THE ISOLATION OF POSSIBLE MITOCHONDRIAL PRECURSOR STRUCTURES FROM AEROBICALLY GROWN BAKER'S YEAST.

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At present, the steps involved in the formation of mitochondria are unknown, primarily because of the lack of a suitable experimental system, since most cells containing mitochondria will not tolerate any extensive modification of their respiratory apparatus. However, such limitations do not apply to the facultative anaerobe, Saccharomyces cerevisiae, whose respiratory system can be drastically altered either by mutation or by environmental conditions, even though the electron transfer steps of aerobic yeast as well as its mitochondria are quite similar to those of mammalian cells (Slonimski 1953; Chance 1959; Vitols et al. 1961).

Results from earlier work failed to provide evidence in favor of a genetic continuity of yeast mitochondria (Schatz et al. 1963). Thus, de novo synthesis of yeast mitochondria appeared possible. In a preliminary communication, Linnane et al. reported the absence of mitochondria in anaerobically grown cells of Torulopsis utilis. An intracellular membrane system was identified in these cells, tentatively designated by the authors as a mitochondrial precursor system (Linnane et al. 1962). The present report deals with the identification and isolation of as yet unknown cytoplasmic particles from aerobic cells of S. cerevisiae. The properties of these particles are consistent with those expected for mitochondrial precursors. The experimental approach was based on previous studies by Ephrussi et al. who had shown that glucose specifically interferes with mitochondrial development in these cells (Ephrussi et al. 1956). Aerobic growth in various concentrations of glucose thus enabled the large scale isolation of cells with a graded development of their mitochondrial apparatus.

Experimental : The previously employed (Schatz et al. 1963; Schatz and Klima 1963) strain W of S. cerevisiae was grown in the medium described by Ephrussi (Ephrussi and Slonimski 1950) which was modified as follows : The amount of yeast extract was lowered to 0.3 % and the amount of glucose was varied (see text). For growth in the absence of glucose, 6 % glycerol was included. For anaerobic growth, the medium contained 10 % glucose

and was supplemented with 0.26 % Tween 80 and 12 ppm ergosterol. All cells were grown at 28° with constant shaking. The aerobic cells were grown in a continuous stream of sterile air (0.5 l/min). All cells were harvested in the stationary phase. The homogenizing procedure, gradient centrifugation, enzymic assays and protein estimation are described elsewhere (Schatz et al. 1963; Schatz and Klima 1963). Specific activities are expressed as μmoles of substrate oxidized per minute per mg protein at 37° in a total volume of 3 ml.

Results : Yeast was grown in varying concentrations of glucose. After harvesting and homogenizing the cells, the specific activities of the various electron transport enzymes in the homogenate were plotted against the concentrations of glucose employed in the growth medium. As seen in figure 1, the specific activities of all enzymes tested were increased upon lowering the glucose concentration in the growth medium. However, the effect of glucose on succinate-cytochrome c reductase activity was particularely pronounced. A rather abrupt rise of this enzymic activity occurs below 2.5 %. In separate experiments it was found that up to this concentration glucose was the limiting factor for growth under the culturing conditions employed. In anaerobic cells, only succinate dehydrogenase was present of the respiratory enzymes tested [+], in confirmation of previous findings (Hebb et al. 1959; Linnane et al. 1962). Specific activity of succinate dehydrogenase was the same as in homogenates of cells grown aerobically in 5.4 % glucose. Thus, of the respiratory enzymes tested, succinate-cytochrome c reductase proved to be the one most sensitive to conditions known to be (Ephrussi et al. 1956) detrimental to mitochondrial development in yeast. In contrast, succinate dehydrogenase was highly active even in the non-respiring anaerobic cells. These experiments suggested that succinate-cytochrome c reductase was likely to be lacking in mitochondrial precursors and succinate dehydrogenase was likely to be present. In addition, although with less certainty, DPNH-cytochrome c reductase and DPNH oxidase could be expected to be associated with such structures, since these enzymic activities responded like succinate dehydrogenase to the inhibiting effect of glucose. Previous studies (Schatz and Klima 1963) have shown that if a homogenate of commercially grown aerobic yeast was layered on top of a linear sucrose gradient and centrifuged for 1-3 hours, all respiratory chain enzymes formed a single band in the gradient. The position of this band depended somewhat on the composition of the homogenizing medium; if 0.25 M sucrose was employed it was found at a density of 1.16 - 1.17 g. cm^{-3}. The particles of this band appeared as well developed mitochondria if viewed in the electron microscope (Schatz et al. 1963). However, if a homogenate of cells grown in 5.4 % glucose was centrifuged in the density gradient, only succinate-cytochrome c reductase equilibrated exclusively at this density. The bulk of succinate dehydro-

[+] The trace of DPNH-cytochrome c reductase present has been shown to be a microsomal enzyme (Schatz and Klima 1963).

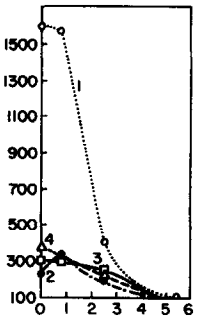

Fig. 1

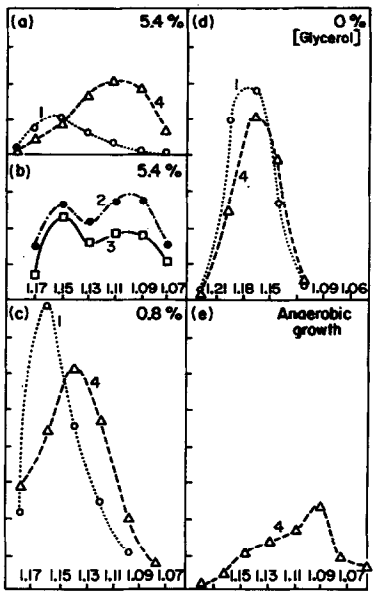

Fig. 2

Figure 1 : Specific activities of succinate-cytochrome c reductase (1), DPNH oxidase (2), DPNH-cytochrome c reductase (3) and succinate-dehydrogenase (4) in homogenates of yeast cells grown in varying concentrations of glucose. Abscissa : % glucose in growth medium. Ordinate : relative specific activity. Absolute specific activities of a homogenate of cells grown in 5.4 % glucose, taken as 100 %, are : (1) : 0.003; (2) : 0.037; (3) : 0.016; (4) : 0.036.

Figure 2 : Distribution of electron transfer enzymes after centrifuging yeast homogenates for 2 hours in a linear sucrose gradient. Abscissa : density in g. cm^{-3}. Ordinate : Enzymic activity in arbitrary units. The various enzymes are represented by the numbers given in the legend of figure 1. The glucose concentrations used for growth are given in the upper right hand corners.

genase and more than half of DPNH-cytochrome c reductase and DPNH oxidase were found at a much lower density[+]. Thus, they must be associated with as yet unknown particles which can be easily separated from typical mitochondria (figure 2a, b). These new particles became less abundant if the cells were grown in decreasing concentrations of glucose. Concomitantly, their band shifted to higher densities (figure 2c). Cells grown on glycerol lacked these structures and thus resembled commercial baker's yeast as well as mammalian cells in that all respiratory chain enzymes were associated with fully developed mitochondria (figure 2d). Preliminary experiments indicate that cells of T. utilis are much less suited to such studies as both the inhibitory effect of glucose and the presence of the aerobic precursors are much less pronounced than in the case of S. cerevisiae. If homogenates of anaerobic yeast were analyzed by following succinate dehydrogenase activity in the gradient, a distribution curve similar to that found after aerobic growth in 5.4 % glucose was observed.

[+] The particles carrying these latter activities had probably not yet reached equilibrium, in contrast to the fully developed mitochondria

However, the distribution curve was less distinct, with indications of a second peak at higher densities (figure 2e). By gradient centrifugation, the new particles from aerobic yeast could be separated from the previously described (Schatz and Klima 1963) microsomal particles rich in TPNH-cytochrome c reductase activity. The possibility, that the new particles from aerobic yeast were artifacts arising from mitochondria during homogenization was rendered unlikely by the following experiments : A homogenate of yeast cells grown in 5.4 % glucose was divided into 4 aliquots : The first aliquot was rehomogenized under the same conditions as before, the second aliquot was sonicated for 90 seconds, the third aliquot was frozen-thawed 3 times whereas the last aliquot served as control. In all four samples, the activity ratio succinate-cytochrome c reductase/succinate dehydrogenase was found to be the same within experimental error. Had the particles been formed artifically from mitochondria, then at least one of these treatments deleterious to mitochondria should have lowered this ratio.

Discussion : The new particles described here have escaped detection in previous studies chiefly because mitochondrial fractions were usually washed repeatedly before gradient centrifugation. The enzymic content of these particles as well as their pattern of occurrence in yeast cells grown under different conditions are compatible with their being precursors to mitochondria. Under the conditions investigated their concentration within the cell always appeared to be inversely related to mitochondrial content. As the precursors decribed here are more fully developed than those found in anaerobic yeast (Linnane et al. 1962), the formation of mitochondria within the yeast cell may be visualized according to the following scheme, in which step 1 is inhibited by anaerobiosis, step 2 by glucose :

Stage I	—1→	Stage II	—2→	Stage III
precursor containing only primary dehydrogenases, but no respiratory chain.		precursor devoid of succinate-cytochrome c reductase.		fully developed mitochondrion.

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