REGULATION OF THE SYNTHESIS OF VARIOUS RIBONUCLEIC ACIDS IN ANIMAL CELLS.

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It has been reported earlier (Kubinski, Koch and Hieronymi, 1963) that in animal cells a highly polymerized RNA is accumulated during the stationary phase of growth in tissue culture. A similar phenomenon has been observed also in vivo: in Ehrlich ascites infected mice the carcinoma cells and the mouse liver cells accumulated high molecular weight RNA during the progress of illness. The RNA in question was identified as early labeled with radioactive precursors and as the one of high molecular weight on the grounds that it eluted after the bulk of ribosomal RNA from the methyl-esterified albumin - kieselguhr (MAK) column (Mandell and Hershey, 1960). There is ample evidence that in animal cells such fast labeled RNA and of this size might be either messenger or ribosomal precursor RNA (Georgiev et al., 1963; Scherrer and Darnell, 1962; Yamane et al., 1964). It was therefore interesting to find out which one of the two kinds of RNA is preferentially synthesized and accumulated in the cell under conditions precluding further cellular division. Both kinds of RNA might be recognized by their characteristic base ratios: messenger RNA would be expected to be similar to DNA, and ribosomal RNA precursor to the ribosomal RNA.

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In the present paper we wish to give a brief report on the synthesis and behavior of this particular RNA and its function in the cell. The cells (human amnion, Fernandez strain) were brought into the stationary phase of growth by suspending and incubating at high concentration (5 x 10^6 cells/ml) in Eagle's medium for various lengths of time. The control culture contained the same cells in Eagle's medium at concentration of 2 x 10^5 cells/ml. The synthesis of RNA was studied by exposing the cells to a pulse of radioactive phosphorus. In some experiments the pulse was followed by chase with "cold" (^{31}P containing) medium. RNA was extracted by hot phenol and chromatographed on MAK. The column effluent was continuously monitored for UV absorbing and

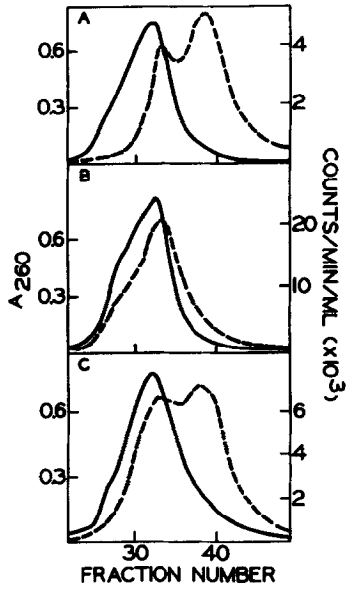

Fig. 1. Elution diagrams of RNA extracted from ^{32}P labeled amnion cells: (A) suspended at concentration of 5 x 10^6 cells/ml. and incubated for 90 minutes in ^{32}P containing medium; (B) the incubation as described in (A) was followed by 120 min. of incubation in "cold" medium at concentration of 2 x 10^5 cells/ml.; (C) the same as (B), except that the cells were incubated in "cold" medium at concentration of 5 x 10^6 cells/ml.

Continuous line: optical density at 260 mu; broken line: radioactivity. Note: the first parts of the elution diagrams (corresponding to the low molecular weight material) are not shown in the figure.

for radioactive materials. Fractions were collected for subsequent base ratio analysis. All experimental methods have been published in detail (Kubinski, Koch and Drees, 1962; Koch and Kubinski, 1964b).

Fig. 1 shows an experiment where the logarithmically growing cells were centrifuged and resuspended in Eagle's medium at concentration of 5×10^6 cells/ml. Radiophosphorus was administered at zero time. Ninety minutes later the cells were centrifuged and the pellet divided into three equal parts: the first one was used for RNA extraction (Fig. 1A) and the rest was resuspended at concentrations of 5×10^6 and 2×10^5 cells/ml of "cold" (^{31}P containing) Eagle's medium. The cells were collected for RNA extraction following additional 120 minutes of incubation under those conditions.

Fig. 1A demonstrates that two high molecular weight RNA fractions are synthesized during the first 90 minutes of incubation in the "hot" medium. The second RNA peak disappears from the elution diagram after transfer of the cells to a fresh medium followed by incubation at low concentrations. The radioactive label is recovered very close to ribosomal RNA (Fig. 1B). On the other hand, RNA synthesized during the first 90 minutes seems to be metabolically stable if the growth conditions continue to be unfavorable (Fig.1C).

The analysis of base composition of RNA synthesized and accumulated during the stationary phase has demonstrated an apparent similarity of this RNA to DNA, while RNA synthesized in logarithmically growing cells (comparison of Figs. 1A and 1B demonstrates that a majority of the labeled RNA observed in Fig. 1B has been synthesized after the transfer to "cold" medium) has a more ribosomal-like base ratio (Table 1).

An RNA of DNA-like base composition and a high molecular weight is also synthesized in the presence of Actinomycin (Table 1). Under our experimental conditions (2 ug/ml of actinomycin), the synthesis of about 95% of high

TABLE I

Nucleotide composition in Mol %.

	C	A	U	G
Ribosomal RNA *	30	17	20	33
DNA **	22	28	29	21
RNA synthesized in dividing cells ***	27	21	23	29
RNA accumulated in nondividing cells ****	22	27	28	23
RNA synthesized in the presence of Actinomycin D (2 ug/ml)	21	27	31	21

* Mean values from several experiments.

** Values computed from data given by Scherrer and Darnell (1962) and by Yamane et al. (1964).

*** Radioactive material eluting last; from experiment shown in Fig. 1B.

**** Radioactive material eluting last; from experiment shown in Fig. 1C.

molecular weight RNA has been inhibited, as compared with the untreated control. The remaining 3%, however, has a base composition closely resembling that of DNA.

In other experiments (to be published elsewhere) we have observed that each treatment of the cells which is likely to interfere with logarithmic growth, resulted in the synthesis and accumulation of this DNA-like RNA. Even such routine treatment as centrifugation and washing of the cells with buffered saline (or medium) or incubation in Eagle's medium with less phosphates than in the normal one (for better incorporation of ^{32}P) triggered the abnormally high accumulation of that particular RNA. RNA newly synthesized after such treatments had base composition markedly shifted from that of ribosomal RNA toward the one of DNA.

The observations described above suggest that under unfavorable growth

conditions messenger RNA is synthesized and stored. It might be expected that in general the chances of recovery for the cell might be considerably increased if the cell had the necessary templates and would be able to start the protein synthesis immediately upon transfer to more favorable conditions. This kind of response to external factors seems to be a rule rather than an exception in nature. In the present report we have described the predominant synthesis of this DNA-like RNA and a parallel inhibition of the synthesis of ribosomal RNA precursor following such diverse treatments as mechanical stress (Centrifugation), chemical (Actinomycin), and depletion of nutrients. Mueller and Kajiwara observed a similar effect in the cells treated with BU (personal communication). Infection with RNA viruses induces the synthesis of an RNA with many features of a messenger and inhibits the synthesis of ribosomal-like RNA (Koch and Kubinski, 1964a).

It might be postulated, therefore, on the basis of the above observations that the synthesis of ribosomal precursor is turned off first under conditions when the over-all metabolism of RNA is partially (or progressively) inhibited. It also implies an independent regulation for the synthesis of various kinds of RNA in the cell. A more detailed account of the above studies will be published elsewhere.

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