# YEAST MITOCHONDRIAL RIBOSOMAL RNA: A NEW EXTRACTION PROCEDURE AND UNUSUAL PHYSICAL PROPERTIES

I.T.FORRESTER, Phillip NAGLEY and Anthony W.LINNANE

*Department of Biochemistry, Monash University, Clayton, Victoria, 3168. Australia*

Received 7 September 1970

The existence of unique species of RNA present in yeast mitochondria is well documented in the literature [1–4]. The high molecular weight components, designated here as 21 S and 15 S, have been shown to have slower sedimentation rates relative to yeast cytoplasmic RNA (26 S and 17 S). Yeast mitochondrial RNA has an especially low G+C content of 26% [4–6], and has recently been shown to be derived from the smaller and larger subunits, respectively, of yeast mitochondrial ribosomal particles [6]. In a recent report [7] the mitochondrial RNA of another fungus, *Aspergillus nidulans*, is described. This RNA has similar sedimentation properties (23.5 S and 15.5 S) and a similar low base composition (G+C = 32%) to yeast mitochondrial ribosomal RNA (rRNA). The behaviour of *Aspergillus* mitochondrial rRNA on polyacrylamide gels was found to be anomalous, in that the RNA had mobilities in the gel expected of more massive molecules, in that case, those of the size of cytoplasmic rRNA (26.5 S and 17.0 S) from the same species. The publication of that report has prompted us to present our own results concerning the paradoxical behaviour of yeast mitochondrial rRNA in gels and in centrifugal fields. In this work we have used a procedure for extracting and purifying RNA from yeast mitochondria, which provides a product showing very little, if any, degradation of the RNA components present. This method is applicable to many other systems where very active nucleases are present in the preparation, and where the regular type of phenol extractions does not lead to wholly satisfactory results.

The extraction procedure is based upon the diethylpyrocarbonate-sodium dodecyl sulphate method described by Solymosy et al. [8] for plant tissues. Mitochondria are homogenised at 0°in 0.01 M tris-0.005 M $MgCl_2$ –2% sodium dodecyl sulphate, pH 8, to which 0.1 vol. diethylpyrocarbonate had been previously added. After incubating at 37° for 5 min, the suspension is rehomogenised and centrifuged at 5000 *g* for 10 min at room temperature. Less than 1% of the RNA is found in the pellet of chemically modified [9] and denatured protein. The supernatant is made 1 M in NaCl by addition of solid crystals and incubated at 37° for 5 min with homogenisation. The suspension is centrifuged at 10,000 *g* for 20 min at 0°, and 2 vols. of ice cold absolute ethanol added to the supernatant (the pellet may be re-extracted if an increased yield is desired), and the nucleic acid precipitate collected by centrifugation after standing for at least 2 hr at –20°. The pellet is washed twice in cold ethanol and dissolved in 0.025 M tris–0.025 M NaCl–0.005 M $MgCl_2$, pH 8.1, and DNAase I (Worthington, RNAase free) added to 100 μg/ml and kept at 0° for 20 min. The solution is deproteinised with methoxyethanol and RNA quantitatively precipitated with cetyltrimethylammonium bromide exactly as described by Bellamy and Ralph [10]. The product, which accounts for 75% of the RNA in the original material, has $\lambda_{max}$ = 259 nm, and $A_{260}$ nm/$A_{235}$ nm ≥2.0 and $A_{260}$ nm/$A_{280}$ nm ≥ 2.2, and may be stored in the medium of choice to which a drop of diethyl pyrocarbonate has been added, at –70°. The sedimentation and polyacrylamide gel profile of the RNA is completely unchanged after nine months storage in this way. This quick and gentle extraction procedure may be used to extract RNA from other subcellular fractions, especially ribosomes, and protoplasts.

For this work, yeast mitochondrial RNA was ex-

tracted from sorbitol gradient purified mitochondria [11] prepared from the haploid grande strain L410 of *Saccharomyces cerevisiae* grown with ethanol as carbon source. Ethanol grown cells are completely derepressed with regard to the formation of mitochondria, a condition which Forrester and Linanne [5] have recently shown to be necessary for minimal contamination of mitochondria by cytoplasmic ribosomes, only trace amounts of cytoplasmic rRNA being detectable in the isolated mitochondrial RNA. RNA was also prepared from cytoplasmic ribosomes of the same yeast strain, and from cytoplasmic ribosomes of rat liver and *Escherichia coli*. The RNA was examined on preparative sucrose gradients and on polyacrylamide gels: in both cases the relative amounts of the larger and smaller rRNA species were in the ratio of 2:1 indicating the absence of any appreciable fragmentation of RNA during extraction.

Table 1
Sedimentation coefficients of various rRNA species in different buffers.

| Species of rRNA | Sedimentation coefficient | | | |
|---|---|---|---|---|
| | E buffer | | Mg buffer | |
| | I | II | I | II |
| Yeast mitochondria | 21.0 | 15.0 | 25.5 | 18.0 |
| Yeast cytoplasmic | 25.0 | 17.5 | 30.5 | 17.5 |
| Rat Liver cytoplasmic | 29.5 | 18.0 | 36.0 | 20.5 |
| *E. coli* | 23.0 | 16.0 | 27.5 | 19.5 |

Sedimentation velocity analyses were carried out in a 2-place rotor in a Spinco model E analytical ultracentrifuge as described [12] at 25° and sedimentation coefficients are uncorrected and rounded off to nearest 0.5 unit. E buffer (tris 40 mM, sodium acetate 20 mM, EDTA 2 mM, pH 7.8) [14] and Mg buffer (tris 30 mM, HCl 16 mM, EDTA 0.1 mM, Mg acetate 2 mM, pH 8.1) [12] are the buffers used for gel electrophoresis.

The sedimentation coefficients of the RNA species as determined in the analytical ultracentrifuge in a buffer lacking magnesium are shown in table 1 (E buffer). The yeast mitochondrial rRNA's appear the smallest in their respective groups. Loening [12] has pointed out that RNA molecules tend to assume a more compact configuration in the presence of magnesium, leading to an increased sedimentation coefficient and increased mobility in polyacrylamide gels. This effect on sedimentation rate is confirmed for all rRNA species studied here (except for the yeast 17 S cytoplasmic rRNA component) as shown in table 1 (Mg buffer); since the rRNA species (except the yeast cytoplasmic 17 S component) all show the same relative increase in sedimentation it would follow that they all undergo similar changes in their overall shape when the magnesium level is adjusted.

The pattern that emerges from polyacrylamide gel electrophoresis studies (fig. 1) is consistent with the sedimentation studies for all the RNA species with the remarkable exception of yeast mitochondrial rRNA. In a standard electrophoresis buffer lacking magnesium, E buffer, the mitochondrial rRNA's now appear to be largest in their respective groups, but where magnesium is present they run almost concurrently with rat liver cytoplasmic rRNA. There are two anomolous features. In neither buffer is the mobility of the mitochondrial rRNA consistent with its sedimentation properties, and secondly, the extent of the change in mobility of mitochondrial rRNA in gels in

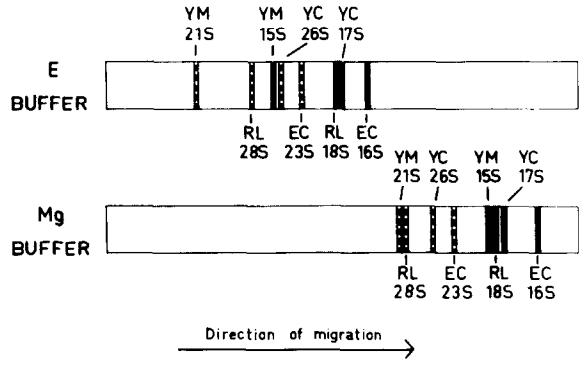


Fig. 1. Diagrammatic representation of results of polyacrylamide gel electrophoresis of various rRNA species in different buffers. RNA samples (30 μg) were layered on 2.4% acrylamide gels [14] in either E buffer or Mg buffer and run for 4 hours at room temperature. Gels were either scanned at 280 nm on a Gilford recording spectrophotometer fitted with a linear transport device, or stained with azure blue to visualise the bands. YM: yeast mitochondrial; RL: rat liver cytoplasmic; YC: yeast cytoplasmic; EC: *E. coli*. Bands are also marked with their designated S values, with the larger rRNA species cross hatched and smaller rRNA species drawn solid. Buffers are detailed in table 1.

the different buffers is far greater than the changes in sedimentation coefficients, in contrast to the behaviour of *Drosophila* rRNA, where changes in gel mobility and sedimentation in the different media are well correlated [12]. Thus yeast mitochondrial rRNA must assume conformations different from those of the other types of RNA studied here. It also appears that in E buffer, at least, the effective negative changes of yeast mitochondrial rRNA is reduced as a result of the conformation assumed since the sedimentation studies show that no drastic changes in shape have occurred. Consequently the affinity of the molecules for the positive electrode is diminished. It is to be noted in this connection that the gel mobilities of the rRNA species studied here are no different when the revised E buffer [12] (containing phosphate in place of acetate) is used – comparisons between our and other results are thus validated.

It is thus not possible to calculate accurate values for the molecular weight of the yeast mitochondrial rRNA components from their behaviour in centrifugal and electric fields. Such values may only be derived from the use of independent means, such as light scattering measurements. It is clear that reliance on the results of sedimentation or gel electrophoretic studies for determination of the molecular weights of RNA species may well lead to serious error.

The biological significance of the unusual conformation of yeast mitochondrial rRNa's may well be related to their role as components of ribosomes which this laboratory has recently suggested [13] form an integral part of mitochondrial membrane structure. The cytoplasmically inherited mutants of yeast which were isolated showed properties which could be explained on the basis of changed *membrane* proteins seriously affecting the characteristics of the mitochondrial protein synthesizing system, viz. the mitochondrial ribosomes.

The extraction procedure reported here and the anomalous mobility of yeast mitochondrial rRNA in polyacrylamide gels yielding a very good separation of mitochondrial from cytoplasmic rRNA, have been used to study the RNA present in yeast mitochondria under a variety of growth conditions; these results are reported elsewhere [5].

## References

[1] P.J.Rogers, B.N.Preston, E.B.Titchener and A.W.Linnane, Biochem. Biophys. Res. Commun. 27 (1967) 405.
[2] S.A.Leon and H.R.Mahler, Arch. Biochem. Biophys. 126 (1968) 305.
[3] W.Wintersberger and G.Viehhauser, Nature 220 (1968) 699.
[4] M.Fauman, M.Rabinowitz and G.S.Getz, Biochim. Biophys. Acta 182 (1969) 355.
[5] I.T.Forrester and A.W.Linnane, submitted for publication.
[6] H.Morimoto, A.H.Scragg, J.Nekhorocheff, V.Villa and H.O.Halvorson in: Autonomy and Biogenesis of Mitochondria and Chloroplasts, eds. N.K.Boardman, A.W.Linnane and R.M.Smillie (North-Holland, Amsterdam, 1970)
[7] M.Edelman, I.M.Verma and U.Z.Littauer, J. Mol. Biol. 49 (1970) 67.
[8] F.Solomosy, I.Fedorcsák, A.Gulyás, G.L.Farkas and L.Ehrenberg, European J. Biochem. 5 (1968) 520.
[9] B.Wolf, J.A.Lesnaw and M.E.Reichmann, European J. Biochem. 13 (1970) 519.
[10] A.R.Bellamy and R.K.Ralph in: Methods in Enzymology, Vol. 12B, eds. S.P.Colowick and N.O.Kaplan (Academic Press, New York, 1968) p. 156.
[11] K.Watson, J.M.Haslam and A.W.Linnane, J. Cell. Biol. 46 (1970) 88.
[12] U.E.Loening, Biochem. J. 113 (1969) 131.
[13] C.L.Bunn, C.H.Mitchell, H.B.Lukins and A.W.Linnane, Proc. Natl. Acad. Sci. U.S., in press.
[14] U.E.Loening, Biochem. J. 102 (1967) 251.