DIFFERENCES BETWEEN THE SEDIMENTATION CHARACTERISTICS OF THE RIBONUCLEIC ACIDS PREPARED FROM YEAST CYTOPLASMIC RIBOSOMES AND MITOCHONDRIA

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The existence of two protein synthesizing systems in Saccharomyces cerevisiae has recently been demonstrated (Huang et al., 1966; Clark-Walker and Linnane, 1966, 1967, Linnane et al., 1967). These systems, one mediated by 80S type ribosomes in the cytoplasm, the other associated with the mitochondria, may be distinguished by their response to chloramphenicol, a known inhibitor of bacterial protein synthesis. While the cytoplasmic system is insensitive to the antibiotic, amino acid incorporation into isolated mitochondria and the in vivo synthesis of the mitochondrial cytochromes a, a_3, b and c_1 are markedly inhibited by chloramphenicol. Similarly, this drug inhibits the in vivo synthesis of chlorophyll in Euglena gracilis (Smillie et al., 1963, Linnane and Stewart, 1967) and amino acid incorporation into isolated chloroplasts (Spencer, 1965). Since chloroplasts and Escherichia coli cells, respond similarly to the antibiotic and both contain 70S type ribosomes, we have earlier suggested that chloramphenicol selectively inhibits protein synthesis, mediated presumably by a bacterial type ribosomal system.

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Although ribosome like particles in mitochondria have been observed from time to time in the electron microscope the occurrence of such particles has not been commonly reported (Kislev et al., 1965, Leduc et al., 1966). The failure to demonstrate the unambiguous presence of ribosomes in the mitochondria together with the low concentrations of RNA found in this organelle led us to attempt the characterisation of the ribonucleoprotein system by determining the sedimentation characteristics of its RNA components. This communication describes the sedimentation behaviour of RNA prepared from the cytoplasmic ribosomes and mitochondria of S.cerevisiae. The $S^{o}_{20,w}$ values of the RNA's prepared from the two sources are different and the isolated mitochondrial RNA has been found to contain species consistent with the presence of a 70S type ribosome in mitochondria.

METHODS

Isolation of Cytoplasmic rRNA and Mitochondrial RNA from Yeast

A diploid strain of S.cerevisiae was grown aerobically on a 1% glucose-yeast extract-salts medium (Clark-Walker and Linnane, 1967). Well washed mitochondria were prepared from yeast protoplasts as described by Duell et al., (1964), homogenized in a 1% solution of sodium dodecyl sulphate (SDS) containing bentonite (5 mg/ml) and the high molecular weight RNA isolated as described by Nievel and Kirby (1966). The purified mitochondrial RNA was stored under absolute ethanol at -15°.

Cytoplasmic ribosomes were isolated from the mitochondria free supernatants by centrifugation at 100,000 x g for 90 min. Ribosomes were also prepared by differential centrifugation of

extracts from mechanically disintegrated yeast cells. The RNA was prepared from the ribosomes using exactly the same methods as indicated above.

Isolation of E.coli rRNA

An E.coli strain was grown under forced aeration at 37°, on a minimal salts medium supplemented with 2% glucose and 0.2% peptone. Cells were harvested in their logarithmic growth phase and lysed in a 1% solution of SDS. The rRNA was again isolated and stored as described above.

Nucleic Acid and Protein Estimations.

RNA was estimated by the orcinol method (Kerr & Serraidonian, 1945), DNA by the diphenylamine method (Burton, 1956) and protein by the method of Lowry et al., (1951). All the RNA samples analysed in the ultracentrifuge contained less than 1% protein and 2% DNA. The mitochondria contained 8-12 µg RNA/mg protein.

Sedimentation Velocity Analysis.

RNA was dissolved in 0.025 M NaCl, 0.01 M Tris buffer pH 7.3. Runs were performed at 20° at 50,740 rpm in a Spinco Model E ultracentrifuge using ultraviolet absorption and schlieren optics. The sedimentation behaviour was studied over an RNA concentration range of 0.03 - 0.3 mg/ml. $S^{o}_{20,w}$ values were obtained by a least mean squares extrapolation to infinite dilution. The concentrations of the RNA components were determined by integration of the peak areas on the schlieren patterns.

RESULTS

At the outset of the work we considered that it would be necessary to demonstrate that the isolated mitochondrial RNA

originated from these organelles and was not due to cytoplasmic contamination. To deal with this possibility the RNA's from the cytoplasmic ribosomes and mitochondria were analysed both individually and as mixtures. In addition E.coli rRNA was included in our studies for two reasons; first to serve as a source of previously well characterized RNA from an acknowledged 70S type

TABLE 1

SEDIMENTATION COEFFICIENTS ($S^{o}_{20,w}$) OF RNA COMPONENTS FROM E.COLI, S.CEREVISIAE CYTOPLASMIC RIBOSOMES AND S.CEREVISIAE MITOCHONDRIA.

SOURCE RNA	$S^{o}_{20,w}$ VALUES (S)				
Yeast rRNA	16.2			24.6	
E.coli rRNA	16.9			22.6	
Yeast rRNA + E.coli rRNA	16.4	22.3		24.5	
Yeast mitochondrial RNA	12.7			17.8	22.4
Yeast mitochondrial RNA + yeast rRNA	12.7	16.0	18.0	22.2	24.1

ribosome; second to serve as a measure of the ability of our method to resolve RNA species with very similar sedimentation characteristics.

The results of the sedimentation experiments are summarized in Table 1. The E.coli rRNA had $S^{o}_{20,w}$ values of 16.9S and 22.6S in good agreement with established figures for this material. The yeast cytoplasmic rRNA was characterized as 16.2S and 24.6S. However, most significantly, the mitochondrial RNA was shown to consist of three high molecular weight species having values of 12.7S, 17.8S and 22.4S. (Table 1, Fig. 1a). To confirm that the

yeast RNA's prepared from the mitochondria and the cytoplasmic ribosomes were not identical, a 1:1 mixture of the two RNA preparations was sedimented. The five components were readily recognized with the two fast moving members partially separating while the slow species completely resolved (Fig. 1c). The $S^{o}_{20,w}$ values of 12.7S, 16.0S, 18.0S, 22.2S and 24.1S determined by the analysis of the mixture were all in good agreement with those

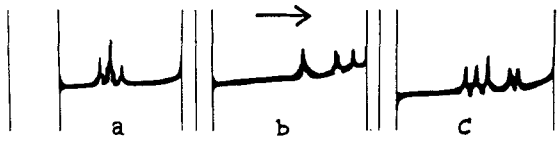

FIGURE 1. Schlieren patterns of, (a) S.cerevisiae mitochondrial RNA (b) E.coli and yeast cytoplasmic rRNA (c) yeast mitochondrial RNA and yeast cytoplasmic rRNA.

obtained from analysis of the two separate RNA preparations.

The resolving power of the procedures adopted to separate species with similar sedimentation behaviour was illustrated by the results obtained from an analysis of a mixture of E.coli and yeast rRNA (Fig. 1b). The fast moving components resolved, but the slower pair moved as a single peak with an apparent $S^{o}_{20,w}$ of 16.4S (Fig. 1b). The shape of this peak suggested that two components were present. It therefore follows that in the system studied differences in S values of 5% are not clearly resolvable but 10% differences are (cf. Table 1).

DISCUSSION

The classification of ribosomes into two size groups has evolved on the basis of their behaviour in the ultracentrifuge. Animal, plant and yeast cytoplasm contain 80S type ribosomes,

while those from bacteria and plant chloroplasts are 70S type (Petermann, 1964).

A further classification is possible when the sedimentation characteristics of the ribosomal RNA components are considered. The values of 16.9S and 22.6S reported here for the rRNA components of E.coli 70S ribosomes are in good agreement with the classical values of 16S and 23 S quoted for bacterial rRNA. However, yeast 80S ribosomes were found to contain a 16.2/24.6S rRNA pair, while the RNA components of rat liver 80S ribosomes, had $S^{o}_{20,w}$ values of 16.2S and 28.9S, under identical experimental conditions (Rogers et al., 1967). These results support the findings of Stutz et al., (1967), who have recently reported the presence of 16/25S rRNA in ribosomes prepared from yeast and plant cytoplasm, and have suggested a close similarity between 80S ribosomes from these two sources. The clear difference in the sedimentation properties of the RNA components of rat liver 80S ribosomes to those of yeast and plant ribosomal RNA, indicates that ribosomes should be classified into at least three classes, two of these identified as 80S, but characterized by different rRNA components, and the third a 70S type ribosome.

The 17.8S and 22.4S RNA components, present in yeast mitochondria, are particularly similar to the E.coli 16.9/22.6S rRNA pair, and strongly suggest the occurrence of 70S ribosomes within the mitochondria. That other mitochondria may contain a 70S type ribosome is supported by our recent findings of 16.6/22.3S RNA species as major components in RNA preparations isolated from rat liver mitochondria (Rogers et al., 1967). The significance of the 12.7S component in the yeast mitochondrial RNA preparations remains to be elucidated.

The results presented above appear to contradict the findings of Eleav (1966) and Wintersberger (1966) who report respectively the presence of 80S ribosomes in rat liver and yeast mitochondria.

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