DIFFERENTIAL INHIBITION OF 28S AND 18S RIBOSOMAL RNA SYNTHESIS BY ACTINOMYCIN

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Summary. Low doses of actinomycin inhibit the synthesis of 28s ribosomal RNA to a greater extent than 18s ribosomal RNA in phytohaemagglutinin stimulated lymphocytes. Under these conditions the excess 18s RNA continues to enter the cytoplasm for extended periods.

Ribosomal RNA in mammalian cells is first synthesized as a 45s molecule, which is then processed by a number of steps to give rise to one molecule each of 28s and 18s RNA, the forms that are found in the mature ribosome (1). The control of ribosomal RNA synthesis could theoretically be exerted either at the level of 45s RNA synthesis or at any of the subsequent processing steps. Evidence that the control can occur at some step during maturation has been obtained in cells that have been incubated with cycloheximide (2-4) or puromycin (5) and in cells that have been starved for methionine (6). In each case the synthesis of 45s RNA continues but its maturation is abnormal. 18s and 28s RNA are not produced in equimolar proportions, there being a deficiency or loss of the 18s RNA. The excess 28s RNA is largely retained in the nucleus and eventually degraded. The recent discovery that a similar situation is found in resting human lymphocytes, and that the deficiency of 18s RNA is rapidly corrected when the lymphocytes are activated with phytohaemagglutinin (PHA) (7,8) suggests that such a control mechanism may be of physiological relevance.

In these situations the synthesis of 18s RNA is inhibited to a greater extent than that of 28s RNA. We report here that under certain circumstances the opposite situation occurs. When lymphocytes activated with PHA are incubated with low doses of actinomycin, which selectively inhibit ribosomal

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RNA synthesis (9-11), the labeling of 28s RNA is inhibited more than that of 18s RNA. In this situation the excess 18s RNA continues to enter the cytoplasm for at least 24 hr.

Methods

The procedures for the preparation, incubation and labeling of cultures of purified lymphocytes from human blood have been described previously (12). When cells were labeled with methyl-^{3}H methionine, 20 mM sodium formate was added to the culture medium to prevent the equilibration of radioactivity with the one-carbon pool of the cells (13).

RNA was extracted either with phenol alone at room temperature, or with phenol at 40° after shaking the cells with 0.5% sodium dodecyl sulphate. The former procedure extracts only cytoplasmic RNA, while the warm phenol method also extracts all the nuclear ribosomal and ribosomal-precursor RNA and a proportion of the nuclear polydisperse RNA (14). RNA was analysed by centrifugation through 5-20% or 8-25% sucrose gradients, as described elsewhere (12).

Results

Lymphocytes that have been incubated with PHA for 18-24 hr incorporate ^{3}H uridine into RNA at 8-20 times the rate of resting lymphocytes, but have not yet begun to synthesize DNA (15,16). The labeling of all classes of RNA is stimulated, but there is a disproportionate stimulation of the labeling of ribosomal and transfer RNA (15,16). Addition of 0.005-0.01 μg/ml actinomycin together with the PHA partially inhibits the rate of RNA synthesis, but has little effect on the PHA-induced stimulation of protein synthesis in the first 24 hr (11).

When lymphocytes were incubated for 24 hr with PHA and ^{3}H uridine, most of the radioactive RNA extracted at 40° (fig. 1a) was 28s or 18s ribosomal RNA or 4s RNA. If 0.01 μg/ml actinomycin was present in the culture medium throughout incubation, then the labeling of both species of ribosomal RNA was reduced much more than that of 4s RNA, as expected, (fig. 1a). However, it was also apparent that the ratio of 28s:18s radioactive RNA was much diminished relative to that of control cultures.

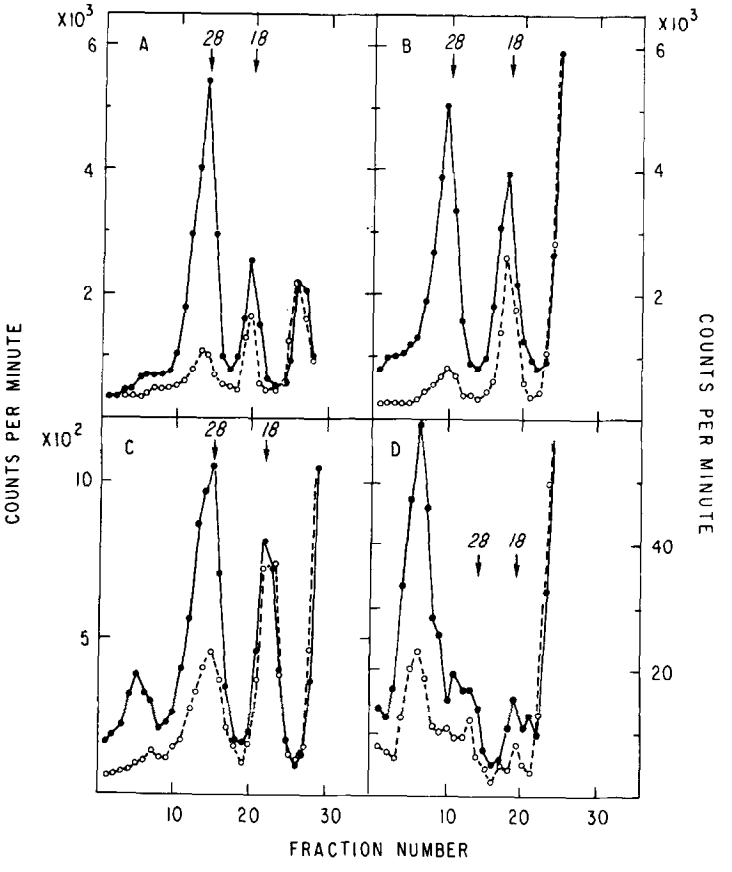

FIG. 1. Effect of low actinomycin concentration on the sedimentation of labeled lymphocyte RNA. The conditions of incubation and labeling of the cultures, and for RNA extraction, are described in the text. The arrows indicate the positions on the gradients of the 28s and 18s peaks of carrier rat liver cytoplasmic RNA. ● Radioactivity of RNA from control cultures. O Radioactivity of RNA from cultures incubated with actinomycin.

To confirm that the incorporation of isotope was not confined to the early part of the culture, and to determine whether the excess 18s RNA entered the cytoplasm, the following experiment was performed. Lymphocytes were incubated with PHA and 0.008 μg/ml actinomycin for 24 hr, and ^{3}H uridine was added only for the final 6 hr. Cytoplasmic RNA was then extracted by the cold phenol method. Fig. 1b shows that the labeling of 28s RNA was inhibited by more than 80%, while the labeling of 18s RNA continued at more than half the control rate.

Two possible explanations for the results shown in fig. 1b were eliminated by the experiment shown in fig. 1c. Firstly, the 18s RNA that continued to be labeled in the presence of actinomycin might represent non-ribosomal RNA species fortuitously sedimenting in the 18s region (17,18). Secondly, actinomycin might slow the transport of ribosomal RNA to the cytoplasm, and as 18s RNA is known to be transported more rapidly than 28s RNA (19), the corresponding 28s RNA might be retained in the nucleus. To examine these possibilities, lymphocytes were incubated with PHA and 0.005 μg/ml actinomycin for 18 hr and then labeled with methyl-^{3}H methionine for 3 hr. RNA was then extracted from these cells with phenol at 40°. Only ribosomal RNA and 4s RNA are methylated in mammalian cells (20). Actinomycin still inhibited the labeling of 28s RNA to a greater extent than 18s RNA (fig. 1c).

These results do not distinguish between the possibilities that actinomycin interfered with the maturation of 28s RNA or that it led to the formation of a defective ribosomal RNA precursor. When the RNA labeled during a 15 min pulse with ^{3}H methionine by cells that had been incubated with PHA and actinomycin for 18 hr was examined (fig. 1d) no indication of the presence of an abnormal precursor was observed. The labeling of the 45s peak was, however, substantially reduced, as it had been after the 3 hr labeling period (fig. 1c). This probably indicates a marked fall in the size of the pool of 45s RNA in the presence of actinomycin, as has been reported in HeLa cells (10). The persistance of 18s RNA labeling at near the control rate suggests that this fall may be due to a more rapid rate of 45s RNA processing rather than a decreased rate of synthesis.

Discussion

In the current model for ribosomal RNA synthesis in mammalian cells, which is supported by extensive evidence, one molecule of 45s RNA gives rise to one molecule each of 28s and 18s RNA (1). Our finding that low doses of actinomycin preferentially inhibit the accumulation of 28s RNA indicates either that a deficient precursor is synthesized in the presence of the drug or that

the 28s RNA or its precursors are rapidly degraded. In the absence of any evidence for the formation of an altered precursor, we favor the latter view, and suggest that abnormal maturation may be the basis for the selective inhibition of ribosomal RNA synthesis by actinomycin.

It seems safe to conclude that the excess 18s RNA is indeed ribosomal RNA, as it comprises up to half the 18s RNA labeled in a 6-24 hr pulse and it can be labeled with methyl-^{3}H methionine as well as ^{3}H uridine. This excess 18s RNA continues to enter the cytoplasm for extended periods. This contrasts with situations in which 18s RNA is preferentially degraded (2-8), when most of the excess 28s RNA seems to be retained in the nucleus and preferentially degraded there. The latter process would seem more suitable for physiological control mechanisms, where coordinate repression of 28s and 18s ribosomal RNA synthesis is presumably required.

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