HETEROGENEITY OF TURNOVER RATES OF 4S RNAs IN FRIEND VIRUS-INFECTED MOUSE LEUKEMIA CELLS

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SUMMARY

Turnover rates of tRNAs in Friend virus-infected mouse leukemia cells are reported. Cells were labeled for one generation with [^{14}C]- or [^{3}H]uridine. ^{3}H-labeled cells were transferred to nonradioactive medium and allowed to grow exponentially for 72 hours. Low molecular weight cytoplasmic RNAs isolated from ^{14}C- and ^{3}H-labeled cells were cochromatographed on reverse-phase columns. The results indicate considerable heterogeneity of turnover rates of 4S RNAs, with the most labile species turning over at least 1.75 times as fast as the most stable species.

In most eukaryotic cells, the relative levels of specific tRNAs are correlated with the relative contents of the cognate amino acids in the protein being synthesized (1–5). As part of a study designed to investigate the mechanism by which tRNA levels are regulated, we have been studying RNA in Friend virus-infected mouse leukemia cells (FL cells). In this paper we show that, during exponential growth of FL cells, 4S RNAs display considerable heterogeneity in their turnover rates.

EXPERIMENTAL PROCEDURES

Cells—FL cells (GM-86, clone 745) were obtained from Dr. J. Papaconstantinou. They were maintained in suspension culture in Eagle's minimal essential medium (Gibco catalogue number F-12) supplemented with 15% fetal calf serum (Gibco) and 20 μg/ml gentamicin. Cells were grown at 37°C in a 5% CO_2 atmosphere. FL cells were labeled with 0.2 μCi/ml [2-^{14}C]uridine (Amersham-Searle, 52 Ci/mole) or 0.5 μCi/ml [5-^{3}H]uridine (Nuclear Dynamics, 370 Ci/mole). To minimize the chance of mycoplasma contamination, [^{14}C]- and [^{3}H]uridine stock solutions were heated at 56°C for 30 min prior to use.

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Labeling was for 18–24 hours. Usually over 80% of [^{14}C]uridine and 50% of [^{3}H]uridine was taken up by the cells under these conditions. When significantly lower uptake occurred, the cells were assumed to be contaminated with mycoplasma (6) and were discarded.

Preparation of cytoplasmic RNA — Cells were suspended in medium containing 5% calf serum at a cell density $\leq 5 \times 10^7$ cells/ml. The cell suspension was added dropwise to a vigorously stirred mixture containing one volume of buffer A (10 mM sodium acetate, 10 mM EDTA, pH 5.1), one volume of phenol saturated with buffer A, and 0.05 volumes of 10% sodium dodecyl sulfate (SDS). After stirring for 10 min at room temperature, the phases were separated by centrifugation (10 min at 10,000 rpm, Sorvall SS-34 rotor). In some cases, a thick protein interface hindered recovery of the aqueous phase. When this happened, one additional volume of buffer A was added and the extraction and centrifugation were repeated. The aqueous phase was extracted with an equal volume of phenol/$CHCl_3$/isoamyl alcohol (100:100:1, v/v/v) and this mixture was centrifuged (2000 rpm for 10 min). RNA was recovered from the aqueous phase (containing 0.2 M NaCl) by precipitation with 2 volumes of ethanol followed by filtration on 24-mm diameter 0.45-μM millipore filters. RNA was eluted from the filters by soaking for 2 hours at 37°C in 2 ml of a solution containing 40 μg/ml pronase (Calbiochem, nuclease-free), 10 mM Tris-Cl^- (pH 7.5), 2 mM EDTA, and 0.2% SDS. If pronase treatment was omitted, recoveries of RNA were often poor, especially when small numbers of cells were processed. Eluates from millipore filters were extracted twice with an equal volume of phenol/$CHCl_3$/isoamyl alcohol.

Analysis of RNA — Electrophoresis on 0.6 X 8 cm 2.6% acrylamide gels was for 80 min at 5 mA per gel in the Tris-phosphate:EDTA-SDS system of Loening and Ingle (7). Gel slices were counted according to Petri (8). Reverse-phase chromatography (RPC-5) was performed according to Kelmers and Heatherly (9).

The 0.6 X 10 cm columns used for Figures 3A and B were eluted at 38°C with 200-ml linear gradients from 0.5–0.7 M NaCl at flow rates of 1.5 ml/min. Gradient buffers also contained 10 mM sodium acetate (pH 4.5), 10 mM $MgCl_2$, 1 mM EDTA, and 2 mM β-mercaptoethanol. 1.5-ml fractions were counted in 15 ml of a scintillation fluid prepared by mixing two volumes of 0.4% 2,5-bis-2-(5-tert.-butylbenzoxazolyl)-thiophene (BBOT) in toluene with one volume Triton X-100. Prior to RPC-5 chromatography, cytoplasmic RNA was adsorbed to and eluted from DEAE-cellulose in order to remove high molecular weight RNA (10).

RESULTS

Average turnover rate of unfractionated 4S RNA — RNA was prepared from cells which had been labeled for one generation with [^{14}C]uridine. Other samples of RNA were prepared from cells similarly labeled with [^{3}H]uridine and then allowed to grow exponentially in nonradioactive medium for various times. Each [^{3}H]RNA preparation was mixed with an appropriate quantity of the [^{14}C]RNA and the mixtures were submitted to electrophoresis on 2.6% polyacrylamide gels. A typical separation is shown in Figure 1. The ratio of radioactivity in the 28S peak to that in the 18S peak was always 2.5, as expected from the relative molecular weights of these two species (11). $^{3}H/^{14}C$ ratios were calculated for the various RNA species. The assumption was made that 18 and 28S rRNAs are stable during exponential growth (12). Hence, the extent of turnover of 4S RNA was taken as the $^{3}H/^{14}C$ ratio for 4S RNA divided by the $^{3}H/^{14}C$ ratio for 18S or 28S species. The data are shown in Figure 2. Because of the long half-life of 4S RNA, the relatively short time over which its decay was followed, and the heterogeneity of turnover rates (see below), the half-life derived from these data is only approximate. However, the value obtained (3.2 ± 0.7 days) is within experimental error of the value of 2.5 days obtained by Abelson et al. (12) for 4S RNA half-life in exponentially growing mouse fibroblasts.

The 2.6% gels do not resolve 5S and 4S RNA. However, since 5S RNA is present only to the extent of 5–10% of the 4S RNA, it was assumed that the influence of 5S RNA on the $^{3}H/^{14}C$ ratio of the "4S" peak was negligible.

Heterogeneity of turnover within the 4S RNA class — Mixtures of the [^{3}H]- and

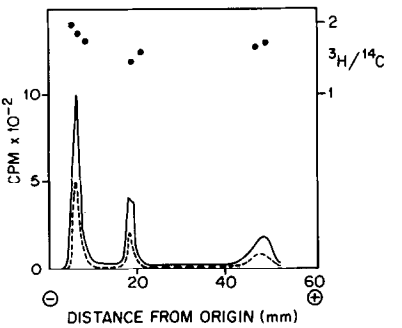

FIG. 1. Electrophoresis on a 2.6% acrylamide gel of a mixture of cytoplasmic RNAs from FL cells labeled for one generation with [^{3}H]- and [^{14}C]uridine. ^{3}H cpm (——), ^{14}C cpm (----), $^{3}H/^{14}C$ (····).

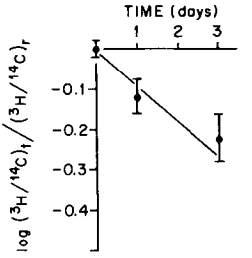

FIG. 2. Decay of 4S RNA in growing FL cells. ($^3H/^{14}C$)t is the $^3H/^{14}C$ ratio for 4S RNA; ($^3H/^{14}C$)r represents the average of the $^3H/^{14}C$ ratios for 18S and 28S rRNAs on the same gel.

[^{14}C]RNA preparations described above were adsorbed to and eluted from DEAE-cellulose, and the eluted low molecular weight RNA was cochromatographed on RPC-5 columns. Figure 3A shows an elution profile of an RNA mixture obtained from cells labeled for one generation with [^{14}C]uridine and from cells labeled for one generation with [^3H]uridine and then grown exponentially in nonradioactive medium for 72 hours. It is clear that this elution profile displays considerable heterogeneity in $^3H/^{14}C$ ratios. Furthermore, the variation of $^3H/^{14}C$ ratios throughout the elution profile was reproducible in two independent experiments.

Figure 3B shows a control experiment in which ^3H- and ^{14}C-labeled RNAs were obtained from parallel cultures, each labeled for one generation before harvest. The relative variation in $^3H/^{14}C$ ratios is clearly much greater for the experimental chromatogram (Fig. 3A) than for the control (Fig. 3B). Furthermore, the largest variations in $^3H/^{14}C$ ratio in the control elution profile occur in the region where the smallest amount of radioactivity eluted; hence, where the precision of the $^3H/^{14}C$ ratio is the poorest.

Since rRNAs are stable in growing cells, the presence of 5S rRNA in these preparations, or their contamination with breakdown products of 18S and 28S rRNA as a result of degradation during preparation, could contribute to the observed heterogeneity. Indeed, I found that 5S RNA (isolated by electrophoresis on 10% polyacrylamide gels) chromatographed on the RPC-5 column of Figure 3A to give peaks at tubes 69–70 and 84–86. 4S RNA, electrophoretically purified on the same gels, chromatographed mostly in the region between tubes 18–53, but also gave rise to several small peaks in the latter half of the profile. I therefore attribute the very high $^3H/^{14}C$ ratios for the material in tubes 65–80 (Fig. 3A) primarily to the presence of 5S RNA in this region. DNA contaminating tRNA preparations from animal cells also elutes in this region and, since it is stable, might also contribute to the high $^3H/^{14}C$ ratio

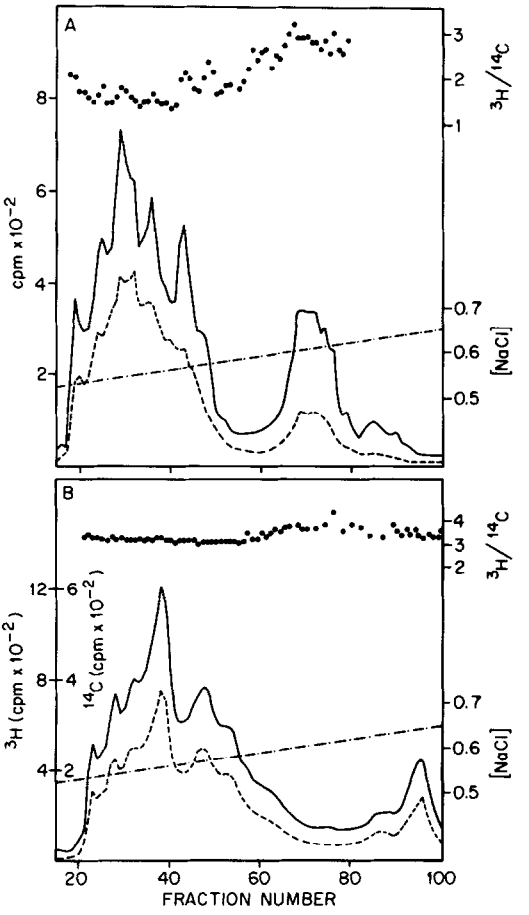

FIG. 3. (A) Cochromatography on RPC-5 of tRNA isolated from cells labeled with [^{3}H]uridine for one generation and then grown exponentially in cold medium for 72 hours, and of tRNA labeled with [^{14}C]uridine for one generation.

(B) Cochromatography on RPC-5 of tRNA isolated from cells labeled with [^{3}H]uridine for one generation and tRNA isolated from cells labeled with [^{14}C]uridine for one generation. Different columns were used for (A) and (B). ^{3}H cpm (——), ^{14}C cpm (----), ^{3}H/^{14}C (····), NaCl molarity (—·—·—).

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observed (L. Waters, personal communication). However, this does not explain the variations in ^{3}H/^{14}C ratio in the region between tubes 18 and 53, where most of the 4S RNA elutes. I feel it is unlikely that these results can be explained by rRNA breakdown during RNA preparation, because the ratio of 28S to 18S rRNA was always 2.5, as expected for undegraded rRNA.

The ^{3}H/^{14}C ratios for species eluting with the majority of 4S RNA species (tubes 18–53

in Fig. 3A) span a range of 1.75-fold. This range represents a minimum estimate for the degree of heterogeneity of turnover rates of 4S RNAs.

DISCUSSION

The results presented here suggest that, during exponential growth of FL cells, different 4S RNA species are degraded at different rates. Other workers have failed to observe heterogeneity of degradation rates for 4S RNAs in mammalian cells. Thus, Hanoune and Agarwal (13) examined the turnover of rat liver 4S RNA species in vivo and found no differences in turnover rates for RNAs separated by BD-cellulose chromatography. However, the resolution of 4S RNAs obtained in their experiment was relatively poor. Abelson et al. (12) measured the turnover rate of total methyl-labeled 4S RNA in cultured mouse fibroblasts in both the growing and resting states. In resting cells, they found a strictly exponential decay of 4S RNA with $t_{1/2}$ = 36 hr, down to less than 5% survival of the initial RNA. As they pointed out, this result implies that all species of 4S RNA turned over at the same rate. Their data for growing cells, which were less extensive, neither support nor exclude a moderate degree of heterogeneity in 4S RNA turnover rates such as that described here.

It is not clear whether my apparently unique finding of heterogeneous turnover rates of 4S RNAs is specific to the FL cell line used or to the condition of exponential growth. Further studies with various cell lines in growing and resting states should resolve this point.

Nomura (14) has shown that, in E. coli, a temperature-sensitive mutant of a suppressor $tRNA^{Tyr}$ is degraded at an abnormally rapid rate at the restrictive temperature. He has suggested the existence of a mechanism for scavenging "deformed" tRNA molecules similar to that which apparently exists for proteins (15). Since normal tRNAs are heterogeneous with respect to their thermal stabilities, it seems reasonable to suppose that they might also be heterogeneous with respect to their turnover rates in vivo.

It should be stated that the data in this paper were obtained with RNA preparations characterized only by their chromatographic and gel electrophoretic properties. Hence no conclusion can yet be drawn about the heterogeneity of turnover rates for functional tRNAs. I am presently attempting to use the method of Klyde and Bernfield (16) to purify specific tRNAs and to estimate their relative rates of turnover in growing FL cells.

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