MITOCHONDRIAL MEMBRANE ORGANISATION, A DETERMINANT OF MITOCHONDRIAL RIBOSOMAL RNA SYNTHESIS.

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SUMMARY: Mitochondria isolated from lipid-supplemented anaerobically grown Saccharomyces cerevisiae contain normal mitochondrial ribosomes while the mitochondrial precursor particles isolated from anaerobic cells, depleted of both unsaturated fatty acid and ergosterol appear to lack mitochondrial rRNA. These findings indicate that the control of mitochondrial rRNA synthesis is directly related to the composition and molecular structure of the mitochondrial membrane.

The morphology and biochemical properties of anaerobically grown Saccharomyces cerevisiae is markedly affected by the lipid composition of the growth medium (1, 2). Cells depleted of lipid contain only primitive mitochondrial structures whereas lipid-supplemented cells possess well defined mitochondrial structures which, in turn, are quite distinct structurally from those in the aerobically grown yeast cell (1). The effect of anaerobic growth and the lipid composition of the growth medium on the development of the mitochondrial protein synthesizing system has been reported by Watson et al. (3). It was found that whereas mitochondria from lipid-supplemented anaerobes have an active amino acid incorporating system in vitro, the lipid-depleted mitochondrial precursor structures appeared to have negligible amino acid incorporating activity in vitro. This communication reports that the lipid composition and therefore molecular architecture of the mitochondrial membrane appears to be a major factor in the control of mitochondrial ribosomal RNA (rRNA) synthesis. The lipid-depleted cells appear to lack mitochondrial rRNA while the lipid-supplemented cells contain normal mitochondrial rRNA and functional ribosomes.

METHODS All experiments were carried out with a locally isolated diploid strain of Saccharomyces cerevisiae, strain M, grown on a basic 0.5% Difco yeast extract - salts medium (1). The carbon source for strict anaerobic growth was galactose (4%), whereas ethanol (1%) was used for aerobic growth. The nomenclature used by Watson et al. (1) has also been adopted: thus (a) An-Gal denotes cells grown anaerobically in 4% galactose-yeast extract medium to yield anaerobic cells deficient in both unsaturated fatty acid (UFA) and ergosterol (E); (b) An-Gal+E denotes cells grown anaerobically in 4% galactose-yeast extract medium supplemented with E to yield anaerobic cells deficient in UFA; (c) An-Gal+T denotes cells grown anaerobically in 4% galactose-yeast extract medium, supplemented with Tween 80, as a source of UFA's to yield E deficient anaerobic cells; (d) An-Gal+T+E denotes cells grown anaerobically in 4% galactose-yeast extract medium, containing Tween 80 and E, to yield lipid-supplemented anaerobic cells; (e) Aer-EtOH denotes cells grown aerobically in 1% ethanol-yeast extract medium to yield aerobic cells with a normal lipid composition. All cells were harvested at early stationary phase and the population shown to be greater than 90% viable; if the lipid deficient cultures are left for some hours the cells begin to die and must be discarded. In this work yeast mitochondria and cytoplasmic ribosomes were isolated as described by Lamb et al. (4). The mitochondria were purified by discontinuous sorbitol gradient centrifugation (1) and the total mitochondrial RNA and cytoplasmic rRNA extracted using diethyl pyrocarbonate-sodium dodecyl sulphate as previously described (5). Fatty acids and ergosterol were determined as described by Jollow et al. (6), RNA base compositions by the method of Katz and Comb (7).

RESULTS Yeast mitochondria contain high molecular weight RNA species

quite distinct, both in basic composition and molecular size, from the rest of the cell (5,8,9,10,11). These include the 21S and 15S components derived from the larger and smaller subunits, respectively, of the mitochondrial ribosome together with a 4-5S component identified with mitochondrial transfer RNA species (11). The presence of these well-defined species in mitochondria isolated from either aerobically grown, or lipid-supplemented anaerobically grown yeast cells is illustrated in Figure 1a, b. The mitochondrial species can be compared with the faster sedimenting cytoplasmic rRNA components (26S and 17S) in Figure 1c. As shown in Figure 1b, the RNA isolated from An-Gal+T+E mitochondria is, to a variable extent, always contaminated with some cytoplasmic rRNA species. However all four cytoplasmic and mitochondrial rRNA species are resolved on linear 15-30% sucrose density gradients, and are individually clearly identifiable.

Morphologically and biochemically the most primitive mitochondria are to be found in anaerobically grown yeast cells which are depleted of both UFA's and E (1). The resulting deficiency in mitochondrial membrane lipid composition is apparent in the mitochondrial structures isolated from An-Gal cells. The fatty acids of the An-Gal mitochondria are made up of about 90% saturated and 10% unsaturated fatty acids which compares with values of about 20% saturated and 80% unsaturated fatty acids for An-Gal+T+E and Aer-EtOH mitochondria. In addition the E content of the An-Gal mitochondria is about 0.4 mg/g dry weight which is considerably lower than that found for An-Gal+T+E mitochondria which is about 2.5 mg/g dry weight. The RNA extracted from An-Gal mitochondrial structures (Figure 1d) contained no detectable mitochondrial rRNA but instead a prominent heterodisperse component with an approximate sedimentation coefficient of 6-14s and in addition small amounts of the two cytoplasmic rRNA species (26S and 17S) contaminated the

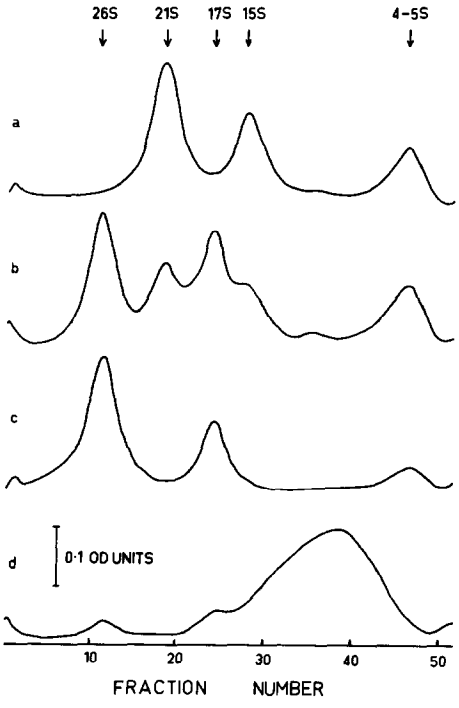

Figure 1. Sucrose density gradient centrifugation of mitochondrial and cytoplasmic RNA from S. cerevisiae. An aliquot containing approximately 30 µg of RNA was layered onto a 5 ml linear gradient of 15 to 30% ribonuclease-free sucrose containing 5mM KCl, 15mM Tris-HCl, pH 7.5 and centrifuged at 32,000 rev/min for 17 hours. The gradient was displaced from the bottom using 70% sucrose and analysed continuously at 254 nm. Direction of sedimentation was from right to left. The S values for the individual RNA species are assigned relative to those obtained for Escherichia coli.

(a) Mitochondrial RNA from Aer-EtOH cells
(b) Mitochondrial RNA from An-Gal+T+E cells
(c) RNA from Aer-EtOH cytoplasmic ribosomes
(d) Mitochondrial RNA from An-Gal cells.

preparations. Mitochondrial rRNA has a G+C (guanine + cytosine) content of 28%, whereas cytoplasmic rRNA is 47% G+C and we have found the 6-14S species to possess a G+C content of about 48%. The heterodisperse RNA species associated with the mitochondria therefore cannot be a simple breakdown product of the mitochondrial rRNA. Considering that yeast mitochondrial DNA has a G+C content of 17-21% (12,13) it is not likely that the 6-14S RNA species are the products

of mitochondrial transcription; these RNA's are currently being further investigated. Both UFA's and E are required simultaneously to promote synthesis of mitochondrial rRNA in the anaerobic yeast cell; neither An-Gal+T nor An-Gal+E mitochondrial structures contain any detectable mitochondrial rRNA species, similar RNA sedimentation profiles as shown in Figure 1d are obtained. The An-Gal+T mitochondria contained about 0.4 mg/g dry weight of E and the fatty acids were 65-70% unsaturated, whereas the An-Gal+E mitochondria had an E content of about 1.2 mg/g dry weight whilst the fatty acid components were 5-10% unsaturated.

DISCUSSION The unique 21S and 15S mitochondrial rRNA species are readily isolated from mitochondria obtained from cells grown aerobically in ethanol medium and from mitochondria of cells grown anaerobically with supplements of UFA's and E. However when the lipid composition and hence molecular structure of the yeast mitochondrial membrane is altered by lipid limiting anaerobic growth conditions, mitochondrial rRNA is apparently not synthesized or at most only formed in amounts which are not detectable by our techniques. The addition separately of a source of UFA or E to the basic anaerobic growth medium, is not sufficient to permit the initiation of detectable mitochondrial rRNA synthesis. We interpret the present results to indicate that mitochondrial rRNA synthesis and ultimate mitochondrial ribosome formation is directly dependent on the lipid composition of the mitochondrial membrane. The lipid-depleted cells are viable and contain normal cytoplasmic ribosomes.

There are a number of possible explanations for the observations reported, two of which may be briefly mentioned and are under investigation. It may be that the mitochondrial membrane of the lipid depleted cells has no suitable receptor binding sites for the mitochondrial DNA-dependent RNA polymerase, which has been

shown to be tightly bound to normal mitochondrial membrane (14). On the other hand we have recently described, both genetically and biochemically, a number of new cytoplasmically determined mutants and these experiments lead us to suggest that the mitochondrial ribosome is in close association with the mitochondrial membrane and may even be an integral part of it (15,16,17). Consequently it could be possible that even though all the biosynthetic enzymes for rRNA and ribosomal protein formation are present in the lipid-depleted organism, the deformed mitochondrial membrane cannot incorporate the complete ribosome and hence a feedback inhibition of ribosome synthesis occurs. This inhibition of ribosome formation is readily reversible and constitutes an ideal system for the investigation of mitochondrial RNA and ribosome synthesis. On aeration of the An-Gal cells mitochondrial rRNA rapidly becomes demonstrable, accompanied by major changes in the mitochondrial membrane composition and structure (Forrester and Linnane, in preparation).

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