CATABOLITE REPRESSION OF THE FORMATION OF PRECURSORS OF ELECTRON TRANSFER COMPLEXES III AND IV IN ADAPTING BAKERS' YEAST: DEPENDENCE UPON A MITOCHONDRIALLY TRANSLATED REPRESSOR[1]

William Rouslin

Department of Biological Sciences,
Douglass Campus, Rutgers University,
New Brunswick, N.J. 08903

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SUMMARY

When bakers' yeast cells which had been grown anaerobically in galactose were aerated in the presence of 10% glucose, they showed a 40% decrease in in vivo [^{14}C]-leucine incorporation into a washed mitochondrial membrane fraction compared with cells which had been aerated in a low glucose medium. The observed catabolite repression of membrane protein synthesis was primarily due to a decrease in cytoplasmic translational activity, but this repression was entirely dependent upon concomitant mitochondrial translation. The inductions of reduced coenzyme Q cytochrome c reductase (complex III) and of cytochrome c oxidase (complex IV) activities were repressed 30 and 60%, respectively, by aeration of the cells for 8 hours in 10% glucose. The catabolite repression of the formation of these two inner membrane complexes was again shown to be dependent upon concomitant mitochondrial translation. Both the amino acid incorporation and enzyme induction data suggest that catabolite repression of both cytoplasmically and mitochondrially translated mitochondrial membrane proteins is mediated through a mitochondrially translated repressor.

A coordination of the activities of the mitochondrial and nucleocytoplasmic genetic systems is presumably required during either the induction or repression of certain mitochondrial inner membrane enzymes. Such "intergenomic" coordination may be envisioned as occurring at a number of levels within the cell. One mechanism of coordination for which there is a currently increasing body of evidence (1-4), involves a mitochondrially translated repressor which acts upon the nucleocytoplasmic genetic system. Earlier studies which proposed the existence of such a repressor demonstrated that, in the presence of the mitochondrial transcriptional and translational inhibitors, ethidium bromide and chloramphenicol, there was a marked increase in the production of components of the mitochondrial genetic system by the

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nucleocytoplasmic genetic system. Thus, Barath and Kuntzel demonstrated that the activities of mitochondrial DNA-dependent RNA polymerase (1), elongation factors and methionyl-tRNA transformylase (2) increased in Neurospora as a result of exposure to either ethidium bromide or chloramphenicol. More recently, Beauchamp and Gross have shown increases in mitochondrial leucyl and phenylalanyl-tRNA synthetases as a result of exposure of Neurospora to these same inhibitors (3).

In the present report evidence is presented for the existence in bakers' yeast cells of a mitochondrially translated repressor which is required for catabolite repression of the formation of mitochondrial inner membrane components produced by both the nucleocytoplasmic and mitochondrial genetic systems. This report diverges from the studies cited above in a number of important aspects: (a) It represents the first such study with fungal cells other than Neurospora. (b) The proteins whose syntheses were observed to be stimulated by chloramphenicol are not components of the mitochondrial genetic system, itself, but include inner membrane respiratory enzyme precursors. (c) While the earlier studies with Neurospora show what are in effect inhibitor mediated "superinductions" of enzymes, the present study with yeast demonstrates, by and large, an inhibitor mediated reversal of catabolite repression. (d) The "intergenomic" repression model presented here tentatively postulates that the proposed mitochondrially translated repressor acts upon both the nucleocytoplasmic and mitochondrial genetic systems, thereby providing a direct means for coordinating the synthetic activities of the two systems during glucose repression. This particular mechanism for "intergenomic" coordination has not been proposed earlier.

METHODS

The wild type Saccharomyces cerevisiae strain D273-10B ($\alpha PET[\rho^+]$) was used. The cells were grown anaerobically in a 0.5% galactose, yeast extract medium as described earlier (5). The cells were harvested in early stationary phase after being chilled to 0°C while still anaerobic in the fermentor carboy. Where appropriate, the cells were poisoned while still in the carboy with cycloheximide (CHI)[2] at a final concentration of 50 µg/ml, or immediately upon harvesting with chloramphenicol (CAP)[2] at 4 mg/ml.

Labeling with [^{14}C]leucine was carried out for 60 min (Table I). The washed mitochondrial membrane fraction was prepared from the labeled cells as follows. At the end of the labeling period the cells were quickly pelleted in the cold and washed twice with a solution containing 10 mM L-leucine, 50 µg/ml CHI, 4 mg/ml CAP, 0.25M mannitol, 20 mM Tris-SO_4, pH 7.4, and 1.0 mM EDTA. Each washed pellet was then resuspended in 5 ml of the same solution and the cells broken by two passes through a French pressure cell. Unbroken cells were removed by centrifugation for 5 min at 4,500g and a membrane pellet was obtained by centrifugation of the low speed supernatant for 30 min at 100,000g. Each such pellet was washed thoroughly in 10 mM L-leucine and resuspended in a small volume of the same. Each sample was then processed for counting as described elsewhere (6).

[2]Abbreviations used: CHI, cycloheximide; CAP, chloramphenicol

Reduced coenzyme Q cytochrome c reductase and cytochrome c oxidase assays were performed as described earlier (5). In the two phase experiments depicted in Fig. 2, phase I incubations were 3 hours long and were terminated by 4 washes in cold phase II buffers. Phase II incubations were 6 hours long and samples were taken at the times indicated for enzyme assays.

TABLE I

In vivo [^{14}C]leucine incorporation into a washed, crude mitochondrial membrane fraction expressed as cpm/mg protein[a]

Antibiotic	% Glucose	Exp. I[b]	Exp. II[c]
NONE	0.3	108,730	51,393
NONE	10.0	62,687	32,822
CAP	0.3	100,842	46,177
CAP	10.0	103,255	47,282
CHI	0.3	10,297	3,906
CHI	10.0	9,158	3,762
CHI+CAP	0.3	1,314	467
CHI+CAP	10.0	1,401	604

[a]The cells were grown anaerobically in galactose and were aerated in 50 ml batches in 250 ml flasks at 28°C in the dark at a cell concentration of 25 mg wet weight/ml. Label was added 15 min after the beginning of the incubation and labeling was carried out for 60 min.

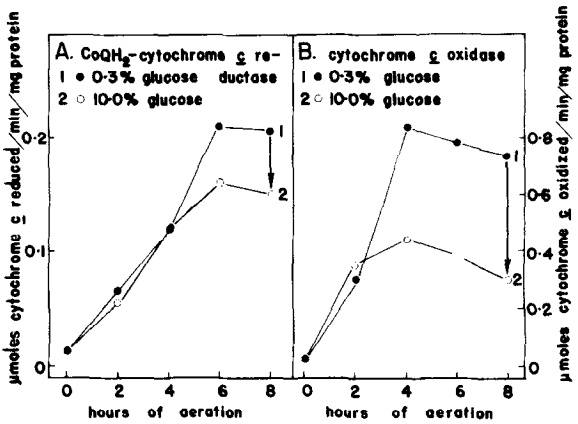
[b]20 μCi [^{14}C]leucine/50 ml of labeling medium

[c]10 μCi [^{14}C]leucine/50 ml of labeling medium

RESULTS AND DISCUSSION

The labeling studies reported in Table I show that, in the absence of antibiotics, the addition of 10% glucose caused a 40% reduction in amino acid incorporation into the membrane fraction. The presence of CAP completely abolished this glucose effect. Moreover, it is evident that the magnitude of the glucose repression (46,000 cpm in Exp. I) is far too great to be accounted for by CAP-sensitive translation alone. It is thus evident that the presence of CAP completely prevented a glucose mediated decrease in cytoplasmic (CHI-sensitive) translation.

Figs. 1A and B depict experiments which show the inductions of reduced coenzyme Q cytochrome c reductase (complex III) and of cytochrome c oxidase (complex IV) activities in the absence of antibiotics. It is evident that the inclusion of 10% glucose in the aeration medium caused a moderate repression (ca 30%) of complex III activity which became obvious only after nearly 6 hours of aeration. The effect of high glucose upon complex IV induction was considerably greater (ca 60% repression)

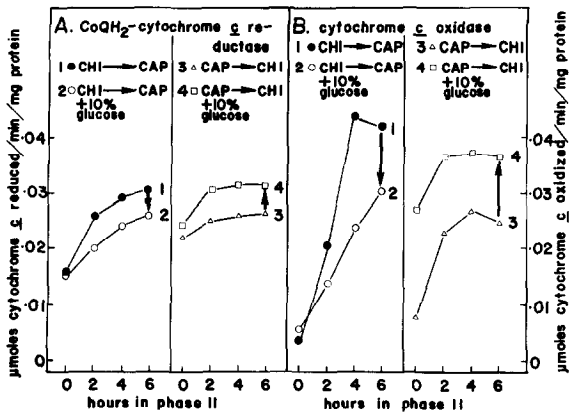


Figures 1A and B
The inductions of (A) reduced coenzyme Q cytochrome c reductase (complex III) and of (B) cytochrome c oxidase (complex IV) activities in the absence of antibiotics. Upon harvesting, the cells were washed once and suspended at 25 mg wet weight/ml in 40 mM KPO_4 at pH 7.4 containing either 0.3% glucose plus 2.0% ethanol (trace 1) or 10% glucose (trace 2). The cells were aerated at 28°C in the dark and samples were removed for enzyme assays at the times indicated.

and was manifested somewhat earlier (after only 4 hours of aeration). This quantitative difference in the effect of high glucose upon the inductions of the two activities correlates well with the degrees to which high glucose repressed the inductions of the two activities in the two phase experiments depicted in traces 1 and 2 of Figs. 2A and B. Thus, while both complexes studied appear to be responsive to catabolite repression control, the levels of complex IV appear to be rather more stringently governed.

The two phase experimental format depicted in Figs. 2A and B was designed to separate in time the translational activities of the mitochondrial and nucleocytoplasmic genetic systems. In traces 1 and 2 where CHI was introduced first, the cells were allowed to accumulate only mitochondrially made precursors in phase I (and cytoplasmically translated components in phase II). On the other hand, in traces 3 and 4 where CAP was introduced first, the cells were allowed to accumulate only cytoplasmically made precursors in phase I (and mitochondrially translated components in phase II).

The inclusion of 10% glucose during phase I had a repressing effect upon the phase II inductions of both enzyme activities studied, but only when the glucose was introduced in the presence of CHI, i.e., only when the mitochondrial system was operating (traces 1 and 2 of Figs. 2A and B). In contrast, and in agreement with the labeling data reported in Table I, when the high glucose was introduced during



Figures 2A and B
The two phase inductions of (A) reduced coenzyme Q cytochrome c reductase (complex III) and of (B) cytochrome c oxidase (complex IV) activities in cells exposed first to CHI and then to CAP (traces 1 and 2), and in cells exposed first to CAP and then to CHI (traces 3 and 4). The cells were aerated during both phases at 28°C in the dark and samples were removed for enzyme assays at the times indicated.

phase I in the presence of CAP (traces 3 and 4 of Figs. 2A and B), it did not cause a repression of the phase II inductions of the two complexes. In fact, there was a marked stimulation of the phase II inductions of both enzyme activities (see vertical arrows between traces 3 and 4 of Figs. 2A and B). It would thus appear that some repression of the formation of both enzyme complexes results when high glucose is present during the operation of the mitochondrial genetic system, but that no repression is possible without concomitant mitochondrial translation.

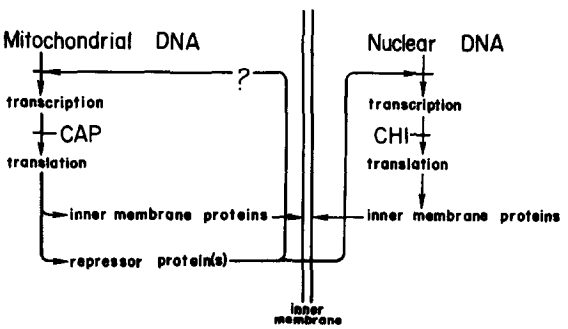


Figure 3
A schematic representation of the role of a mitochondrially translated repressor in coordinating the activities of the mitochondrial and nucleocytoplasmic genetic systems during the catabolite repression of the formation of mitochondrial membrane components.

Fig. 3 depicts an "intergenomic" repression control model which bears some resemblance to models proposed earlier (1,2). However, in addition to postulating a mitochondrially translated repressor which acts upon the nucleocytoplasmic genetic system, the present model also tentatively proposes the operation of a mitochondrially translated control element which acts upon the mitochondrial genetic system itself. This latter aspect of the model is suggested by the ability of the cells to be repressed by glucose even in the presence of CHI as observed in traces 1 and 2 of Figs. 2A and B and, to some extent, in Table I.

The model of Barath and Kuntzel predicts two phases of mitochondrial biogenesis within each cell cycle. In the first phase there is a buildup of cytoplasmically translated mitochondrial membrane proteins and enzymes of the mitochondrial genetic apparatus. Their buildup stimulates a second phase in which intramitochondrial protein synthesis occurs including the production of a repressor. This second phase is then terminated due to the effect of the repressor upon the pool of cytoplasmically translated proteins. According to the earlier model, the pool of cytoplasmically translated proteins is necessary for continued mitochondrial translation. While this may, in fact, be the case, the present model additionally suggests that the buildup of repressor is itself sufficient for the slowing of mitochondrial translation whether or not the pool of cytoplasmically translated proteins has been fully exhausted. The postulation of what might be a single control element for the repression of mitochondrially directed synthesis by both genetic systems provides a more direct mechanism for the coordination of the activities of the two genetic systems during the catabolite repression of the formation of mitochondrial respiratory enzymes.

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