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THE SIZE OF YEAST MITOCHONDRIAL RIBOSOMAL RNAs

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SUMMARY

The sizes of mitochondrial rRNAs of Saccharomyces cerevisiae and Torulopsis glabrata have been investigated by agarose gel electrophoresis after glyoxal treatment. The large and small mt rRNAs from S. cerevisiae were found to be 3100 and 1460 bases respectively, values which are considerably smaller than previous estimates (1). The corresponding lengths for T. glabrata mt rRNAs are 2700 and 1400 bases, indicating that the size of mitochondrial rRNAs in yeasts is not conserved.

INTRODUCTION

The molecular weights of mitochondrial rRNAs of Saccharomyces carlsbergensis, as determined by four methods, have been reported to be 1.3×10^6 daltons for the large and 0.7×10^6 daltons for the small species (1). In terms of bases, the same laboratory quoted the large mt rRNA as having 3700 bases (2). If we assume that this value was calculated from the molecular weight, then a similar calculation shows that the small mt rRNA has 1990 bases from the ratio of the previously quoted molecular weights. However we have discovered in Torulopsis glabrata, a petite positive yeast with a small mitochondrial genome (3), that the lengths of the large and small mt rRNAs are 2700 bases and 1400 bases respectively (4). Prompted by the large difference in lengths between the mt rRNAs of T. glabrata and those reported for S.carlsbergensis (1), we undertook a reinvestigation of the sizes of mt RNAs from S. cerevisiae, a yeast which cross hybridizes to

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S. carlsbergensis (5). We had in mind that the large difference in the mitochondrial genomes of S. cerevisiae (70-75 Kb) and T. glabrata (18.9 Kb) may in part be mirrored by the size of the genes they possess and that there may indeed be large differences in the sizes of the mt rRNAs. However although our data indicate that there are real differences in the sizes of the mt rRNAs from T. glabrata and S. carlsbergensis/S. cerevisiae, nevertheless we find that the sizes of the latter yeasts mt rRNAs have previously been overestimated.

MATERIALS AND METHODS

S. cerevisiae 4342-2B, a ade 8-18, lys2, was obtained from S. Fogel and T. glabrata CBS138 is the same strain used before (3). Yeast strains were grown on a rich medium containing yeast extract, peptone and 2% ethanol as carbon source (for details see (3)). Strain C600 was used for the preparation of E. coli rRNAs and was grown on Luria broth.

The details of mt rRNA preparations have been described elsewhere (4). In short, twice washed mitochondria were lysed with 2% sarkosyl and extracted with phenol-chloroform. The nucleic acids were then recovered by ethanol precipitation and the rRNAs selectively isolated from the total nucleic acid by precipitation from 2.5 M NaCl. After two repetitions of NaCl fractionation the large and small species of rRNAs were separated on sucrose density gradients in a two step procedure. First, the mixture of rRNAs were sedimented through a 5-25% sucrose gradient and fractions containing the peaks of small and large rRNAs were separately pooled and precipitated with ethanol. As the large rRNA pool still contained aggregates of low molecular weight RNAs, these were removed by a second sucrose gradient after heating the sample at 65° for 3 min.

For cytoplasmic rRNA from S. cerevisiae we used a petite mutant lacking mt DNA. The cells were broken with glass beads in a Braun homogeniser. E. coli cells were broken by sonication. Isolation procedures of rRNA from these cultures were the same as described above except that the large and small rRNA species were not separately isolated.

For size estimation, the nucleic acids were denatured and reacted with glyoxal essentially as described by McMaster and Carmichael (6). Dimethylsulphoxide and the 30% solution of glyoxal were freshly deionised by passing through a dry bed of Biomineralit (Purmutit). To 1-3 µg RNA in 6 µl, was added 15 µl of dimethylsulphoxide and 3 µl of 100 mM sodium phosphate buffer pH 7.0. After 10 min. at 65°, 6 µl of 30% glyoxal was added and the incubation continued for 1 h at 50°. The RNA samples were then electrophoresed in a 200 mm x 200 mm x

2.5 mm 1.2% agarose gel at 30 mA/170 volts for 2 h at room temperature. The gels were stained with methylene blue and photographs were taken through a red filter on positive-negative Polaroid Land film type 665. Influenza RNA was a gift from G. Air.

RESULTS AND DISCUSSION

Electrophoretic mobility of denatured RNAs are shown in Figure 1. As the lengths of the RNAs from influenza virus, and *S. cerevisiae* cytoplasmic rRNAs have been well established (Table 1), they were used for calibration (Fig. 1, channels 6 and 7). The mobility of the RNA molecules, under the conditions described, is strictly proportional to the logarithm of their sizes (Fig. 2). From the graph, the lengths of the large and small mt rRNAs from *T. glabrata* were estimated to be 2700 and 1400 bases respectively (Fig. 1, channels 4 and 5), in agreement with our earlier determination (4). However, the large and small mt rRNAs from *S. cerevisiae* measured 3100 and

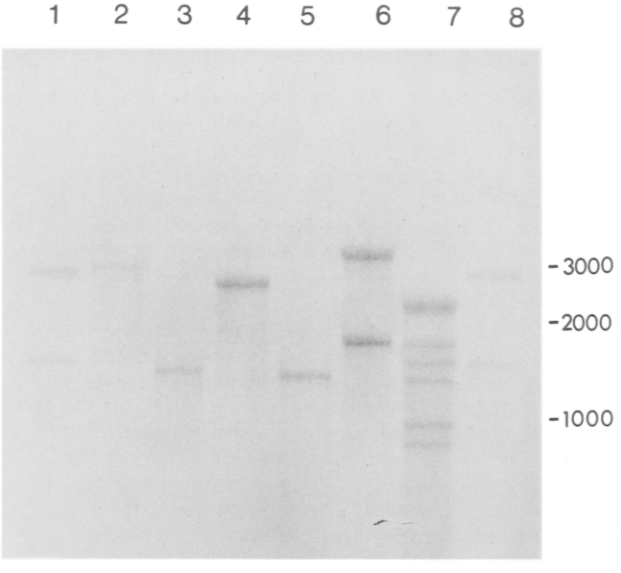

FIGURE 1. Agar gel electrophoresis of RNAs. Channels 1 and 8, are *E. coli* rRNAs; channel 2, *S. cerevisiae* large mt rRNA; channel 3, *S. cerevisiae* small mt rRNA; channel 4, *T. glabrata* large mt rRNA; channel 5, *T. glabrata* small mt rRNA; channel 6, *S. cerevisiae* cytoplasmic rRNAs and channel 7, Influenza Virus RNAs.

Table 1: Sizes of RNAs

RNA	Size (bases)	Reference
Influenza virus		
fragment 1 and 2	2390	14
fragment 3	2290	14
fragment 4	1760	14
fragment 5	1560	14
fragment 6	1480	14
fragment 7	1060	14
fragment 8	890	14
S. cerevisiae cytoplasmic		
large rRNA	3360	15
small rRNA	1710	15
T. glabrata large mt rRNA	2700	This study and 4
small mt rRNA	1400	This study and 4
S. cerevisiae		
large mt rRNA	3100	This study
small mt rRNA	1460	This study
E. coli large rRNA	3053	See below[a]
small rRNA	1541	10
E. coli large rRNA	2950	This study
small rRNA	1540	This study

(a) This value was calculated from the ratio of molecular weights of large and small rRNAs from *E. coli* (16) and size of small rRNA (10).

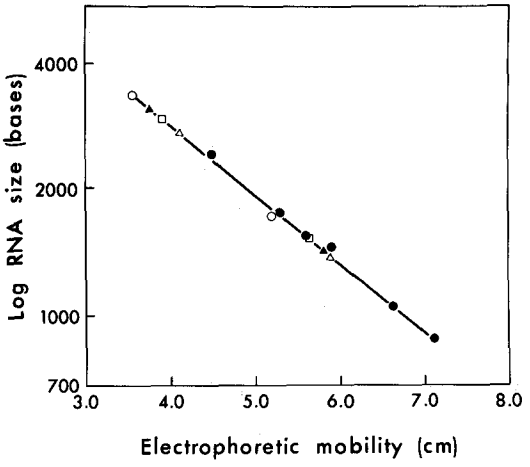

FIGURE 2. Semilogarithmic plot of electrophoretic mobility versus size of RNAs (Table 1). Filled circles, are Influenza RNAs; open circles, cytoplasmic rRNAs from S. cerevisiae; squares, rRNAs from E. coli; open triangles, T. glabrata mt rRNAs; filled triangles S. cerevisiae mt rRNAs.

1460 bases (Fig. 1, channels 2 and 3 and Fig. 2). Additionally coelectrophoresis of the large rRNAs from T. glabrata and S. cerevisiae (Fig. 3, see channels 4,5 and 6) confirmed that the large mt rRNA of T. glabrata is smaller than the large mt

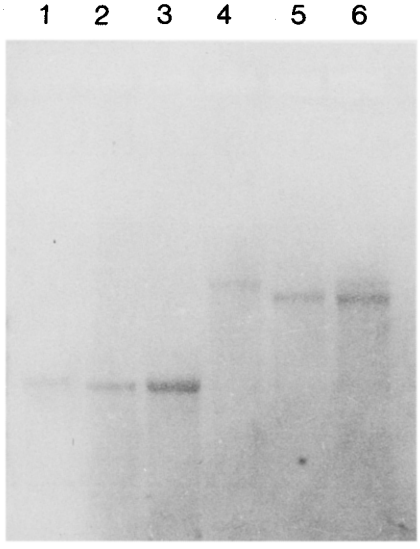

FIGURE 3. Agar gel electrophoresis of mt RNAs from S. cerevisiae (channels 1 and 4) and T. glabrata (channels 2 and 5). In channels 3 and 6 are mixtures of the small (3) and large (6) mt rRNAs of the two yeasts.

rRNA of S. cerevisiae. On the other hand, the difference between the two small mt rRNAs is not great enough to see a clear separation on co-electrophoresis (Fig. 3, see channels 1, 2 and 3). In summary, these results demonstrate firstly, that the size of mt rRNAs from S. cerevisiae are considerably smaller than previously reported, and secondly, the sizes of these mt rRNAs are greater than their counterparts in T. glabrata.

In relation to the size of the S. cerevisiae mt rRNAs we wish to make a number of points. Firstly, mt rRNAs from S. cerevisiae and S. carlsbergensis have been shown to have identical electrophoretic mobilities, but absolute sizes were not determined by these authors (7). Secondly, the smaller mt rRNA sizes are more in accord with DNA-mt rRNA hybridization data where saturation levels of 2.5% were found (8), whereas 3% would be expected from our size estimates (3100 and 1460 bases/75000 bp) and 3.8% from the previous values (3700 + 1990). Thirdly, based on hybridization to DNA fragments of known length, doubt was expressed that the large mt rRNA size had been overestimated (9). Our size estimate for the large mt rRNA resolves this difficulty. Finally, we consider it probable that the previous values for the sizes of the mt RNAs had been overestimated because of incomplete denaturation of the E. coli rRNAs used for calibration. We consider that this problem has now been surmounted because under the present denaturing conditions we find an excellent agreement of the estimated size of the small rRNA molecule from E. coli (Table 1) and its length obtained by sequence analysis (10). Also this rRNA in our hands migrates slower than the small mt rRNA from S. cerevisiae, suggesting that denaturation is more complete

than that obtained in the previous studies where the electrophoretic mobilities of these two molecules were always reversed (1) (11).

The second observation from the present investigation is that the mt rRNAs of T. glabrata are smaller than their counterparts in S. cerevisiae. From the data of rRNA/DNA hybridization in different yeasts, it was concluded (12) that ribosomal rRNA cistrons in both nuclear and mitochondrial genomes have been conserved during evolution. However, it appears that there is more variation in the sequences of mt rRNAs than in the cytoplasmic rRNAs of different yeasts. Size differences observed by us between mt rRNAs of S. cerevisiae and T. glabrata seem to support the idea that mt rRNA cistrons have evolved more rapidly than their nuclear encoded rRNA counterparts (12). This is in accord with data suggesting that animal mitochondria genomes undergo a higher rate of evolution than their corresponding nuclear genomes (13).

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