A NOVEL METHOD FOR DEMONSTRATING A NUCLEAR 30-32S PRECURSOR OF THE RNA OF THE SMALLER RIBOSOMAL SUBUNIT IN AMOEBA PROTEUS

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Received November 18, 1970

SUMMARY

Nuclei containing ^{3}H-RNA were transplanted into unlabeled cytoplasms. The next day the nuclei were separated from the cytoplasms and analyses were made of the ^{3}H-RNA that had left the nucleus and the ^{3}H-RNA that had appeared in the cytoplasm. The sucrose density patterns of these 2 kinds of RNA were not alike and the simplest explanations of the difference appears to be that nuclear 30-32S RNA contains a precursor of cytoplasmic 19S ribosomal RNA.

Evidence from many sources (see refs. 1 and 2 for detailed discussion) indicates that the immediate nuclear precursor of the smaller cytoplasmic ribosomal RNA (rRNA) subunit has a sedimentation constant of 37-39S (and perhaps higher for mammalian cells). There are some reports (cf. 3 and 4) that the 37-39S precursor is sometimes cleaved to yield within the nucleus a 20-23S precursor of the smaller cytoplasmic rRNA subunit. The finding reported here, that the Amoeba proteus nucleus contains a 30-32S precursor of the cytoplasmic 19S RNA (the rRNA of the smaller ribosomal subunit in A. proteus), seems to be a significant exception to previous findings. There is some uncertainty as to whether a 37-39S rRNA precursor is present in A. proteus (6) and, thus, there may be some relationship between the absence of that kind of precursor and the presence of an unusual precursor of the smaller rRNA subunit.

MATERIAL AND METHODS

Details of culture methods, cell labeling, nuclear transplantation, nuclear isolation, RNA extraction, sucrose density centrifugation, and radioactivity assay have been reported elsewhere (7).

Ribosomal subunit particles were prepared by Dounce homogenizing cells in a solution composed of 10^{-3} M $MgCl_2$, 0.1 M KCl, and 0.1 M Tris at pH 7.5 in an ice bath. The homogenate was centrifuged at 10,500xg for 10 min at 0°C. The supernatant was centrifuged in a 60 Ti rotor in a Spinco L2-65B at 60,000 rpm for 145 min at 0°C. The pellet, which contained the ribosomal material, was resuspended in a solution composed of $5x10^{-3}$ EDTA, 0.1 M KCl, and 0.1 M Tris at pH 7.5 (the ribosomes were dissociated to 60S and 40S subunits by the presence of EDTA) and the suspension was Dounce homogenized. The homogenate was centrifuged at 15,000xg for 5 min at 0°C to remove the material that did not go into solution. The supernatant containing the ribosomal subunits was centrifuged in a 15 to 30% w/v sucrose gradient in a SW-27 rotor of a Spinco L2-65B centrifuge for 15 hr at 18,500 rpm at 5°C. At the end of the centrifugation the fractions were collected and assayed as were those of the other gradient analyses (see Fig. 1a legend).

RESULTS

In the experiments reported here rRNA precursor-product relationships were studied in a relatively direct manner. Nuclei containing ^{3}H-RNA (from cells that had reached "steady-state" labeling with ^{3}H-uridine and then were chased) were transplanted into unlabeled cytoplasms and sometime later the pattern of ^{3}H-RNA that was determined to have left the nuclei was compared with the pattern of ^{3}H-RNA that appeared in the cytoplasm.

Figure 1a shows the sucrose density pattern of nuclei just prior to transplantation of ^{3}H-RNA nuclei into unlabeled, enucleate cytoplasms and Fig. 1b shows the pattern of ^{3}H-RNA remaining nuclei after residence in unlabeled cytoplasms for 1 day. Fig. 2a illustrates the sucrose density pattern of the cytoplasmic ^{3}H-RNA after the ^{3}H-RNA nuclei had been resident in the unlabeled cytoplasms for 1 day. Fig. 2b shows the pattern obtained by subtracting (on a cpm/nucleus basis) the pattern of Fig. 1b from the pattern of Fig. 1a. Fig. 2b thus represents the material that had left the nucleus and can be assumed to be, at least in part, the material that is

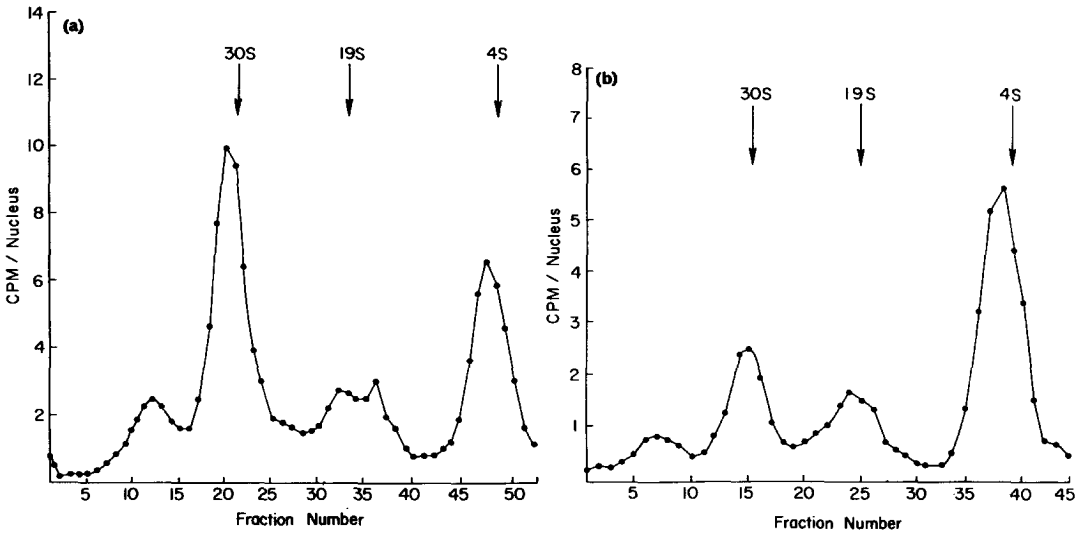

Figure 1a--Sixty (60) nuclei were isolated in a solution of spermidine and Triton X-100 from ^{3}H-RNA cells that had been fed unlabeled food for 1 day. The nuclei were extracted by an adaptation of the method of Fedorcsák et al. (7) and the extracted ^{3}H-RNA, along with added unlabeled carrier RNA extracted from A. proteus cytoplasm, was centrifuged in a 15-30% w/w sucrose gradient at 27,000 rpm in a Spinco SW27 rotor for 22 hr. The fractions were collected through a Gilford recording spectrophotometer, precipated in 10% trichloroacetic acid (TCA) solutions onto type HA Millipore filters, and assayed for radioactivity in a liquid scintillation system.

Figure 1b--Nuclei from the same cell population as those used for experiment shown in Fig. 1a were transferred into unlabeled, enucleate cells which were then fed unlabeled food. For convenience sake rather than isolating the nuclei, the next day the nuclei were transferred to a new group of unlabeled cells which immediately after the operation were placed in the extraction medium. (The enculeate cytoplasms in which the ^{3}H-RNA had been for 1 day also were immediately extracted to determine what ^{3}H-RNA had appeared in the cytoplasm. That part of the experiment is illustrated in Fig. 2a.) The extracted nuclear ^{3}H-RNA was processed as described in the legend for Fig. 1a. The sample contained material extracted from 81 nuclei.

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a precursor of the ^{3}H-RNA that appeared in the cytoplasm. If all of the ^{3}H-RNA that left the nucleus appeared unchanged in the cytoplasm, the pattern in Fig. 2b should be the same as the pattern for cytoplasmic ^{3}H-RNA in Fig. 2a. Clearly, however, the patterns shown in Figs. 2a and 2b are not identical and we must conclude that some change in nuclear RNA must occur before it appears in the cytoplasm. I believe that the simplest explanation that fits these

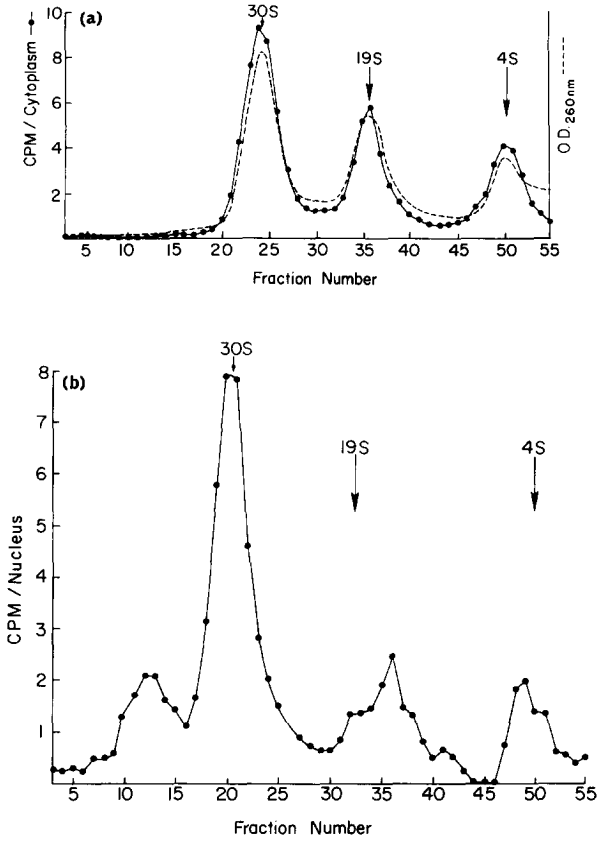

Figure 2a--The cytoplasms into which the ^{3}H-RNA nuclei had been implanted for 1 day were extracted (as described in the Fig. 1 legend) after the nuclei had been removed. The remainder of the processing was as for nuclear RNA. Sample contained 160 cytoplasms.

Figure 2b--After making suitable adjustments for the difference in number of fractions, the difference between the pattern in Fig. 1a and the pattern in Fig. 1b was determined and plotted as shown. This represents ^{3}H-RNA that had left the nucleus during the 1 day residency in unlabeled cytoplasm and is to be compared with what appeared in the cytoplasm, as shown in Fig. 2a.

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data is that there is a conversion of 30-32S nuclear RNA to 19S cytoplasmic rRNA. The 30-32S nuclear RNA thus may contain precursors of both 30S and 19S rRNA. (In A. proteus the larger subunit rRNA is 30S and the smaller 19S (5).)

Since breaks in some of the 30S rRNA may exist, the removal of protein may cause an apparent shift in the proportion of the 30S to 19S peaks in cytoplasmic RNA and thus result in a misinterpretation of these results.

Accordingly the basic experiment was repeated but, instead of extracting cytoplasmic RNA, ribosomal particles were examined. After pelleting the ribosomes as described in the MATERIALS and METHODS section, the ribosomes were resuspended in an EDTA solution to separate the ribosomes into 60S and 40S subunits, which then were centrifuged in a sucrose gradient as described. The analysis of such a preparation showed the ratio of larger to smaller subunit radioactivity increased somewhat but still remained substantially less than would be predicted if 30-32S RNA of the nucleus gave rise only to 30S cytoplasmic RNA.

DISCUSSION

Although there is reason to doubt that the nuclear 39S shown in Figs. 1a and 1b contains rRNA precursors (6), we can make the conservative assumption that it does and that approximately 1/4 of it would become cytoplasmic 19S rRNA (2). (Perry et al. report that in general about 1/2 of the 39S rRNA precursor is converted to 28S rRNA, 1/4 to 18S rRNA, and 1/4 to unknown products.) If that is so, we estimate from Fig. 2b that the ratio of all the 30-32S nuclear RNA to 1/4 of the 39S RNA plus the nuclear 19S RNA is somewhere between 4.5 and 5 to 1--depending on how one measures the area under each curve. (The material in the 16S region is not included.) In measurements of the most extreme cytoplasmic case, which is for the RNAs of the intact ribosomal subunits, we obtain a ratio between 2.3 and 2.9 to 1, 30S to 19S. This discrepancy is the basis for suggesting that the nuclear 30-32S region contains more than precursors of cytoplasmic 30S rRNA. I suggest that this "excess" 30-32S material contains precursors of cytoplasmic 19S rRNA--although it may be that the material turns-over within the nucleus or is converted to something else that does not appear in the cytoplasm.

An alternative explanation is that there is breakdown and reutilization of ^{3}H-precursors that could produce the kinds of patterns reported here, even assuming that a typical maturation from the 39S RNA has occurred. This seems unlikely. Not only were the cells given unlabeled food for 1 day

prior to the onset of the experiment, but ^{3}H-RNA nuclei are transplanted to unlabeled cytoplasm (which made the "new" cell 98% unlabeled) and the chase was continued with unlabeled food. This would seem to be as thorough a chase as it is possible to administer to a eukaryotic nucleus. It must be admitted, however, that it is extremely difficult to determine such things as pool sizes and turnover rates in A. proteus and thus establish the true effectiveness of the chase.

The suggestion that there is a 30-32S nuclear RNA precursor of the cytoplasmic 19S rRNA raises the question as to whether a similar precursor relationship has been missed in other eukaryotic systems. The answer is probably "No"--at least for HeLa cells. Jeanteur and Attardi (8) report that, whereas cytoplasmic 28S rRNA competes with nuclear 32S RNA for hybridization with particular DNA fragments as is expected (1,3), the cytoplasmic 18S rRNA competed not at all with the nuclear 32S RNA. There is, however, evidence from bacteria (cf. 9) that the smaller rRNA subunit derives from a larger precursor molecule, which itself is apparently unrelated to the precursor of the larger rRNA subunit.

There is a suggestion that the A. proteus nucleus does not contain a rRNA precursor larger than 30-32S (6). If true, that condition may be related to the presence of a 30-32S precursor of the cytoplasmic 19S rRNA. It may be that the 2 rRNA subunits of A. proteus do not derive from a common large precursor molecule as is the case for many other cell types.

The data reported here lead to the prediction that the nuclear 30-32S material is composed of more than one kind of RNA molecule and that is the next thing we will attempt to determine.

ACKNOWLEDGEMENTS

This work could not have been completed without the assistance, at one time or another, of Mary Beeson, Ellen Codner and Olive H. Trescott. The project was supported by a United States Public Health Service research grant No. 5 R01 GM 15156.

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