CHARACTERIZATION OF THE RAPIDLY LABELED HYBRIDIZABLE RNA SYNTHESIZED IN L5178Y MOUSE LEUKEMIC CELLS

HYBRIDIZATION LIFETIME STUDIES

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ABSTRACT An attempt is made to characterize the rapidly labeled hybridizable RNA of L5178Y mouse leukemic cells which has been shown to have similar base sequences when synthesized in two different stages of the cell cycle. The size of rapidly labeled RNA molecules was heterogeneous. For labeling times of 20 min or less, the per cent of hybridization was maximal. With longer labeling times, the per cent of hybridization decreased as radioactivity appeared in long-lived species of low hybridization efficiency; the radioactivity profile resembled the optical density profile in sucrose gradients. The lifetime of newly synthesized hybridizable RNA was studied by pulse labeling exponentially growing cells and then "chasing" with nonradioactive uridine. The per cent of hybridization was studied as a function of chase time. Three RNA groups, which comprised different proportions of rapidly labeled hybridizable RNA, were distinguished. The short-lived group had a half-life of 10 min, much less than the values reported in the literature for messenger RNA of mammalian cells. The half-life of 1–1½ hr observed for a medium-lived group more closely corresponds to that of messenger RNA. A long-lived group had a half-life of approximately 20 hr. Specific activity measurements during chase indicate the presence of a "pool" of labeled uridine derivatives. The uridine of this pool appears to be nonexchangeable with but dilutable by exogenous uridine. A nontoxic concentration of actinomycin D was added to the chase media in an attempt to block the "pool effect". A rapidly degradable RNA was demonstrable both by specific activity and per cent of hybridization measurements.

INTRODUCTION

It has recently been shown in L5178Y mouse leukemic cells that hybridizable RNA is synthesized throughout the cell cycle as a constant proportion of the total RNA

synthesized (1). Also, no dissimilarity could be detected between rapidly labeled hybridizable RNA sequences synthesized at two different times in the cell cycle. An attempt was therefore made to characterize the RNA which contributes to rapidly labeled hybridizable RNA on the basis of the lifetime of the hybridizable RNA. A rapidly degradable hybridizable RNA was demonstrable without using actinomycin D.

METHODS

Cell Line

The mouse leukemic cell line L5178Y (near-diploid) was maintained in exponential growth by continuing serial dilution in Fischer's Medium for Leukemic Cells of Mice (Grand Island Biological Co., Grand Island, N.Y.) with 10% horse serum (2, 3).

Biochemical Isolation Procedures

A modified Marmur procedure was used to isolate the DNA from the L5178Y cells (4). A modified hot phenol technique was used to isolate total cell RNA (5, 6).

RNA/DNA Hybridization

DNA filters were prepared using the standard technique of Gillespie and Spiegelman (7). Usually, 25 μg of L5178Y DNA were deposited on nitrocellulose membrane filters (Schleicher and Schuell Inc., Keene, N.H., Type B-6, 25 mm diameter). After hybridization, the filters were washed, treated with ribonuclease, and dried (7). The empirically determined hybridization conditions were as follows: (*a*) hybridization time: 36 hr; (*b*) temperature: 65°C (with shaking in a water bath); (*c*) volume of RNA solution: 1 ml; (*d*) RNA:DNA ratio usually 1:1.

After drying, the filters were dissolved in 14 ml of Bray's solution (8) plus 1 ml of distilled water. The per cent of hybridization is defined as the percentage of the radioactivity (disintegrations per min, dpm) of the RNA bound to the DNA on the filters over the radioactivity (disintegrations per min) of the total RNA added.

Lifetime Studies: Pulse Label and Cold Uridine or Actinomycin D Chase

An exponentially growing population of L5178Y cells was mass-cultured to a concentration of 2–4 $\times$ 10^5 cells/ml (approximately 4 $\times$ 10^8 cells). The cells were centrifuged in sterile containers for 5 min at 1000 rpm in a Sorvall centrifuge (Model RC-2, Ivan Sorvall, Inc., Norwalk, Conn.) at room temperature. The old media was decanted, and the cells were resuspended in a small volume (less than 50 ml) of fresh warm media containing approximately 10^{-4} M thymidine and 10^{-4} M deoxycytidine. After incubation for 3–5 min at 37°C, the cell suspension was transferred to a flask containing a small volume of fresh warm media and an appropriate volume of 500 μCi/ml 5-uridine-^{3}H solution (20 Ci/mmole, Schwarz BioResearch Inc., Orangeburg, N.Y.). The volumes chosen were such that the final concentration was always 50 μCi/ml, and the concentrations of thymidine and deoxycytidine were both 10^{-4} M. The cells were pulse labeled for the desired time at 37°C and immediately centrifuged at 1000 rpm for 5 min at room temperature. The media was removed and the cells resuspended with an equal volume of fresh warm "chase media" containing 10^{-4} M uridine, 10^{-4} M thymidine, and 10^{-4} M deoxycytidine. An aliquot of the labeled cells was immediately

centrifuged, the cells were washed three times with saline and frozen by immersion in absolute ethyl alcohol dry ice (zero chase time). The remainder of the pulse-labeled cells were decanted into 175 ml of chase media. Since the cells were still highly concentrated, the pH of the media was subject to rapid change; fresh warm chase media was added to the cell suspension to compensate for this during the chase period. At different times, appropriate aliquots of the cell suspension were removed, centrifuged, washed, and frozen.

In actinomycin D chase experiments, the procedure was essentially the same, except that the chase media also contained actinomycin D (Mann Research Labs, Inc., New York) at 0.5 μg/ml.

Sucrose Gradient Centrifugation

Sucrose (crystalline, ribonuclease-free: Mann Research Labs, Inc.) was dissolved in 0.01 M Tris-Cl buffer (pH 7.4). Linear concentration gradients (5–20%) were prepared with a peristaltic pump and mixing chamber (Buchler Instruments, Inc., Fort Lee, N.J.). Usually, 0.1–0.3 ml of solution containing 100–300 μg of RNA were carefully layered on 4.5 ml of gradient solution, and the gradients were centrifuged in a SW39 or SW-65-Ti rotor in a Beckman Model L-2 Ultracentrifuge (Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.) at 33,000 rpm for 4½ hr at 5°C. After centrifugation, the tubes were refrigerated until punctured at the bottom; ten-drop fractions were collected. Distilled water (0.5 or 0.6 ml) was added and the optical densities were read at 260 nm. Aliquots (0.2 ml) of each fraction were counted in 10 ml of Bray's solution plus 1 ml of distilled water in a Nuclear-Chicago Scintillation Counter (Nuclear-Chicago Corporation, Des Plaines, Ill.).

RESULTS

Characteristics of Rapidly Labeled RNA as a Function of Labeling Time

The RNA in exponentially growing L5178Y cells was labeled by incubating the cells with high specific activity 5-uridine-^{3}H (50 μCi/ml, 20 Ci/mmole: Schwarz BioResearch Inc.) for 10, 20, 30, or 60 min. The specific activity (dpm/μg) of the rapidly labeled RNA increased with labeling time (Table I, "No actinomycin D"), indicating that most of the newly synthesized RNA accumulated in the cells. The per cent of hybridization was maximal at 20 min of pulse labeling and declined thereafter (Table I).

The sucrose gradient centrifugation patterns of RNA isolated from cells pulse labeled for 10, 30, or 60 min indicate the heterogeneous size distribution of rapidly labeled RNA and the alteration in distribution of incorporated radioactivity with increasing labeling time (Fig. 1). Since 5-uridine-^{3}H appears as rapidly in larger molecules as in smaller molecules (Fig. 1 *a*) and since the optical density profiles do not indicate an abundance of these larger molecules, we must assume that the larger molecules are either completely degraded or rapidly broken down into smaller molecules. If the larger molecules are broken down into smaller ones, small rapidly labeled and rapidly degradable molecules would sediment to the same positions in sucrose gradients as the fragments and could not be distinguished.

TABLE I

PULSE LABELING OF L5178Y CELL RNA (WITH OR WITHOUT ACTINOMYCIN D)

Pulse time	Specific activity		
	No actinomycin D	0.5 μg/ml actinomycin D	$\left(\frac{\text{dpm/μg with act. D}}{\text{dpm/μg no act. D}}\right) \times 100$
min	*dpm/μg*	*dpm/μg*	%
10	17,281	6,157	36
20	39,858	8,529	21
30	84,616	9,043	11
60	208,387	25,895	12

Pulse time	Hybridization*					
	No actinomycin D			0.5 μg/ml actinomycin D		
	Filter 1	Filter 2	Average	Filter 1	Filter 2	Average
min	%	%	%	%	%	%
10	1.56	1.14	1.35	3.43	1.91	2.67
20	1.60	1.48	1.54	2.05	2.39	2.22
30	0.92	1.15	1.04	2.84	1.79	2.32
60	0.89	0.83	0.86	2.56	1.85	2.21

* (dpm bound/dpm added) × 100.

Half-Life of Newly Synthesized RNA

Exponentially growing L5178Y cells were pulse labeled with 5-uridine-^{3}H for intervals of from 5 to 25 min in a series of experiments (Table II), and then incubated (chased) for varied lengths of time in the presence of 10^{-4} M nonradioactive uridine. The specific activity immediately after pulse labeling is proportional to the ratio of newly synthesized RNA molecules remaining undegraded during the pulse interval to the total cell RNA. If the nonradioactive uridine in the fresh chase media immediately diluted the labeled uridine available in the cell for incorporation, no further increase in specific activity would be expected. Because of a pool effect, this was not the case, and the specific activity increased gradually in the initial 1–4 hr of cold uridine chase (Table II). After the time of maximal specific activity, there was a gradual decline with chase time. The rates of decline appeared to be independent of the initial labeling interval and sometimes had half-lives greater than 30 hr.

In an effort to determine the fate of newly synthesized RNA, the per cent of hybridization of RNA isolated from cells with increasing chase time was measured. Initially, the per cent of hybridization decreased quickly; this was followed by a more gradual decline (Fig. 2), indicating that the hybridizable material consists of

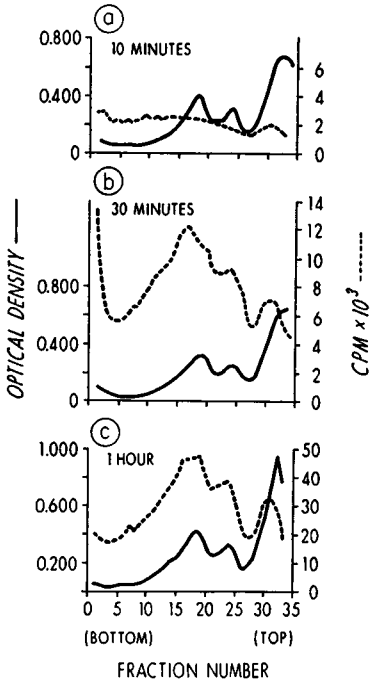

FIGURE 1

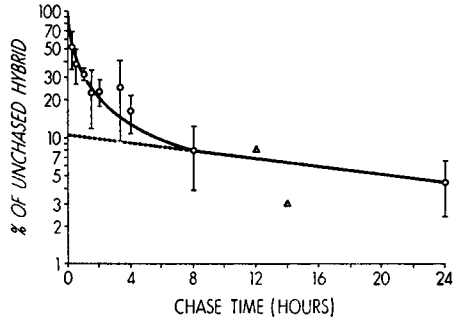

FIGURE 2

FIGURE 1 (*a*) Sucrose gradient centrifugation optical density and radioactivity profiles of RNA isolated from L5178Y cells pulse labeled for 10 min with 5-uridine-^{3}H (50 μCi/ml). (*b*) Sucrose gradient centrifugation optical density and radioactivity profiles of RNA isolated from L5178Y cells pulse labeled for 30 min with 5-uridine-^{3}H. (*c*) Sucrose gradient centrifugation optical density and radioactivity profiles of RNA isolated from L5178Y cells pulse labeled for 60 min with 5-uridine-^{3}H.

FIGURE 2 Hybridization lifetime study of the rapidly labeled RNA of exponentially growing L5178Y cells: pulse label and cold uridine chase. Per cent of hybridization of RNA isolated from cells incubated in the presence of 10^{-4} M nonradioactive uridine for different times after pulse labeling as compared to the per cent of hybridization of RNA isolated from cells immediately after pulse labeling. O = average values (and standard deviation) for cells labeled for 5–25 min (2–5 experiments per point). $\triangle$ = values from individual experiments.

several types of RNA with different half-lives. Although the average curve could be analyzed in different ways, the simplest method has been chosen. The curve is considered to be a summation of short-lived, medium-lived, and long-lived groups.

The initial extrapolation for half-life analysis of the long-lived group appears in Fig. 2. The residual curves for medium- and short-lived groups appear in Fig. 3. The results are summarized in Table III.

Since the curve in Fig. 2 is comprised of components of very different and always declining slopes, the conversion of RNA of one group to another (e.g., short-lived to medium-lived) seems unlikely. Were it not for the pool effect, the change in specific activity with chase time would have paralleled the changes in the per cent

TABLE II

PER CENT (%) OF UNCHASED SPECIFIC ACTIVITY: PULSE LABEL AND COLD URIDINE CHASE

Experiment No.:	3A	5A	9	13	19
Pulse time, *min*:	15	15	5	15	25
Chase time					
min	%	%	%	%	%
0	100	100	100	100	100
15			142	110	114
30	154	126	203	175	114
60	151	177	251		
90	178	153	238	208	123
120	156	120	239		
200	179	127			
hr					
4			238	198	137
8				186	123
12					106
14				175	
24				100	60

of hybridization. In other words, because of the pool effect, the short-lived component could be demonstrated by the hybridization technique, but not by the specific activity measurement.

In brief summary, the hybridization lifetime experiments have demonstrated the possible existence in cultured mammalian L5178Y cells of three independent groups of hybridizable RNA of different half-lives, with these groups contributing different amounts to the total hybridizability of newly synthesized RNA.

Characterization of Labeled RNA Present in the Cell after Different Chase Times

Immediately after pulse labeling, the majority of rapidly labeled hybridizable RNA in the cell was rapidly degraded. Since the half-life of this material was only 10 min, the RNA isolated after a chase of 1 hr would be expected to contain label predominantly in the medium-lived group. Similarly, after a 24 hr chase, label would be expected only in the long-lived group.

The sucrose gradient centrifugation pattern of the RNA isolated immediately after a 20 min pulse label (Fig. 4) shows the heterogeneous distribution of radioactivity in RNA. After a chase of 1 hr, the radioactivity profile becomes similar to that of the optical density, but even after a chase of 24 hr, the patterns are not identical. It was hoped that molecules of specific sizes could be assigned to each of the three groups of RNA with different lifetimes. The probable existence of precursor pathways, whereby unknown amounts of newly synthesized molecules are

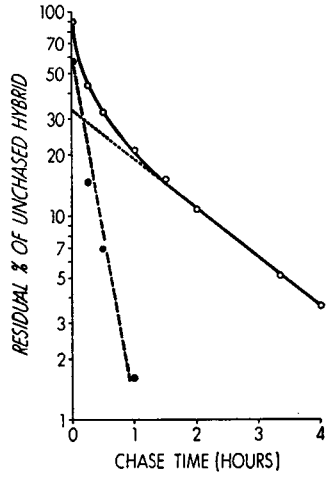

FIGURE 3

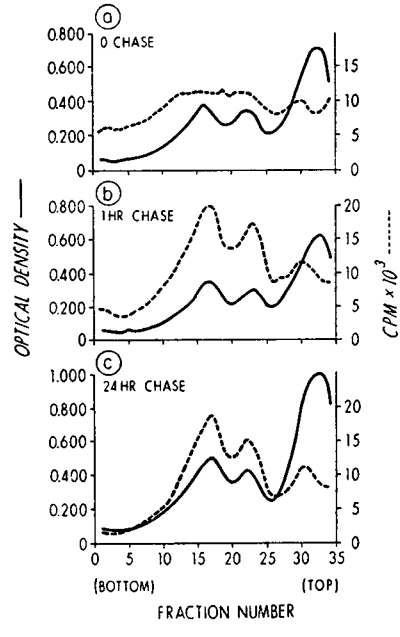

FIGURE 4

FIGURE 3 Hybridization lifetime study; residual per cent of unchased hybrid. —○— = residual curve after subtracting the contribution of the long-lived component to the total average curve (Fig. 2). - -●- - = residual curve after subtracting the contribution of the medium-lived component to the above residual curve.

FIGURE 4 (*a*) Sucrose gradient centrifugation optical density and radioactivity profiles of RNA isolated from L5178Y cells pulse labeled for 20 min with 5-uridine-^{3}H (50 μCi/ml). (*b*) Sucrose gradient centrifugation optical density and radioactivity profiles of RNA isolated from L5178Y cells pulse labeled for 20 min with 5-uridine-^{3}H and subsequently incubated in the presence of 10^{-4} M nonradioactive uridine for 1 hr. (*c*) Sucrose gradient centrifugation optical density and radioactivity profiles of RNA isolated from L5178Y cells pulse labeled for 20 min with 5-uridine-^{3}H and subsequently incubated in the presence of 10^{-4} M nonradioactive uridine for 24 hr.

converted into smaller molecules during the chase period, e.g. 45S ribosomal precursor into 28S and 18S ribosomal RNAs, made this impossible.

It is noteworthy that after a 24 hr chase (Fig. 4 *c*), when the radioactivity profile appears similar to the optical density profile, the hybridization efficiency is very low. This indicates that the RNA molecules which are long-lived in the cell hybridize with low efficiency.

Effect of Actinomycin D

In an attempt to eliminate the pool effect and stop the incorporation of 5-uridine-^{3}H into hybridizable species, actinomycin D, an inhibitor of RNA synthesis, was added to the nonradioactive uridine chase media. The concentration of actinomycin D chosen was 0.5 μg/ml, which blocks the synthesis of the RNA necessary for

TABLE III

HALF-LIFE ANALYSIS: SUMMARY OF HALF-LIVES OF HYBRIDIZABLE RNA GROUPS AND THEIR PER CENT CONTRIBUTION TO RAPIDLY LABELED HYBRIDIZABLE RNA*

Group	Contribution to unchased hybrid	Half-life
	%	
Long-lived	10	20 hr
Medium-lived	33	1–1½ hr
Short-lived	56	10 min

* Averaged for labeling times less than 25 min.

TABLE IV

PER CENT (%) OF UNCHASED SPECIFIC ACTIVITY: PULSE LABEL AND ACTINOMYCIN D CHASE

Chase time	Experiment No.: 18 Pulse time, *min*: 25	20 30
min	%	%
0	100	100
15	75	77
30	70	73
90	57	67
240	55	53

L5178Y cells to move from late S or early G_2 into M (9). The inhibition of 5-uridine–H^3 incorporation by 0.5 μg/ml of actinomycin D depended on the incubation time, amounting to 64% in 10 min and 88% by 30 min (Table I).

The specific activities resulting from two such pulse label and actinomycin D chase experiments (Table IV) appear to indicate elimination of the pool effect; the specific activity does not increase with chase time, but declines almost immediately. This decline has at least two components over the 4 hr chase interval studied. The rapidly decreasing component, amounting to 32% of the initial specific activity, has a half-life of 10 min. The half-life of the remaining 68% is approximately 12 hr.

Similarly, the hybridization lifetime study with actinomycin D chase demonstrates a rapidly decreasing component, amounting to 58% of the initial hybridizable material, with a half-life of 20 min (Fig. 5). The half-life of the long-lived component, which contributed 42% of the initial hybridizable material, could not be determined as it became increasingly difficult to isolate RNA from cells which had been incubated in the presence of actinomycin D at this concentration for more than 4 hr.

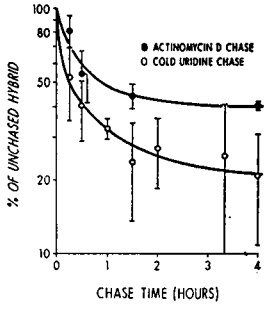

FIGURE 5

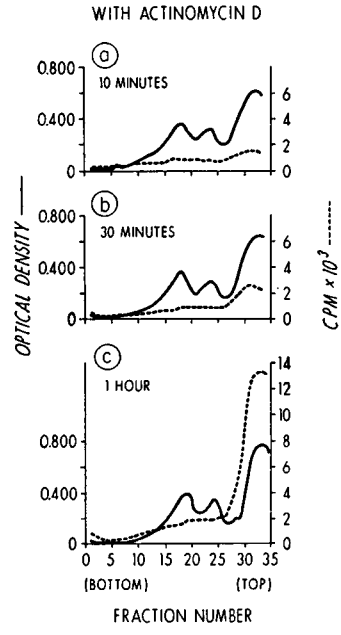

FIGURE 6

FIGURE 5 Effect of chase in the presence of actinomycin D (0.5 μg/ml) on the hybridization lifetime study of the rapidly labeled RNA of exponentially growing L5178Y cells. Per cent of hybridization of RNA isolated from cells incubated in the presence of 10^{-4} M nonradioactive uridine and 0.5 μg/ml actinomycin D for different times after pulse labeling as compared to the per cent of hybridization of RNA isolated from cells immediately after pulse labeling. ● = actinomycin D chase, ○ = initial 4 hr of cold uridine chase.

FIGURE 6 (*a*) Sucrose gradient centrifugation optical density and radioactivity profiles of RNA isolated from L5178Y cells pulse labeled with 5-uridine-^{3}H (50 μCi/ml) in the presence of 0.5 μg/ml of actinomycin D for 10 min. (*b*) Sucrose gradient centrifugation optical density and radioactivity profiles of RNA isolated from L5178Y cells pulse labeled with 5-uridine-^{3}H (50 μCi/ml) in the presence of 0.5 μg/ml of actinomycin D for 30 min. (*c*) Sucrose gradient centrifugation optical density and radioactivity profiles of RNA isolated from L5178Y cells pulse labeled with 5-uridine-^{3}H (50 μCi/ml) in the presence of 0.5 μg/ml of actinomycin D for 60 min.

While these results again indicate the presence of a rapidly degradable group in newly synthesized RNA, the hybridization lifetime pattern was sufficiently different after an actinomycin D chase (Fig. 5) to warrant a brief investigation of the effect of actinomycin D on incorporation of label (Table I) and on the ability of the labeled material to hybridize (Table I). In contrast to the normally pulse-labeled cells, the per cent of hybridization did not decrease significantly with increasing labeling times in the presence of actinomycin D, but remained nearly constant for up to 1 hr at a per cent of hybridization higher than the maximum for pulse-labeled cells. Sucrose gradient analysis of this RNA (Fig. 6) showed that the majority of label was in low molecular weight species.

DISCUSSION

The per cent of hybridization measured in an experiment will depend on (*a*) the availability of DNA genome for hybridization of a particular RNA species or group, (*b*) the abundance of the RNA molecules of that species or group, (*c*) the "relative radioactivity" of each of the hybridizable groups compared to each other and to less efficiently hybridizable species, and (*d*) the specific activity of total RNA. The latter two vary with the relative rates of synthesis and degradation, the length of the labeling interval, and the availability of radioactive precursors from continually changing cellular pools.

Only the effect of the length of the labeling interval on per cent of hybridization was investigated here, even though the half-life estimates of the pulse and chase experiments must include all factors. The initial rapid approach of the per cent of hybridization to its maximum value (Table I) most likely results from a rapid increase to maximal specific activity of the short-lived group with high hybridization efficiency. With increasing labeling time, as the total specific activity continues to increase, the per cent of hybridization decreases. Even though radioactivity may be entering several hybridizable groups, the decrease must result from a predominant accumulation of radioactivity in long-lived species of very low hybridization efficiency.

Effect of Cellular Pool on Half-Life and its Estimate

The half-life estimates of the three hybridizable groups are related to the cellular pool in different degrees, in each case also depending on the specific activity of the RNA in each group at the end of the labeling interval. The estimates for the medium- and long-lived groups are especially dependent on the labeling conditions; they should only be taken as qualitative indications of the existence of subgroups of hybridizable RNA. The measured value for the half-life of the short-lived hybridizable RNA, although a fortuitous result of the rapid labeling of this group, is a valid approximation for just this reason. The specific activity of the cellular pool during the labeling interval is higher than or equivalent to that of the short-lived group for the labeling times employed here, but because of its rapid approach to maximal specific activity, the subsequent chase must dilute the specific activity of the cellular pool to a value much lower than that of the short-lived group. Reutilization from the pool probably yields only a slight overestimate.

In an attempt to determine a "true" half-life, a cold uridine chase was performed in the presence of actinomycin D. The concentration used was not sufficient to inhibit completely the incorporation of 5-uridine-^{3}H into hybridizable RNA species (Table I). Since higher concentrations, which would be able to shut off total RNA synthesis, were toxic to the cells, analysis of the medium-lived and long-lived groups free of the pool effect was not possible. In the presence of actinomycin D, a short-lived group, with a half-life of 10 min (if calculated from the specific activity de-

crease) to 20 min (if calculated from the per cent of hybridization decrease) was distinguishable from other groups.

In summary, for shorter pulse intervals, the majority of rapidly labeled hybridizable RNA (as much as 75% after a 5 min pulse label) belongs to the rapidly degradable RNA of the short-lived group, with an average half-life of 10 min. The remainder of the hybridizable radioactivity is contributed by the RNA of the medium- and long-lived groups.

Characterization of the Rapidly Degradable Group of Rapidly Labeled Hybridizable RNA

The short-lived group has been found to be a major component of rapidly labeled hybridizable RNA in L5178Y cells for labeling times less than 30 min. Because of this, the findings of cell cycle studies (1) can be related to the short-lived group. The RNA(s) of the short-lived group appear to be synthesized throughout the cell cycle, except in M stage (10). The short-lived RNA synthesized at one time of the cell cycle (G_1) is of the same type (i.e., similar in base sequence) as that synthesized at a different time (S). The rate of synthesis of RNA of the short-lived group most likely doubles as L5178Y cells leave G_1 and enter S stage. The DNA which acts as template for the short-lived group of RNA seems to be synthesized throughout S (1).

The size of the RNA molecules of the short-lived group (Figs. 1 *a* and 4 *a*) could be heterogeneous. In radioautographic studies of 5-uridine-^{3}H incorporation into L5178Y cells during a 10 min labeling period, most of the label was found in the nucleus (11). During such a short labeling period, the RNA of the short-lived group is therefore likely to be confined to the nucleus.

Biological Characterization of Unstable RNA

Since the early pulse and chase experiments of Harris (12, 13), the existence of a short-lived (rapidly-turning-over) RNA confined to the nucleus of the cell has been suggested in a variety of cell systems (12, 14–25). These results are in accord with those reported here for L5178Y cells. If rapid degradation of an RNA species occurs in the nucleus immediately after synthesis, it probably does not function as a "messenger RNA".

In mammalian tissues, the literature values for the half-lives and lifetimes of messenger RNA are of the magnitude of hours whether the messenger is defined (*a*) as polyribosomal- or ribosomal-associated messenger RNA (26–29), (*b*) in relation to specific enzymes (30, 31), (*c*) as DNA-like RNA (32), or for various proteins in an isolated perfused liver system (33). Stable messenger RNA has been reported in an in vitro system (34) and in tissues in vivo (35, 36).

The literature values for the half-lives and lifetimes of messenger RNAs in mammalian cells are summarized in Table V. Again, the values can be determined in a variety of ways. While no average values can be defined, the magnitudes reported are of the order of hours.

TABLE V

HALF-LIFE AND LIFETIME ESTIMATES OF MESSENGER RNA IN MAMMALIAN CELLS

RNA	Reference
Polyribosomal-associated messenger RNA (sucrose gradient analysis)	
HeLa cells: half-life = 3–4 hr	44
Don C Chinese hamster cells: persistence through mitosis	45
HeLa S3 cells: persistence through mitosis	46
Messenger RNA of specific enzymes	
Chang liver cells; arginase mRNA lifetime = greater than 4 hr	47
HeLa S3 cells; alkaline phosphatase mRNA lifetime = 12 hr	48
Messenger RNA for protein synthesis	
3T6 mouse fibroblast cells in stationary phase; mRNA for collagen synthesis, half-life = 3 hr	49
Chinese hamster cells; mean functional lifetime of mRNA species coding for protein essential for division = 1 hr	50
Stable messenger RNA	
L-929 mouse fibroblast cells	51
Rabbit reticulocytes	52

In contrast to rapidly-turning-over and/or messenger RNA, the ribosomal RNA of rapidly proliferating animal cells has been considered to be stable (15, 37). This was demonstrated in HeLa cells (38) and in mouse L-cells (39). Thus, the half-life reported here for the short-lived hybridizable RNA group does not correspond to that reported for mammalian messenger or ribosomal RNA.

A recent report (40) described a heterogeneous molecular weight rapidly-turning-over RNA which was found only in the nucleus of HeLa cells. A possibly unrelated RNA with similar properties was identified in the cytoplasm. Since both types of RNA showed labeling characteristics different from polyribosomal messenger RNA, it was suggested that these two species are not messenger RNA.

We similarly conclude that the rapidly degradable hybridizable RNA, with a half-life of 10 min in L5178Y cells, is probably not messenger RNA. The biological role of rapidly degradable, readily hybridizable RNA remains to be determined. The long-lived RNA indicated by the analysis may represent both hybridization of RNA species synthesized due to the pool effect, and the hybridization of 28S and 18S ribosomal (or their precursor) and transfer RNA. The latter are included because the per cent of hybridization is very low as expected for these species in rapidly proliferating or cultured animal cells (41–43), and the radioactivity profile of RNA isolated after 24 hr chase and subjected to sucrose gradient centrifugation (Fig. 4 *c*) is similar (but not identical) to the optical density profile.

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