Intracellular Distribution of Low Molecular Weight RNA Species in HeLa Cells

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ABSTRACT   Intracellular distributions of the low molecular weight RNA species of HeLa cells were determined by a nonaqueous method of cell fractionation, in which lyophilized cells were homogenized and centrifuged in anhydrous glycerol. The nonaqueous method was used to avoid artifactual extraction of weakly bound nuclear RNA during cell fractionation.

We found that the mature small RNA species K, A, C, and D were almost entirely (>95%) nuclear, and that mature 4S tRNA was partially (5–10%) nuclear. Our results gave higher nuclear content of the mature species K, A, C, and 4S than was shown previously with conventional aqueous cell fractionation.

The nonaqueous method also gave higher nuclear proportions of some short-lived precursors to mature small RNAs. We found that approximately one-half of recently synthesized pre-4S RNA and more than one-half of recently synthesized 5S RNA were nuclear, whereas these species had been thought to be cytoplasmic from previous work. The species C' and D', precursors to the stable nuclear species C and D respectively, were found to be partially nuclear, also in contrast to earlier work. The stable cytoplasmic species L (oncornavirus 7S RNA) was found to be mostly nuclear shortly after synthesis.

Mammalian cells contain at least 13 electrophoretically distinct, long-lived, abundant, small RNA species of sizes between 75 and 300 nucleotides (recently reviewed in references 10, 15, 25, and 41). The small RNAs include three cytoplasmic classes: the 4S transfer RNAs, the 5S ribosomal RNA, and the 7S RNA of retroviruses (which has been called L RNA).[1] The other small RNA species are partially or entirely nuclear. Of the nuclear species, A has been found in isolated nucleoli and the others in isolated nucleoplasm (41). Two of the nucleoplasmic species, C and D, have cytoplasmic precursors (5, 7, 8, 41), which in turn are probably derived from much larger nuclear initial transcripts (6). The species A, C, and D have 5' cap structures similar to those of messenger RNAs (28, 29, 34). A small fraction of C, D, and G' may be covalently linked to components of chromatin (26). The species C, D, and H have been found in nuclear RNA-protein complexes (3, 11, 12, 16,

[1] *Abbreviations used in this paper:* Small RNA species A, C, D, F, G', H, K, and L refer to the nomenclature of Weinberg and Penman (38), and species C' and D' to that of Zieve et al. (40). The species U1, U2, and U3, in the nomenclature of Busch and colleagues (31), refer to D, C, and A, respectively. PBS denotes physiological isotonic phosphate-buffered saline; PPO denotes 2,5-diphenyloxazole; and rRNA denotes any or all of the 5S, 18S, 28S, or 45S ribosomal RNA species.

33). Recently, it has been observed that the species C and D contain nucleotide sequences that might function in the splicing of nuclear RNA in the formation of messenger RNA (22, 32). Except for 4S transfer RNA, however, the functions of the small RNAs are not yet known.

To evaluate evidence for the functions of the small RNAs, we must know with confidence the nuclear-cytoplasmic partitions of the RNAs. The partitions have been determined to date by conventional aqueous cell fractionation, which may give artifactual extraction of weakly bound nuclear RNA into the cytoplasmic fraction. Indirect evidence for the extraction of nuclear small RNA during fractionation was given by Lönn (24) who showed that the precursors to 4S RNA and newly synthesized 5S RNA of the insect *Chironomus tentans* were nuclear when examined by anhydrous cytological fixation and manual dissection, whereas the two species had appeared to be cytoplasmic by conventional aqueous cell fractionation. The relevance of Lönn's observations on the giant polytene cells of *Chironomus* to the much smaller mammalian cells is not known.

To avoid the above objections to conventional cell fractionation, we have now reexamined the nuclear-cytoplasmic distributions of the small RNAs of HeLa cells, using an alternate bulk nonaqueous cell fractionation technique. The nonaqueous

technique employs quick-freezing of cells, lyophilization, homogenization of the dried cells in anhydrous glycerol, and finally sedimentation of nuclei from the glycerol homogenate (13, 18). The same method has been used to show that DNA polymerase-α, formerly thought to be cytoplasmic, was actually weakly bound to the nuclei of cultured mouse and human cells (9). In this report, we show that seven species were more nuclear by nonaqueous fractionation than had been found previously with aqueous fractionation. We extended the observations of Lönn, mentioned above, to HeLa cells, among other findings. Our conclusions for two species, C and D, differ from those recently reported by Frederiksen and Hellung-Larsen (10), who used an alternate method of nonaqueous fractionation.

MATERIALS AND METHODS

Cell Culture

HeLa S-3 cells (27) and a monolayer HeLa strain used in adenovirus research (39) were both grown in monolayers in Dulbecco's modification of Eagle's medium plus 10% bovine serum to a density of 2×10^7 cells per 100-mm petri plate. Some cultures were labeled for 15 or 40 min with [5-^{3}H]uridine (20 μCi/ml, 25 Ci/mmol, 2 ml per plate in fresh growth medium) and were harvested immediately, or else were incubated further for 11 h or 24 h after addition of 15 ml of fresh medium per culture. Other cultures were labeled for 15 or 40 min with [methyl-^{3}H]methionine (65 μCi/ml, 13 Ci/mmol, 2 ml per plate, in growth medium lacking nonradioactive methionine).

Nonaqueous Fractionation

A more detailed description of the procedure has been published elsewhere (13). Two to four cultures were chilled to 2°C with cold PBS and then were treated with 2 ml of 100 μg/ml crystalline trypsin in PBS for 5 min at 2°C. The trypsin was neutralized by a brief rinsing with 2 ml of soybean trypsin inhibitor in PBS (20 μg/ml) at 2°C. After being rinsed twice more with PBS at 2°C, the cells were detached from the petri plates by gentle pipetting and were sedimented (2,000 g, 2°C, 1 min). The pellet of cells was resuspended at 2°C in two pellet volumes of hypotonic phosphate (10 mM sodium phosphate, pH 7.0). The cells were frozen within 5 min of resuspension by dripping the concentrated hypotonic suspension into melting Freon 12 (dichlorodifluoromethane) (−158°C). The frozen cells were then lyophilized (3 torr, −28°C, 18 h). Dried cells were suspended at 2°C in spectral-grade glycerol and homogenized by a 1-cm rotating blade at 6,000 rpm for 15 min. The homogenate was then centrifuged (140,000 g, 0°C, 3 h), and the nuclear pellet and cytoplasmic supernate were separated at 2°C. The cell fractions were stable for several months at −35°C.

Purification of RNA from Cell Fractions

We precipitated RNA from the nonaqueous supernatant fractions by diluting glycerol to 20% (vol/vol) in 0.15 M sodium acetate, 1 mM EDTA, pH 7.0, and then adding ethanol to 70% (vol/vol). After chilling at −20°C for 18 h, the precipitated crude RNA was recovered by sedimentation (10,000 g, 0°C, 30 min).

The nonaqueous nuclear pellets and the precipitated material from the nonaqueous cytoplasmic supernate were suspended separately in 30 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.3% (wt/vol) SDS, 100 μg/ml self-digested pronase (17) at 5 ml per 2×10^7 cell equivalents. The mixtures were first incubated at 37°C for 30 min and then extracted at 23°C for 2 min with an equal volume of 1:1 mixture of liquified water-saturated phenol and chloroform. The aqueous phases of the emulsions were adjusted to 0.15 M sodium acetate and then to 70% (vol/vol) ethanol to precipitate the nucleic acids (−20°C, 18 h). The precipitated nucleic acids were recovered by sedimentation (10,000 g, 0°C, 30 min) and dissolved at 2°C in 2 ml of 0.2 M sodium acetate, pH 7.0, 10 mM MgCl$_2$ per 2×10^7 cell equivalents. The dissolved nucleic acids were digested at 2°C for 60 min with 10 μg/ml bovine pancreatic DNase I that had been treated with iodoacetate to inactivate contaminating ribonuclease activity (42). The mixtures were then adjusted to 100 μg/ml self-digested pronase (17), 10 mM EDTA, 0.3% SDS, pH 8.0, incubated, and extracted with phenol-chloroform as described above. After ethanol precipitation as above, the purified RNA from 2×10^7 cell equivalents was dissolved in 50 μl of 10 mM triethanolamine-HCl, pH 7.4, 1 mM EDTA, 0.5% SDS, and was stored at −20°C.

Electrophoresis, Fluorography, and Quantitation of Small RNA

RNA was electrophoresed in 6–15% polyacrylamide linear gradient slab gels (Fig. 1) according to the methods of Studier (35) with the modifications of Zieve and Penman (41). Alternatively, RNA was electrophoresed in 10% polyacrylamide slab gels according to the method of Loening (23). The dimensions of the 10% polyacrylamide slab were $14 \times 20 \times 0.12$ cm. Electrophoresis was in the long dimension (20 cm) for 10–12 h at 90 V. After electrophoresis, the gels were prepared for fluorography by impregnation with the fluor PPO in dimethyl sulfoxide, according to the methods of Bonner and Laskey (1) and Laskey and Mills (20). Impregnated gels were used to expose prefogged Kodak XR X-ray film at −80°C for 1– 40 d. The developed X-ray film was photographed with low-contrast film (Kodak Panatomic-X) and printed on low-contrast paper (Kodak RC-soft).

RNA was also electrophoresed in tandem cylindrical gels to quantitate large RNAs (45S, 28S, 18S) and small RNAs (C, D, 5S, 4S) simultaneously in a single preparation. The cylindrical gels consisted of 8 cm of 2.4% polyacrylamide (to resolve large RNAs) over 12 cm of 10% polyacrylamide (to resolve small RNAs). The electrophoresis, slicing, and liquid scintillation counting were carried out as described previously (4, 5).

RESULTS

Fractionation

To test fractionation of well-described RNA species under our experimental conditions, HeLa cells were labeled for 11 h with [^{3}H]uridine and fractionated. RNA extracted from whole cells and from the nuclear and cytoplasmic cell fractions was electrophoresed in 2.4%–10% polyacrylamide tandem cylindri-
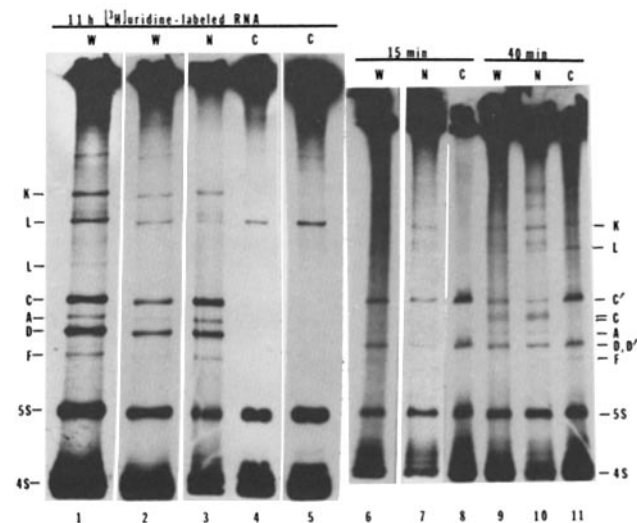

FIGURE 1 6–15% polyacrylamide gradient gel resolution of low molecular weight RNAs extracted from whole cells and nonaqueous cell fractions. Each lane contained RNA from cells labeled with [^{3}H]uridine for 11 h, 15 min, or 40 min. The figure is a composite of several film exposures. (1) Whole-cell (W) RNA labeled for 11 h. (2) Whole-cell RNA labeled for 11 h; shorter film exposure to show large species. (3) Nuclear (N) RNA labeled for 11 h. (4 and 5) Cytoplasmic (C) RNA labeled for 11 h. (6) Whole-cell RNA labeled for 15 min. (7) Nuclear RNA labeled for 15 min. (8) Cytoplasmic RNA labeled for 15 min. (9) Whole-cell RNA labeled for 40 min. (10) Nuclear RNA labeled for 40 min. (11) Cytoplasmic RNA labeled for 40 min. Lanes 1–5 are from one gel electrophoresis; lanes 6–11 are from another one. In Figs. 1, 3, and 4, the number of cell equivalents in gel tracks from the same cell harvest are not necessarily similar. For example, there are considerably more cell equivalents of cytoplasmic RNA in lane 11 than cell equivalents of nuclear RNA in lane 10 (in comparison to the number of cell equivalents of whole-cell RNA in lane 9).

cal gels. The radioactivities of the RNA species 45S, 18S, 5S, 4S, C, and D from a typical experiment are shown in Table I.

In computing the extent of fractionation we have assumed that 45S rRNA was 100% nuclear and that 18S rRNA was ~1.2% nuclear (38). Our nuclear cell fraction contained 15% of the cellular 18S rRNA labeled in an 11-h exposure of cultured cells to [³H]uridine, and the cytoplasmic fraction contained no detectable 45S rRNA of the same cells. By comparison with the aqueous fractionation (38), we conclude that the cytoplasmic fraction described in Table I did not contain detectable nuclear material (<1%) and that the nuclear fraction contained 14% of the cytoplasmic material of the cells. The results of Table I are typical of several fractionations of HeLa cells.

Our cells were used in rapidly dividing state, ~4% being mitotic cells without nuclear membrane. The majority of these mitotic cells were broken during homogenization, and their nuclear low molecular weight RNAs would then contaminate the cytoplasmic fraction (30).

Distributions of the Small RNA Species

K RNA: Using aqueous fractionation, Zieve and Penman (41) found that after 10 min of labeling, K RNA was totally cytoplasmic; after 90 min, K RNA was 10% nuclear; and after 18 h, K RNA was 60–95% cytoplasmic. In contrast, we found that K RNA was almost exclusively nuclear in both short- (15 min) and long-term (40 min) labeling (Figs. 1 and 2).

L RNA: L RNA (or oncornavirus 7S RNA) is found both in the virion of oncornaviruses and in the cytoplasm of mammalian cells (36, 41). In nondenaturing gel electrophoresis, it appears as a major band plus a family of faster-running conformers (41). We found that the main L RNA band was mostly nuclear after 15 min of labeling, about half nuclear after 40

min of labeling, and mainly, or exclusively, cytoplasmic after long-term incubations with [³H]uridine (Figs. 1 and 2). After long-term labeling, a minor nuclear species of unknown origin comigrated with the main L RNA band in gel electrophoresis (Figs. 1 and 2).

A RNA (OR U3 RNA): Zieve and Penman (41) found A RNA only in the aqueous cytoplasmic fraction during the first 15 min of labeling, then found equal amounts in the cytoplasmic and nuclear fractions after 2 h of labeling, and finally found >90% of A RNA in the nucleolus after 16 h of labeling. In contrast, using nonaqueous fractionation, we found A RNA
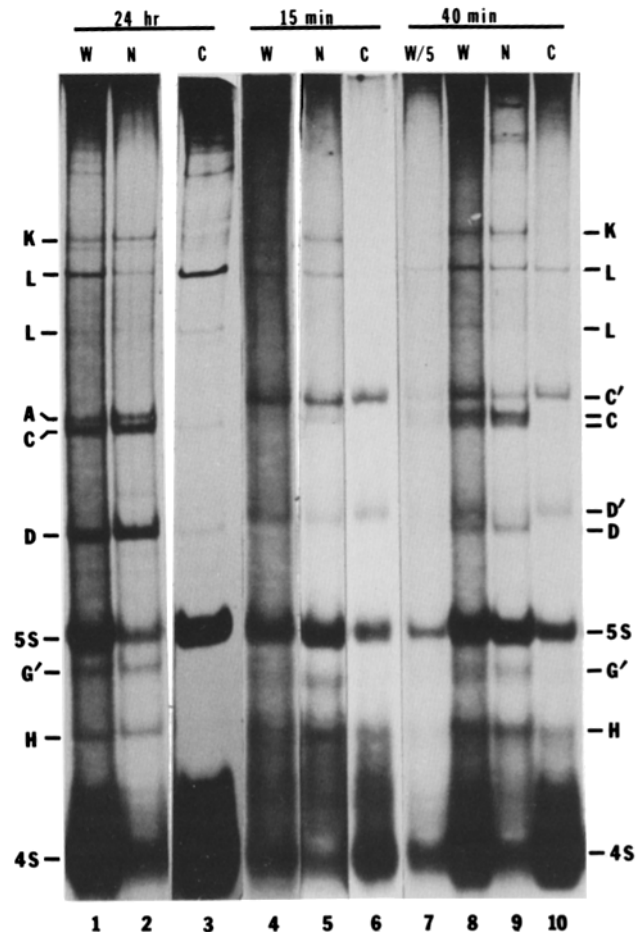

FIGURE 2 10% polyacrylamide gel resolution of small RNAs from nonaqueous cell fractions. Samples were prepared as in Fig. 1; the 15-min and 40-min samples are from the same preparation as in Fig. 1. The figure is a composite of 2-d, 4-d, 6-d and 11-d film exposures, all from the same slab gel. (1) Whole-cell RNA labeled for 24 h. (2) Nuclear RNA labeled for 24 h. (3) Cytoplasmic RNA labeled for 24 h. (4) Whole-cell RNA labeled for 15 min. (5) Nuclear RNA labeled for 15 min. (6) Cytoplasmic RNA labeled for 15 min. (7) Whole-cell RNA labeled for 40 min, one-fifth as much as 8. (8) Whole-cell RNA labeled for 40 min, five times as much as 7. (9) Nuclear RNA labeled for 40 min. (10) Cytoplasmic RNA labeled for 40 min. We assume that the number of cell equivalents in lane 6 (cytoplasmic RNA labeled for 15 min) is comparable to that in lane 4 (whole cell RNA labeled for 15 min), because the level of labeled 4S RNA of mature size (a predominantly cytoplasmic species) is similar in both gel lanes. Because the main L RNA band cannot be seen in lane 6, this species should be mostly nuclear after 15 min of labeling. Therefore, we also assume that the number of cell equivalents in lane 5 (nuclear RNA labeled for 15 min) approximates that in lane 4, because the level of the labeled main L RNA band is similar in both gel lanes.

TABLE I

Subcellular Levels of Long-labeled Low Molecular Weight RNA Species: Quantitation by Liquid Scintillation Counting

Experi-ment	RNA species	Whole cell	Nuclear fraction	Cytoplasmic fraction
			Radioactivity, cpm	
1*	45S	3,000	3,000*	0
	18S	49,7000	7,600*	49,700*
	5S	3,880	1,330*	—
	4S	32,130	6,950*	30,600*
2‡	C		1,960	19
	D		2,360	0

RNA was extracted from cells labeled for 11 h with [³H]uridine and analyzed by electrophoresis on 2.4–10% polyacrylamide tandem cylindrical gels, which were sliced and counted by liquid scintillation. Radioactivity was summed over the peaks representing the species of interest.

* The nuclear radioactivities were normalized to a complement of 45S rRNA of 3,000 cpm, which was the measured 45S radioactivity in the whole cell sample. The cytoplasmic radioactivities were likewise normalized to an 18S rRNA radioactivity of 49,700 cpm measured in the whole cell sample. If ~1.2% of the cellular 18S rRNA is nuclear (38), in this experiment the cytoplasmic contamination of the nuclear preparation was ~14%. The radioactivity found in 4S RNA in the nuclear fraction (6,950 cpm) minus 14% cytoplasmic contamination (4,280 cpm) equals 2,670 cpm; that is, ~8% of the cellular 4S RNA would be nuclear. The radioactivity detected in 5S RNA in the nuclear fraction (1,330 cpm) minus 14% cytoplasmic contamination (420 cpm) gives 910 cpm; therefore, ~23% of the cellular 5S RNA appears to be nuclear. (RNA species G′ was not separated from 5S rRNA in these gel electrophoreses; however, the HeLa cell content of 5S rRNA is ~27-fold higher than G′ RNA (37, 38).

‡ Radioactivities were normalized as above, to 6,000 cpm of 45S rRNA and 193,000 cpm of 28S rRNA found in the whole cell RNA sample of this experiment.

to be nuclear at all times of labeling, 15 and 40 min, and 11 and 24 h (Fig. 1). A RNA exhibited the same difference in electrophoretic mobility relative to C RNA in the two electrophoresis systems (Figs. 1 and 2) as reported by Zieve and Penman (41).

C RNA (OR U2 RNA) AND ITS PRECURSORS: Zieve and Penman (41) reported that 25–40% of mature C RNA appeared in the aqueous cytoplasmic fraction. By contrast, we found that essentially no C RNA could be detected in the nonaqueous cytoplasmic fraction (Table I; Fig. 1, lanes 4 and 5). Long exposures of the cytoplasmic fraction (Fig. 2, lane 3) showed faint bands at the mobilities of C and D RNA, but we believe that the origin of this "cytoplasmic" C and D RNA was broken mitotic cells that were present at frequencies between 1% and 5% in homogenates of our unsynchronized cultures. Our results do not agree with those of Frederiksen and Hellung-Larsen (10), who reported that rat liver C and D were 40–60% cytoplasmic, using another method of nonaqueous fractionation.

The dimeric appearance of C RNA after short-term labeling (Figs. 2 and 3) has been noted previously (37, 41), with the slower component of the doublet being called B (37).

The distribution of C' RNA, a precursor to C RNA (8) was reexamined by use of nonaqueous fractionation and the superior resolution afforded by long-slab gel electrophoresis. Based on the data given in the legend to Fig. 2, lanes 4, 5, and 6 of Fig. 2 represent similar numbers of cell equivalents. The radioactivity of C' RNA in the nuclear fraction (Fig. 2, lane 5) was approximately equal to the radioactivity of C' in the cytoplasmic fraction (Fig. 2, lane 6). The rather high level of nuclear C' radioactivity could not be attributed to cytoplasmic contamination of nuclei, as the level of contamination measured as described above was only 14%. We therefore believe that nuclei contain a substantial fraction (30–50%) of C' RNA.

D RNA (OR U1 RNA) AND ITS PRECURSORS: D RNA was essentially all nuclear, as was C RNA. Traces of D RNA radioactivity in the cytoplasmic fraction (Fig. 2, lane 3) could be explained as arising from broken mitotic cells, as discussed above for C RNA.

We reexamined the distribution of D' RNA, the precursor to D RNA (8), by nonaqueous fractionation and long-slab gel electrophoresis. The electrophoresis has resulted in better resolution of D and D' RNAs than available previously. As with the case of C' RNA, some nuclear D' RNA radioactivity could be detected in short-term-labeled material, and the level of nuclear D' RNA radioactivity was higher than expected from known contamination of nuclear RNA with cytoplasmic material (Fig. 2, lane 5; Fig. 3, lane 3).

5S rRNA: 5S RNA is the nonmethylated small RNA species that is present in the large subunit of cytoplasmic ribosomes. In some gel electrophoresis systems, 5S rRNA has a mobility similar to that of the nuclear methylated RNA species G' (also called 5S RNAIII) (31). In 10% polyacrylamide gels (Figs. 2 and 3) G' RNA and 5S rRNA were well separated and we found that about one-fourth of the uniformly labeled cellular 5S rRNA was nuclear after nonaqueous fractionation (Table I), in agreement with the aqueous fractionation values of Knight and Darnell (19). Weinberg and Penman (38) estimated that only 10% of mature 5S RNA was nuclear. After 15 min of incubation with [³H]uridine, essentially all of the labeled cellular 5S rRNA was found in the cytoplasmic fraction after aqueous cell fractionation (21), whereas the majority was in the nuclear fraction after nonaqueous fractionation (Fig. 2). A large fraction of short-term-labeled 5S RNA could be removed

from nonaqueous nuclei by rinsing with an aqueous buffer (not shown).

A precursor of mammalian 5S rRNA has been detected that is eight nucleotides longer than the mature form at the 3' end (14). Gel electrophoresis in the presence of 7 M urea showed that after 15 min of labeling, cytoplasmic 5S rRNA and nuclear 5S rRNA were both just slightly larger than cytoplasmic 5S rRNA from cells labeled for 24 h (not shown).

G' RNA (OR 5S RNA III): G' RNA is methylated (38) and has a very different base sequence from that of cytoplasmic 5S rRNA (31). In agreement with aqueous fractionation data (41),
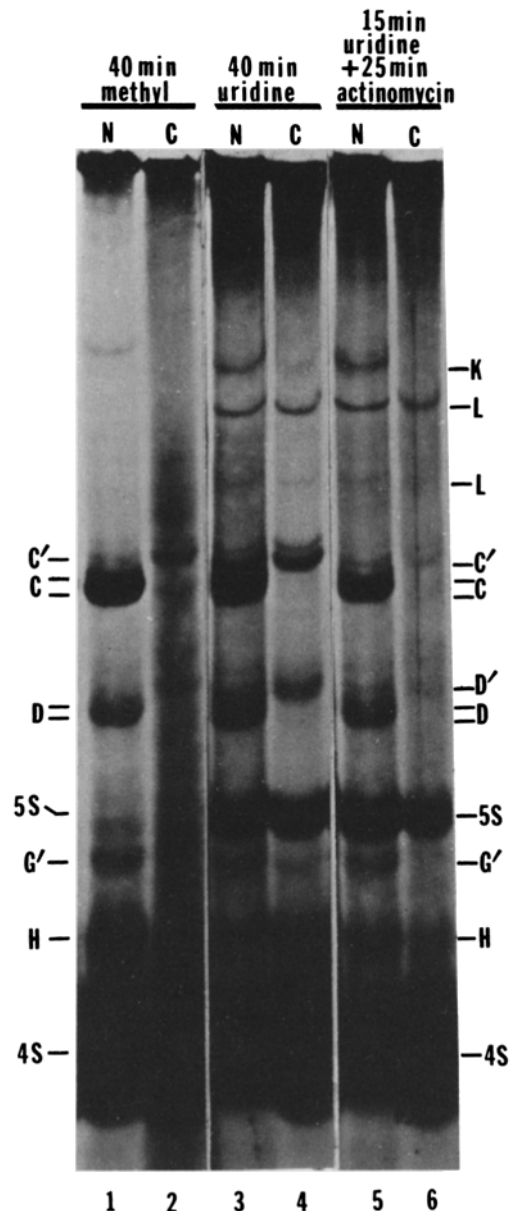

FIGURE 3 Distributions of methylated species and of unstable species. (1) Nuclear RNA labeled for 40 min with [methyl-³H]methionine. (2) Cytoplasmic RNA labeled for 40 min with [methyl-³H]methionine. (3) Nuclear RNA labeled for 40 min with [5-³H]uridine. (4) Cytoplasmic RNA labeled for 40 min with [5-³H]uridine. (5) Nuclear RNA labeled for 15 min with [5-³H]uridine and then chased for 25 min with 5 μg/ml actinomycin D. (6) Cytoplasmic RNA labeled and chased as in 5. All lanes are from the same gel slab.

this species was nuclear by nonaqueous fractionation (Fig. 2). Fig. 3 shows the resolution of the methylated G′ RNA ([methyl-³H]methionine labeling in lane *1*) from the nonmethylated 5S rRNA ([³H]uridine labeling in lane *3*).

PRECURSORS TO 4S RNA: We assume that the briefly labeled RNA species migrating in gel electrophoresis between H RNA and mature 4S RNA are mostly 4S RNA precursors (Fig. 2). (The basis for this assumption has been reviewed by Burdon [2]). After aqueous fractionation, most, if not all, of the population of apparent 4S RNA precursors is found in the cytoplasm (2). In contrast, perhaps as much as half of the 4S RNA precursors were present in nuclei prepared by the non-aqueous method (Fig. 2, lanes *4–6*). The majority of the presumptive pre-4S nuclear RNA could be removed from the nuclei by washing in an aqueous buffer (not shown).

4S RNA: Nuclei prepared in aqueous medium are esentially free of mature 4S RNA. For example, Weinberg and Penman (38) estimated their level to be below 0.2% of the total cellular 4S RNA content. Instead, by nonaqueous fractionation, ~8% of the cellular 4S RNA appeared to be present in the nuclear fraction (Table I).

OTHER RNA SPECIES: F RNA and H RNA (41) were found to be nuclear, in agreement with aqueous fractionation (41).

After long-term (11 or 24 h) labeling, we detected three or more cytoplasmic species migrating more slowly than K RNA (Figs. 1 and 2). After 40-min [³H]uridine labeling, we detected two nuclear species that comigrated with two of these long-term-labeled cytoplasmic species (Fig. 1). After 40 min [methyl-³H]methionine labeling, we detected a minor methylated nuclear species that migrated slightly more slowly than K RNA (Fig. 3, lane *1*).

We detected a nuclear species of mobility between K and L RNA, which was detected after both short-term and long-term labeling. This species was not detectably methylated.

A minor cytoplasmic species was observed migrating slightly more slowly than C′ in short-term labeled material (Fig. 3, lane *4*). Long film exposures showed that this species was methylated (not shown).

A minor nuclear methylated species was observed migrating between 5S RNA and G′ RNA (Fig. 3, lane *1*).

The possibility exists that some of the minor species not previously described could be conformers of other species.

Refractionation Experiment

To examine the possible redistribution of small RNAs during nonaqueous fractionation, nuclei from cells labeled for 40 min were first isolated by the methods given above, then suspended again in glycerol, rehomogenized, and centrifuged as in the first fractionation. Samples of RNA from whole cells, once-fractionated nuclei, cytoplasm, twice-fractionated nuclei, and the glycerol supernate from the second centrifugation were electrophoresed on a 10% polyacrylamide gel slab. The patterns of radioactivities from nuclei once- and twice-fractionated were essentially identical (not shown). The glycerol supernate from the second fractionation contained 2.2% of the radioactivity of twice-sedimented nuclei and gave an electrophoretic pattern essentially identical to that of the cytoplasmic fraction after the first centrifugation. This experiment therefore showed that the species identified as nuclear by the first fractionation sedimented again with nuclei in the second fractionation.

DISCUSSION

Nonaqueous fractionation showed that most of the newly synthesized 5S rRNA and about one-half of the 4S RNA precursors were nuclear in HeLa cells. Lönn (24) has shown by other methods that essentially all newly synthesized 5S rRNA and 4S RNA precursors of polytene insect cells were nuclear. We have no direct evidence to address the possibility of partial elution of 5S rRNA and 4S RNA prescursors, but we can say from the refractionation experiment above that the RNA of isolated nuclei was not eluted further during a second round of nonaqueous manipulations. The evidence available therefore supports two interpretations: either the fractionation results represent actual distributions of 5S rRNA and 4S RNA precursors in HeLa cells; or there exist two populations of nuclear 5S rRNA and 4S RNA precursors, one of which can be eluted into the cytoplasmic supernate in nonaqueous fractionation.

Some RNA species were more nuclear than has been reported previously by aqueous fractionation. These included long-term-labeled RNA species K, A, C, and D and newly synthesized L, C′, and D′. Our results differ from those of Frederiksen and Hellung-Larsen (10), who observed, by use of an alternate non-aqueous method, that 40–60% of C and D from rat liver was cytoplasmic. We cannot explain the disagreement except to suggest that the differences in biological material or in methods may have been responsible.

It is estimated from our nonaqueous fractionation data that ~8% of the total cellular 4S RNA may be nuclear. This value represents ~8 × 10⁶ molecules of 4S RNA per HeLa cell nucleus (38). Because of this result, the possibility of a nuclear function of 4S RNA should not be ruled out. It should be noted also that our estimations of the nuclear content of 4S and 5S RNA depend on the assumption that ~1.2% of the total cellular 18S rRNA is nuclear (38). The latter value was obtained by aqueous cell fractionation, which tends to give artificially low nuclear concentrations of various in vivo nuclear components. If the nuclear level of 18S rRNA in the living cell were actually higher, our estimates of nuclear content of 4S and 5S RNA would be also higher.

Several subcellular distribution patterns are found during the processing of the various small RNA species. The majority of the nuclear RNA species (A, K, H, and G′) remain in the nucleus of the interphase cell at all times, even within minutes after their transcription. The exceptions seem to be nuclear RNA species C and D, which are found briefly in the cytoplasm shortly after their synthesis. Some newly made cytoplasmic RNAs (e.g., L and 5S RNA) have a fairly long nuclear phase before appearing in the cytoplasm, whereas 15 min after addition of [³H]uridine most of the labeled 4S RNA is already of mature size and cytoplasmic.

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