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MITOCHONDRIAL PHENOTYPES AND GENOTYPES IN YEAST

Technical Annual Progress Report

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ABSTRACT

An eight component yeast complex III preparation has been obtained which contains up to 6.2 nanomoles of heme b per mg protein. Studies in which the enzyme activity associated with the complex (reduced CoQ-cytochrome c reductase) was monitored, and in which in vivo ^{14}C -leucine incorporation into whole washed mitochondrial membranes was carried out suggest that (1) entrance of mitochondrially translated proteins into the mitochondrial inner membrane is not dependent upon a prior build up of cytoplasmically made precursors, and (2) that catabolite repression of the formation of cytoplasmically made precursors is mediated by a mitochondrially translated repressor protein.

NARRATIVE

A large scale procedure (using commercially grown bakers' yeast) has been further refined and yields an eight component yeast complex III which contains up to 6.2 nanomoles of heme b per mg protein. Preliminary molecular weight estimates of the components are: 44,000, 34,000, 30,500, 27,000, 23,000, 15,000, 12,000 and 9,500 daltons.

Parallel studies, to be submitted for publication shortly (preprints will be sent along at that time), have dealt with aspects of the regulation of the biogenesis of the complex. These studies include two types of experiments: (a) those in which the enzyme activity associated with the complex was monitored and (b) those in which in vivo labeling of mitochondrial membranes with ^{14}C -leucine was carried out. These regulation studies have been primarily concerned with two questions. The first is whether or not membrane attachment of inner membrane enzyme subunits made by either the mitochondrial or by the nucleocytoplasmic genetic system is at all stringently delimited by a prior depletion of partner subunits (which might be thought of as binding sites) made by the "other" genetic system. This question was approached by aerating anaerobically grown cells for 3 hours in a preincubation medium (called phase I) containing either no antibiotic, chloramphenicol (CAP) alone, cycloheximide (CHI) alone, or both together as shown below:

TABLE I

In vivo ^{14}C -leucine incorporation during phase II incubations into a washed, crude mitochondrial membrane fraction expressed as cpm/mg protein.

<u>Phase I</u>	<u>Phase II</u>	<u>Exp. I</u>	<u>Exp. II</u>
NONE $\longrightarrow$ CHI		5,306	1,224
CAP $\longrightarrow$ CHI		5,969	1,350
CHI+CAP $\longrightarrow$ CHI		6,291	1,421
CHI+CAP $\longrightarrow$ CHI+CAP		1,648	304
CHI $\longrightarrow$ CAP		36,945	17,776
CHI+CAP $\longrightarrow$ CAP		40,242	15,939

¹⁴C-leucine was then added (60 min labeling period) after the cells had been thoroughly washed in Phase II media containing a change of antibiotic. It may be seen that the synthesis and membrane attachment of mitochondrially translated proteins is essentially unaffected by a prior depletion of cytoplasmic proteins (due to inclusion of CHI in the Phase I incubation). Similarly, there is no significant change in the synthesis or membrane attachment of cytoplasmically translated proteins due to a depletion of mitochondrially made subunits (due to the inclusion of CAP in the Phase I incubation). The general conclusion is that, at least for cells undergoing respiratory adaptation, there is little direct control of entry into the membrane of the translational products of either genetic system by the availability of "partner" proteins.

A second approach to this same question was made by monitoring the emergence of reduced CoQ cytochrome c reductase (Complex III) and cytochrome c oxidase (Complex IV) activities in anaerobically grown cells undergoing aeration in the presence of CHI and/or CAP during an initial incubation, followed by a new antibiotic exposure in a second incubation (see figure on following page). The experiment is similar in design to those reported earlier (1), except that here the cells were exposed to CHI and CAP in both orders, i.e., CHI then CAP, and CAP then CHI. The object of this design was to settle a potential disagreement between my earlier observation that yeast cells are capable of accumulating substantial levels of mitochondrially translated complex III precursors in the absence of concomitant cytoplasmic protein synthesis (1), and a report from Beattie's group to the effect that reduced CoQ cytochrome c reductase activity emergence is especially enhanced if the cells are preincubated in CAP (2) (in order to build up cytoplasmically translated subunits which allegedly stimulate the synthesis of the mitochondrially translated subunits of complex III - after the ideas of Tzagoloff on the regulation of the formation of rutamycin-sensitive ATPase) (3). It can be seen from an examination of the figure (on the following page) that the greatest level of induction of both complex III and complex IV associated enzyme activities is obtained when the exposure is to CHI first followed by CAP in phase II. Thus the products of both genetic systems may be stored to a substantial degree in the absence of concomitant synthetic activity by the other, but the products of the mitochondrial system appear to be able to accumulate to somewhat greater levels for both of the electron transfer complexes studied.

While it may be seen (Fig A on page 4 of this progress report) that exposure of the cells to CHI before CAP (as in trace 2) results in a higher level of delayed complex III activity induction (compared with that in trace 3), it may also be seen (in trace 3) that exposure of the cells to CAP first elicits an elevated phase II zero time activity value (see arrow at zero time between traces 2 and 3). This elevated zero time activity observed for complex III, but not for complex IV, is attributable to a complex III precursor already present in the anaerobic cells, but whose "expression" requires aeration under conditions which allow cytoplasmic translation, i.e., in the absence of CHI (see Fig on page 5 of this progress report). Examination of the left half of Fig 1A, on page 5, shows that an induction of reduced CoQ cytochrome c reductase activity is observed which amounts to about 50% of that which is attained later in phase II of Fig A. This 50% induction required no new mitochondrial protein synthesis and is, therefore, presumed to be the expression of an anaerobically formed mitochondrially translated precursor, perhaps the apoprotein of one of the cytochromes b.

Fig B (on page 5) shows that a reintroduction of anaerobic conditions results in a rapid decay of oxygen induced complex III activity, but only to a level roughly equal to that which is the expression of the anaerobic precursor developed in phase I in the presence of CAP. The experiment suggests that there are two mitochondrially synthesized components, one made anaerobically which supports the level of enzyme induction seen in phase I, and another made only upon aeration which supports the level of induction seen in phase II.

μmoles cytochrome c reduced/min/mg protein

A. CoQH₂-cytochrome c re- phase I phase II ductase

- 1 ● none → none
2 ○ CHI → CAP
3 △ CAP → CHI
4 □ CHI+ → CHI

CAP
galactose-grown
cells

B. cytochrome c oxidase phase I phase II

- 1 ● none → none
2 ○ CHI → CAP
3 △ CAP → CHI
4 □ CHI+ → CHI

CAP
galactose-grown
cells

The second problem addressed by my just completed work concerns the mechanism by which the effects of glucose repression are coordinated between the two genetic systems. Experiments in which anaerobically grown cells were sequentially exposed either first to CHI and then to CAP, or first to CAP and then to CHI (similar to the experiment just described) were performed in which the activities of reduced CoQ cytochrome *c* reductase and of cytochrome *c* oxidase were monitored (see figure on page 7). The induction of both enzyme activities was repressible by exposure to 10% glucose, but only when the glucose was present during mitochondrial translation, i.e., in the presence of CHI. These results suggest that glucose repression of the formation of these two enzyme complexes is mediated through a mitochondrially translated control element.

Further experiments dealing with the effects of high glucose upon amino acid incorporation into membranes show that high glucose does substantially repress incorporation into membranes in the absence of antibiotic, and that the magnitude of this inhibition is such that it must be largely accounted for by a reduction in cytoplasmic translation (see table below):

In vivo ¹⁴C-leucine incorporation into a washed, crude mitochondrial membrane fraction expressed as cpm/mg protein.

Antibiotic	% Glucose	Exp. I	Exp. II
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

Most importantly, however, is the observation that this glucose repression of cytoplasmic translation is entirely abolished in the presence of CAP, again demonstrating that glucose repression is dependent upon concomitant mitochondrial protein synthesis. Moreover, the fact that the repression of cytoplasmic translational activity is dependent upon a product of mitochondrial translation leads to the postulation of an "intergenomic" repression model in which a mitochondrially translated repressor protein is required for the repression of synthetic activity by the nucleocytoplasmic genetic system. Such an intergenomic model is analogous to one reported by Barath and Kuntzel (4) which they proposed to explain an ethidium bromide/chloramphenicol induced build up of nuclearly encoded proteins of the mitochondrial genetic apparatus.

Literature Cited

1. Rouslin, W. 1975. Arch. Biochem. Biophys. 168: 685.
2. Lin, L.-F., Kim, I.-C. and D.S. Beattie, 1974. Arch. Biochem. Biophys. 160: 458.
3. Tzagoloff, A. 1971. J. Biol. Chem. 246: 3050.
4. Barath, Z. and H. Kuntzel. 1972. Proc. Nat. Acad. Sci. 69: 1371.

A. CoQH₂-cytochrome c reductase

1 ○ CHI → CAP
2 ○ CHI → CAP
+ 10% glucose

3 △ CAP → CHI
4 □ CAP → CHI
+ 10% glucose

B. cytochrome c oxidase

1 ○ CHI → CAP
2 ○ CHI → CAP
+ 10% glucose

3 △ CAP → CHI
4 □ CAP → CHI
+ 10% glucose