TURNOVER OF HeLa RIBOSOMAL RNA

The Characterization of a Class of RNA in HeLa Cytoplasm Derived from 28S RNA

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ABSTRACT

A class of RNA in HeLa cytoplasm with a sedimentation value of 22S is described. This RNA is a true cytoplasmic component and is not an artifact of cell rupture or of the method of RNA preparation. The 22S RNA sediments in a sucrose gradient with those ribosomal structures containing 28S ribosomal RNA (60S and 74S particles and polyribosomes). It has a base composition and methyl content similar to those of 28S RNA. The kinetics of formation of 22S suggest that it is not a direct product of transcription but is derived in vivo from "old" molecules of 28S RNA.

INTRODUCTION

The sedimentation profile of total cytoplasmic RNA from HeLa cells is characterized by the two predominant peaks of the 28S and the 18S ribosomal RNA and a small peak in the 4–5S region due to the transfer RNA (tRNA)[1] and 5S ribosomal RNA. However, under conditions of gradient analysis permitting high resolution, other minor RNA peaks can be observed. The origin and the nature of these minor RNAs have not been carefully studied. We report here the results of our investigation of one such minor RNA of HeLa cell cytoplasm which sediments between the 18S and the 28S RNA with a relative S value of 22. We conclude that it is a derivative of the 28S RNA arising as a consequence of turnover of the 28S ribosomal RNA. We report here some physical and chemical properties of the 22S RNA.

[1] *Abbreviations used in this paper*: EDTA, ethylenediaminetetraacetate; rRNA, ribosomal RNA; SDS, sodium dodecyl sulfate; SSC, standard sodium citrate; TCA, trichloroacetic acid; tRNA, transfer RNA.

MATERIALS AND METHODS

Materials

Uridine-^{3}H (20 Ci/mmole), methyl-^{3}H-methionine (100 mCi/mmole) and carrier-free phosphoric acid-^{32}P were obtained from New England Nuclear Corp., Boston, Mass.

Triton X-100 was obtained from Rohm and Haas Co., Philadelphia, Pa. Deoxycholic acid, sodium salt, and sodium dodecyl sulfate (SDS) were obtained from the Sigma Chemical Co., St. Louis, Mo. Millipore filters, DA 0.65 μ, white, plain 25 mm were obtained from the Millipore Corporation, Bedford, Mass.

T_1 ribonuclease and pancreatic ribonuclease were obtained from Worthington Biochemical Corporation, Freehold, N. J.

Cell Culture

HeLa and L cells were grown in suspension culture as previously described (1).

Cell Fractionation

Cell pellets were washed twice with cold Earle's solution and suspended in a buffer containing 0.01 M Tris, pH 7.4, 0.01 M NaCl, and 0.0015 M $MgCl_2$. The cell suspension was homogenized in a stainless steel-ball homogenizer or by lysis with the nonionic detergent Triton X-100 (2, 3). In the latter case, the cell suspension in Earle's solution containing 0.05% Triton X-100 (v/v) was allowed to stand for 10 min with occasional stirring in an ice bath. The homogenate prepared by either method was centrifuged at 1000 *g* for 3 min to remove the nuclei. The supernatant is designated the cytoplasm.

Extraction of RNA

RNA was extracted from nuclei-free cytoplasm or from ribosomal subunits at room temperature or at 60°C by the phenol-SDS procedure (4). RNA was precipitated overnight at −20°C. with 2 vol of ethanol in the presence of 0.2 M NaCl and then purified by sedimentation through a sucrose gradient containing SDS. In some experiments ribosomal RNA (rRNA) was released from ribosomal structures by 1% SDS only, then purified by sedimentation through a sucrose gradient. Radioactive RNA in fractions from sucrose gradients was precipitated with trichloroacetic acid (TCA), collected on a Millipore filter, and counted in a liquid scintillation counter after addition of Bray's liquid scintillator solution.

Isolation of Ribosomal Subunits

Ribosomes and polysomes in the cytoplasmic extract were dissociated into the 50S and 30S subunits with ethylenediaminetetraacetate (EDTA) added to 10 mM final concentration. The subunits were isolated by centrifugation at 4°C through a sucrose gradient containing 0.01 M Tris, 0.01 M NaCl, and 0.01 M EDTA. The native subunits, 60S and 45S, were isolated by adding Brij-58 (0.5% w/v) and sodium deoxycholate (0.5% w/v) to the cytoplasmic extract and sedimenting it through a sucrose gradient in the above buffer without EDTA and with 1.5 mM $MgCl_2$ at 4°C.

Specific Radioactivity of RNA

LONG TERM: Uridine-^{3}H was added to a culture of cells to a concentration of 0.5 μCi/ml; the cells were allowed to grow for 6–14 days with the cell concentration doubling each 24 hr. Virtually all the uridine-^{3}H was incorporated during the first 24 hr of growth and no uridine-^{3}H was supplied after the initial addition. RNA was released from cytoplasmic structures with 1% SDS and purified on sucrose-SDS gradients. The 22S RNA was further purified on a second sucrose gradient as described in Fig. 1. The specific radioactivity of RNA was obtained by determining acid-precipitable counts per unit of OD 260 of RNA.

SHORT TERM: Cells were labeled with uridine-^{3}H, 0.2 μCi/ml, and samples were taken at 6, 30, 60, and 144 hr. RNA was released from the 60S subunit and purified on sucrose-SDS gradients, and the radioactivity of each fraction was determined as acid-precipitable counts.

Base Composition of RNA

A culture of cells in medium containing the normal amount of phosphate was labeled with carrier-free phosphate-^{32}P (1 μCi/ml). The cells were allowed to double four times with no further phosphate-^{32}P supplied after the initial addition. Total cytoplasmic RNA was extracted with SDS-phenol and purified on sucrose-SDS gradients. Peak fractions of 18S and 28S were taken for base composition analysis; 22S RNA was further purified on a second sucrose gradient before base composition analysis. RNA labeled with ^{32}P was hydrolyzed for 18 hr in 0.3 M KOH, and the base composition was determined by a modification of the paper electrophoresis technique previously described (5). The alkaline hydrolysate of RNA was neutralized and desalted by passing through a small Dowex 50-NH_4^+ column. Electrophoresis of the salt-free hydrolysate was carried out in a buffer of 0.1 M sodium acetate, 0.01 M EDTA, pH 3.5 for 5 hr at 800 v.

Methyl Content of RNA

The relative methylation of 18S, 22S, and 28S RNA was indirectly determined by uniformly labeling the phosphate groups with ^{32}P and the methyl groups with methyl-^{3}H–methionine (6). Labeling of methyl groups was carried out in the presence of 10^{-4} adenosine and guanosine to prevent the entry of ^{3}H label into the purine ring system through one carbon transfer. The ratio of ^{3}H/^{32}P represents the methyl content of the RNA.

DNA-RNA Hybridization

Hybridization experiments were performed by the technique of Gillespie and Spiegelman (7). DNA from HeLa cells was extracted and purified by the procedure of Marmur (8). The DNA was denatured by heating at 100°C for 5 min. The DNA in 2 × SSC (SSC is standard sodium citrate; 0.15 M NaCl, 0.015

M sodium citrate) was passed through a Millipore filter, soaked in the same buffer, at a rate of 2 ml/min. The filters were blotted on paper, then heated at 80°C in an oven for 16 hr. Filters were stored in a vacuum desiccator at room temperature until used for hybridization.

Radioactive 28S was prepared from cells grown in medium containing 20 μCi/ml of uridine-^{3}H. The specific activity of the 28S was 1×10^8 cpm/mg RNA. The ^{3}H-labeled as well as unlabeled 28S was prepared from the EDTA-induced 50S ribosomal subunit. The RNA in the 50S subunit was extracted with SDS-phenol and purified by sedimentation through SDS-sucrose gradients. Peak fractions of 28S were used for hybridization.

The hybridization reaction was carried out at 60°C or 68°C for 16–20 hr in screw-capped vials, each vial containing a DNA filter and RNA-^{3}H in a total volume of 0.75 ml 2 × SSC containing 0.1% SDS. After 16–20 hr at the designated temperature the filters were washed with 2 × SSC at room temperature. Each filter was treated with 1 ml of a ribonuclease mixture (5 units/ml of T_1 RNAase and 2.5 μg/ml of pancreatic RNAase) for 45 min at 37°C in 2 × SSC. The filters were again washed with 2 × SSC and dried in air at room temperature; the radioactivity was counted in a liquid scintillation counter.

RESULTS

22S RNA in HeLa Cytoplasm

EFFECT OF CONDITIONS OF CENTRIFUGATION Fig. 1 *a* illustrates the usual pattern observed when total cytoplasmic RNA from HeLa cells is centrifuged through a 30 ml sucrose-SDS gradient in a Spinco SW25.1 rotor. Under these conditions a shoulder is always observed on the faster sedimenting side of the 18S RNA. This region corresponds to an S value of 22. Hence the RNA of this region was designated 22S RNA and will be referred to as such throughout this paper. If total cytoplasmic RNA is centrifuged through a 37 ml sucrose-SDS gradient in the Spinco SW27 rotor (Fig. 1 *c*), better resolution of the region between 18S and 28S RNA is obtained than in the 30 ml gradient. Moreover, the RNA sedimenting at 22S is resolved into a discrete peak in the 37 ml gradient. This procedure permitting higher resolution was therefore routinely used in the separation of cytoplasmic RNA.

EFFECT OF CELL RUPTURE PROCEDURE: The possibility that the 22S RNA might be an artifact of the homogenization procedure was investigated. Cytoplasmic RNA from cells ruptured by homogenization in a stainless steel–ball homogenizer was compared to cytoplasmic RNA prepared from cells which were lysed by the detergent Triton X-100. Cytoplasm prepared by detergent lysis of the cell membrane has been shown to contain less RNA resulting from nuclear leakage or rupture than cytoplasm prepared by the homogenization procedure (3). Therefore, the detergent method by minimizing nuclear rupture would also help decide whether the 22S RNA is a truly cytoplasmic RNA. Figs. 1 *a* and 1 *b* illustrate that the two procedures are identical with respect to the yield of 22S RNA, suggesting that the 22S RNA is not an artifact of homogenization and further that it is a cytoplasmic RNA.

EFFECT OF RNA PREPARATION PROCEDURE: Another possibility was that the 22S is a degradation artifact of 28S RNA occurring during the preparation of RNA. Cytoplasmic RNA was prepared by releasing RNA from ribosomal structures with 1% SDS (Fig. 1 *c*) and compared to RNA prepared by lysis of a cell pellet directly with 1% SDS and 0.01 M EDTA (Fig. 1 *d*). The same number of cells was used for both preparations. The amount of 22S RNA in both preparations is approximately the same. When RNA extracted from a cell pellet or cytoplasm with hot phenol-SDS is sedimented through sucrose, a profile identical to that in Fig. 1 *c* or 1 *d* is obtained. Furthermore, no change in sedimentation profile of cytoplasmic RNA or increase in 22S RNA is observed when a nuclei-free cytoplasm is allowed to remain at 4°C for 1 hr after rupture of the cells.

Purification of 22S RNA

When the fractions containing 22S RNA are pooled (Fig. 1 *d*) and the RNA is resdeimented through a sucrose gradient, a well resolved peak of 22S RNA is obtained which is completely free of 28S RNA and largely free of 18S RNA (Fig. 1 *e*). Contamination by 18S RNA can be reduced to a negligible level either by sedimentation through a third sucrose gradient or by using only the peak fractions of 22S RNA of the second gradient. Calculations made using amounts of 22S and 28S obtained after purification indicate that the amount of 22S in HeLa cytoplasm is approximately 2–4% of the 28S.

22S RNA in L Cells

Before investigating the properties of the 22S RNA of HeLa cells, it was of interest to know if it

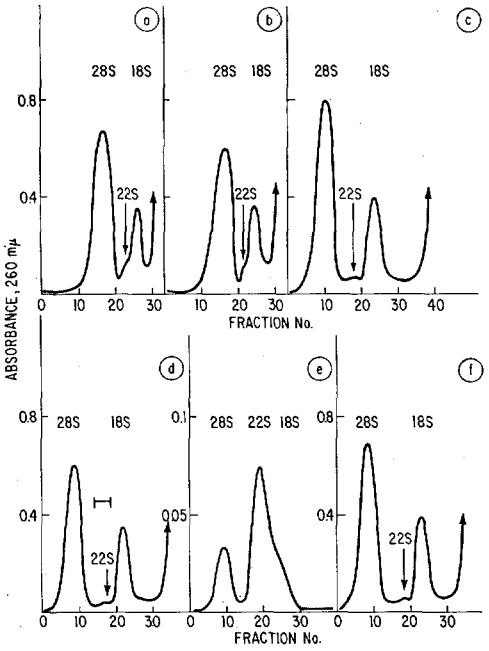

FIGURE 1 Analysis of HeLa cytoplasmic RNA on sucrose gradients. Effect of conditions on cell rupture, RNA preparation, and RNA sedimentation. In panels *a*, *b*, *c*, and *d*, RNA was prepared from 3–4 × 10^7 cells. *a*. Cytoplasm was prepared by homogenization in a stainless steel–ball homogenizer, SDS was added to 1%, and the RNA was analyzed on a 30 ml, 15–30%, w/w, sucrose gradient prepared in 0.01 M Tris, pH 7.2, containing 0.1 M NaCl, 0.01 M EDTA, and 0.2% SDS; centrifugation was carried out for 16 hr, at 26°C and 23,000 rpm, in a Spinco SW25.1 rotor. RNA was pumped from the bottom through a flow cell at the rate of 3 ml/min, and the OD 260 mμ was recorded with a Gilford Model 2000 recording spectrophotometer. *b*. Cytoplasm was prepared by detergent lysis of cells, SDS was added to 1%, and the RNA was analyzed as in *a*. *c*. Sedimentation analysis was carried out in a Spinco SW27 rotor using 37 ml sucrose gradients; other details were as in *a*. *d*. A cell pellet was lysed at 37°C for 10 min in a buffer of 0.1 M EDTA, pH 7, containing 1% SDS. The RNA was analyzed as in *c*. *e*. The RNA from the 22S region of panel *d* was precipitated with ethanol and analyzed on a sucrose gradient as in *c*. *f*. A cytoplasm from 3 × 10^7 L cells was prepared by lysis with Triton X-100 as described in Methods; SDS was added to 1%, and the RNA was analyzed on a sucrose gradient as in *c*.

could be observed in another cultured cell. L cells were chosen and a cytoplasm was prepared by lysing the cell membrane with Triton X-100 as described in Methods for HeLa cells. RNA was released from ribosomal structures with 1% SDS and analyzed by sedimentation through a sucrose gradient (Fig. 1 *f*). Clearly, an RNA sedimenting at 22S is present, indicating that it is not unique to HeLa cells.

Localization of 22S RNA in HeLa Cytoplasm

The results presented above indicate that 22S RNA is present in the cell before homogenization and is not derived during preparation of the RNA. Therefore, it was of interest to ask where it is localized in the cytoplasm, i.e., does the 22S RNA sediment with ribosomal structures? Derived ribosomal subunits were prepared and isolated as described under Methods, and the RNA from the 50S and 30S particles was analyzed by sedimentation through a sucrose gradeient. Fig. 2 *a* shows the preparation of 30S and 50S particles and Fig. 2 *b* shows the RNA obtained from the 50S region of the sucrose gradient. It can be seen that the 22S RNA is associated with a structure which sediments with a relative S value of 50. Only negligible amounts of 22S RNA were found in the 30S region of the gradient. In a similar experiment not presented here, the 22S RNA content of polyribosomes and structures sedimenting at 74S, 60S, and 45S was determined. The 22S RNA was found in polyribosomes and at 74S and 60S, but not at 45S. For hybridization, base composition, and methylation experiments, 22S RNA was prepared from the 50S region of a sucrose gradient containing 0.01 M EDTA.

Base Composition of 22S RNA

The association of 22S RNA with ribosomal structures containing 28S RNA or with different structures of similar S values made it necessary to investigate its relationship to the 28S RNA. Hence, the base composition of 22S RNA was determined as well as that of 28S and 18S RNA. Table I summarizes the results from three separate experiments. It can be seen that the base compositions of the 28S and the 18S RNA are in agreement with published data (9–11). The base composition of the 22S RNA is quite similar to that of the 28S RNA and dissimilar to that of 18S or messenger RNA (9–11).

DNA-RNA Hybridization

The foregoing experiments suggested that the 22S RNA is similar to 28S RNA in base composi-

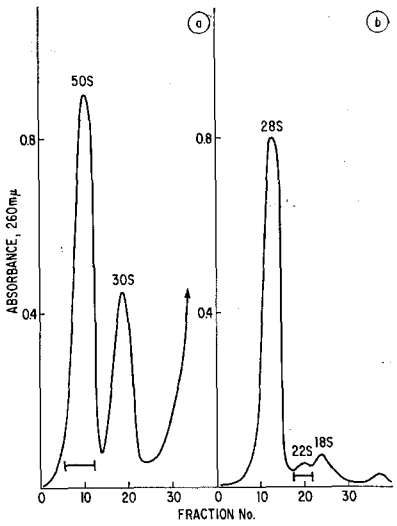

FIGURE 2 *a*. Isolation of 50S and 30S ribosomal subunits. The subunits were prepared as described in Methods and separated by sedimenting the cytoplasm through a 37 ml, 15–30%, w/w sucrose gradient prepared in 0.01 M Tris, pH 7.2, 0.01 M NaCl, and 0.01 M EDTA; 16 hr, at 24,000 rpm, 4°C, Spinco SW27 rotor. The fractions were collected into SDS (1% final concentration) and the RNA in the 50S subunit was precipitated with ethanol. *b*. RNA of the 50S subunit. The RNA in the 50S subunit (*a*) was analyzed on a sucrose-SDS gradient as described in Fig. 1 *c*.

tion. The extent of the similarity of 22S and 28S could be reflected in base sequence homology which can be indirectly measured by hybridization competition experiments. The results of annealing experiments are summarized in Table II and Fig. 3. Unlabeled 28S RNA inhibited the annealing of 28S RNA-^{3}H to the DNA by 63% (Table II). Under the conditions of the experiment, 80% inhibition was predicted. Unlabeled 22S RNA competed with 28S RNA-^{3}H as effectively as unlabeled 28S RNA (Table II, Fig. 3), suggesting that the 22S RNA has sequence homology with the 28S RNA. To a certain extent, unlabeled 18S RNA inhibited annealing of 28S RNA-^{3}H to DNA. It is believed that most, if not all, of the observed competition by 18S RNA was a consequence of contamination of the 18S RNA by 28S RNA and/or its derivatives (12, 13).

Methyl Content of 18S 22S, and 28S RNA

The methyl contents of 18S and 28S RNA have been reported (6). The methylation of the 18S, 22S, and 28S RNA was determined as described in Methods. Table III shows the ratio of ^{3}H label in methyl groups to ^{32}P label in each RNA. It is clear that the 22S RNA is quite similar in methylation to 28S RNA but not to 18S RNA which is more extensively methylated. This method is valid only if negligible ^{3}H enters the purine ring via one-carbon transfer. A convenient internal control is the 5S rRNA which contains no methyl groups (14). In the methylation experiments reported here, negligible ^{3}H entered the 5S RNA.

Kinetics of Formation of 22S RNA

The kinetics of formation of 22S RNA was compared to that of 28S RNA by determining the specific radioactivity of the two RNA molecules after various periods of labeling. Since it was suspected that 22S might be derived from 28S in vivo, radioactive labeling experiments were designed using uridine-^{3}H so that the majority of the ^{3}H was incorporated into RNA during the first 24 hr of growth. Thus, formation of radioactive RNA after the first 24 hr would result either from the degradation of higher molecular weight labeled RNA or from resynthesis from the radioactive precursors arising from completely degraded RNA. The latter possibility is considered unlikely but cannot be rigorously excluded.

The results of long term (6–14 days) labeling of 28S, 22S, and 18S RNA are presented in Table IV. The specific activities of 18S RNA and 28S RNA are similar; the specific activity of the 22S, however, increases as the period after labeling increases. 6–7 days after labeling, the specific activity of the 22S RNA is approximately twice the specific activity of the 28S, and at 10–14 days after labeling the specific activity of the 22S is threefold greater than that of the 28S (Table IV). These differences in specific activities (22S vs. 28S) have been reproduced in a number of similar experiments and are not the result of experimental variability. This is consistent with the 22S being derived from old 28S RNA in vivo and suggests that it is not formed as a result of random degradation, in vivo or in vitro, of 28S RNA. Random degradation of all 28S molecules would produce, at any time after labeling, a 22S with the same specific activity as the 28S. Old 28S RNA is defined as that radioactive 28S synthesized during exposure of a culture to radioactive uridine and present as radioactive 28S RNA after a period of cell growth (chase) when negligible radioactivity is incorporated into newly synthesized RNA.

TABLE I

Base Composition of HeLa Cytoplasmic RNA

Base composition was determined by high-voltage electrophoresis of an alkaline digest of purified RNA-^{32}P, as described under Methods.

Experiment	RNA	Mole per cent of base				G + C (Moles per cent)
		A	C	G	U	
1	22S	16.1	27.4	38.0	18.5	65.4
2	22S	15.3	28.1	39.2	17.4	67.3
3	22S	18.6	27.6	36.2	17.7	63.8
1	28S	16.3	30.2	36.3	17.2	66.5
1	18S	20.6	27.6	30.4	21.4	58.0

TABLE II

Hybridization of 28S RNA-^{3}H to HeLa DNA with Simultaneous Competition by HeLa 18S, 22S, and 28S RNA and E. coli RNA

The hybridization reaction was carried out in screw-capped vials as described under Methods. Each competition system contained a DNA filter (20 μg DNA) RNA-^{3}H (28S-^{3}H, 2.0 μg, 5.62 × 10^5 cpm) and 1 unlabeled RNA (12.0 μg) in 0.75 ml 2 × SSC, containing 0.1% SDS. The blank vials contained RNA-^{3}H and blank filters (no DNA) and the control vials contained DNA filters and RNA-^{3}H in 2 × SSC, 0.1% SDS. The vials were incubated at 60°C for 20 hr.

Hybridization system	cpm bound		Average of cpm bound, less blank	Percentage hybridization
	Filter No. 1	Filter No. 2		
Blank filter (no DNA) + 28S-^{3}H	38	38	0	0
DNA filter + 28S-^{3}H	644	676	622	100
DNA filter + 28S-^{3}H + unlabeled 28S	270	265	230	37
DNA filter + 28S-^{3}H + unlabeled 18S	521	453	449	72
DNA filter + 28S-^{3}H + unlabeled 22S	234	280	219	35
DNA filter + 28S-^{3}H unlabeled *E. coli* RNA	676	684	642	>100

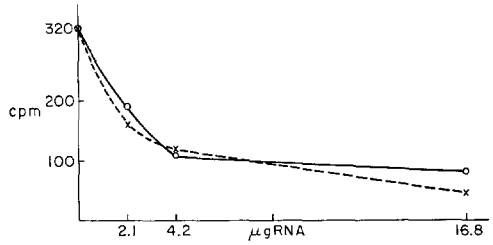

FIGURE 3 DNA-RNA hybridization: competition of unlabeled 28S RNA and 22S RNA with labeled 28S RNA. Each competition system contained 10 μg of DNA, 2.1 μg of labeled 28S RNA, and unlabeled RNA as indicated. Other details are described in Table III and in Methods. Unlabeled 28S, O——O; unlabeled 22S, ×——×.

If the 22S RNA is derived in vivo from old 28S RNA, it would be predicted that the specific activity of the former would be less than that of the latter during relatively short periods of growth after labeling. Experiments were designed to examine the labeling of 22S RNA by isolating it from the native 60S ribosomal subunit. The 60S subunit was chosen to minimize contamination by 18S and messenger RNA. The results of these experiments are shown in Fig. 4. It can be seen that there is little radioactivity and no significant peak of radioactivity corresponding to 22S at 6 and 30 hr after the addition of uridine-^{3}H. However, at 60 and 144 hr (Fig. 4 *d* and 4 *e*) after the addition of uridine-^{3}H a significant peak of radio-

TABLE III

Relative Methyl Content of 18S, 22S, and 28S RNA of HeLa Cell Cytoplasm

A culture of HeLa cells was grown for two doublings in the presence of 10^{-4} M guanosine and adenosine. During the second doubling 500 μCi of each of methyl-^{3}H-methionine and phosphate-^{32}P was present. RNA was extracted and purified as described under Methods, and the acid-precipitable counts of ^{3}H and ^{32}P were determined for each RNA.

RNA	Ratio, ^{3}H/^{32}P
18S	0.96
22S	0.59
28S	0.57

TABLE IV

Specific Radioactivity of 18S, 22S, and 28S RNA after Prolonged Growth

Experiment	Days after labeling	Specific radioactivity of RNA		
		18S	22S	28S
		cpm/OD	*cpm/OD*	*cpm/OD*
1	6	17,000	25,200	13,500
2	7	9,100	17,400	9,800
3	10	860	2,407	695
4	14	105	313	116

activity had formed at 22S. The specific activity of the 22S RNA thus would increase at a slower rate than that of 28S RNA. These data are also consistent with the hypothesis that the 22S RNA is a result of the in vivo turnover of old 28S RNA. The 60S region of a ribosome gradient contains about 10% of the total cytoplasmic 28S and 22S RNA. It is possible that the 22S RNA in the 60S region of a ribosome gradient is different from the 22S in the other regions of the gradient, although we have no data which suggest that more than one class of 22S RNA exists in HeLa cytoplasm.

DISCUSSION

The experiments reported here indicate that the 22S RNA of HeLa cells is not an artifact of isolation procedures, since neither the methods used for breaking the cell nor extracting the RNA significantly altered the relative proportion of this component. A 22S RNA has been observed in the cytoplasm of L cells by use of identical isolation procedures. We have concluded that the 22S RNA is a real constituent of HeLa and L cell cytoplasm.

Our data also show that the 22S RNA is similar to the 28S RNA by all but one criterion employed, i.e., sedimentation rate. The two RNAs have similar base compositions (Table I), similar base sequences (Table II), and similar methyl contents (Table III). Since 22S RNA behaves like 28S RNA in hybridization-competition experiments, we have concluded that the 22S RNA is in fact 28S RNA fragmented into a number of polynucleotides which sediment at 22S and contain

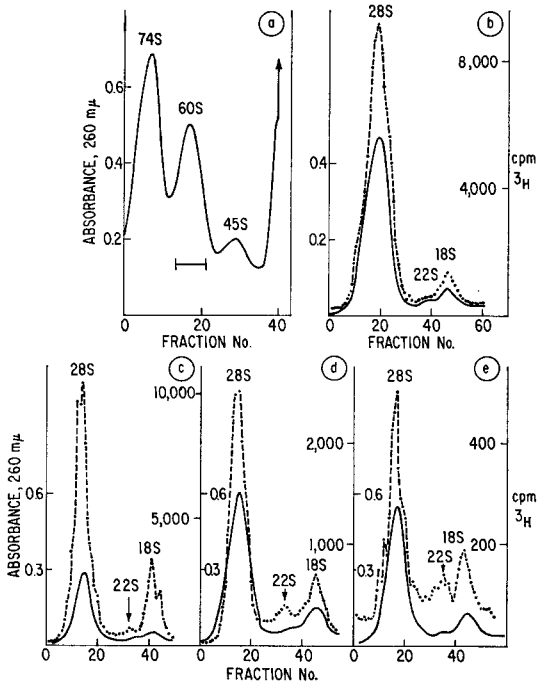

FIGURE 4 The short-term labeling of 22S RNA. To a 120 ml culture of HeLa cells was added 20 μCi of uridine-^{3}H; 40-ml samples were taken at 6 hr and 30 hr, and cytoplasms were prepared. At 30 hr an equal volume of fresh medium was added to the remaining 40 ml, and the cells were allowed to grow an additional 114 hr with the cell concentration doubling each 24 hr. Samples of cells were taken at 60 hr and 144 hr. All cytoplasms were prepared with Triton X-100 and layered onto 37 ml, 5–20%, w/w, sucrose gradients prepared in 0.01 M Tris, pH 7.2, 0.01 M NaCl, 0.0015 M $MgCl_2$. Centrifugation was at 4°C, 16 hr, 18,000 rpm, in the Spinco SW27 rotor. The RNA in the 60S region was precipitated with ethanol and analyzed on a sucrose-SDS gradient as described in Fig. 1 *c*. Fractions of 0.5 ml were collected. *a*. Isolation of 60S ribosomal subunit. *b*. RNA from 60S subunit after 3 hr. *c*. RNA, 60S subunit after 30 hr. *d*. RNA, 60S subunit after 60 hr. *e*. RNA, 60S subunit after 144 hr. OD 260 mμ —; Radioactivity, ^{3}H, ●– – –●.

collectively all the sequences of the 28S. We cannot exclude the possibility that the 22S is solely a conformational variant of the 28S. We have referred throughout this paper to 22S RNA, but we recognize that the RNA sedimenting at 22S may not be one homogeneous polynucleotide chain.

The kinetics of labeling with radioactive uridine indicate that the 22S RNA is labeled at a slower rate than the 28S RNA. This is reflected in the short- and long-term specific activity data (Table IV, Fig. 4) obtained by exposing the cells to radioactive uridine for a period of time and then allowing cell growth to continue (chase) for various periods of time before analysis of the cytoplasmic RNA. We believe that these data suggest that the 22S RNA is a product of in vivo turnover of the 28S RNA in HeLa cytoplasm and is not a direct product of transcription. Moreover, the cell selects for degradation only the old 28S RNA. The old 28S in these experiments is the 28S that is radioactively labeled. Alternatively, the 22S may be the result of fragmentation of old 28S after extensive participation in translation with turnover starting at the 22S. It is impossible at present to experimentally distinguish between the two mechanisms. It is suggested that the amount of 22S in HeLa cytoplasm represents its steady-state concentration with "newly-formed" 22S entering the pool and 22S leaving the pool by degradation at all times. We have not searched for fragments of 28S with S values less than 22.

That ribosomal RNA turnover is slow in animal cells in culture has already been demonstrated (15). A half-life of 4–6 days for ribosomal RNA has been reported for nonproliferating liver cells (16). Our present data do not allow us to calculate a half-life for 28S RNA in HeLa cells.

The 22S is associated with structures in HeLa cytoplasm which cosediment with those ribosomal structures that contain the 28S RNA. The simplest explanation is that the 22S resides in the larger ribosomal subunit. Whether the larger subunit containing only 22S can participate in translation is unknown at present.

Further studies on the origin and degradation of the 22S RNA may provide insight into the role and mechanism of turnover of ribosomal RNA. Physical and chemical studies on the 22S may provide useful information concerning the structure of the 28S RNA.

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