RNA SYNTHESIS IN THE ULTRASTRUCTURAL AND BIOCHEMICAL COMPONENTS OF THE NUCLEOLUS OF CHINESE HAMSTER OVARY CELLS

ANDRE ROYAL and RENE SIMARD

From the Département de Biologie Cellulaire, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec, Canada

ABSTRACT

A correlated autoradiographic and biochemical study of RNA synthesis in the nucleoli of chinese hamster ovary cells has been made. Quantitative analysis of the labeling indicates that the fibrillar ribonucleoprotein (RNP) component is labeled faster than 80S RNP and 45S RNA molecules, but approaches simultaneously a steady-state ^{3}H to ^{14}C ratio or grains/μm^2 after 30 min of [^{3}H]uridine incorporation. On the other hand, the 55S RNP, the 36S + 32S RNA, and the granular RNP components have the same kinetic of labeling with [^{3}H]uridine. These results suggest that the fibrillar and granular RNP components of the nucleolus are the ultrastructural substratum of, respectively, the 80S RNP (45S RNA) and 55S RNP (36S + 32S RNA). The possibility that precursors to 80S RNP exist also in the fibrillar region of the nucleolus is strongly suggested by the rapid labeling of the fibrils on the autoradiographs.

It is generally admitted that the synthesis and assembly of mammalian ribosomes take place in the nucleolus (15). Biochemical studies have shown that ribosomal RNA precursors exist in the nucleolus as ribonucleoproteins (RNP) which are direct precursors of cytoplasmic ribosomal subparticles (8, 20, 22). These RNP particles can be visualized by electron microscopy in the form of fibrils and granules which are sensitive to pronase and ribonuclease (9) and can be labeled selectively with [^{3}H]uridine (6).

Essentially, two different RNP particles have been described in isolated nucleoli: an 80S RNP containing the 45S RNA, and a 55S RNP containing the 32S RNA (20). A rapidly labeled low molecular weight population of RNP has also been reported (2).

Since pulse-chase experiments have suggested a precursor-product relationship between the fibrillar and the granular ultrastructural components (5), it has been proposed that newly synthesized preribosomal RNA first appears in the nucleolar fibrils as a low molecular weight RNP, eventually to be completed and associated with more proteins to form the 80S RNP particles (2). The nucleolar granular component could then be the ultrastructural substratum of both 80S and 55S RNP particles or only the latter.

In order to assign an ultrastructural compartment to each RNP and RNA molecule in the nucleolus, we compared the incorporation of RNA precursors in both the ultrastructural and biochemical subfractions of the nucleolus. The kinetics of incorporation of [^{3}H]uridine in the fibrillar component is grossly similar to that of the 80S RNP and 45S RNA molecules, although there

seems to exist a precursor to the 80S RNP particles, as already proposed (2). On the other hand, there exists a close correlation between the kinetics of labeling of the granular component and that of the 55S RNP and the 36S + 32S RNA molecules.

MATERIALS AND METHODS

Cell Cultures

Chinese hamster ovary cells (CHO), routinely grown at 37°C in glass bottles in α-medium (Flow Laboratories, Rockville, Md.) supplemented with 10% fetal calf serum (Flow Laboratories), were harvested by trypsinization and transferred to 500-ml spinner flasks in α-medium supplemented with 10% fetal calf serum and 0.075% kanamycin sulfate. The cell density was maintained between 3×10^5 and 6×10^5 cells/ml.

Autoradiography

3-cm^2 plastic petri dishes containing $3-4 \times 10^6$ cells growing exponentially were labeled by replacing the medium with fresh α-medium containing 100 μCi/ml of [5-^{3}H]uridine (20–30 Ci/mmol; Amersham/Searle Corp., Oakville, Ontario). After labeling, the incorporation was stopped by thorough washing with ice-cold phosphate-buffered saline (PBS). After a double fixation in 2.5% glutaraldehyde (30 min) and 1% OsO_4 (30 min), the cells were scraped from the petri dishes, pelleted, and dehydrated with alcohol (70%, 90%, 100%) and propylene oxide before embedding in Epon 812.

Ultrathin sections were prepared for autoradiography with Ilford L4 emulsion (Ilford Ltd., Ilford, Essex, England). They were exposed at 4°C for 30 days and developed with the gold latensification Elon-ascorbic acid method (GEA) as described by Wisse and Tates (21). The autoradiographs were contrasted with 5% uranyl acetate (10 min) followed by lead citrate (10 min). Quantitative data were obtained by counting the grains on micrographs and approximating the area by superposition of a transparent sheet calibrated in μm^2 (16). Statistical treatment of the data was done and the student's t test proved significant over 1/1,000 for each value.

Labeling Procedure for Biochemical Studies

Spinner cultures containing approximately 6×10^5 cells/ml were diluted to 3×10^5 cells/ml and labeled with 0.05 μCi/ml of [2-^{14}C]uridine (50–60 mCi/mmol, Amersham/Searle Corp.). After 8–9 h, [5-^{3}H]uridine was added (1 μCi/ml) for periods of 2.5–60 min. The incorporation was stopped by dilution of the cell suspension with 1 vol of ice-cold PBS and 1 vol of crushed frozen PBS and vigorous shaking in an ice-water bath.

Cell Fractionation

The cells were harvested by centrifugation for 5 min at 600 *g* and the nuclei prepared immediately according to a method based on the use of detergents (1). The cell pellet was resuspended in 10–15 vol of Tris 10 mM (pH 7.4) containing 0.25 M sucrose, 2.5 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 20 μg/ml of polyvinylsulfate, and homogenized with an UltraTurrax (Janke and Kunkel, Staufen, Germany) after the addition of collagenase (0.5 mg/ml) (Calbiochem, Los Angeles, Calif.), 0.4% Cemulsol NPT 10 (vol/vol), and 0.05% Celanol 251 (vol/vol) (Melle-Bezons, Neuilly-sur-Seine, France). The nuclei were collected by centrifugation (600 *g* for 5 min) and washed once in the same solution without detergents and collagenase. The nucleoli were then obtained by ultrasonic disintegration of the nuclei, as described by Zalta et al. (23). Occasionally, nucleoli were prepared by the method of Penman (13) as modified by Warner and Soeiro (20).

RNA Extraction

Nucleoli from $1-2 \times 10^8$ cells were resuspended in 2 ml of 20 mM sodium acetate (pH 5) containing 10 μg/ml polyvinylsulfate and 0.5% sodium dodecyl sulfate (SDS). An equal volume of water-saturated phenol containing 0.1% 8-hydroxyquinoline was then added and the mixture gently homogenized before extraction at 55°C for 3 min. The aqueous phase and the interphase layer were reextracted together at 4°C for 30 min, first with 1 vol of phenol and a second time with 0.5 vol of phenol. The RNA solution was brought to 0.2 M NaCl and precipitated with 2 vol of cold ethanol at −20°C overnight (7, 19). The RNA was then separated by electrophoresis on 10-cm gels containing 2.3% acrylamide (Eastman Kodak Co., Organic Chemicals Div., Rochester, N.Y.) and 0.6% agarose (Calbiochem), as described by Peacock and Dingman (12). The gels were subsequently cut into 2-mm slices with a Gilson gel slicer and incubated in 0.6 N NH_4OH at 67°C for 2 h. 12 ml of Aquasol (New England Nuclear, Boston, Mass.) were then added and the radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer (Packard Instruments, Downer's Grove, Ill.).

Extraction of Nucleolar RNP

Nucleolar RNP were prepared essentially as described by Warner and Soeiro (20) and by Mirault and Scherrer (11). Nucleoli pellets (from $1-2 \times 10^8$ cells) were resuspended with 2 ml of 10 mM NaCl, 10 mM ethylenediaminetetraacetic acid (EDTA), 10 mM Tris (pH 7.4), 5 μg/ml polyvinylsulfate, 10 mM dithiothreitol (Calbiochem), and 0.1% Brij 35; the RNP particles were then extracted by homogenization at 25°C for 10 min in a water bath. The suspension was centrifuged for 10 min at 20,000 *g* and the RNP were separated by ultracentrifugation on 5–20% sucrose gradients (40,000 rpm, rotor SB-283, International ultracentrifuge B-60, 2.6 h) or on

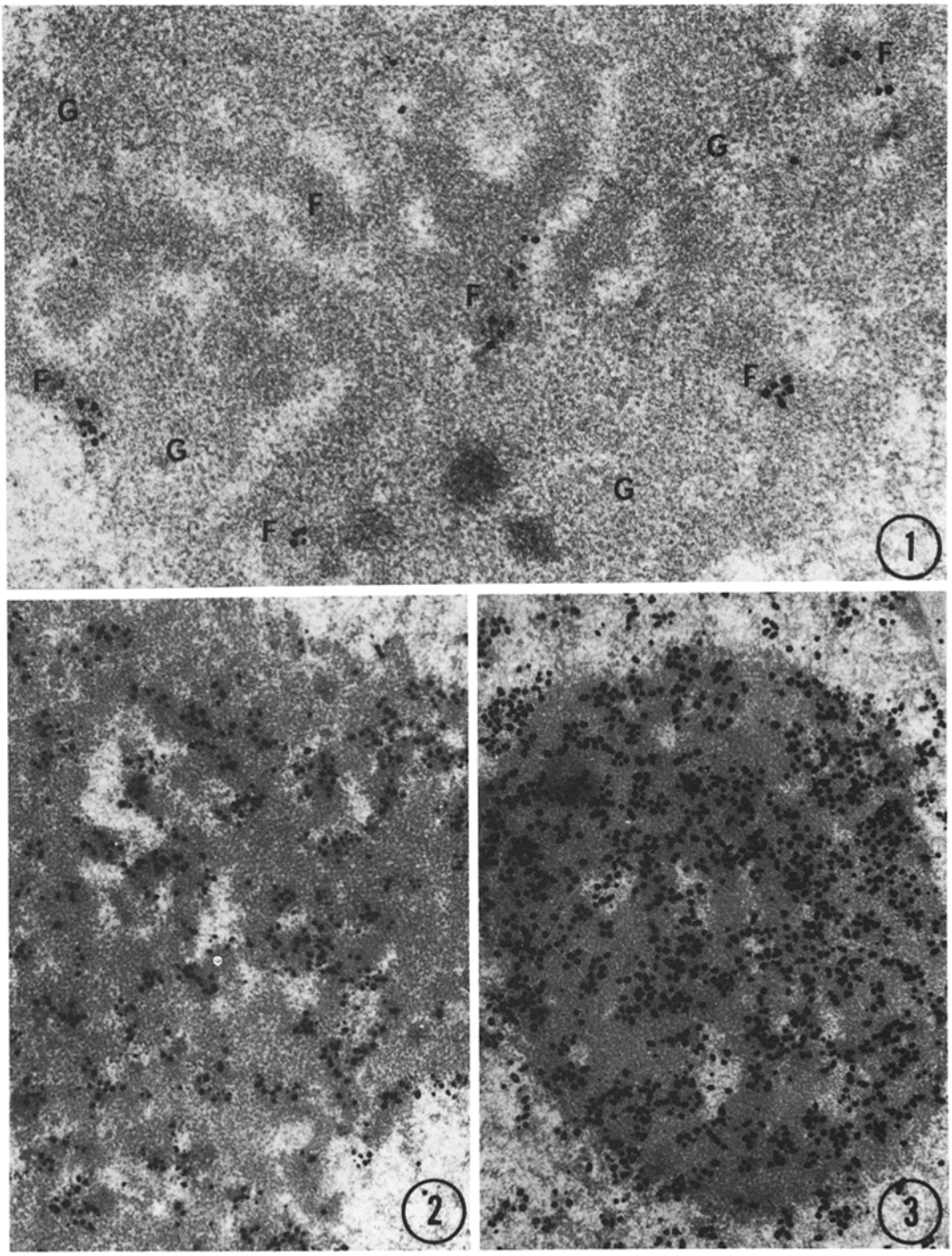

FIGURE 1 Nucleolus of CHO cell pulse labeled with [^{3}H]uridine for 2.5 min. The radioactivity is located exclusively on the fibrillar RNP component (*F*) of the nucleolus, while the granules (*G*) are unlabeled. Double fixation and staining. Ilford L-4 emulsion with GEA development. × 36,000.

FIGURE 2 Nucleolus of CHO cell pulse labeled with [^{3}H]uridine for 30 min. The fibrillar zone is heavily labeled while the granular area displays some activity. Same as in Fig. 1. × 24,000.

FIGURE 3 Nucleolus of CHO cell pulse labeled with [^{3}H]uridine for 60 min. The whole nucleolus is studded with silver grains. Same as in Fig. 1. × 24,000.

15–30% sucrose gradients (23,000 rpm, rotor SW27, Beckman ultracentrifuge L2-65B, 18 h). The sucrose gradients were made in the same solution except for dithiothreitol (1 mM) and Brij 35 (0.01%). Fractions were collected from the bottom of the tubes directly into scintillation vials for radioactivity measurements after the addition of 0.5 ml of H_2O and 12 ml of Aquasol. When needed, RNA was extracted from fractions of the gradients by the addition of 0.25 vol of 5% SDS, 2.5% sodium deoxycholate, 2.5 M urea, and 0.05M EDTA with agitation for 10 min at room temperature. Then, 50 μg of yeast-soluble RNA (Calbiochem) and NaCl to 0.2 M were added and the RNA was precipitated with 2 vol of ethanol at −20°C. It was analyzed by gel electrophoresis as described above.

Counting Efficiencies

For determination of counting efficiency, a known quantity of [^{3}H]- or [^{14}C]toluene was added, before mixing with the scintillator, to a fraction of gradient or to a slice of gel containing unlabeled RNP or RNA. The counting efficiency for ^{3}H was 9% in polyacrylamide gels and 29% in sucrose gradients while it was, respectively, 12% in gels and 27% in gradients for ^{14}C. These data were used to determine ^{3}H to ^{14}C ratios independent of the counting efficiencies.

RESULTS

Autoradiography

In order to compare quantitatively biochemical and autoradiographic data, it appeared important to choose an autoradiographic method that would provide good sensitivity and resolution while permitting quantitative evaluation.

Our choice of the Ilford-L4 emulsion and GEA development was motivated by the fact that this combination allowed easy localization of grains within small adjacent organellar compartments along with short exposure times. Provided adequate fixation and staining are carried out, the fibrillar and granular RNP components of the nucleolus can be mapped on micrographs and the surface occupied by each, approximated. We have calculated that the fibrillar component comprises around 16% of the total nucleolar surface in CHO cells growing exponentially. This is in close agreement with the values reported for the fibrillar component of rat liver nucleoli (18).

For short periods of [^{3}H]uridine incorporation (2.5 and 5 min), radioactivity is found in the fibrillar component of almost all nucleoli ($\geq$90% after 2.5 min and 100% after 5 min) while the granules remain unlabeled (Fig. 1). After 30 min of incorporation, the fibrillar zones are heavily labeled while the granules show a definite increase in radioactivity (Fig. 2). After 60 min, both the granular and fibrillar areas are filled with grains (Fig. 3). Similar autoradiographs were used to determine the concentration of radioactivity in both the granules and the fibrils as a function of time; over 3,000 grains were counted for each point (see Table I).

Biochemistry

The best way to characterize the content of both the granular and fibrillar components of the nucleolus would be to extract RNA and RNP from isolated fibrils and granules. In the absence of a method that can yield such fractions without concomitant processing of the RNA, the incorporation of labeled uridine was studied as a function of time in whole nucleolar RNA (Fig. 4) and nucleolar RNP (Fig. 5). In order to compare the various values obtained, the cells were grown for 9 h in the presence of [^{14}C]uridine and pulse labeled with [^{3}H]uridine for periods varying from 2.5 to 60 min. In each case, the ^{3}H to ^{14}C ratios were determined by measuring the area under each peak.

After 2.5 min of incorporation, nucleolar RNA is heterogeneous, but at 5 min, a peak of [^{3}H]uridine can be identified in the 45S RNA region. Then, the 36S RNA is labeled (15 min) followed by the 32S RNA (30 min) and the 28S RNA (45 min) (Fig. 4). These results are similar to those described for HeLa cells (14). Within the RNP particles, a significant amount of incorporation is observed in both the 80S and 55S RNP (Fig. 5). Since, for short pulse-labeling experiments, the radioactivity is found mostly within the 45S RNA species, the possibility exists that the 80S RNP undergoes degradation during the extraction and separation procedures or that the 55S RNP are preferentially extracted. To clarify this situation, the RNA was extracted from fractions of a sucrose gradient containing RNP prepared from cells labeled for 15 min with [^{3}H]uridine.

In these conditions, the 80S peak contains 45S RNA (Fig. 6 *a*) and the 55S peak contains mostly 36S RNA (Fig. 6 *b*). However, the ^{3}H to ^{14}C ratio of the 36S RNA was found to be 6.3, a value similar to that of nucleolar 45S RNA (6.8) and of the 80S RNP (7.0), but unlike the isotopic ratio of nucleolar 36S RNA (2.2) (see Table I). This suggests that part of the 45S RNA population is degraded into 36S RNA during the preparation and/or separation of the RNP. It is not clear

TABLE I

Duration of incorporation	Fibrillar region	Granular region	80S RNP	55S RNP	45S RNA	36S + 32S RNA
min	*grains/μm²*	*grains/μm²*	*$^3H/^{14}C$*	*$^3H/^{14}C$*	*$^3H/^{14}C$*	*$^3H/^{14}C$*
2.5	14.4	0.4	—	—	—	—
5	47.2	2.4	1.4	0.2	1.2	—
15	90.0	10.0	7.0	1.9	6.8	1.3
30	144.4	36.4	19.8	5.9	20.0	6.3
45	156.0	37.2	—	—	28.6	13.8
60	191.2	91.6	35.0	14.0	30.6	16.5

Incorporation of [^{3}H]uridine into nucleolar ultrastructural components, nucleolar RNP, and nucleolar RNA. The patterns of [^{14}C]- and [^{3}H]uridine incorporation shown in Figs. 4 and 5 were used to determine the isotopic ^{3}H to ^{14}C ratios in nucleolar RNA and RNP. The values obtained were corrected as stated in Materials and Methods. The ratios, grains/μm^2, were determined from autoradiograms similar to those shown in Figs. 1–3.

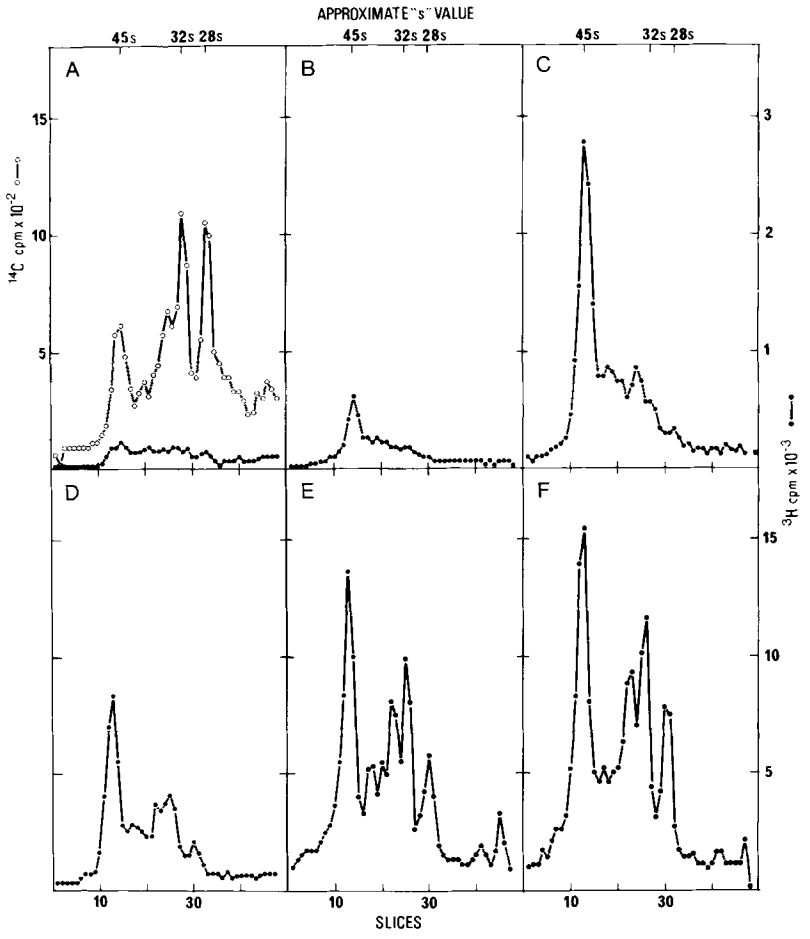

FIGURE 4 Incorporation of [^{14}C]- and [^{3}H]uridine in nucleolar RNA. 8–10 × 10^7 CHO cells labeled with 0.05 μCi/ml of [^{14}C]uridine for 8–9 h were labeled with 1 μCi/ml of [^{3}H]uridine for: A, 2.5 min; B, 5 min; C, 15 min; D, 30 min; E, 45 min; and F, 60 min. Nucleolar RNA was prepared as described in Materials and Methods and electrophoresed for 5 h at 8 V/cm and gels were cut into 2-mm slices. ^{3}H radioactivity (—●—); ^{14}C radioactivity (—○—); the 9-h [^{14}C]uridine pattern was the same in all cases and accordingly is shown only in the first frame. Direction of electrophoresis is from left to right.

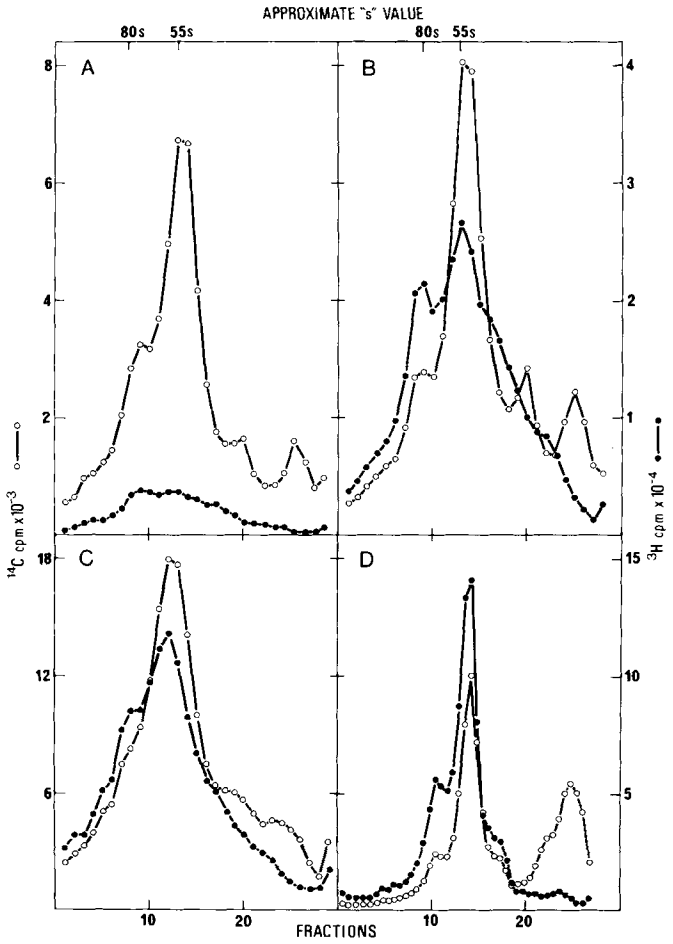

FIGURE 5 Incorporation of [^{14}C]- and [^{3}H]uridine in nucleolar RNP. 8–10 × 10^7 CHO cells labeled with 0.05 μCi/ml of [^{14}C]uridine for 8–9 h were labeled with 1 μCi/ml of [^{3}H]uridine for: A, 5 min; B, 15 min; C, 30 min; and D, 60 min. Nucleolar RNP were prepared as described in Materials and Methods. Ultracentrifugation was carried out for 18 h on 15–30% sucrose gradients at 23,000 rpm in the SW27 rotor (A, B, and C) and for 2.6 h on a 5–20% sucrose gradient at 40,000 rpm in the SB-283 rotor (D). ^{3}H radioactivity (—●—); ^{14}C radioactivity (—○—). Direction of sedimentation is from right to left.

whether this degradation affects the 45S RNA population at large or only a limited portion of it. One could imagine that the RNA of newly synthesized RNP particles is more easily broken down because, possibly, of a lower protein content of the RNP (2), while older molecules about to be cleaved would also represent good candidates. It is not possible, however, to discriminate between these possibilities in these experiments, although the appearance of the label in the 55S peak after 5 min of incorporation suggests that at least some of the newly synthesized molecules are degraded.

The kinetics of labeling of the various nucleolar components were compared in cells pulse labeled with [^{3}H]uridine. The concentration of radioactivity as expressed in grains/μm^2 in the fibrillar and granular RNP was studied in parallel with the ^{3}H to ^{14}C ratio in the 80S and 55S RNP and in the 45S and 36S + 32S RNA. The results are summarized in Table I and Fig. 7. For short periods of labeling (2.5–15 min), the major portion of [^{3}H]uridine incorporated is located in the fibrillar ultrastructural component of the nucleolus, and in the 45S RNA within the 80S RNP particles. With time, there is an increased proportion of radioactivity in the granular component in parallel

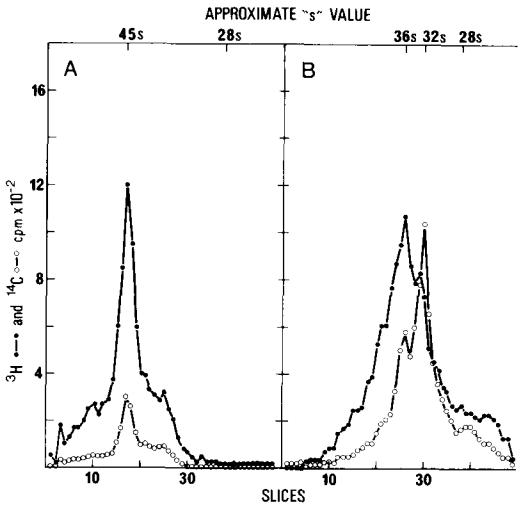

FIGURE 6 Analysis of the RNA contained in the isolated RNP particles. 1.5–2 × 10^8 CHO cells labeled with 0.05 μCi/ml of [^{14}C]uridine for 8.75 h were labeled with 1 μCi/ml of [^{3}H]uridine for 15 min. RNP was prepared as described in Materials and Methods. They were separated on 5–20% sucrose gradients by centrifugation for 2.6 h at 40,000 rpm in the SB-283 rotor, fractionated, and 25% of each fraction was used to determine the position of the peaks while the remaining RNP were extracted as described in Materials and Methods. The extracts corresponding to the 80S peak and the 55S peak were pooled separately and the RNA was electrophoresed for 5 h at 8 v/cm. The gels were cut into 2-mm slices. A, RNA extracted from the 80S peak; B, RNA extracted from the 55S peak. ^{3}H radioactivity (—●—); ^{14}C radioactivity (—○—). Direction of electrophoresis is from left to right.

with an increase in the specific activities of the 55S RNP and the 36S + 32S RNA. In Fig. 7, these results are expressed as percent of the maximum value. On examination of the data, we found that three curves could be drawn: one through the points describing the kinetics of incorporation of the fibrillar ultrastructural component; a second through the points for the 80S RNP and the 45S RNA; and finally, a third accommodating the values obtained for the granular component, the 55S RNP, and the 36S + 32S RNA.

DISCUSSION

The kinetic data reported in this paper suggest that the 80S RNP containing the 45S RNA are localized in the fibrillar ultrastructural compartment of the nucleolus. However, from Fig. 7 it can be seen that the labeling increases faster in the fibrillar component than in the 80S RNP and 45S RNA. It should be remembered that the incorporation of precursors into the fibrils as studied by autoradiographic methods measures all growing molecules as well as completed ones. Hence, some differences are expected between ultrastructural and biochemical data. In fact, a rapidly labeled RNP population has been described in ascites tumor cell nucleoli. These RNP are believed to contain newly synthesized RNA molecules not yet stabilized by the addition of a full complement of proteins. They are found in sucrose gradients after 3 min of precursor incorporation and are rapidly chased into the 80S RNP particles (2). These RNP could represent the material that is rapidly labeled in the nucleolus and, therefore, the fibrillar compartment of the nucleolus would be the structural counterpart not only of the 80S RNP (and 45S RNA) but also of a rapidly labeled precursor of these species. Furthermore, our results are consistent with other studies indicating that the fibrillar region of the nucleolus is concerned with the transcription of rRNA (5, 6) and they imply that it is also concerned with the assembly of the ribosomes.

It is also of interest to note that the 80S RNP, the 45S RNA, and the fibrillar component simultaneously approach a steady-state ^{3}H to ^{14}C ratio or grains/μm^2 after 30 min of [^{3}H]uridine incorporation.

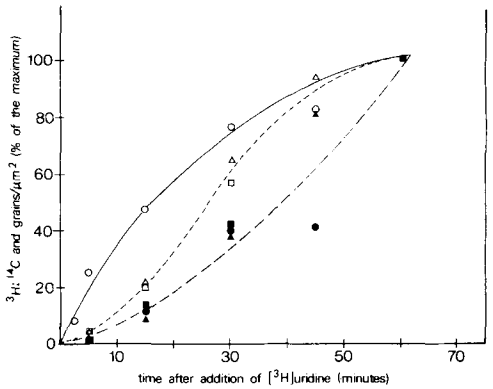

FIGURE 7 Kinetics of incorporation of [^{3}H]uridine into nucleolar components. The ratios shown in Table I were expressed as the percent of the maximum. Fibrillar region (○); granular region (●); 80S RNP (□); 55S RNP (■); 45S RNA (△); 36S + 32S RNA (▲). On inspection, it is found that three curves can be drawn. First, one that fits the granular region, the 55S RNP and the 36S + 32S RNA (---); a second that fits the 80S RNP and the 45S RNA (----); a third for the fibrillar region (——).

On the other hand, the 55S RNP and the 36S + 32S RNA appear to be localized in the granular compartment of the nucleolus, since they have the same kinetics of labeling with [^{3}H]uridine. Although no correction has been made for the artifactual processing of the 80S RNP particles occurring during the preparation, the ^{3}H to ^{14}C ratio in the 55S RNP increases significantly only after 45 min, as in the nucleolar 36S + 32S RNA, in parallel with the increase in radioactivity observed in the granular component.

Although the function of the nucleolus has been fairly well elucidated (15), the interplay between its different ultrastructural components and their respective functions has been very difficult to study mainly because of the lack of an adequate method of nucleolar fractionation. However, studies of the role of the cell protein-synthesizing machinery in the control of ribosome biosynthesis have hinted that such controls may be not only at the level of the synthesis and assembly, but also at the level of the release of the 55S precursors from the nucleolus (3, 4). Our results would be consistent with this view of the granular region as a pool of nearly completed 55S subunits and could be relevant to the observation that nucleoli of tumor cells have a greater proportion of granules than normal cells (18).

In conclusion, even though the presence of some 55S RNP in the fibrillar region or the presence of some 80S RNP in the granular region cannot be excluded, our experiments strongly suggest that the 80S RNP is localized within the fibrillar compartment and the 55S RNP within the granular compartment. The possibility that precursors to 80S RNP exist also in the fibrillar region of the nucleolus is suggested by the rapid labeling of the fibrils on the autoradiographs. The configurational changes that occur when fibrils are converted into granules probably correspond to the metabolization of 80S RNP into 55S RNP in association with the processing of the ribosomal RNA precursors. Recent descriptions of the ultrastructure of the isolated nucleolar RNP (10, 17) are in agreement with this interpretation.

We would like to thank Drs. J. P. Bachellerie, C. Kedinger and J. P. Zalta for many helpful discussions.

This research was supported by the Medical Research Council of Canada.

Received for publication 7 March 1975, and in revised form 27 May 1975.

REFERENCES

1. AMALRIC, F., R. SIMARD, and J. P. ZALTA. 1969. Effet de la température supra-optimale sur les ribonucléoprotéines et le RNA nucléolaire. II. Etude biochimique. *Exp. Cell Res.* **55**:370–377.
2. BACHELLERIE, J. P., C. MARTIN-PREVEL, and J. P. ZALTA. 1971. Cinétique de l'incorporation d'uridine-(^{3}H) dans des fractions subnucléolaires de cellules d'hépatome ascitique du rat. *Biochimie (Paris).* **53**:383–389.
3. COOPER, H. L., and E. M. GIBSON. 1971. Control of synthesis and wastage of ribosomal ribonucleic acid in lymphocytes. II. The role of protein synthesis. *J. Biol. Chem.* **246**:5059–5066.
4. CRAIG, N. C., and R. P. PERRY. 1970. Aberrant intranucleolar maturation of ribosomal precursors in the absence of protein synthesis. *J. Cell Biol.* **45**:554–564.
5. GEUSKENS, M., and W. BERNHARD. 1966. Cytochimie ultrastructurale du nucléole. III. Action de l'actinomycine D sur le métabolisme du RNA nucleolaire. *Exp. Cell Res.* **44**:579–598.
6. GRANBOULAN, N., and P. GRANBOULAN. 1965. Cytochimie ultrastructurale du nucléole. II. Etude des sites de synthèse du RNA dans le nucléole et le noyau. *Exp. Cell Res.* **38**:604–619.
7. KEDINGER, C., and R. SIMARD. 1974. The action of α-amanitin on RNA synthesis in Chinese hamster ovary cells. *J. Cell Biol.* **63**:831–842.
8. LIAU, M. C., and R. P. PERRY. 1969. Ribosome precursor particles in nucleoli. *J. Cell Biol.* **42**:272–283.
9. MARINOZZI, V. 1964. Cytochimie ultrastructurale du nucléole. RNA et protéines intranucléolaires. *J. Ultrastruct. Res.* **10**:433–456.
10. MATSUURA, S., T. MORIMOTO, Y. TASHIRO, T. HIGASHINAKAGAWA, and M. MURAMATSU. 1974. Ultrastructural and biochemical studies on the precursor ribosomal particles isolated from rat liver nucleoli. *J. Cell Biol.* **63**:629–640.
11. MIRAULT, B. E., and K. SCHERRER. 1971. Isolation of preribosomes from HeLa Cells and their characterization by electrophoresis on uniform and exponential-gradient-polyacrylamide Gels. *Eur. J. Biochem.* **23**:372–386.
12. PEACOCK, A. C., and C. W. DINGMAN. 1968. Molecular weight estimation and separation of ribonucleic acid by electrophoresis in agarose-acrylamide composite gels. *Biochemistry.* **7**:668–674.
13. PENMAN, S. 1966. RNA metabolism in the HeLa cell nucleus. *J. Mol. Biol.* **17**:117–130.
14. PENMAN, S., I. SMITH, and E. HOLTZMAN. 1966. Ribosomal RNA synthesis and processing in a particulate site in the HeLa cell nucleus. *Science (Wash.D.C.).* **154**:786–789.
15. PERRY, R. P. 1969. Nucleoli: the cellular sites of

ribosomes production. *In* Handbook of Molecular Cytology. A. Lima-de-Faria, editor. North-Holland Publishing Co., Amsterdam. Chap. 25. 620–636.

16. SIMARD, R., and R. CASSINGENA. 1969. Actinomycin resistance in cultured hamster cells *Cancer Res.* **29**:1590–1597.
17. SIMARD, R., F. SAKR, and J. P. BACHELLERIE. 1973. Ribosomal precursor particles in ascites tumor cell nucleoli. *Exp. Cell Res.* **81**:1–7.
18. SMETANA, K., K. SHANKAR NARAYAN, and H. BUSCH. 1966. Quantitative analysis of ultrastructural components of nucleoli of the Walker tumor and liver. *Cancer Res.* **26**:786–796.
19. TIOLLAIS, P., F. GALLIBERT, and M. BOIRON. 1971. Effect of valine deprivation on the biosynthesis of ribosomal and messenger RNA in a mammalian cell line (L-5178Y). *Eur. J. Biochem.* **18**:35–45.
20. WARNER, J. R., and R. SOEIRO. 1967. Nascent ribosomes from HeLa cells. *Proc. Natl. Acad. Sci. U. S. A.* **58**:1984–1990.
21. WISSE, E., and A. A. TATES. 1968. A gold latensification Elon-ascorbic acid developer for Ilford L-4 emulsion. Proceedings of the 4th Conference on Electron Microscopy. Tipografia Poliglotta Vaticana, Rome. II:465–466.
22. YOSHIKAWA-FUKADA, M. 1967. The intermediate state of ribosome formation in animal cells in culture. *Biochem. Biophys. Acta.* **145**:651–663.
23. ZALTA, J., J. P., ZALTA, and R. SIMARD. 1971. Isolation of nucleoli: a method that combines high yield, structural integrity, and biochemical preservation. *J. Cell Biol.* **51**:563–568.