Attachment "A"

MITOCHONDRIAL GENOTYPES AND PHENOTYPES IN YEAST

Technical Annual Progress Report

William Rouslin

Rutgers, The State University
New Brunswick, New Jersey 08903

Reporting Period: September 1, 1973 - August 31, 1974

Prepared for the U.S. ATOMIC ENERGY COMMISSION under
Contract No. AT(11-1)-3516

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

"This report was prepared as an account of Government-sponsored work. Neither the United States, nor the Atomic Energy Commission, nor any person acting on behalf of the Commission:

- A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus method, or process disclosed in this report may not infringe privately owned rights; or
- B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of, any information, apparatus, method, or process disclosed in this report.

As used in the above, 'person acting on behalf of the Commission' includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor."

ABSTRACT

Two projects were continued during the past year. The first one concerns the regulation of the formation of electron transfer complex III (which includes cytochromes b and c_1) during the differentiation of promitochondria into mitochondria in adapting bakers' yeast cells. We have shown for the first time that the mitochondrially translated portion(s) of the complex (possibly the apoprotein of cytochrome b) requires oxygen for either its synthesis or for its integration into the inner mitochondrial membrane (see preprint which is included with this renewal application). Moreover, we have demonstrated that the cytoplasmically translated portions of electron transfer complexes III and IV (i.e., cytochromes aa_3 , b and c_1) all require oxygen for their synthesis or integration into the membrane. We have developed a large scale procedure for the preparation of complex III from commercially grown bakers' yeast. These large scale preparations will be used in subsequent studies described in the renewal proposal. The second project concerns the three dimensional structure, membrane dimensions and number per cell of the promitochondria present in anaerobically grown cells. These data are to be compared with the corresponding ones for the single giant mitochondrion present in aerobically grown cells. Thus far we have run the definitive experiment yielding promitochondrial and mitochondrial material from the same starting culture. Samples taken at zero time and during adaptation have been embedded for electron microscopy. It remains for us to make serial sections and construct models.

NARRATIVE

Anaerobically-grown bakers' yeast cells were aerated first in the presence of cycloheximide (CHI) and then in the presence of chloramphenicol (CAP). During the CHI incubation they accumulated mitochondrially made precursors of electron transfer complexes III and IV. During the CAP incubation which followed, the cells completed the formation of the electron transfer complexes by adding cytoplasmically made components to the mitochondrially made precursors which had formed earlier in the presence of CHI. This completion of electron transfer complexes III and IV was monitored by following the "delayed inductions" of coenzyme QH_2 -cytochrome c reductase and of cytochrome oxidase activities, respectively.

which occurred during the CAP incubation. The imposition of anaerobiosis during either the CHI incubation (period of mitochondrial translation) or during the CAP incubation (period of cytoplasmic translation) completely abolished the delayed inductions of both coenzyme QH₂-cytochrome c reductase (complex III) and of cytochrome oxidase (complex IV). It was thus established that components of electron transfer complexes III and IV of both mitochondrial and nuclear origin require oxygen for their synthesis.

Thus far, the only component of complex III known to be made upon mitochondrial ribosomes is the apoprotein of cytochrome b. It is thus quite possible (and remains for us to determine) that one site of action of oxygen as inducer of the formation of electron transfer complex III is in the induction of the synthesis of cytochrome b apoprotein by the mitochondrial genetic system. The information we have thus far obtained (see preprint included herein) indicates that this control by oxygen over the synthetic activity of the mitochondrial genetic system is exerted independently of any control oxygen may have over the nucleocytoplasmic system.

In order to make positive identifications of just which complex III polypeptides are controlled by oxygen, we have begun an experimental approach which begins with the purification of electron transfer complex III. To this end we have now set up equipment and established a procedure for the large-scale preparation of electron transfer complex III from commercially grown bakers' yeast. The procedure involves the lysing of 10 pound batches of commercially grown yeast cells with a Gaulin homogenizer (this is, in effect, a continuous flow French press), the preparation of submitochondrial particles and their subsequent fractionation with ammonium sulfate, DEAE-cellulose and sephadex all in the presence of bile salt containing buffers. The large-scale procedure is required for the production of enough material so that specific antisera can be prepared against each of the major components of the complex. The use of these antisera are discussed in the renewal proposal.

As reported by us in Science 181: 749 (1973), the aerobically grown bakers' yeast cell contains only one mitochondrion. The organelle is a very large (by earlier standards) continuous system of branching and, anastomosing tubular membranes most of which are localized close to the

cell's plasma membrane. The organelle appears to be constructed and positioned within the aerobically grown cell so as to present maximum surface area to the extra-mitochondrial cytoplasm close to the cell's plasma membrane, i.e., close to the source of diffusing oxygen. This new conception of the mitochondrion as being an extensive system of membranes leads one to conclude that what one isolates during cell fractionation experiments are not whole organelles, but pieces of these very large (and fragile) organelles broken off by shearing forces.

If one compares the fragments of the large mitochondrial organelles isolated from bakers' yeast cells grown under different conditions one observes that the organelle pieces from cells grown under glucose repression are generally larger than those isolated from fully respiring cells. This observation suggests that the mitochondrion present within the glucose repressed cell may not be as extended and branching in its structure. The glucose-repressed mitochondrion may be more condensed and have a lower surface area (lower surface to volume ratio) than the aerobic organelle. Such a "rounded-up" structure would be less fragile and would thus tend to form larger fragments during cell fractionation.

The observation of different sizes of mitochondrial fragments from cells grown under different conditions suggested that the mitochondria present in yeast cells in different metabolic states might have different three-dimensional structures, membrane dimensions and might possibly be present in different numbers per cell. Accordingly, bakers' yeast cells were grown under strict anaerobiosis in a medium containing Tween 80 and ergosterol so as to produce cells containing promitochondria with sufficient unsaturated fatty acid content to be visualized with electron stains. An aliquot of these anaerobic cells was harvested directly into glutaraldehyde, formaldehyde fixative. Other aliquots of the same culture were harvested and aerated either in 0.3% glucose, 1% ethanol (adaptation medium) or in 5% glucose (glucose-repression medium). Samples from each incubation were fixed at intervals up to six hours of aeration. These samples were embedded for electron microscopy. They will be serially sectioned and photographed during the next three months and three-dimensional models constructed of (1) the promitochondrion, (2) the glucose-repressed mitochondrion and (3) the fully adapted organelle.

Articles and abstracts published during this support year or expected to be published as a result of work completed during this support year:

Hoffmann, H.-P. and C.J. Avers. 1973. Mitochondrion of yeast: Ultrastructural evidence for one giant, branched organelle per cell. *Science* 181: 749-751. (submitted with last year's renewal in preprint form as document no. COO-3516-3; resubmitted here in reprint form under the same document no., i.e., COO-3516-3).

Cooper, C.S. and C.J. Avers. 1974. Evidence of involvement of mitochondrial polysomes and messenger RNA in synthesis of organelle proteins. In: *Mitochondrial Biogenesis* (A.M. Kroon and C. Saccone, eds.), pp. 289-303. Academic Press, Inc., New York. (conference paper presented in June, 1973 at an international conference on The Biogenesis of Mitochondria in Rosa Marina near Bari, Italy, and submitted here as document no. COO-3516-5).

Rouslin, W. 1974. Regulation of the biosyntheses of electron transfer complexes III and IV in adapting yeast. *Fed. Proc.* 33: 1269. (Abst). (abstract of paper presented in June, 1974 at the Biochemistry/Biophysics Meetings in Minneapolis, and submitted here as document no. COO-3516-6).

Rouslin, W. 1974. Oxygen dependence of promitochondrial and cytoplasmic protein synthesis in the formation of electron transfer complexes III and IV in adapting yeast. *Arch. Biochem. Biophys.* Submitted (preprint of paper submitted to *Arch. Biophys. Biochem.* submitted here as document no. COO-3516-7).

Percentage time/effort devoted to project by Principal Investigators from September 1, 1973 to August 31, 1974 = (C.J. Avers) 15% of academic year; (W. Rouslin) 20% of academic year and 100% of two summer months.