THE RIBOSOMAL SUBUNITS OF HAMSTER CELL MITOCHONDRIA

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Summary. The "13s" and "17s" RNA's previously shown to be specifically associated with the mitochondrial fraction of cultured hamster cells have been shown to occur as components of submitochondrial particles, presumably mitochondrial ribosomal subunits, sedimenting at approximately 25s and 33s, respectively. Reconstruction experiments indicate that these particles are not artifacts of the isolation procedure.

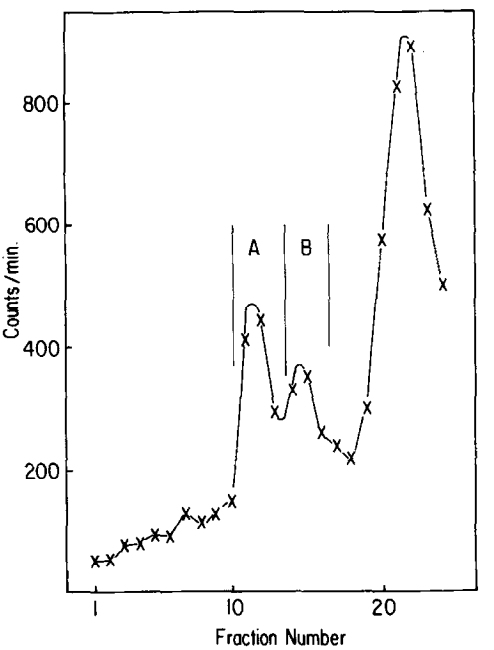
We have described two discrete RNA species, sedimenting at about 13s and 17s, specifically associated with the mitochondrial fraction of cultured hamster cells. These RNA's were present in approximately equimolar amounts, and we proposed that they were the structural RNA's of mitochondrion-specific ribosomes. However, they appeared unusually small for ribosomal RNA's, and were unusually low in methylated nucleotides and in GC content (1,2,3). Furthermore, similar RNA species observed in HeLa cell mitochondria did not appear to be present in equimolar amounts, and were thought not to be ribosomal (4).

The aim of the present studies was to examine this question further; the results indicate that the 13s and 17s RNA's of hamster mitochondria are indeed components of ribosomal subunits.

METHODS

Cultured hamster (BHK-21) cells were labeled with ^{14}C-uridine for 18-22 hours, as previously described (2). Actinomycin D, 0.1 µg/ml, was routinely added to preferentially suppress incorporation into cytoplasmic ribosomes (5) and unlabeled deoxycytidine and thymidine were added to prevent labeling of DNA.

To minimize opportunity for ribonuclease action during isolation of mitochondria, an abbreviated version of an earlier procedure ("C" of ref.2) was used. Cells were harvested and disrupted as before, but the crude 10,000 xg pellet was used directly as the source of submitochondrial particles. (Such a pellet, when dissolved in 1% SDS, was found to yield 13s and 17s RNA patterns similar to those obtained using more rigorously purified mitochondria.) In addition, it was found necessary to add polyvinyl sulfate to all solutions used in processing submitochondrial particles (cf. ref. 6). Further details are described in the legends to the figures and in the text.

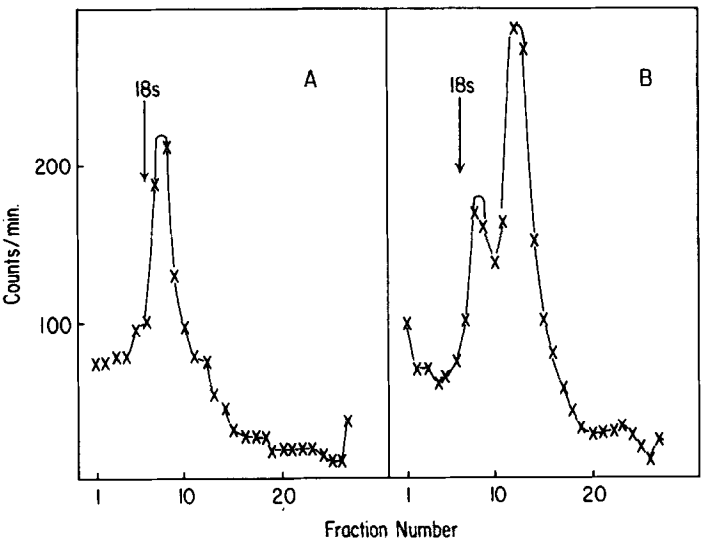


Legend to Figure 1. Cells were labeled with ^{14}C-uridine (0.2 μc/0.01 μmole /ml, 18 hrs.) and a crude mitochondrial pellet was obtained, as described in Methods. This pellet was treated at 5° with 2% Triton X-100 in buffer containing 0.5 NaCl, 0.01 M Tris, pH 7.4, 0.0015 M $MgCl_2$ and 20 μg/ml polyvinylsulfate. The preparation was then layered onto a gradient of 5-20% sucrose in the same buffer, and centrifuged for 90 min. at 56,000 rev/min. at 5° in a Spinco SW56 rotor. Samples were obtained and assayed as previously described (2).

RESULTS AND DISCUSSION

Figure 1 shows the pattern obtained when a ^{14}C-uridine-labeled mitochondrial pellet was disrupted with 2% Triton X-100, and sedimented through a sucrose density gradient, both in the presence of 0.5N NaCl. About 30% of the labeled RNA sedimented as two fairly discrete peaks, whose estimated sedimentation constants were 25s and 33s (range, 24-26s and 32-34s). For markers, either 28s RNA in "acetate-NaCl" buffer (7) or 50s cytoplasmic ribosomal subunits in 0.02 M EDTA (8), were run in parallel tubes.

The yield of labeled RNA in these particles approximated the yield of 13s and 17s RNA obtained when mitochondrial pellets were lysed in 1% SDS. Similar particles were obtained when mitochondria were disrupted and sedimented in buffer containing a low concentration (0.01 N) of NaCl. However, under these conditions the yield of particles was greatly



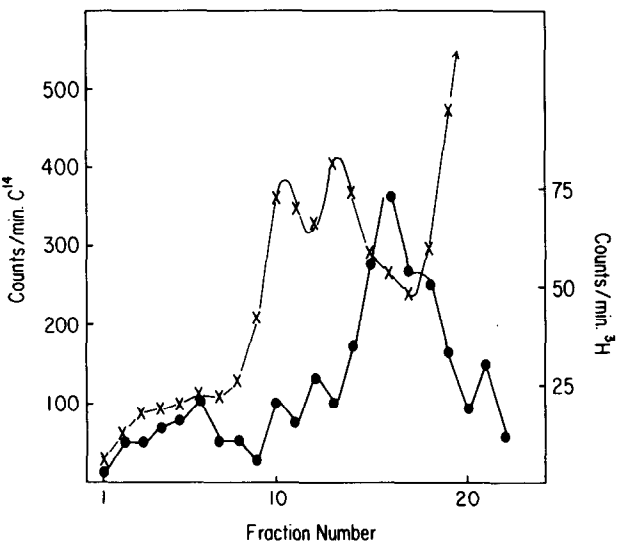
Legend to Figure 2. The fractions within the vertical lines of Figure 1 were pooled, and precipitated with 2 volumes of ethanol. The resulting pellets were dissolved in buffer containing 0.5% SDS, 0.01 N NaCl, 0.01 M Tris, pH 7.4 and centrifuged through a 5-20% sucrose gradient containing the same 0.5% SDS buffer at 27,000 rev/min. for 17 hours in an SW41 rotor at 23°C. The position of the 18s RNA marker is indicated by the arrow.

Abbreviation: SDS, sodium dodecylsulfate

diminished, with increased amounts of RNA appearing in the pellet after density gradient centrifugation.

RNA extracted from fractions corresponding to the 25s and 33s peaks yielded density gradient patterns such as that shown in Fig. 2. The 25s particle yielded largely 13s, and the 33s particle 17s, RNA.

It was possible that the particles were not native structures, but rather resulted from artifactual association between free RNA and protein (cf. ref. 8). To test this possibility, trace amounts of ^{3}H-uridine-labeled 13s and 17s RNA were added to ^{14}C-uridine-labeled mitochondrial pellets, either just prior to or just after addition of Triton-X, and using either low or high salt conditions. A representative "high salt" experiment is shown in Fig. 3. The added RNA did sediment somewhat faster under these, than under standard (ref. 7) conditions, perhaps because of some aggregation of the RNA (cf. ref. 9) or limited association with protein. However, in no case did a significant proportion of the added RNA



Legend to Figure 3. Cells were labeled with ^{14}C-uridine, and a crude mitochondrial pellet was obtained and treated with Triton-X, as for Figure 1. Immediately after addition of the Triton-X, trace amounts of purified 17s plus 13s RNA, labeled with ^{3}H-uridine, were added and the mixture was fractionated as described for Fig. 1, except that centrifugation was for 100 min. ^{14}C, (x-x); ^{3}H, (-•-•-).

sediment with the 25s or 33s particles. The pattern of Fig. 3 also illustrates another property of our system. Namely, the relative amounts of 25s and 33s particles varied somewhat from one experiment to another. Although generally the 33s particle was the more heavily labeled (Fig. 1), occasionally this situation was reversed. The reason for this variability is not known.

There are several other reports of distinctive ribosome-like particles in animal mitochondria. Obrien and Kalf (10) and Ashwell and Work (11) have described a particle in liver mitochondria that sedimented at about 55s and that was preferentially labeled when isolated mitochondria were "pulsed" with radioactive leucine. If, as was suggested, this particle represents the mitochondrial ribosomal monomer, its low sedimentation constant would be in accord with the unusually low values of our putative ribosomal subunits. Swanson and Dawid (12) have recently presented evidence that in Xenopis the mitochondrial ribosomal monomer and subunits sediment at 60s, and 43s and 32s, respectively--values also in reasonable agreement with our results. On the other hand, Perlman and Penman (13) have described a "protein-synthesizing structure" in HeLa mitochondria that sedimented at about 95s, as well as "45s" particles that contained the bulk of their mitochondrion-specific high-molecular-weight RNA; the relationship between these particles and ours is at present not clear.

In summary, we have shown that the 13s and 17s RNA's of hamster mitochondria are intimately associated with particles, sedimenting at approximately 25s and 33s respectively, that appear to be analogous to ribosomal subunits. Together with other recent observations, these findings suggest that, like the mitochondrial ribosomal RNA (3), the ribosomal particles of animal cell mitochondria have unusually low sedimentation values, even compared to ribosomal particles from mitochondria of lower organisms (14-19). A more detailed physicochemical characterization of the 25s and 33s particles is in progress.

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REFERENCES

1. Dubin, D. T. and Brown, R.E., Biochem. Biophys. Acta 145:538 (1967).
2. Dubin, D. T. and Montenecourt, B. S., J. Mol. Biol. 48:279 (1970).
3. Montenecourt, B. S., Langsam, M., and Dubin, D. T., J. Cell Biol. 46:241 (1970).
4. Vesco, C. and Penman, S., Proc. Natl. Acad. Sci., Wash. 62:218 (1969).
5. Dubin, D. T., Biochem. Biophys. Res. Commun. 29:655 (1967).
6. Rifkin, M. R., Wood, D. D. and Luck, D. J. L., Proc. Natl. Acad. Sci., Wash. 58:1025 (1967).
7. Dubin, D. T., J. Mol. Biol. 8:749 (1964).
8. Girard, M. and Baltimore, D., Proc. Natl. Acad. Sci., Wash. 56:999 (1966).
9. Vaughan, M. H., Warner, J. R., and Darnell, J. E., J. Mol. Biol. 25:235 (1967).
10. Obrien, T. W. and Kalf, G. F., J. Biol. Chem. 242:2180 (1970).
11. Ashwell, M. A. and Work, T. S., Biochem. Biophys. Res. Commun. 39:204 (1970).
12. Swanson, R. F. and Dawid, I. B., Proc. Natl. Acad. Sci., Wash. 66:117 (1970).
13. Perlman, S., and Penman, S., Nature 227:133 (1970).
14. Küntzel, H. and Noll, H., Nature 215:1340 (1967).
15. Küntzel, H., J. Mol. Biol. 40:315 (1967).
16. Vignais, P. V., Huet, J., and André, J., FEBS Letters 3:177 (1969).
17. Schmitt, H., FEBS Letters 4:234 (1969).
18. Stegeman, W. J., Cooper, C. S. and Avers, C. J., Biochem. Biophys. Res. Commun. 39:69 (1970).
19. Edelman, M., Verma, I. M. and Littauer, U. Z., J. Mol. Biol. 49:67 (1970).