21,328

MITOCHONDRIAL PHENOTYPES AND GENOTYPES IN YEAST

Technical Annual Progress Report

Charlotte J. Avers

Rutgers, The State University

New Brunswick, New Jersey 08903

Reporting Period: August 1, 1972 - July 31, 1973

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

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.

ABSTRACT

Three projects were continued during the year and definitive data were collected in each case. (1) Mitochondrial polysomes in isolated organelle preparations were identified as sites of protein synthesis. The individual ribosomes of the polysomal aggregates are held together by a strand of messenger RNA, which guides the translation into particular proteins. These proteins were of comparable molecular weight to those described by others for yeast adapting to oxygen, while our system involved respiratory adaptation during glucose derepression. (2) The DNA molecules of yeast mitochondria replicate according to a Cairns-type mode and not according to a "rolling circle" model. The replicating molecules were of varied contour length, thus substantiating their reality and casting further doubt on the claim by Borst that all yeast mitochondrial DNA is 25-26 μ m long. (3) During our investigation of mitochondrial biogenesis we discovered that all the separate profiles of a yeast cell actually constitute sections through a single giant mitochondrion. This discovery must lead to an extensive reevaluation of data collected from many studies of mitochondrial genetics and biogenesis. A new perspective is required since our model of the mitochondrion shows that previous notions of form, size, and number per cell were totally wrong.

NARRATIVE

Mitochondria were isolated from yeast spheroplasts undergoing glucose derepression and incorporation of radioactive uracil into RNA and amino acids into proteins was followed in organelles devoid of cytoplasmic contamination or contribution. Radioactive uracil was incorporated into polysomal RNA and not into 4S or 5S species in short pulses; and the polysomal RNA species was not ribosomal. The one remaining RNA type which is known is messenger, and we tentatively have concluded that the incorporation was indicative of a mitochondrially-synthesized messenger RNA. Experiments designed to determine sites of protein synthesis within mitochondria revealed that radioactive amino acids were incorporated preferentially in polysomes, the synthesis could be abolished by erythromycin but not by cycloheximide, and puromycin treatment led to release of nascent polypeptides from the polysomes. Using polysome size as a measure of messenger RNA length, we calculated that proteins which were preferentially synthesized during respiratory adaptation on these polysomes had molecular weights of 27,000-30,000 and 34,000-40,000, respectively. Some polysome fractions were inactive in protein synthesis at these

times during adaptation which led us to suggest that regulation of protein synthesis may occur at the level of translation rather than transcription exclusively. Proteins of similar molecular weights were described by Schatz as being synthesized preferentially in yeast adapting to oxygen, a system comparable to our own. The evidence we have collected from electron microscopy of polysome fractions from gradients, incorporation of radioactive precursors, sedimentation and electrophoretic analyses, and physical studies all lead us to conclude that protein synthesis in the mitochondrion occurs specifically on polysomes under the guidance of messenger RNA transcripts of mitochondrial DNA information, for some proteins unique to the organelle during respiratory adaptation. We are the first to demonstrate the existence of functional polysomes in the mitochondrion.

Electron microscopy of mitochondrial DNA released from intact isolated organelles by osmotic lysis was accomplished to determine the mode of replication of these molecules and whether or not the DNA was a heterogeneous population of circles. The replicating molecules were present at one specific brief interval during synchronous cell growth; they formed replicating topologies of the type described by Cairns for E. coli and not the "rolling circle" type; and the DNA contour lengths varied between 0.5 and 10.0 μm . Physical studies indicated that the DNA molecules did not include significant levels of circular forms as a result of hydrogen bonding of cohesive termini, but that covalently closed circles predominated. Since molecules of varying lengths were found in the process of replication, we believe that Borst's claim is unsubstantiated and that yeast mitochondrial DNA exists as a heterogeneous collection of circular molecules rather than as a homogeneous population with a contour length of 25-26 μm , as we have claimed all along. The genetic analysis of the yeast mitochondrion will require different assumptions for heterogeneous DNA than for a homogeneous 25- μm population, which is the major implication of our data.

During the course of our study of mitochondrial biogenesis in synchronous cell cultures we perceived that all the separate mitochondrial profiles in electron micrograph thin-sections actually were cuts through a single giant mitochondrion per cell. We have reconstructed models of the organelle to scale. The mitochondrion grows during 80-90% of the cell cycle, flowing into the developing bud cell, and then is pinched in two when the new wall is completed between mother and bud cells. The questions about mode of biogenesis must be recast in new terms since the older notions about growth-and-division versus addition of new membranes from

non-mitochondrial sources all were based on intracellular populations containing numerous, small mitochondria. Indeed, reevaluations of many kinds of mitochondrial studies would seem to be required because of our proposed model of the organelle; especially in relation to genetics and the physiological modifications during growth in various environments. We believe that the proposed model will have a profound effect on the future course of investigations of mitochondria in many kinds of cells in addition to yeast. We expect to complete autoradiographic analyses concerning mitochondrial DNA synthesis and thus to add data to our overall study of the mitochondrion in synchronously growing cell cultures.

Articles published or expected to be published during this support year:

- Hoffmann, H.-P. and C.J. Avers. 1973. Mitochondrion of yeast: Structural model of the one giant, branched organelle per cell. Science, submitted.
- Cooper, C.S. and C.J. Avers. 1973. Evidence of involvement of mitochondrial polysomes and messenger RNA in synthesis of organelle proteins. Second Conference on Mitochondrial Biogenesis, Bari, Italy. (to be published in the symposium volume; will be submitted at the time of the conference June 25-28, 1973)

Percentage time/effort devoted to project by Principal Investigator (Charlotte J. Avers) from August 1, 1972 until July 31, 1973 = 20%, academic year; 50% two summer months.