Activities of Mitochondrial Enzymes during Aerobic Synchronous Growth of Aerobically and Anaerobically Grown *Saccharomyces cerevisiae*

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ABSTRACT. The mitochondrial enzymes cytochrome-c : O₂-oxidoreductase (E.C.1.9.3.1), NADH : cytochrome-c-oxidoreductase (E.C.1.6.2.1), NADH : ferricyanide oxidoreductase (E.C.1.6.2.99), L-malate hydrolyase (E.C.4.2.1.2) and L-malate : NADH-oxidoreductase (E.C.1.1.3.7) increase their activities during the aerobic synchronous growth of aerobically grown *Saccharomyces cerevisiae* in discrete steps and only once during the cell cycle. An identical phenomenon was observed during the aerobic synchronous growth of anaerobically grown yeast. The mechanism of completion of mitochondrial membranes is thus likely to be discontinuous and the same during both mitochondrial multiplication and the conversion of promitochondria to fully functioning mitochondria.

An understanding of the organization and sequence of individual processes during mitochondrial formation requires an insight into the properties of separate enzyme systems involved as well as an appraisal of the conditions of cell integrity which impose metabolic and physiological control on the participating systems. Synchronous yeast cultures, in which most cells are at the same time in the same physiological and metabolic state, represent a suitable model for the study of temporal organisation of mitochondria-forming processes. Moreover, the close relation between the position of the corresponding gene on the chromosome and the expression of the given enzymic activity (Tauro and Halvorson, 1966; Tauro et al., 1969) makes it possible to explore the genetic background of nucleo-cytoplasmic interactions in the process of mitochondrial completion.

The formation of mitochondria is a process both complex and time-organized. This is seen from the fact that the activities of typical mitochondrial enzymes such as cytochrome oxidase (Cottrell and Avers, 1970), succinate dehydrogenase (Greksák and Hanicová, 1973), NADH cytochrome c oxidoreductase (Poole and Lloyd, 1973) and respiratory activity (Scopes and Williamson, 1964; Osumi and Sando, 1969; Greksák and Hanicová, 1973; Dharmalingam and Jayaraman, 1973) of aerobic synchronous yeasts of different species exhibit a discontinuous and, within the cell cycle, precisely time-defined increase.

The present paper is concerned with the study of the activities of some membrane and matrix mitochondrial enzymes during a synchronous aerobic growth of both aerobically and anaerobically grown yeast *Saccharomyces cerevisiae*. 
Fig. 1. Activities of NADH:cytochrome c oxidoreductase (NCR) and NADH:ferriyvanide oxidoreductase (NDH) during aerobic synchronous growth (C) of aerobically grown yeast cells.

MATERIALS AND METHODS

Microorganisms. All experiments were performed with a wild-type diploid strain of *Saccharomyces cerevisiae* Hansen DT XII. The stock culture was maintained on agar slopes at 2 °C.

Preparation of a synchronous yeast population. Aerobic yeast was grown on semi-synthetic growth medium (Kovác et al., 1968) with 0.25% glucose at 30 °C for 16 h in Erlenmayer flasks filled to 1/10 total volume, using a reciprocal shaker. Anaerobic yeast was prepared under strictly anaerobic conditions in an identical manner; the growth medium contained in this case 3% glucose, and over 2.5 ml Tween 80, 0.05 g ergosterol, and 2.5 ml ethanol in 1000 ml. When they stopped growing, the cells were spun down in the cold, washed with ice-cold sterile water and used immediately for preparing the synchronous culture.

Selection of synchronously growing yeast cells. The selection of these cells from asynchronous population was carried out by a slightly modified (Greksák and Hanicová, 1973) method of isopycnic centrifugation in discontinuous Ficoll gradient (Wiemken et al., 1970). Synchronous aerobic cells collected from 21% (w/v) Ficoll layer showed, during the first cell cycle, an average synchronisation index of 0.65 (Williamson and Scopes, 1962). With anaerobically grown yeast cells the highest synchronisation index, 0.55-0.60, was found in cells from the layer of 23% Ficoll. In this case the selection was carried out under anaerobic conditions at 2-4 °C.

Aerobic synchronous growth of yeast cells. The growth was accomplished in a 6-litre flask at 30 °C under intensive aeration. The flask was equipped with air inlet, mech-