THE NUMBER OF 4-S RNA GENES ON YEAST MITOCHONDRIAL DNA

L.REIJNDERS AND P.BORST

Department of Medical Enzymology*, Laboratory of Biochemistry, University of Amsterdam, Amsterdam (The Netherlands)

Received March 1, 1972

SUMMARY

Hybridization of mitochondrial 4-S RNA from Saccharomyces carlsbergensis with mtDNA in the presence of excess unlabelled high-molecular-weight mtRNA gave a plateau of 0.9 μg RNA hybridized per 100 μg DNA. This indicates that yeast mtDNA contains at least 20 genes for tRNA.

INTRODUCTION

Mitochondria contain tRNA species that differ from the corresponding cell-sap tRNAs in their behaviour in various chromatographic systems[1-5], their acylating properties[6], the presence of a fMet-tRNA[7-10] and the absence of a fluorescent base[11]. Both in rat liver[12] and in yeast[13], some of the mitochondrial tRNAs have been shown to hybridize specifically with mtDNA, by utilizing aminoacyl-tRNAs labelled in the amino acid moiety. The number of tRNA genes present on mtDNA in animal tissues has been determined by hybridization of 4-S RNA in the presence of excess cold mitochondrial high-molecular-weight RNA. The hybridization

---

* Postal address: Jan Swammerdam Institute, Eerste Constantijn Huygensstraat 20, Amsterdam (The Netherlands).

Abbreviations: SDS, sodium dodecylsulfate; 1 x SSC, 0.15 M NaCl-0.015 M sodium citrate (pH 7.0).

plateaus obtained indicate that only 11 tRNA cistrons are present on HeLa cell mtDNA[14] and 15 on Xenopus mtDNA[15,16]. The questions raised by this intriguing result have been discussed in detail elsewhere[17].

Although the mitochondrial tRNAs of Neurospora have been studied in detail[1-4], no attempt has yet been made to determine the number of tRNA genes on mtDNA in this organism or in the related ascomycete yeast. The results in this paper fill this gap for yeast and show that in this case mtDNA can in principle code for a full set of tRNAs.

METHODS

Preparation of mtDNA and total mtRNA from S. carlsbergensis NCYC 74

[2,4-^{3}H]Adenine-labelled mtDNA was obtained from purified mitochondria[18], incubated with 100 µg pancreatic deoxyribonuclease (Sigma) per ml at 0°. The DNA was extracted with phenol: cresol from a detergent-lysate[18] and purified by chromatography on a column of methylated albumin on Kieselguhr[19]. Remaining nuclear fragments were removed by selecting DNA sedimenting faster than 21 S on sucrose gradients.

Total mtRNA was prepared as described by Grivell et al.[18].

Hybridization[20]

Filters (0.1 µ pore size, Sartorius Membranfilter GmbH) were pre-soaked in 2 x SSC-0.1% SDS before loading with DNA. DNA was denatured at pH 13, neutralized and brought onto the filter in a concentration of 0.1 µg/ml at 5°. Three filters were always incubated together in 4 ml 3 x SSC-0.1% SDS; one containing ^{3}H-labelled mtDNA, one containing a corresponding amount of

Escherichia coli DNA and one without DNA. The incubation was carried out in a shaking water bath for 20 h at 63°. The filters recovered from the vessels (containing crude hybrids) were treated as follows: a wash at 63° for 15 min in 3 x SSC (5 ml), a wash at 25° for 15 min in 5 ml 3 x SSC, an incubation in 4 ml 2 x SSC, containing 10 μg ribonuclease A (Worthington) and 25 units T_1 ribonuclease (Worthington) (pre-heated for 10 min at 80°) per ml for 2 h at 10°, a wash at 25° for 15 min in 5 ml 3 x SSC-0.1% SDS, a wash at 63° for 15 min in 5 ml 3 x SSC-0.1% SDS, and a wash at 25° for 15 min in 5 ml 3 x SSC. The filters were dried and counted in a liquid scintillation counter. Values for hybridization with mtDNA are corrected for hybridization with E. coli DNA. Blank filters (containing no DNA) did not bind more than 0.01% of the input ^{32}P radioactivity.

The concentration of DNA was determined by the method of Burton[20] using calf-thymus DNA as standard.

The concentration of RNA was determined spectrophotometrically assuming that 1 mg RNA has an absorbance at 260 nm of 24/cm.

Specific activities of RNA and DNA samples used in hybridization were determined by counting a known amount of RNA or DNA spotted and dried on the membrane filters used for hybridization experiments.

RESULTS

Yeast mtDNA was obtained from purified mitochondria, incubated with deoxyribonuclease to degrade the contaminating nuclear DNA. Remaining nuclear DNA fragments were removed by selecting DNA sedimenting faster than 21 S on sucrose gradients. The DNA preparation used contained no detectable nuclear DNA contamination in analytical CsCl gradients. This procedure avoids the

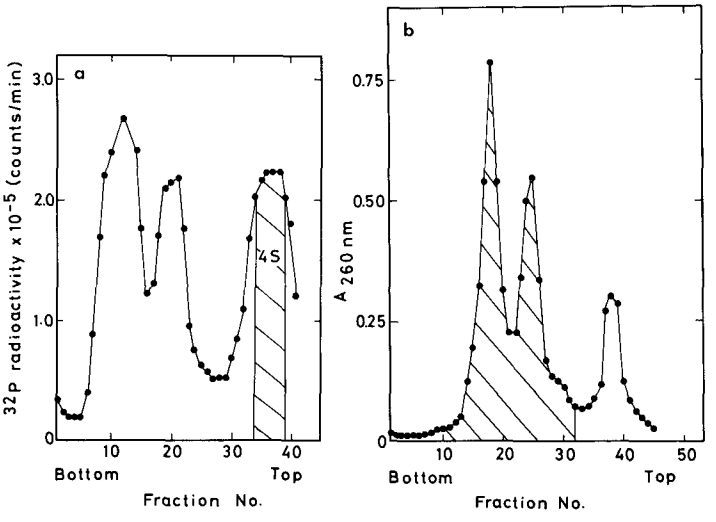

Fig. 1a. Preparation of ^{32}P-labelled 4-S mtRNA. - Total ^{32}P-labelled mtRNA (specific activity about 11 000 counts/min per µg) was spun for 14 h at 33 000 rev./min in the Spinco SW-41 rotor into an isokinetic 5-25% sucrose gradient, containing 100 mM NaCl, 10 mM Tris-HCl (pH 7.6). Fractions were collected and monitored for ^{32}P radioactivity by Cerenkov counting. The cross-hatched region indicates the fractions pooled to obtain 4-S RNA. Before hybridization the pooled fractions were dialysed against 3 x SSC.

Fig. 1b. Preparation of high-molecular-weight RNA. - Total mtRNA was spun for 13 h under the same conditions as described in the legend to Fig. 1a. The cross-hatched region indicates the fractions pooled to obtain high-molecular-weight RNA. Before hybridization the pooled fractions were dialysed against 3 x SSC.

---

usual preparative CsCl density gradients, which could lead to loss or enrichment of tRNA genes.

^{32}P-labelled yeast mtRNA was fractionated by sucrose gradient centrifugation (Fig. 1a) and the 4-S fraction was used for hybridization with yeast mtDNA. In initial experiments hybridization continued to increase with increasing RNA input and even when 9% of the DNA was converted into hybrid no plateau was reached (not shown). The experiments were, therefore, repeated in the presence of excess cold high-molecular-weight RNA (Fig. 1b) to compete out the contribution of fragments of mRNA and

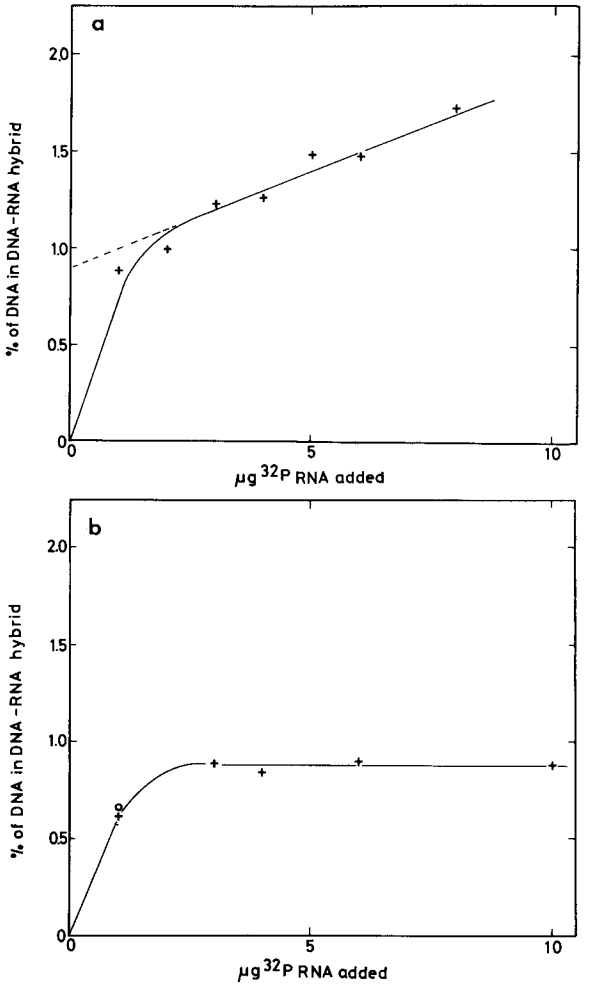

Fig. 2. Hybridization of 4-S mtRNA with mtDNA. - Increasing amounts of 4-S RNA were hybridised to filters containing 0.6 μg mtDNA, as described in METHODS. a) In the presence of a 20-fold excess of high-molecular-weight RNA (+ — +). b) In the presence of a 200-fold (+ — +) or 400-fold (0) excess of high-molecular-weight RNA. The specific activity of the 4-S RNA was 9050 (Fig. 2a) and 7060 (Fig. 2b) counts/min per μg, respectively. The specific activity of the mtDNA was 820 counts/min per μg.

rRNA to the hybridization of the 4-S RNA. At 20-fold excess competition was incomplete (Fig. 2a), but at a 200-fold excess the hybridization level became independent of RNA concentration above an input of 3 μg RNA (Fig. 2b). The plateau in Fig. 2b is at 0.89% and by extrapolating the level in Fig. 2a back to zero

RNA concentration a level of 0.92% is obtained. Since two independent high-molecular-weight RNA preparations were used in these two experiments the agreement is satisfactory.

DISCUSSION

Our experiments show that mitochondrial 4-S RNA in yeast hybridizes to an extent of 0.9% with yeast mtDNA, if the hybridization of contaminating fragments of high-molecular-weight RNA is suppressed by excess cold RNA. The translation of this hybridization plateau into the number of tRNA genes is complicated by a number of factors that require brief discussion:

1. Yeast mtDNA consists of twisted circles with a measured contour length of 25 μm (ref. 21). Since the length of twisted circles tends to be underestimated we have previously concluded that 55×10^6 is a more realistic value for its molecular weight than the 49×10^6 calculated on the basis of a mass/length ratio of 1.96×10^6 per μm. (For other uncertainties in the molecular weight of yeast mtDNA see ref. 17).

2. In addition to tRNA, mitochondrial 4-S RNA could contain other small RNAs, not derived from the high-molecular-weight RNA added as competitor. The mitochondrial equivalent of ribosomal 5-S RNA has not been found in yeast (see ref. 22) or Neurospora (ref. 23), but the possibility that it is hidden in the 4-S RNA peak has not been rigorously excluded. However, one gene for such an RNA would contribute less than 0.05% to the hybridization plateau observed.

3. If the competing high-molecular-weight RNA contained tRNA sequences, this could result in an underestimation of the number of tRNA genes. The fact that the extrapolated hybridization levels in Figs. 2a and 2b are identical makes this unlikely

and suggests that any high-molecular-weight tRNA precursor, if existing at all, is present in a very low concentration.

On the basis of these considerations and with the reservations mentioned, we conclude that the hybridization plateau found corresponds to about $0.009 \times 55 \times 10^6 \times 25\ 000^{-1} \approx 20$ tRNA genes. The plateau previously obtained for rRNA was 2.4%, rather than the 3.1% expected if one gene for each of the two rRNAs is present on mtDNA[24]. If this is due to an underestimation of the molecular weight of yeast mtDNA, the number of tRNA genes would rise to $20 \times 3.1/2.4 = 26$. We interpret these results to mean that yeast mtDNA, in contrast to animal mtDNA, contains genes for a full set of tRNAs for 20 amino acids. In addition, our results are compatible with the idea that yeast mitochondria do not contain several tRNAs for one amino acid, as found in prokaryotes or eukaryotic cell-sap. We cannot exclude, however, that additional tRNAs, specified by nuclear genes, are imported into the mitochondria.

ACKNOWLEDGEMENTS

This work was in part supported by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organisation for the Advancement of Pure Research (Z.W.O.).

REFERENCES

1. Epler, J.L., Biochemistry, 8, 2285 (1969).
2. Barnett, W.E., Brown, D.H. and Epler, J.L., in Teas, H.J., Genetics and Developmental Biology, 1969, University of Kentucky Press, p. 100.
3. Epler, J.L., Shugart, L.R. and Barnett, W.E., Biochemistry, 9, 3575 (1970).
4. Epler, J.L. and Barnett, W.E., Biochem.Biophys.Res.Commun., 28, 328 (1967).
5. Buck, C.A. and Nass, M.M.K., J.Mol.Biol., 41, 67 (1969).
6. Ashwell, M. and Work, T.S., Ann.Rev.Biochem., 39, 251 (1970).
7. Galper, I.B. and Darnell, J.E., Biochem.Biophys.Res.Commun., 39, 205 (1969).
8. Smith, A.E. and Marcker, K.A., J.Mol.Biol., 38, 241 (1968).

9. Halbreich, A. and Rabinowitz, M., Proc.Natl.Acad.Sci.U.S., 68, 294 (1971).
10. Bianchetti, R., Lucchini, G. and Sartirana, M.L., Biochem. Biophys.Res.Commun., 42, 97 (1971).
11. Fairfield, S.A. and Barnett, W.E., Proc.Natl.Acad.Sci.U.S., 68, 2972 (1971).
12. Nass, M.M.K. and Buck, C.A., J.Mol.Biol., 54, 187 (1970).
13. Casey, J., Cohen, M., Rabinowitz, M., Fukuhara, H. and Getz, G.S., J.Mol.Biol., in press.
14. Aloni, Y. and Attardi, G., J.Mol.Biol., 55, 271 (1971).
15. Dawid, I.B., J.Mol.Biol., 63, 201 (1972).
16. Dawid, I.B., in Miller, P.L., Control of Organelle Development, 1970, Cambridge: Cambridge University Press, p. 227.
17. Borst, P., Ann.Rev.Biochem., in press.
18. Grivell, L.A., Reijnders, L. and Borst, P., Europ.J.Biochem., 19, 64 (1971).
19. Cohen, L.H., Hollenberg, C.P. and Borst, P., Biochim.Biophys. Acta, 224, 610 (1970).
20. Burton, K., Biochem.J., 62, 315 (1956).
21. Hollenberg, C.P., Borst, P. and Van Bruggen, E.F.J., Biochim Biophys.Acta, 209, 1 (1970).
22. Grivell, L.A., Reijnders, L. and Borst, P., Biochim.Biophys. Acta, 247, 91 (1971).
23. Lizardi, P.M. and Luck, D.J.L., Nature New Biol., 229, 140 (1971).
24. Reijnders, L., Kleisen, C.M., Grivell, L.A. and Borst, P., Biochim.Biophys.Acta, submitted for publication.