THE CONTROL OF MITOCHONDRIAL ENZYME SYNTHESIS IN YEAST: A NEW HYPOTHESIS

A. J. S. BALL and E. R. TUSTANOFF
Department of Pathological Chemistry, University of Western Ontario, London, Canada

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It has been well documented that either an excess of glucose or a lack of oxygen [1] causes respiration to cease in the facultative anaerobe, *Saccharomyces cerevisiae*. This 'repression' phenomena is characterized by a decrease in mitochondrial enzyme activity which is closely coupled to a loss in mitochondrial profiles [2]. It has been strongly suggested by a number of investigators [3-5] that the concentration of glucose alone determined whether these cells will be regimented into an anaerobic or aerobic type of metabolism. The experiments reported here show that it is possible to differentiate between repression due to anaerobiosis from that due to carbon source. A possible system of this control is discussed.

Yeast were grown aerobically to mid exponential fermentative phase in a medium and manner described previously [6]. The harvested cells which were strongly repressed when transferred from glucose and slightly repressed when transferred from galactose (i.e., an excess of hexose still present in each culture medium) (cf. [7]), were used to inoculate a second batch of medium in the manner described in table 1. At various times after transfer, dry weight, respiratory ability, malic dehydrogenase (MDH) and cytochrome c oxidase (cyt. oxidase) activities of the yeast were determined. The respiratory ability which was determined polarographically on isolated culture samples aerated for one minute prior to measurement, reflected the oxidative capability of the yeast cell in the culture at the time of sampling.

Since yeast utilize glucose and galactose at different rates the results of these experiments are expressed in terms of activity versus change in dry weight in order to normalize these activities to the same stage in their physiological development. Fig. 1A illustrates the change in total respiratory ability observed during the four different transfer experiments. All curves show an increase in total respiratory ability, indicating that there is no net destruction of oxidative activity (i.e., mitochondria). Curves A3 and A4 demonstrate that the respiratory ability is governed by the nature of the fermentable carbon source. The change in rate which occurs late in the growth phase in each medium occurs only when the concentration of the carbon source falls below the level which is repressive (cf. curves A1 and A2). The specific activities of MDH (fig. 1B) under these same conditions are repressed after transfer (conditions $3 > 2 > 1$). The specific activities of the cyt. oxidase, however, are markedly different. Curves C2 and C3 demonstrate that anaerobiosis is controlling the level of the oxidase rather than by the carbon source. Anaerobiosis does not affect enzyme activity after transfer 4.

Since the curves in fig. 1A suggest there is no net degradation of existing mitochondrial activity, it is difficult to differentiate whether the increase during anaerobic growth is due to metabolic de-repression or to net enzymic synthesis. Dilution curves, based on dry weight calculated for MDH and cyt. oxidase [8] showed that the observed values are higher than those predicted in transfers 1, 2 and 4 for MDH and for transfers 1 and 4 for cyt. oxidase; while the other transfers were identical with those predicted. The maintenance of specific activity in curves B4 and C4 implies that net synthesis is occurring during these conditions. The decrease in specific activity of the

Table 1

Transfer no.	1st growth condition	2nd growth condition	Condition tested
1	2.7% galactose aerobic	2.7% galactose aerobic	slight repression aerobic control
2	2.7% galactose aerobic	2.7% galactose anaerobic	slight repression + anaerobiosis
3	2.7% galactose aerobic	2.7% glucose anaerobic	strong repression + anaerobiosis
4	2.7% glucose aerobic	2.7% glucose anacrobic	anaerobiosis on repressed cells

cyt. oxidase (curves C2 and C3) is not sufficient to attain this lower steady-state level. This suggests that the synthesis of the cyt. oxidase is turned off until the specific activity falls to a level appropriate to the new growth conditions, when synthesis should resume. Further studies carried out in this laboratory on transfer 4 [9] have shown net increases in the total activity of MDH, cyt. oxidase, succinic dehydrogenase and succinic oxidase. Chapman and Bartley [5] have recently reported net synthesis of MDH and cyt. oxidase for conditions analogous with our transfer 4. This synthesis of mitochondrial enzymes under anaerobic conditions suggests that oxygen is not necessary as an inducer of mitochondrial enzyme synthesis. The effects of carbon source in these transfers show that the levels to which the cyt. oxidase decreased are not conditioned by the carbon source *per se*. In contrast, the level of MDH is affected by both the atmospheric en-

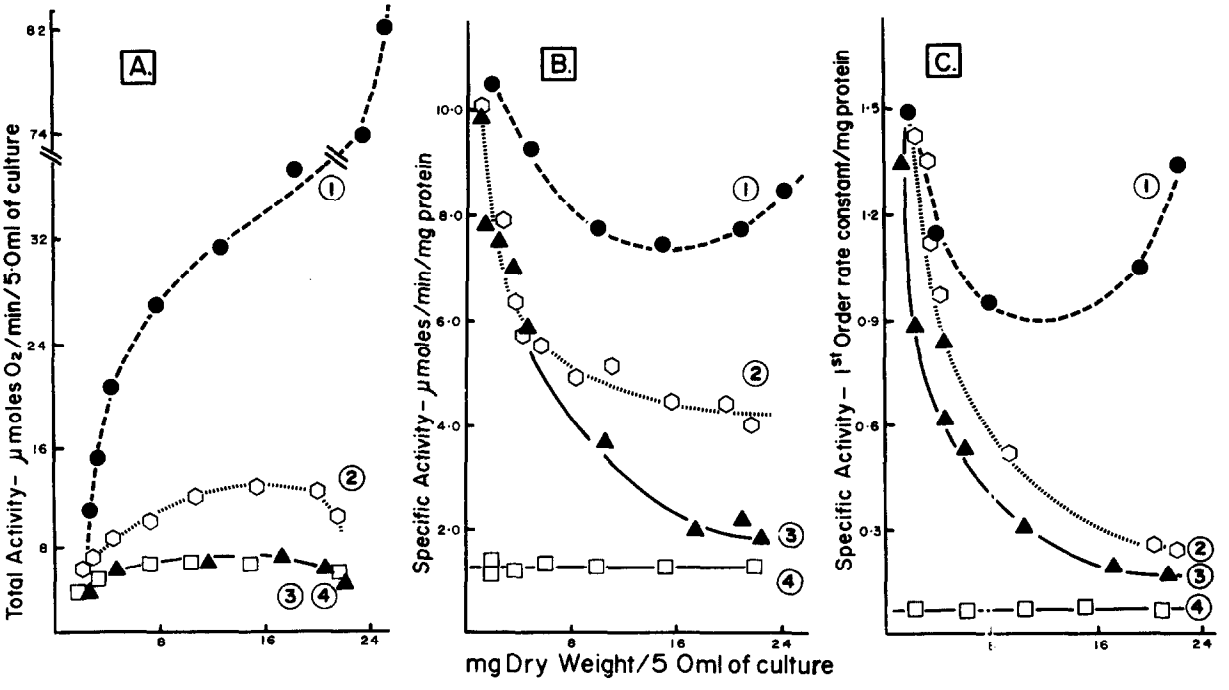

Fig. 1. Effects of various transfer conditions on (A) total respiratory ability, (B) malic dehydrogenase and (C) cytochrome c oxidase activities in yeast.

vironment and the carbon source (curves B2, B3 and B4). The effects here are isolated but additive, whereas in the former case they are non-additive. The current hypotheses regarding the control of mitochondrial proliferation in yeast do not adequately explain the data provided by our experiments. Neither the withdrawal of induction by oxygen [10] nor control by carbon source alone can explain the manner in which MDH and cyt. oxidase activities are altered in response to the transfer conditions described above.

In order to explain the similar effects of glucose and anaerobiosis on cyt. oxidase activity and the varied effects of anaerobiosis, galactose and glucose on MDH activity a different mechanism for the control of mitochondrial genesis must be considered. Recent studies [3] have shown that yeast mitochondria possess DNA. Mounolou et al. [11] demonstrated that the ability to synthesize cytochromes a, b and c_1 by 'petite' yeast mutants is directly correlated to lesions in their mitochondrial DNA. The possibility exists that the control mechanism for mitochondrial synthesis is a complimentation of a feedback to and a synthesis from both nuclear DNA and mitochondrial DNA. Clark-Walker and Linnane [12] reported that the synthesis of cytochromes a, b and c_1 can be inhibited by chloramphenicol (CAP) in yeast grown on 1% glucose. Further studies on this system carried out in this laboratory [13] have established that only D(-)CAP prevents the synthesis of respiratory enzymes under all growth conditions, and that neither D(−) nor L(+) threo-CAP directly inhibit respiration in whole cells or isolated mitochondria. These observations substantiate the hypothesis that CAP is directly interfering with mitochondrial protein synthesis. The circumstantial evidence provided by the genetic studies suggests that cytochromes a, b and c_1 are coded by mitochondrial DNA and synthesized in the mitochondrion. When yeast cells were grown aerobically in 2.7% galactose and transferred to 2.7% galactose medium containing CAP (cf. transfer 1), it was possible to demonstrate by measuring the total activity, that the rate of synthesis of cyt. oxidase is inhibited by 50%. This inhibition affects the rate of increase of respiratory ability and delays the rate of change (curve A1). The synthesis of MDH is not affected by CAP, which inhibits mitochondrial protein synthesis. MDH, however, is a mitochondrial enzyme which is probably coded for by the nucleus (not affected by 'petite' mutation [12]), synthesized in the cytoplasm and transported to the mitochondrion [14]. This experiment with CAP implies that there is no direct positive or negative feedback existing between mitochondrial and nuclear DNA. Thus it is possible that mitochondrial DNA proliferates via its gene → gene product → gene feedback mechanism, independently of the nucleus, in response to the micro-environment inside the cell. Nuclear DNA would also replicate in response to the macro-environment outside the cell. Thus, the anabolic development of the cell would only become dependent upon mitochondrial proliferation when growth conditions forced the cell to rely upon respiration as the source of metabolic energy production.

When actively respiring yeast are subjected to anaerobiosis or excess glucose, respiration in the cell is inhibited. The metabolic repression due to glucose is independent of the concentration of the respiratory enzymes present in the cell at the time of exposure (curves A2 and A3), but is conditioned by the level of the fermentative enzymes [15]. Transfers C2 and C3 show that anaerobiosis overrides the expected differential repression of synthesis due to the different rates of fermentation of glucose and galactose, while transfers A2 and A3 show that the respiration of galactose cells is initially conditioned by the carbon source. If mitochondrial protein synthesis was coupled to the ability of the organelle to produce energy for synthesis, a slow rate of catalytic protein production would be observed during extreme respiratory inhibition utilizing ATP sequestered from the cytoplasm. This is in fact observed during transfer 4, both before and after transfer; as well as in curves B1 and C1 after transfer. On the other hand, it would be expected that enzymes of the citric acid cycle (e.g., MDH) would be controlled by the metabolic needs of the cell. As most of these enzymes are involved in anapleurotic pathways as well as respiration, it would seem optimistic to expect the levels of these enzymes to be controlled solely by the need for mitochondrial genesis. As illustrated by fig. 1B, the level of specific activity of MDH is conditioned by the carbon source which is mediated by different rates of fermentation of galactose and glucose. The hypothesis that the mitochondria proliferate in response to the micro-environment inside the cell, independent of any direct nuclear control, successfully explains the phenomena described above.

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