SMALL RNAS IN THE NUCLEUS AND CYTOPLASM OF HELA CELLS

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SUMMARY: The possible relationship between two small RNAs (A and B), which are quickly labeled and are briefly found in the cytoplasm of HeLa cells, and two of the small, metabolically stable nuclear RNAs (C and D, nomenclature of ref. 1), has been studied. They were compared by polyacrylamide gel electrophoresis, actinomycin D chases of [^{3}H]uridine pulses, and two-dimensional fingerprinting of their ribonuclease T_1 digests. Taken together, the data suggest that species A and B, found in the cytoplasm, are precursor forms of nuclear RNAs C and D, respectively.

Recently we have been studying two quickly labeled, homodisperse, low molecular weight, methylated RNA species, which have half-lives of a few minutes in the cytoplasm of HeLa cells (2), and whose accumulation in the cytoplasm is enhanced during suppression of protein synthesis (3). A series of metabolically stable, small, homodisperse, nuclear RNAs has been known for several years (4). We wanted to test the possibility that these RNAs found in the cytoplasm might be related to two of the nuclear RNAs, and in this report evidence is presented suggesting that they are precursor forms of two nuclear RNAs.

MATERIALS AND METHODS: Camptothecin (sodium salt) was kindly supplied by Dr. H.B. Wood, Jr., Drug Development Branch, Division of Cancer Treatment, NCI, Bethesda, Md. The conditions for HeLa cell growth in spinner culture and for the experiments with [^{3}H]uridine, including cell labeling and harvest, isolation of cytoplasmic RNA, and analysis by 10% polyacrylamide gel electrophoresis, were as described before (5,2). Nuclear RNA was prepared as described by Penman (6). For ^{32}P labeling, HeLa cells were washed twice with phosphate-free Joklik-modified minimum essential medium, (Grand Island Biological Co., Grand Island, N.Y.), they were then resuspended at 4×10^6 cells/ml of the same medium with 7% dialyzed horse serum, and preincubated at 37°C for 15 min. Carrier-free ^{32}P (as orthophosphoric acid) was then added (0.16 mCi/ml of medium) and the cells were harvested after 2, 3 or 4 hr. For the 22 or 48 hr experiments, cells were resuspended at a concentration of 2×10^5 cells/ml, in medium containing 0.05-0.1 mM phosphate, and ^{32}P was added (20 µCi/ml). The nuclear and cytoplasmic RNA fractions were isolated and extracted as indicated above. The 28 S-18 S rRNA was removed from the cytoplasmic preparation by centrifugation through a sucrose gradient (5-20% linear sucrose gradient in 0.01 M sodium acetate, pH 5.1, 0.05 M NaCl, 0.1 mM $MgCl_2$). The nuclear RNA sample, and a pool of the fractions lighter than 18 S rRNA and up to 4 S RNA, from the gradient of

the cytoplasmic sample, were loaded onto 10% polyacrylamide gels (5) of 6.5 mm in diameter and 20 cm in length, and electrophoresis was carried out at 5 mA/gel for 18 hr. One-mm thick slices were cut and monitored by Čerenkov counting, the slices with the desired peaks were pooled, and RNA was eluted as described by Ikemura and Dahlberg (7). The conditions of RNA fingerprint analysis by digestion with ribonuclease T_1 and two-dimensional electrophoresis were as described by Sanger et al (8).

RESULTS AND DISCUSSION: Two small RNA species, which we have been calling A and B, can be detected in the cytoplasm of HeLa cells as early as 4 min after addition of [^{3}H]uridine (2), the shortest time tested. Their half-life in the cytoplasm is about 10 min (2), and perhaps even less in the case of RNA B (3). The small nuclear RNAs C and D, which are metabolically stable, can be detected in nuclei 15 min after addition of [^{3}H]uridine (9). (We are calling nuclear RNAs C and D those species which migrate like the C and D of Weinberg and Penman (1), in gel electrophoresis). When the electrophoretic mobilities of nuclear and cytoplasmic small RNAs were compared, cytoplasmic species A moved considerably more slowly than nuclear species C, and cytoplasmic species B appeared to be slightly slower than nuclear species D, in sodium dodecyl sulfate-acrylamide gels (Fig. 1a). This was also the case when gels had 8 M urea, therefore disrupting hydrogen bonding (Fig. 1b). As in these urea gels the migration of RNAs is inversely proportional to the logarithm of their molecular weights (3), it can be estimated that the differences in mobilities in Fig. 1b could be due to RNA A having about 10 more nucleotides than RNA C, and RNA B about 3 more nucleotides than RNA D. These differences still existed when both the nuclear and cytoplasmic preparations had been phenol extracted at 55°C, and the isotope labeling of the nuclear fraction had been carried out for shorter times (nuclear RNA labeled for 90 min with ^{32}P vs. cytoplasmic RNA labeled for 40 min with [^{3}H]uridine) (Fig. 1c).

It has been reported that ultraviolet irradiation inhibits the accumulation of nuclear RNAs C and D but not of cytoplasmic RNAs like tRNA (9), and we have found that the accumulation of cytoplasmic RNAs A and B is also particularly sensitive to ultraviolet irradiation. We had previously reported that the

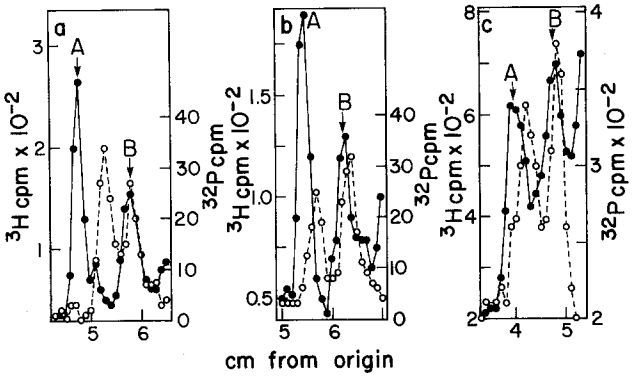

Fig. 1. Comparison of the mobilities of small RNAs from the nucleus and cytoplasm of HeLa cells, in polyacrylamide gel electrophoresis. (a) nuclear RNA from cells incubated with ^{32}P for 4 hr, plus cytoplasmic RNA from cells incubated with [^{3}H]uridine for 35 min, were co-electrophoresed in a 10-cm long, 10% polyacrylamide gel. (b) the same as (a) except that the gel had 8 M urea (3). (c) nuclear RNA from cells incubated with ^{32}P for 90 min, plus cytoplasmic RNA from cells incubated with [^{3}H]uridine for 40 min and phenol extracted at 55°C, were co-electrophoresed in a gel like the one in (a). The migration is from left to right. The cytoplasmic RNA peaks A and B are labeled in the figure. ^{3}H cpm, ●—●; ^{32}P cpm, 0---0.

TABLE 1. EFFECT OF CAMPTOTHECIN ON THE ACCUMULATION OF SMALL RNAS

HeLa cells were incubated with [^{14}C]uridine (53 Ci/mol, 50 nCi/ml of medium) for 4 hr at 2x10^6 cells/ml, and after a 10-fold dilution the incubation was continued for about 20 more hours. Then, the cells were incubated with (b) or without (a) camptothecin (2 µg/ml, sodium salt) for 10 min at 2x10^6 cells/ml of medium. [^{3}H]uridine (28 Ci/mmol, 10 µCi/ml of medium) was then added, and the incubation continued for 30 more minutes. The ^{3}H incorporation figures have been corrected by the ^{14}C incorporation into 4 S RNA for the cytoplasmic samples and into RNA D for the nuclear samples.

	Cytoplasmic (^{3}H cpm)		Nuclear (^{3}H cpm)	
	A	B	C	D
(a) Control	1010	520	322	322
(b) Camptothecin	0	17	51	74

accumulation of cytoplasmic species A and B was very sensitive to low concentrations of camptothecin, while that of other small RNAs like 5 S rRNA and 4 S RNA was considerably less sensitive (3). Table 1 shows that the

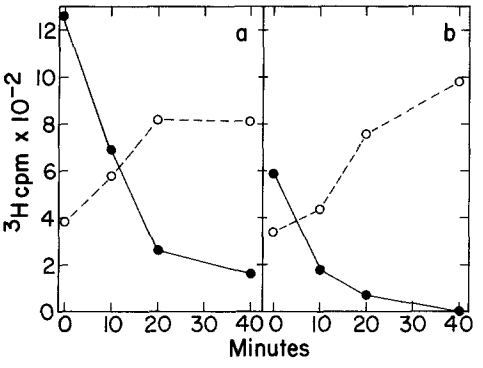

Fig. 2. Fate of the radioactivity from a [^{3}H]uridine pulse after an actinomycin D chase, in small RNAs. (a) cytoplasmic RNA A (●—●), nuclear RNA C (0---0). (b) cytoplasmic RNA B (●—●), nuclear RNA D (0---0). HeLa cells were incubated with [^{14}C]uridine for about 24 hr as indicated in the legend to Table 1, and then with [^{3}H]uridine (28 Ci/mmol, 10 μCi/ml of medium) for 20 min at 2x10^6 cells/ml. A cell aliquot was then harvested, actinomycin D (5 μg/ml) was added to the rest of the cells (time zero in the figure), and aliquots were harvested at the indicated times. The ^{3}H counts in various cytoplasmic aliquots were corrected by their endogenous steady-state labeled [^{14}C]4 S RNA. From the data of Weinberg and Penman (1) it can be calculated that the mass ratio of cytoplasmic 4 S RNA vs. nuclear RNA D is about 53 in HeLa cells. Using this figure, the ^{3}H counts in various nuclear aliquots were corrected by their endogenous steady-state labeled [^{14}C]D nuclear RNA, with respect to the cytoplasmic [^{14}C]4 S RNA values.

accumulation of both cytoplasmic species A and B and nuclear species C and D was very sensitive to camptothecin.

When a pulse of [^{3}H]uridine was chased with actinomycin D (5 μg/ml, to suppress further RNA synthesis), there was at least a qualitative correlation between the disappearance of cytoplasmic RNAs A and B, and the accumulation of nuclear RNAs C and D, respectively (Fig. 2).

We then compared the oligonucleotide fingerprint patterns of these RNAs, after digestion with ribonuclease T_1. Fig. 3 (a and b) shows that the fingerprints of nuclear species C and cytoplasmic species A are quite similar, with some extra nucleotide spots in RNA A (two of the most reproducible ones are indicated by the arrows in Fig. 3b). Fig. 3a also shows that the fingerprint of HeLa nuclear RNA C is similar to that of nuclear RNA C from KB cells (human) (10) and of rat hepatoma nuclear RNA U-2 (11). Fig. 3 (c and

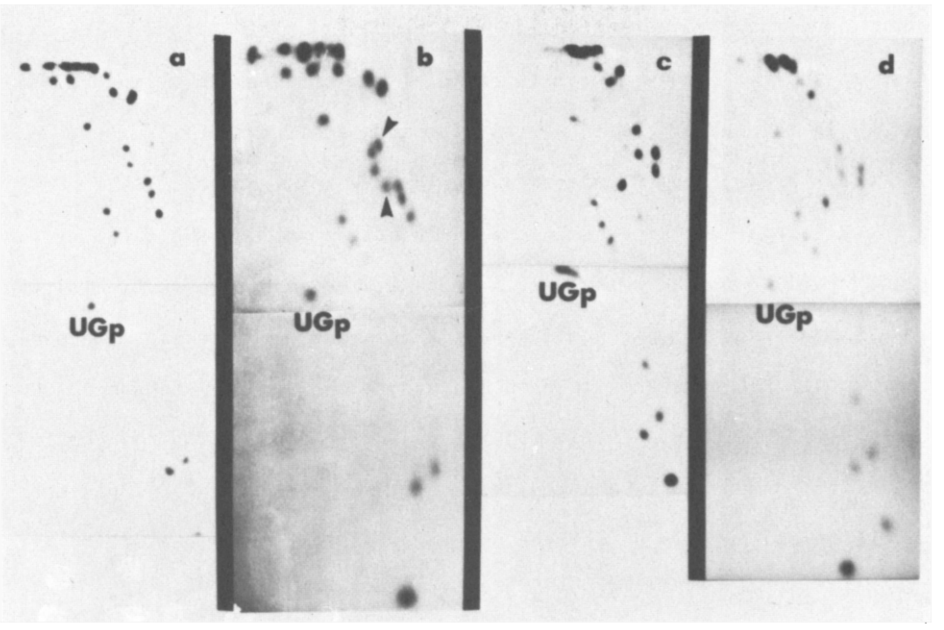

Fig. 3. Autoradiograms showing two-dimensional electrophoresis fingerprints of ribonuclease T_1 digests of nuclear RNA C (a), cytoplasmic RNA A (b), nuclear RNA D (c), and cytoplasmic RNA B (d). HeLa cells were incubated with ^{32}P for 48 hr in (a), 2 hr in (b) and (d), and 22 hr in (c). The direction of migration is from right to left in the first dimension, and from top to bottom in the second dimension.

d) shows that the fingerprint patterns of HeLa nuclear RNA D and cytoplasmic RNA B are quite similar, and also similar to KB nuclear RNA D (10) and rat hepatoma nuclear RNA U-1 (12). For the fingerprints of the cytoplasmic preparations, to avoid the possibility of nuclear contamination, the cells were broken with few strokes of a ball homogenizer, so that many nuclei were still surrounded by some cytoplasm and were discarded with the nuclear pellet. The fingerprints, in conjunction with the migration of these RNAs in gel electrophoresis and their behavior in pulse chase studies, suggest that species A and B, found in the cytoplasm, are precursor forms of nuclear RNAs C and D, respectively.

RNAs C and D are transcribed in the nucleus (they hybridize to nuclear DNA (13)), we propose that then they appear immediately in the cytoplasm where they remain for a few minutes and finally they end in the nucleoplasm, where

they have been shown to be metabolically stable (9). This cytoplasmic phase could be an in vivo phenomenon or it could be nuclear leakage during cell handling. In the latter case a series of events lasting several minutes might need to occur after these RNAs are transcribed, before they can be "fixed" in the nucleus, as perhaps happens in the early stages of 5 S rRNA (14). As the half-life of RNA B in the cytoplasm is markedly lengthened during inhibition of protein synthesis (3), it would appear that newly made proteins are necessary for this RNA to reach its proposed mature nuclear form as species D.

Based mostly on electrophoretic mobility and pulse chase studies, conclusions similar to ours have been reached about the existence of cytoplasmic precursors of nuclear RNA C (15) and nuclear RNA D (16). By nuclei implantation studies in amoeba, Goldstein and Ko (17) recently concluded that some RNA species, which they identify as U-1 and U-3, shuttle between nucleus and cytoplasm, while an RNA band which they term U-2 stays in the nucleus and does not shuttle.

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