TECHNICAL ANNUAL PROGRESS REPORT**MASTER**

During the past year we have pursued four particular problems: (1) analysis of mitoribosomal proteins, with reference to their translation as products of nuclear and mitochondrial gene action; (2) characterization of putative messenger RNA populations transcribed from mitochondrial DNA templates; (3) analysis of mtDNA populations, with reference to heterogeneity, topology, and replicative mode; and (4) analysis of mitochondrial formation in synchronous cell cultures. Each of these problems was resolved into specific questions from which experiments were designed and executed. A portion of the data were sufficiently fruitful to pursue the analysis in greater detail, and we anticipate publication of two of these lines of investigation within the next 4-6 months. The remaining studies were valuable in directing our efforts further so that we have planned additional experiments, and we expect to derive levels of information which will lead to significant publication.

1. Mitoribosomal proteins were resolved into about 16 bands for smaller subunits and about 19 bands for the larger subunits of the 73s organelle ribosome. Considerable overlap occurred among these bands, but in comparisons with 78s cytoribosomes from these same cells we found that at least 4 bands of the mitochondrial particles were unique and about 8-10 bands were unique to the cytoribosomes. Insufficient resolution prevented us from deriving firm conclusions in these preliminary experiments. From our studies of synthesis of mitoribosomal proteins we found that cycloheximide was almost as effective in inhibiting incorporation of ^3H -amino acids into these proteins as cycloheximide plus chloramphenicol or erythromycin. The possibility exists that one or more mitoribosomal proteins may be translated in the organelle rather than in the cytoplasm, but we could not interpret our data well enough to state this conclusion. Recent studies from Halvorson's group indicates that one mitoribosomal protein is missing in erythromycin-sensitive as compared with drug-resistant strains. Whether this protein is translated from mitochondrial messengers or from nucleocytoplasmic messengers that function within the mitochondrion is unknown at present.

Further characterization of the rRNA from mitoribosomes confirmed the fact that there is no 5s component in these particles. In this way, mitoribosomes differ from prokaryotic particles, which have a 5s RNA, as they differ in sedimentation values shown in our earlier work.

2. Mitochondrial messenger RNAs were characterized further in new experiments. Previously we showed their probable presence as a function of polysome isolation in density gradients and the disappearance of these aggregates (with concomitant increase in monomers) after brief treatment with RNase. From studies of uracil incorporation and char-

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acterization of RNAs extracted from the gradient fractions using gel electrophoresis, we found that the RNAs fit the predictions made for messengers transcribed from mtDNA in the isolated intact mitochondrial test system, namely: (a) synthesis occurs in mitochondria essentially free of cytoplasmic and bacterial contamination; (b) the newly-synthesized RNAs are rapidly labeled; (c) these RNAs show a broad range of molecular weights, hence lengths; (d) the rapidly-labeled RNAs are associated predominantly with the polysomes in the gradient; and (e) the sizes of putative mRNAs correlate exactly with the range of polysome sizes. In addition, we have excellent electron micrographs showing polysomes (some with obvious strand connections) of different sizes that correspond with the region of the density gradient from which they were isolated. We believe that we have demonstrated the synthesis of messenger RNA in mitochondria, and their participation in polysome-directed activities. Our next experiments will attempt to locate organelle protein synthesis predominantly or exclusively with these polysome populations.

3. Physical studies of mtDNA have proceeded, using mitochondria isolated from synchronously-growing cells selected from appropriate intervals in successive cell cycles. The electron photomicrographs have been analyzed in part, and show a bimodal distribution of replicative forms. A group of 1-3 μ m-long circular molecules appears to replicate in a manner suggestive of the "rolling circle" model as proposed for various viruses; a group of 3-6 μ m-long circular forms show rather complex topologies, reminiscent of the forms reported for mouse L-cells from Vinograd's laboratory. We have seen no 25-26 μ m-long circular forms as reported from Borst's laboratory. The only replicative forms have come from one specific brief interval in the cell cycle during which mtDNA replicates. This observation has been verified from chemical analyses of mtDNA during the cell cycle and from kinetics of incorporation of labeled thymidine-5-monophosphate. At other times during the cell cycle, samples show a collection of mtDNA lengths and conformations, but not one of these shows evidence of replication forks or complex topology indicative of replication. There is no cytoplasmic or bacterial contamination in these preparations, although we cannot rule out viral contamination. It seems unlikely that we would observe substantial viral populations precisely at a time known to be the mtDNA synthesis period of 5-10 minutes in the yeast cell cycle, and we expect no synchrony in contaminating viruses even if they were present. These analyses will be continued and we hope to discern replicative modes from these data.

4. Mitochondrial formation in synchronous cultures still appears to occur by some mechanism different from simple growth-and-division of the mitochondrial complement. There is mitochondrial synchrony, monitored by various biochemical and physiological tests,

So that any sample represents an fairly accurate portion of any mitochondrial population of any cell growing synchronously with all the others in the culture. There is a gradual increase in mitochondrial numbers per cell throughout the cycle, and a restoration of the mean number when the bud separates from the mother cell. These observations have been verified from light microscopic cytochemistry as well as from electron micrographs. Morphometric analyses have been initiated since mitochondrial morphology may be such that our two-dimensional photographs are deceiving in relation to number of profiles versus whole mitochondria in sections and whole cells. We have a huge amount of fixed material sampled at 10-15 min intervals from about 5-6 hours of successive cell cycles, and will analyze this material in great detail during the coming year.

Articles published in 1971, from this work:

Cottrell, S.F., and C.J. Avers. 1971. Mitochondrial activities in synchronous cultures of baker's yeast. in Autonomy and Biogenesis of Mitochondria and Chloroplasts, eds. N.K. Boardman, A.W. Linnane, and R.M. Smillie. North-Holland, Amsterdam. pp. 481-491.

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