DEGRADATION OF 28S RNA LATE IN RIBOSOMAL
RNA MATURATION IN NONGROWING LYMPHOCYTES
AND ITS REVERSAL AFTER GROWTH STIMULATION

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INTRODUCTION

In physiologically nongrowing lymphocytes, the rate of entry of new ribosomal RNA (rRNA) molecules to the cytoplasm is only half the rate at which rRNA is synthesized in the nucleus, although there is no net nuclear accumulation of rRNA (6). When total cell RNA is extracted from intact lymphocytes after moderately short labeling periods, the amount of radioactivity appearing in 18S rRNA is only half that anticipated from the labeling observed in 32S + 28S molecules. However, new 28S and 18S molecules appear in the cytoplasm in the expected equimolar ratio (2–4, 6). After growth stimulation with phytohemagglutinin (PHA), both of the above disparities are rapidly eliminated.

We attributed these findings to the degradation or "wastage" of half of the newly synthesized rRNA molecules in resting lymphocytes. Elimination of such wastage was proposed as an important early step in the shift from the resting to the growing condition (2–4, 6). Evidence indicated that the aberrant (32S + 28S):18S labeling ratio noted above was due to synthesis and rapid degradation of new 18S RNA while coordinately synthesized 32S + 28S molecules still survived (2–4, 6). Since no excess of labeled 28S rRNA appeared in the cytoplasm, it was reasoned that the extra 32S + 28S molecules were restricted to the nucleus. Maintenance of cellular integrity would not permit continued accumulation of excess 32S + 28S RNA in the nucleus, and so it was predicted that degradation of these molecules would also be seen, but only after some delay. Failure to confirm

this prediction would cast doubt on the wastage interpretation of our data.

In the experiments to be described, delayed degradation of 32S and 28S molecules was demonstrated, together with its reversal after PHA stimulation.

MATERIALS AND METHODS

Human peripheral blood lymphocytes were prepared and placed in culture as described elsewhere (1, 5). Labeling of rRNA by methylation with [methyl-^{3}H]-methionine, extraction of RNA, electrophoresis of RNA in agarose polyacrylamide gels, and counting techniques have all been described (2, 4, 7, 9). Further details are given in figure legends.

RESULTS

New rRNA molecules were methyl-labeled for a short period, using [methyl-^{3}H]methionine as a precursor, and the fate of labeled molecules was followed during an extended chase with unlabeled methionine. RNA was extracted and analyzed by electrophoresis in agarose-acrylamide gels (Figs. 1 and 3). Radioactivity in the various rRNA species was measured at each point.

In resting cells, the combined total of 32S + 28S radioactivity reached a peak after 1–2 h of chase incubation (Fig. 2). If no subsequent wastage occurs, this peak should remain constant, with label shifting from 32S to 28S RNA. However, between the 2nd and 4th h of chase incubation, a marked fall in the 32S + 28S total occurred in resting lymphocytes (Fig. 2). During this same period,
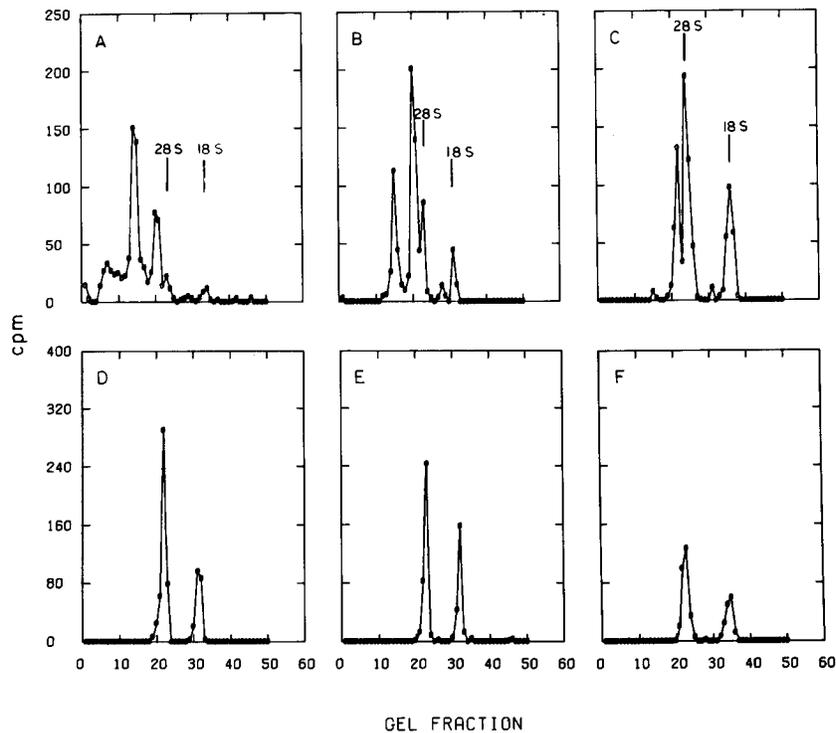
GEL FRACTION

FIGURE 1 Electrophoretic distribution of radioactive rRNA species after pulse labeling and prolonged chase incubation in resting lymphocytes. Replicate lymphocyte cultures, containing 5×10^7 cells (2×10^6 cells per ml of Eagle's MEM (Grand Island Biological Co., Grand Island, N. Y.) with 10% autologous plasma) were labeled with [methyl-^{3}H]methionine (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y., 50 μCi/ml) for 1 h, then incubated in fresh medium containing a 100-fold excess of unlabeled methionine. At various times, duplicate cultures were sacrificed and RNA was extracted with phenol sodium dodecyl sulfate at 50°C (7). Electrophoresis in 1.75% acrylamide-0.5% agarose gels (9) was performed in 13-cm cylinders for 2.0 h at 2.5 mA/gel. The first 10 cm of gel was scanned in a recording spectrophotometer at 260 nm (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) and then fractionated into 2-mm segments. After mixing with Aquasol (New England Nuclear, Boston, Mass.), radioactivity was assayed. For each time period, only one of the duplicate cultures is shown. Position of 28S and 18S rRNA determined by A_{260} measurements is indicated. Origin is to the left. (A) 1-h pulse. (B) 1-h chase. (C) 2-h chase. (D) 4-h chase. (E) 6-h chase. (F) 12-h chase.

the level of 18S label remained constant, which serves as an internal control to eliminate a large, generalized turnover of cytoplasmic rRNA as the cause of the fall. The size and discontinuity of this fall also speak against its being part of a random cytoplasmic turnover of rRNA.

When lymphocytes were incubated with PHA for 10 h before labeling, a time when the 28:18S labeling ratio approaches equimolarity most closely (3), the abrupt fall in the 32S + 28S total was absent (Fig. 4).

It should be noted that resting lymphocytes do not synthesize DNA or divide. After 10 h of PHA

treatment, the stimulated cells are well into the G_1 phase of their first mitotic cycle, but DNA synthesis has not begun. Mitosis begins to occur only after about 24 h of growth. Therefore, in the above experiment, after 10 h of PHA stimulation followed by a 12-h chase incubation, the first round of cell division has not yet begun.

DISCUSSION

These results demonstrate that in resting cells a large portion of newly synthesized 32S + 28S rRNA is eventually degraded in a manner unre-

30 MIN PULSE, LONG CHASE
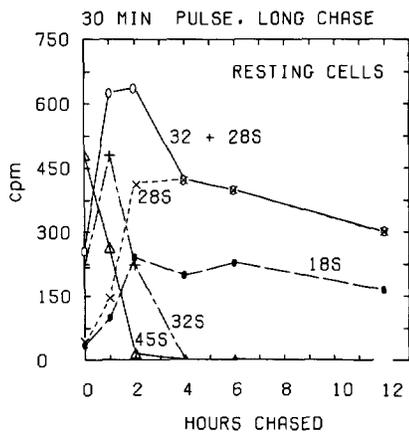
FIGURE 2 Fate of labeled rRNA species during prolonged chase incubation in resting lymphocytes. Radioactivity in each rRNA species after varying chase periods was determined from the experiment illustrated in Fig. 1. Data were normalized with respect to the A_{260} of 28S and 18S rRNA in each electrophoretic gel, determined by planimetry of A_{260} scans, to correct for the slight variation among samples due to recovery factors. Each point is the mean of determinations from duplicate cultures. Duplicate values varied by <10% of the mean. ($\triangle$—$\triangle$), label in 45S RNA; (+— - - -+), label in 32S RNA; (×- - -×), label in 28S RNA; (●——●), label in 18S RNA; (○—○), total label in 32S + 28S RNA.

lated to ribosomal turnover, and that this wastage is eliminated during growth stimulation.

No evidence of early degradation of 18S RNA in resting lymphocytes, which has been shown previously (6), was obtained in this study. This is consistent with the very early degradation of any susceptible 18S RNA molecules. Because of the experimental design, the only labeled 18S molecules appearing have already escaped degradation either through some type of stabilization, or by transport away from the site of nuclease activity.

Thus, while the overall result is the loss of half of all newly synthesized rRNA molecules, there is a temporal dissociation in the degradation of the two portions of each 45S rRNA precursor molecule. The basis of this dissociation becomes evident on consideration of the process of rRNA maturation. After synthesis of a 45S precursor, cleavage into 32S and 18S pieces occurs. The 18S portion is rapidly transported to the cytoplasm with little accumulation in the nucleus while the 32S form enters a sizeable pool of such molecules in the nu-

cleolus. Its appearance in the cytoplasm is delayed while conversion to the 28S form occurs (8). During a relatively short labeling period the small number of nuclear 18S molecules quickly will be replaced by new radioactive ones. The accompanying labeled 32S molecules, however, will enter and form a small part of the nucleolar pool of preexisting nonradioactive molecules. Degradation of 18S molecules has been seen to occur soon after cleavage. Degradation of the corresponding labeled 32S + 28S molecules may occur by random selection of molecules from the nucleolar 32S + 28S pool, or at a specific point in a sequential maturation process. If degradation of 32S + 28S is random, then admixture with preexisting unlabeled molecules will reduce the number of labeled molecules degraded at any time. Loss of labeled 32S + 28S molecules will be exponential and prolonged, while loss of 18S molecules will be immediate, producing the aberrant 28:18S labeling ratio observed previously. Alternatively, 32S + 28S degradation may occur at a specific point in the maturation sequence of 32S to 28S RNA. In this case, loss of 32S + 28S molecules would be rather abrupt, but lagging behind that of 18S. The further along a sequential pathway the degradation of 32S + 28S RNA occurs, the greater the lag and consequently the greater the expected effect on the labeling ratio. If degradation occurs very late in a sequential process, a short pulse will produce an 18S labeling deficit which gives an estimate of the proportion of rRNA molecules being wasted.

Our reported values for the 18S labeling deficit in resting lymphocytes approximate quite closely the degree of rRNA wastage evident in this study and in our previous study of the disparity between rRNA synthesis and accumulation (6). This suggests, therefore, that the site of 32S + 28S degradation is very late in processing and favors a point in a sequential process near the completion of 28S rRNA maturation.

The conclusion that 32S + 28S degradation occurs at a particular point late in maturation is also supported by the abrupt fall observed between 2 and 4 h of chase incubation (Fig. 2). It is possible that molecules become susceptible to RNase attack only after reaching the 28S form.

A small, parallel fall in both 28S and 18S radioactivity occurred between 6 and 12 h of chase incubation in resting lymphocytes. A similar fall occurred between 4 and 12 h in PHA-stimulated
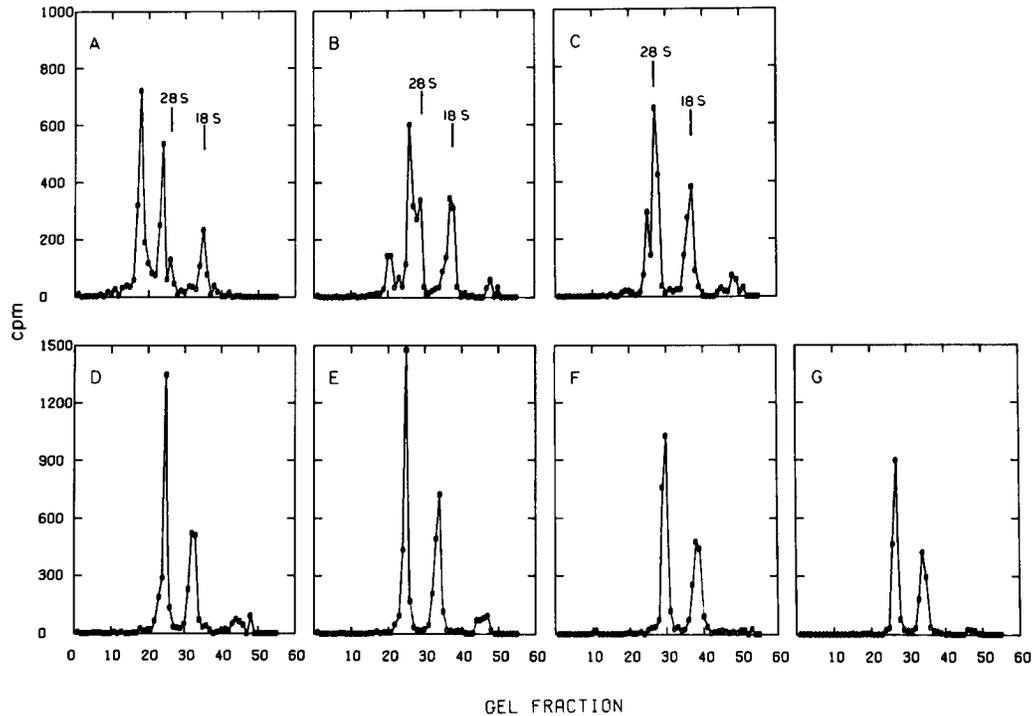
252 BRIEF NOTES

GEL FRACTION

FIGURE 3 Electrophoretic distribution of radioactive rRNA species after pulse labeling and prolonged chase incubation in growing lymphocytes. Cultures were stimulated to grow by incubation with PHA for 10 h before labeling. Other experimental details as in Fig. 1. (A) 30-min pulse. (B) 30-min chase. (C) 1-h chase. (D) 2-h chase. (E) 4-h chase. (F) 6-h chase. (G) 12-h chase.

cells. These observations suggest that mature rRNA undergoes turnover in both resting and growing lymphocytes. The present data are insufficient for detailed examination of this point, which will be considered in a future publication. In any case, it should be emphasized that rRNA turnover occurs late and involves both forms of rRNA in parallel, while the wastage phenomenon occurs early and involves a temporal dissociation in the degradation of the two forms.

These results confirm the existence of wastage of newly synthesized rRNA during maturation in resting lymphocytes and its reversal following growth stimulation. The magnitude of the wastage phenomenon suggests that its reversal plays an important role in the progressive ribosome accumulation characteristic of growing lymphocytes. Whether changes in cytoplasmic rRNA turnover, which are reported to occur in density inhibited chick embryo fibroblasts (10), also occur in the lymphocyte will be the subject of a future report.

SUMMARY

The fate of pulse-labeled rRNA was followed over a prolonged period of chase without radioactivity in resting and in PHA-stimulated lymphocytes. In resting cells, a marked loss of prelabeled 32S + 28S rRNA was observed between 2 and 4 h of chase. This fall was eliminated by PHA stimulation and was not paralleled by 18S loss. Later parallel loss of both 28S and 18S rRNA also occurred in both resting and growing cells, and was interpreted as rRNA turnover. The findings confirm predictions based on earlier work which concluded that degradation (wastage) of a large portion of newly synthesized rRNA occurs in resting lymphocytes and that reversal of this wastage was an important early step in cell growth induction.
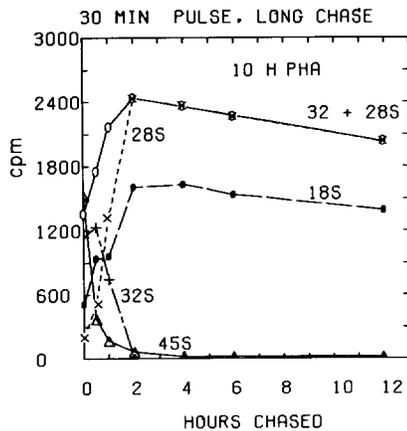
30 MIN PULSE, LONG CHASE

FIGURE 4   Fate of labeled rRNA species during prolonged chase incubation in growing lymphocytes. Radioactivity in each rRNA species after varying chase periods was determined from the experiment illustrated in Fig. 3. A_{260} measurements of total rRNA obtained from electrophoretic gels over the course of the experiment indicated an increase of 4% of the initial value per hour in rRNA content of growing cells. The increase was effectively limited to unlabeled molecules synthesized during the chase since the net synthesis during the 30-min pulse is negligible. This rRNA increase was taken into account when normalizing the data to correct for recovery variation by the following formula:

Normalized cpm =
(Observed cpm) $(1 + .04t)/A_{260}$ of total rRNA

Other details and symbols as in Fig. 2.

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