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SUBCELLULAR LOCATION OF PRECURSORS TO SMALL NUCLEAR RNA SPECIES C AND D AND OF NEWLY SYNTHESIZED 5 S RNA IN HELA CELLS

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SUMMARY: The nuclear-cytoplasmic partition of newly synthesized C, D and 5 S RNAs of HeLa cells was studied with aqueous and non-aqueous methods of cell fractionation. The level of briefly labeled 5 S RNA in cytoplasmic fractions prepared by a non-aqueous method is lower than in cytoplasmic fractions obtained by an aqueous method. The reverse is true in nuclear fractions. At the same time, with both aqueous and non-aqueous cell fractionation, the level of briefly labeled precursors to RNAs C and D is similarly high in cytoplasmic fractions and low in nuclear preparations. This suggests that the previously reported finding that RNA species C and D are cytoplasmic during the first minutes of their lifetime, may represent an in vivo phenomenon, instead of an artifactual elution from nuclei during fractionation.

RNA species C and D (nomenclature of Weinberg and Penman (1), or U2 and U1, respectively, in the nomenclature of Busch and colleagues (2)), the most abundant of the small nuclear RNAs, are derived from short-lived cytoplasmic precursors(3). They are transcribed in the nucleus (they hybridize to nuclear DNA (4)), their precursors are quickly found in the cytoplasm, where they remain for a few minutes (3,5,6), and finally they appear in the nucleoplasm, where they are long lived (7).

It is possible that the apparent cytoplasmic location of newly synthesized RNA was derived from nuclear leakage of briefly labeled species C and D during aqueous cell fractionation. The same reservation can be applied also to the cytoplasmic location of newly synthesized 5 S ribosomal RNA (8). We have therefore reexamined the nuclear-cytoplasmic partition of these small RNAs using an alternate non-aqueous fractionation procedure in which lyophilized cells are homogenized and centrifuged in anhydrous glycerol. The non-aqueous method retains soluble weakly-bound nuclear constituents within the nucleus during fractionation. DNA poly-

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merase-α, for example, is quantitatively nuclear by non-aqueous fractionation, and cytoplasmic by aqueous fractionation (9,10).

While the RNA species that turned out to be cytoplasmic precursors to C and D RNA were of unknown identity, they were called A and B respectively, in some of our previous publications (5,11,3). In this report we are calling them C' and D', respectively, adopting the nomenclature of Zieve et al (12).

MATERIALS AND METHODS: Monolayer cultures of HeLa S3 cells were grown at 37°C in 100 mm-diameter plates, in Dulbecco-modified minimum essential medium supplemented with 10% bovine serum. The cells were incubated with [^{14}C]uridine (0.1 μCi/ml, 58 Ci/mol) for 29 hours, and after changing the medium, they were labeled with [^{3}H]uridine (10 μCi/ml, 27 Ci/mmol) for 15 minutes. To obtain pellets of non-aqueous nuclear and cytoplasmic fractions and of whole cells, a portion of the culture was processed as described previously (13). The non-aqueous method gives typically 5-15% cross-contamination of the two fractions (13). For aqueous cell fractionation, another aliquot of the culture was washed and removed from the plates as described before (13), the cells were then resuspended in RSB (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 2 mM $MgCl_2$), adjusted to 1% Triton X-100, broken with a ball homogenizer, and the nuclei were pelleted by centrifugation. The cytoplasmic fractions prepared by aqueous and non-aqueous methods, after being adjusted to 1% SDS, were extracted three times at room temperature with a mixture of one volume of phenol and one volume of a mixture of chloroform and isoamyl alcohol (99:1 ratio). The whole cells and the nuclear fractions obtained by aqueous and non-aqueous methods were treated with deoxyribonuclease and extracted at 55°C with phenol and chloroform, as described by Penman (14). Gel electrophoresis in 9.5 cm-long 10% acrylamide gels was as described before (15).

RESULTS AND DISCUSSION: HeLa cells that had been labeled for 29 hours with [^{14}C]uridine were incubated with [^{3}H]uridine for 15 minutes, separate cultures were then fractionated into nuclei and cytoplasm by aqueous and non-aqueous methods, and their extracted RNAs were finally analyzed by polyacrylamide gel electrophoresis. Fig. 1 shows the electrophoresis patterns of these preparations, and Table 1 lists the number of counts calculated from the various RNA bands in Fig. 1.

The level of newly made 5 S RNA (expressed as the [^{3}H]5 S/[^{14}C]D RNA cpm ratio) was higher in non-aqueous nuclear material than in aqueous nuclear material (ratios of 3.5 vs. 1.3). In agreement with this result, the level of newly synthesized 5 S RNA (expressed as the [^{3}H]5 S/[^{14}C]D RNA cpm ratio) was lower in non-aqueous cytoplasmic material than in aqueous cyto-

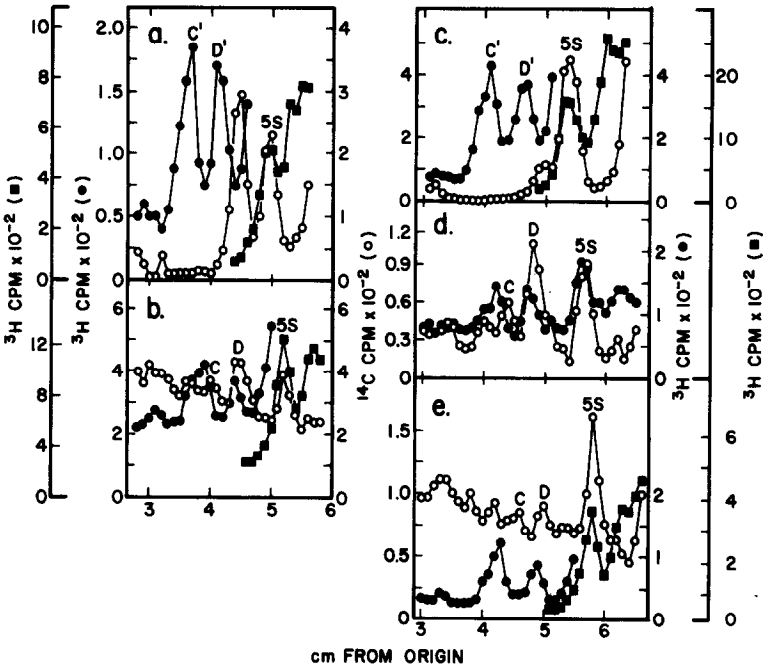

Fig. 1. Polyacrylamide gel electrophoresis patterns of small RNAs from cellular subfractions. The samples are: cytoplasmic (a) and nuclear (b) fractions obtained in a non-aqueous system, cytoplasmic (c) and nuclear (d) fractions prepared by an aqueous method, and whole cells (e). The long-term labeling with [^{14}C]uridine, the 15 minute pulse with [^{3}H]uridine, the cellular fractionations, RNA extractions, and analysis by polyacrylamide gel electrophoresis were all as indicated in Materials and Methods. Both the cytoplasmic precursor species to RNAs C and D, particularly C, are larger (and migrate more slowly in gel electrophoresis) than their nuclear mature counterparts (3). C' and D' refer to the cytoplasmic precursors of nuclear RNAs C and D, respectively (nomenclature of Zieve et al (12)). The ^{14}C-labeled peak between D' and 5 S in (a) is 5.8 S ribosomal RNA, produced by denaturation of 28 S ribosomal RNA during handling of the non-aqueous cytoplasmic fraction. The nuclear contamination of cytoplasmic preparations was estimated from their level of ^{3}H-labeled 45 S ribosomal RNA precursor. The cytoplasmic contamination of nuclear preparations was determined from their content of ^{14}C-labeled 18 S ribosomal RNA. The cytoplasmic contamination of nuclear fractions prepared by the non-aqueous method was below 10%. The cytoplasmic contamination of nuclear fractions prepared in the aqueous system, and the nuclear contamination of the cytoplasmic preparations obtained with aqueous and non-aqueous procedures, were negligible. (■——■) ^{3}H (contracted scale); (●——●) ^{3}H (expanded scale); (o——o) ^{14}C.

plasmic material. Using aqueous fractionation of HeLa cells, Leibowitz et al (8) found that, after 15 minutes of labeling with [^{3}H]uridine most of the labeled 5 S RNA was in the cytoplasm. Our results indicate that with non-aqueous cell fractionation the nuclear/cytoplasmic proportion of

TABLE 1. DISTRIBUTION OF SMALL RNAS IN CELLULAR SUBFRACTIONS

	Radioactivity (expressed in cpm)									
	[^{3}H]C	[^{3}H]D	[^{3}H]5S	[^{14}C]D	[^{14}C]5S	$\frac{[^3H]D}{[^{14}C]D}$	$\frac{[^3H]5S}{[^{14}C]D}$	$\frac{[^3H]C}{[^{14}C]5S}$	$\frac{[^3H]D}{[^{14}C]5S}$	$\frac{[^3H]5S}{[^{14}C]5S}$
Cytoplasm (non-aqueous)	404	256	506		513			0.79	0.50	0.99
Nuclei (non-aqueous)	*	186	1,480	420	326	0.44	3.5			
Cytoplasm (aqueous)	1,040	623	3,340		1,400			0.74	0.44	2.4
Nuclei (aqueous)	183	175	245	189	220	0.93	1.3			
Whole cell	217	113	536	39	179					

These numbers were taken from the gel electrophoresis patterns in Fig. 1.
* The radioactivity in the ^{3}H-labeled RNA species C of this gel could not be estimated due to its poor resolution.

briefly labeled 5 S RNA is higher than with aqueous methods. This suggests that the high cytoplasmic level of briefly labeled 5 S RNA in aqueous cytoplasm is derived at least partially from extraction of weakly-bound nuclear 5 S RNA into the cytoplasmic supernatant during aqueous fractionation. Our finding of partial cytoplasmic location of recently labeled mammalian 5 S RNA differs from insect cells, where Lönn recently found exclusive location of briefly labeled 5 S RNA in non-aqueous nuclear preparations (16). However, we cannot exclude the possibility that newly synthesized mammalian 5 S RNA may be partially eluted from the nucleus despite use of a non-aqueous procedure.

In contrast to our results for 5 S RNA, we found that the level of briefly-labeled species C and D was largely cytoplasmic to similar extents in both fractionation methods. In fact, the level of briefly-labeled [^{3}H]D, normalized to long-term labeled nuclear[^{14}C]D, was somewhat lower in non-aqueous nuclei than in aqueous nuclei (Table 1).

It should be noted that the radioactivity counts of the three RNA species in question in Table 1 do not reflect their mass ratios in the cell. According to data of Weinberg and Penman (1) the steady state mass ratio of cytoplasmic 5 S RNA/nuclear RNA D is about 4 in HeLa cells. Using this correction factor and also correcting for approximately 10% cytoplasmic

contamination of the nuclear preparations, it can be estimated from the data in Table 1 that the cytoplasmic/nuclear ratio of ^{3}H-labeled RNA D in non-aqueous systems was about 8-9 in this experiment. As the cytoplasmic half life of D is about 10 minutes (5,6), the 11-13% of ^{3}H-labeled D found in nuclei after 15 minutes of labeling could simply represent the fraction of the D population that has become stably nuclear after the cytoplasmic precursor stage.

With aqueous systems, briefly labeled RNAs C and D had only been found in cytoplasmic fractions (6,3,7). Our present results,which use an alternate fractionation method and confirm earlier results, suggest that the precursors are indeed cytoplasmic in living cells. The precursors to RNAs C and D would then be the first examples of RNAs synthesized in the nucleus, spending a few minutes in the cytoplasm, and finally returning to the nucleus for a long lifetime there.

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