INTERDEPENDENCE BETWEEN PROMITOCHONDRIAL AND CYTOPLASMIC PROTEIN SYNTHESIS DURING RESPIRATORY ADAPTATION IN BAKER'S YEAST.

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SUMMARY

When anaerobically-grown yeast cells were aerated in the presence of cycloheximide, they accumulated intermediate(s)for respiratory adaptation. The formation of these intermediates was dependent upon promitochondrial protein synthesis and independent of cytoplasmic protein synthesis. The cytoplasmic system was unable to accumulate significant amounts of intermediates for adaptation in the absence of protein synthesis by the promitochondrial system.

Aeration of anaerobically-grown yeast in the presence of an energy source results in the rapid formation of a respiratory system (1) and the differentiation of promitochondrial bodies present in the unadapted cells (2). This adaptive transition involves the cooperation of two distinct protein synthesizing systems (3). One of these resides within the promitochondria and can be selectively inhibited by chloramphenicol (CAP). The other, associated with the cytoplasmic ribosomes, is specifically blocked by cycloheximide (CHI). Inhibition of either system alone prevents respiratory adaptation (4).

Previous studies with adapting yeast cells have suggested that the effect of oxygen on the cytoplasmic protein synthesizing system is in part dependent upon synthesis by the promitochondrial system. Thus, the adaptive formation of the insoluble mitochondrial cytochromes (presumably by the cytoplasmic system) (5) requires protein synthesis by the promitochondrial system (6). This dependence of oxygen-induced cytoplasmic protein synthesis upon the operation of the promitochondrial system raises important questions: How does the promitochondrial protein synthesizing system exert this control over the cytoplasmic system? Can the promitochondrial system respond to oxygen while cytoplasmic protein synthesis

is inhibited?

The present study addresses itself to these questions. Although the results reported are preliminary, we consider them of sufficient interest to warrant their presentation now.

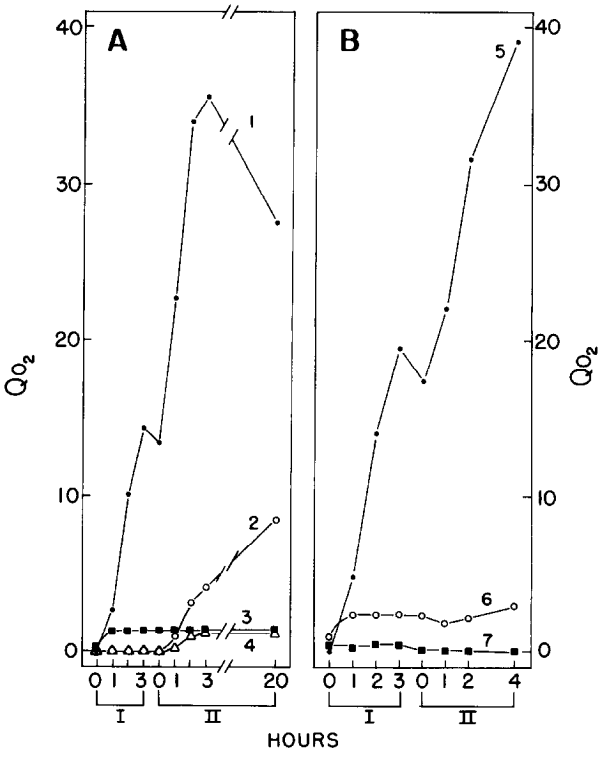
METHODS

All experiments were carried out with the wild-type Saccharomyces cerevisiae strain D 273-10B ($\alpha P \wp^{+}$, haploid). Growth media and harvesting procedures were as described elsewhere (7), except that the concentration of glucose in the growth medium was lowered to 0.3%, and CHI-poisoning was carried out only where indicated. Upon harvesting, the cells were washed twice by centrifugation (5 min at 1500 x g) with cold adaptation buffer consisting of 40 mM KPO_4, pH 7.4, 0.3% glucose and 1% ethanol. Subsequent incubations were carried out in the buffer at 28° in the dark and at a cell concentration of 20 mg wet weight per ml. Where indicated, CHI was present at a concentration of 25 µg/ml and CAP at a concentration of 4 mg/ml. The experiments described here were carried out in two incubation phases which differed from each other in oxygen supply and/or in the antibiotic present. After the first incubation phase, the cells were washed four times by centrifugation (cf. above) with cold adaptation buffer containing the appropriate antibiotic for the second phase. The cells were then further incubated at 28° under the conditions of the second phase. $Q0_2$ is expressed as µl of 0_2 consumed per hour per mg dry weight at 25°. It was determined polarographically with cells suspended in adaptation buffer and was corrected for oxygen uptake insensitive to 0.5 mM NaCN (7). Although this assay does not yield information on the assembly of specific respiratory components, it is the most meaningful measure of the overall adaptation process.

RESULTS AND DISCUSSION

When anaerobically grown yeast cells were aerated in the absence of antibiotics, they rapidly acquired cyanide-sensitive respiration (Fig 1A, trace 1). The discontinuity in trace 1 observed at the end of phase I reflects the temporary

interruption of adaptation which occurred when the cells were washed in the cold. No adaptation was observed when the cells were aerated in the presence of CHI (Fig 1A, phase I of trace 2). However, when they were subsequently washed free of CHI and aerated further in the presence of CAP, a "delayed adaptation" was observed (Fig 1A, phase II of trace 2). This delayed response suggests that the products of a CHI-insensitive process accumulated during phase I, were conserved during the washing procedure and expressed during phase II. The formation of these CHI-insensitive products is blocked by CAP and, therefore, dependent on promitochondrial protein synthesis (Fig 1A, phase 2 of trace 4). The very low level of adaptation which did occur in the presence of CAP alone (Fig 1A, phase I of trace 3 and phase II of trace 4) may reflect either an incomplete inhibition



Figures 1A and 1B

Respiratory adaptation of anaerobically-grown yeast cells aerated in the absence and presence of antibiotics. 1: phase I, no antibiotic; phase II, no antibiotic. 2: phase I, cycloheximide (CHI); phase II, chloramphenicol (CAP). 3: phase I, CAP; phase II, CAP. 4: phase I, CAP + CHI; phase II, CAP. 5: phase I, no antibiotic; phase II, no antibiotic. 6: phase I, CAP; phase II, CHI. 7: phase I, CAP + CHI; phase II, CHI.

of promitochondrial protein synthesis by CAP, or the presence of low levels of mitochondrial adaptation components in the unaerated cells. It should be mentioned that CAP at a concentration of 4 mg per ml did not inhibit cyanide-sensitive respiration in fully adapted cells.

When cells were adapted in the presence of CAP alone (Fig 1B, phase I of trace 6), the small CAP-insensitive response was seen. No additional delayed adaptation was observed, however, when the cells were further aerated in the presence of CHI alone (phase II of trace 6). It appears, therefore, that the inhibition of promitochondrial protein synthesis prevented the formation or accumulation of at least one product of the cytoplasmic system essential for adaptation. Trace 7 of Figure 1B illustrates the complete inhibition of adaptation which occurred when CHI was present in both phases I and II. Control experiments (not shown here) indicated that poisoning by either CHI or CAP could indeed be reversed by washing, although the rate and degree of reversal were somewhat lower for CAP than for CHI. Additional experiments demonstrated that the formation of adaptation-associated products by both the CHI-and CAP-sensitive systems required oxygen. Thus, if either phase I or phase II of the experiment illustrated in Fig 1A, trace 2 was carried out anaerobically, the delayed adaptation response was abolished.

It was of interest to determine how rapidly promitochondrial products were elaborated upon aeration of the cells (Fig 1A, trace 2), and, once made, how stable they might be upon the removal of O_2. Figure 2A shows that an increase in delayed adaptation (above the CAP-insensitive level) began after 20 minutes of aeration and became maximal after 60 minutes. Figure 2B shows that the oxygen-induced promitochondrial products decayed when the cells were further incubated under N_2. The decay was approximately linear and complete in about 4 hours; after this time only the small CAP-insensitive response remained which then decayed more slowly. Although the delayed adaptation response is relatively small, it was observed in many separate experiments. Moreover, its magnitude is consistent with the half life of the oxygen-induced promitochondrial products and the rate of adaptation.

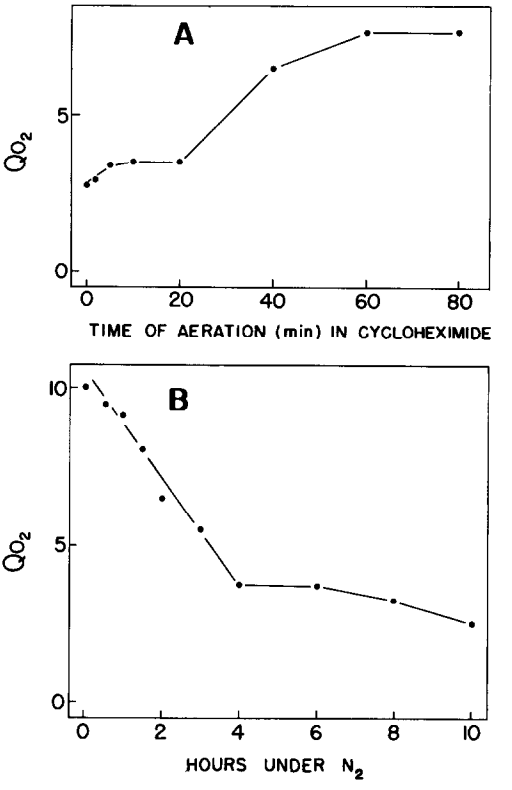


Figure 2A

Time course for the onset of the delayed adaptation response. Yeast cells were grown anaerobically, aerated in the presence of CHI for the various times indicated, then washed and further aerated in the presence of CAP for 2 hours. The QO_2 of the cells was measured at the end of the experiment.

Figure 2B

Decay of the delayed adaptation response. Yeast cells were grown anaerobically, aerated in the presence of CHI for 1 hour, made anaerobic again for the times indicated and washed and further aerated in the presence of CAP for 4 hours. The QO_2 of the cells was measured at the end of the experiment.

In conclusion, the results suggest that aeration of anaerobically-grown yeast cells induces the formation of intermediates required for adaptation by means of promitochondrial protein synthesis. These intermediates can be formed in the absence of cytoplasmic protein synthesis and exhibit a half life of about two hours. They can be utilized for adaptation only when complemented with products of the cytoplasmic protein synthetic system. Although the formation of these intermediates requires promitochondrial protein synthesis, it remains

to be shown whether or not these intermediates are themselves proteins, and whether their oxygen-induced accumulation reflects an increase in their synthesis or an inhibition of their breakdown. In contrast to the independence of the promitochondrial system, at least one essential adaptation product of the cytoplasmic system cannot accumulate while the promitochondrial system remains inhibited.

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