DEGREE OF METHYLATION OF MITOCHONDRIAL RIBOSOMAL RNA

Donald T. DUBIN and Deborah A. FRIEND
Department of Microbiology, Rutgers Medical School, New Brunswick, N.J. 08903, USA

Received 25 August 1971

1. Introduction

We have reported that the ribosomal RNA of hamster cell mitochondria is very poorly, if at all, methylated. When cells in culture were labeled with Me-^{14}C-methionine and ^{32}P-phosphate, the ^{14}C/^{32}P ratio of an unresolved mixture of the mit 17 S and 13 S rRNA components was approx. 4% that of cyt tRNA, or approx. 0.3 putative methyl groups per 100 nucleotides [1]; it was unclear whether this low level actually represented methylation. In the present work the degree of methylation of hamster mit rRNA was studied in greater detail. The 13 S and 17 S components were examined separately, and their apparent degrees of methylation were compared with those of other methyl-poor RNA fractions. The value for 17 S RNA was found to resemble that of cyt 5 S RNA, and that for 13 S RNA was only very slightly higher. Even if these mit rRNA values are *not* corrected for the presumed background of incorporation from methyl-labeled methionine into rings, they remain considerably lower than values reported from HeLa mit rRNA [2], or in fact for any other discrete high molecular weight cellular RNA species.

2. Methods

Cells were grown as previously described [1], except that uridine (10^{-5} M) as well as purine nucleosides were added to the medium to minimize leakage of radioactivity from methionine into the carbon skeletons of nucleic acid bases. Cells were labeled in medium with lowered levels of methionine and phosphate [1], and in the presence of 0.1 μg/ml of actinomycin to preferentially suppress incorporation into cyt rRNA [3]. Mit and cyt RNA were prepared by Procedure C of [1], and RNA was fractionated and assayed as indicated in the legends to figs. 1 and 2.

Abbreviations:
mit : mitochondrial
cyt : cytoplasmic
SDS: sodium dodecyl sulfate

3. Results

Fig. 1 shows the density gradient pattern obtained by centrifuging a mit RNA sample so as to achieve good separation of the 17 S and 13 S peaks. There was, as expected, a substantial ^{14}C-methyl-labeled peak associated with the 4 S fraction, and in addition small quantities of ^{14}C sedimented further down the tube. In some experiments this ^{14}C appeared to contain small peaks corresponding at least roughly to mit rRNA peaks (cf. [1]), while in some, as here, it appeared rather heterogeneous. The values for apparent degree of methylation were uniformly low, however (table 1 and [1]). The ^{14}C peak at 18 S may represent a small amount of label in contaminating cyt 18 S RNA, perhaps due to methylation at a late stage of maturation [4].

In this experiment the cyt 4 S RNA fraction was separated into 5 S and transfer RNA by acrylamide gel electrophoresis (fig. 2). As we had observed for such preparations [1], approx. 6% of the ^{32}P was in 5 S RNA. In addition, small amounts of ^{14}C traveled in the 5 S region of the gel.

The ^{14}C/^{32}P ratios of the various cyt and mit RNA fractions are summarized in table 1, columns 1 and 2, and are expressed relative to cyt tRNA in column 3.

North-Holland Publishing Company – Amsterdam

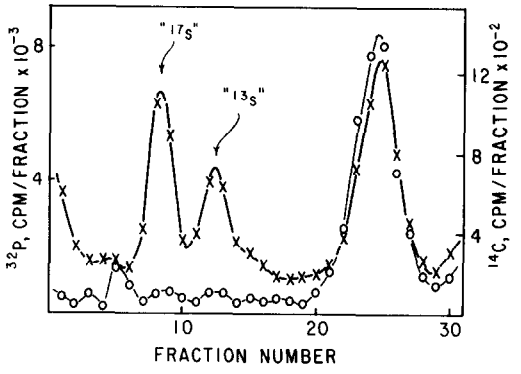

Fig. 1. Zonal sedimentation pattern of ^{14}C-methyl-, ^{32}P-labeled mitochondrial RNA. Cells were labeled with ^{32}P-phosphate, 6 μCi/1.2 μmoles/ml, and Me-^{14}C-methionine, 1 μCi/3 μg/ml, for 21 hr in the presence of actinomycin D, 0.1 μg/ml. Mit and cyt RNA were purified as described in Methods, and were fractionated by centrifugation through a sucrose gradient (5–20% sucrose in 0.5% SDS, 0.005 M Tris HCl, pH 7.4; Spinco SW 41 rotor, 27,000 rpm for 17 hr at 23°). Samples of each fraction were assayed for ^{32}P and ^{14}C by a combination of differential screening and counting at separated time intervals [1]. Radioactivity values here and throughout this paper have been corrected for ^{32}P decay and for differing efficiencies between gradients and gels. ^{32}P. (x—x); ^{14}C, (o—o).

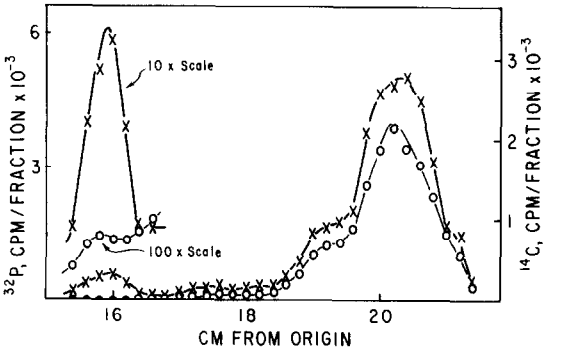

Fig. 2. Acrylamide gel electrophoresis of ^{14}C-methyl, ^{32}P-labeled cytoplasmic 4 S RNA. The cyt RNA from the culture of fig. 1 was electrophoresed in 10%, 0.6 × 30 cm gel, at 7 mamp for 20 hr in buffer containing 0.5% SDS [4]. Fractions corresponding to 2 mm segments were collected using a Savant fractionator, were dried on planchettes, and were counted differentially for ^{32}P and ^{14}C. ^{32}P, (x—x); ^{14}C, (o—o).

Table 1
Apparent degree of methylation of hamster mitochondrial and cytoplasmic RNA.

	^{14}C:^{32}P ratio		^{14}C:^{32}P, % of tRNA	[Me] per 100 nucleotides
	Gradient	Gel		
Cyt				
4 S	0.426		–	
tRNA	0.455*	0.443	(100)	8.3*+
5 S	–	0.015	3.3	0.27
heterodisperse	0.018		4.0	0.33
18 S	–		–	2.0+
28 S	–		–	1.4+
Mit				
4 S	0.191		43.	3.6
13 S	0.026		5.8	0.48
17 S	0.016		3.6	0.30
heterodisperse	0.015		3.4	0.28

Except as noted, the values were obtained from the experiment described in figs. 1 and 2. The mit heterodisperse RNA value was determined using fractions 1–3 of fig. 1, and corresponding fractions were used for the cyt heterodisperse RNA.

* Correcting for 6.5% 5 S RNA in centrifugal 4 S peak. + From ref. [1].

The value for cyt 5 S RNA (which is unmethylated [6]) was 3% of that cyt tRNA. This is somewhat less than the presumed background observed in comparable experiments on HeLa cells [7]. The values for the heterogeneously sedimenting RNA fractions – which are also generally considered to be unmethylated – were not significantly higher, nor was the value for mit 17 S RNA. The value for mit 13 S RNA was regularly, as in this experiment, slightly but significantly higher than those of the other methyl-poor fractions.

4. Discussion

Even if one assumes that all of the ^{14}C in our 17 S and 13 S peaks represents methyl groups, our values for the degree of methylation of the larger and smaller mit rRNA components – 0.3 and 0.48 methyl groups/100 nucleotides – are considerably lower than corresponding values reported for human cells. Attardi and Attardi have recently stated [2] that the "16 S" and "12 S" mit rRNA species of HeLa cells – which in most respects closely resemble the BHK mit rRNA species – were substantially methylated, containing 0.9–1.0 methyl groups per 100 nucleotides. However, their degrees of methylation as estimated from their actual density gradient patterns (fig. 7 of [2]) appear to be approx. 0.4 and 0.5 methyl groups/100 nucleotides for the larger and smaller rRNA components. Vesco and Penman [8] have also studied the methylation of mit RNA in HeLa cells and detected no peaks labeled from Me-^{3}H-methionine corresponding to their rRNA peaks. In Vesco and Penman's patterns, however, the background level of radioactivity from Me-^{3}H-methionine was relatively high, perhaps because their cells were labeled in the absence of actinomycin and there was considerable methyl-label in contaminating cyt rRNA. This makes it difficult to assess accurately the apparent degree of methylation of their methyl-poor species. Thus, it is possible, despite the discrepant statements in the literature (cf. [9]), that the degrees of methylation of HeLa and BKH mit rRNA are not very different.

The precise nature of the ^{14}C in our mit rRNA remains unclear. If the ^{14}C in our 5 S RNA represents leakage into carbon skeletons, and this leakage equally affects mit RNA, then we can say that mit 17 S RNA contains less than 0.1 methyl groups per 100 nucleotides (or <2 per molecule), and 13 S contains less than 0.3 per 100 nucleotides (or <4 per molecule). However, if the ^{14}C in the 5 S region represents contaminating methylated species (such as, say, aggregates of tRNA or tRNA precursors), then it is possible that most or all of the ^{14}C in the 13 S and 17 S peaks does represent methylation; the corresponding values would then be approx. 6 methyl groups per molecule for both components. In either case, the levels are substantially less than any previously documented for a discrete high molecular weight cellular (as opposed to viral) RNA species. Experiments in progress are aimed at accumulating sufficient radioactivity in the methyl-poor species for more detailed studies, such as methylated base analysis.

Acknowledgements

This work was supported by Grant GM 14957 of the National Institute of General Medical Sciences, U.S. Public Health Service.

References

[1] D.T. Dubin and B.S. Montenecourt, J. Mol. Biol. 48 (1970) 279.
[2] B. Attardi and G. Attardi, J. Mol. Biol. 55 (1971) 231.
[3] D.T. Dubin, Biochem. Biophys. Res. Commun. 29 (1967) 655.
[4] E.F. Zimmerman, Biochemistry 7 (1968) 3156.
[5] U.E. Loening, Biochem. J. 102 (1967) 251.
[6] B.G. Forget and S.M. Weissman, Science 158 (1968) 1695.
[7] R.A. Weinberg and S. Penman, J. Mol. Biol. 38 (1968) 289.
[8] C. Vesco and S. Penman, Proc. Natl. Acad. Sci. U.S. 62 (1969) 218.
[9] P. Borst and L.A. Grivell, FEBS Letters 13 (1971) 73.