochondria and the accompanying succinate dehydrogenase is clearly subject to a catabolite repression (cf. also Linnane, 1965).

For anaerobic growth of yeast cells a source of unsaturated fatty acids and ergosterol is required. In the present experiments, cells grown anaerobically without the addition of Tween 80 and ergosterol obtain the necessary trace amounts of their lipid requirements from the Difco Yeast Extract of the medium.

The cells grown anaerobically on galactose without lipid supplements contain only traces of succinate dehydrogenase (Table I) and when examined in

TABLE I

Succinate Dehydrogenase Activity of Total Particle Fractions From S. cerevisiae Grown Aerobically and Anaerobically.

Additions to Media	Mitochondrial Profiles	Succinate Dehydrogenase Activity: Specific Activity	Succinate Dehydrogenase Activity: Total Activity
		Aerobic Growth	
Glucose	++	0.095	3.3
Galactose	++++	0.310	10.8
		Anaerobic Growth	
Glucose	-	< 0.002	< 0.07
Glucose+T+E	+/-	< 0.002	< 0.06
Galactose	+/-	0.004	0.09
Galactose+T+E	++	0.021	0.57

The cells were grown aerobically or anaerobically on a basic inorganic salts - 0.5% Difco Yeast Extract medium (Linnane, 1965) with 5% glucose or galactose, 0.5% Tween 80 (T) and 0.002% ergosterol (E) added as indicated.

The cells were harvested, washed, suspended in 0.4 M mannitol - 0.001 M EDTA - 0.02 M tris buffer pH 7.4 and quantitatively smashed by two passages through a French pressure cell operated at 9 tons/square inch pressure. The cell debris was removed by centrifugation at 2000 x g and the resultant whole homogenate recentrifuged at 105,000 x g for 90 mins. yielding a particulate fraction designated "total particle fraction".

The occurrence of mitochondrial profiles in yeast as shown by electron microscopy (Linnane, 1965; Morpurgo, 1964; Polakis et al, 1964, 1965) are tabulated as; ++++ equivalent to numerous profiles, perhaps 200 per cell, +/- equivalent to the order of 0 to 5 per cell and - signifying mitochondrial profiles not recognised.

Succinate dehydrogenase was assayed using a phenazine methosulphate-dichlorophenolindophenol dye mixture as electron acceptors (Arrigoni and Singer 1962). Specific activities are expressed as µmoles succinate oxidized/mg. protein/min. and total activities as µmoles succinate oxidized/min./100 mg. whole homogenate protein.

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the electron microscope, only an occasional single membrane vesicle is observed within the cytoplasm. The vesicles possess only traces of internal membrane and have an electron density similar to that of the cytoplasm,

presumably they may represent a primitive mitochondrial profile. The inclusion of Tween 80 and ergosterol in the galactose medium produces a profound change in the succinate dehydrogenase content and in the cytological characteristics of the cells. These cells possess numerous well defined mitochondrial profiles and the particulate fraction isolated therefrom contains considerable amounts of succinate dehydrogenase, although the level is only respectively about 5 and 15% of that achieved by the aerobically grown galactose and glucose cells (Table I).

In support of the earlier electron microscope studies reporting the absence of mitochondrial profiles from S. cerevisiae grown anaerobically on the glucose medium without lipid supplementation; a particulate succinate dehydrogenase was not detected in sub-cellular fractions (Table I). It is difficult, if not impossible to sustain an argument for the complete absence of an enzyme from any given system. However, using a Gilford 2000 spectrophotometer, our assay can record amounts of particulate enzyme activity as low as 0.002 μmoles succinate oxidized/mg. protein/min., and as a consequence at least, it can be reported that the activity of the fraction from these cells was less than this value.

Cells grown anaerobically on the glucose medium supplemented with Tween 80 and ergosterol also did not contain detectable amounts of succinate dehydrogenase, although the presence of the lipids in the medium leads to some slight cytological differentiation. These cells contain an occasional single membrane vesicle similar to those observed in the cells cultured on galactose.

Schatz (1965) has suggested that our electron microscope studies (Wallace & Linnane 1964, Linnane 1965) reporting the absence of recognizable mitochondrial profiles from anaerobically grown cells must be in error as his work demonstrates the presence of substantial amounts of particulate succinate dehydrogenase in anaerobically grown cells. The present work reporting at best extremely low values for succinate dehydrogenase in cells grown anaero-

bically on glucose supports our earlier claim for the possible absence of mitochondrial membrane from these cells. However, it is also apparent that the formation of succinate dehydrogenase and associated mitochondrial profiles in the anaerobic cells is influenced by a catabolite repression and the lipid composition of the medium. It is therefore possible to achieve a wide range of enzyme contents determined by the growth conditions. No doubt, much of the conflicting evidence in the literature on the presence or absence of succinate dehydrogenase in anaerobic cells has arisen through the various growth conditions employed (Hebb et al, 1959; Rossi et al, 1964; Schatz, 1965 and Linnane et al, 1962). Strain and certainly species differences also probably influence the extent of catabolite repression of mitochondrial profile and succinate dehydrogenase formation.

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