RNA SYNTHESIS DURING PREPARATION FOR GROWTH IN A RESTING POPULATION OF MAMMALIAN CELLS*

Sherman Bloom**, George J. Todaro and Howard Green

Department of Pathology

New York University School of Medicine

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Cultures of an established line of mouse fibroblasts, 3T3, grow with a doubling time of 19 hours in sparse culture. However, they are very susceptible to contact inhibition of cell division and become arrested at confluence in the G_1 period of the growth cycle (Nilausen and Green, 1965). Cells in this state are not nutritionally limited and will resume cell division when they are separated from their neighbors, or when a macromolecular substance present in dialyzed serum is added to temporarily diminish the effectiveness of contact inhibition (Todaro, Lazar and Green, 1965). When cell divisions are induced in this way there occurs within 15 minutes a rapid increase in the rate of RNA synthesis; this is followed within two hours by an increase in the rate of protein synthesis, and many hours later by the initiation of DNA synthesis and cell division (Todaro, et al, 1965). In the present communication it will be shown that the increase in the rate of RNA synthesis involves, in a coordinate fashion, all of the major classes of RNA, and can occur in the absence of protein synthesis.

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**Present address: Dept. of Pathology, University of Utah College of Medicine

1. Nature of the RNA synthesized by stationary cultures and cultures stimulated by serum:

A stationary culture was provided with fresh medium (the Dulbecco-Vogt modification of Eagle's medium) containing 10 per cent calf serum*; 20 minutes later C^{14} labelled uridine (uridine-2-C^{14}, 30 μc/μmole, New England Nuclear Corp.) was added to a final concentration of 10 μc/ml, and allowed to remain for 30 minutes. A control (stationary) culture, provided with medium depleted of the serum factor (Todaro and Green, 1966), was otherwise treated in the same way, except that the uridine was tritium-labelled (uridine-6-H^3, 7.8 mC/μmole, New England Nuclear Corp.), and was added to a final concentration of 40-120 μc/ml. At the end of the 30 minute incubation period, the cultures were pooled, unlabelled cellular RNA was added as carrier, and the RNA was extracted by the method of Scherrer and Darnell (1962), modified in that a Sephadex G-25 column was used to remove small molecular weight material (Perry, 1965). The result of sucrose density gradient centrifugation is shown in Figure 1a. The RNA synthesized was distributed across a single broad peak which was identical for the two cultures. When, following the incorporation of labelled uridine, the cells were incubated in an excess of cold uridine for four hours before isolation of the RNA, the labelled RNA was resolved into 4S, 16S, and 28S peaks, more or less coincident with the optical density distribution (Fig. 1b),and a heavier peak, probably of ribosomal precursor RNA (Scherrer and Darnell, 1962). The radioactivity incorporated into each of the peaks was in the same proportion for stationary phase cells and serum-stimulated cells. Since the overall rate of uridine incorporation, measured in separate cultures, was 10-15 times greater following exposure to the serum factor (see also Table 1), it may be concluded that the rate of synthesis for each of the major

*For convenience in these experiments. All the effects described may be produced by the simple addition of dialyzed serum.

classes was increased proportionally. This was also found to be true in experiments comparing cells in a steady state of exponential growth with stationary phase cells, and comparing cells 30 minutes and four hours after serum stimulation.

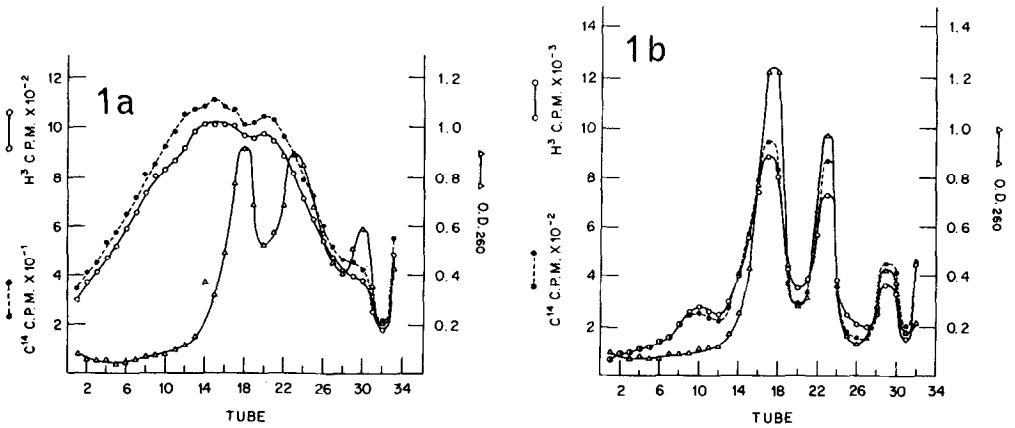

Fig. 1. Sucrose gradient analysis of RNA synthesized by stationary cultures (tritium-labelled uridine) and serum stimulated cultures (C^{14} - labelled uridine).

All cultures were exposed to labelled uridine for 30 minutes. In the experiment shown in a, the RNA was then extracted, while for b, a four-hour chase of cold uridine was given before extraction. The RNA was centrifuged at 25, 000 r.p.m. through a 5-20 per cent sucrose gradient for 12 hours at 4°C. in the SW 25.1 head of a Spinco Model L2. One ml. fractions were collected from the bottom of the centrifuge tube. 0.5 ml aliquots of each fraction were used for the simultaneous determination of H^3 and C^{14} content in a liquid scintillation spectrometer, while the remainder of the sample was used for the determination of the OD_{260}.

2. Protein synthesis is not required for the increase in rate of RNA synthesis:

Though the addition of serum increases the rate of RNA synthesis in resting cells within 15 minutes, this increase might depend on an even earlier increase in the rate of synthesis of some protein, perhaps specifically related to the control of genetic transcription. This possibility was examined by adding cycloheximide, an inhibitor of protein synthesis (Ennis and Lubin, 1964; Widuczynski and Stoppani, 1965), to cultures 30 minutes before they received fresh medium. The rates of protein synthesis and RNA synthesis were determined in control and serum-stimulated cultures by incorporation of labelled proline and uridine. The result is shown in Table 1. It can be seen that 30 minutes after addition of cycloheximide to a final

concentration of 250 μg/ml, protein synthesis was reduced to a few per cent of the control rate. Fifty μg/ml was somewhat less effective. In each of the experiments shown, however, the addition of serum elicited the same rise in RNA synthesis in the presence or absence of cycloheximide. Puromycin (50 μg/ml), like cycloheximide, was unable to prevent the rise in RNA synthesis while actinomycin (5 μg/ml), as expected, very effectively prevented it.

Table 1

Effect of Cycloheximide on the Serum Induced

Stimulation of RNA Synthesis

Expt. No.	cycloheximide concentration (μg/ml)	C^{14} uridine incorporated (relative isotope concentration)		H^3 proline incorporated (relative isotope concentration)	
		non-stimulated	serum stimulated	non-stimulated	serum stimulated
1	0	100	678	100	52
	250	70	609	2	3
2	0	100	2, 368	100	102
	50	167	1, 840	---	13
3	0	100	1, 358	100	89
	50	86	1, 194	9	25

Stationary cultures of 3T3 were incubated with and without cycloheximide for 30 minutes; then at time zero 0.4 ml of serum was added to each culture. The unstimulated cultures were exposed for ten minutes to C^{14} labelled uridine or to tritiated proline (L-proline-3, 4-H^3, 5 C/m mole, New England Nuclear Corp.) at time zero, and the serum stimulated cultures were similarly exposed thirty minutes later (when the rate of RNA synthesis is maximal).

When mammalian cells pass from exponential growth to stationary phase as the result of contact inhibition, the rate of RNA synthesis is reduced to about one tenth and protein synthesis to one third (Levine, Becker, Boone and Eagle, 1965;

Todaro et al, 1965). When 3T3 cells go from the resting to the growing state, the rates of these syntheses are restored, but not concurrently; the rate of RNA synthesis rises first, then the rate of protein synthesis, and finally DNA synthesis begins (Todaro et al, 1965). The transition from resting to growing cells has been investigated in a variety of other cell systems. Lymphocytes stimulated with phytohemagglutinin (Mueller and Le Mahieu, 1965; Rubin and Cooper, 1965) and liver cells following partial hepatectomy (Tsukada and Lieberman, 1964) also increase their rate of RNA synthesis in preparation for cell division. In these cases a much longer time elapses between the stimulus and the rise in the rate of RNA synthesis. In the phytohemagglutinin stimulated lymphocyte, the initial rise in RNA synthesis has been ascribed by Rubin and Cooper (1965) mainly to polydisperse and 4S RNA, while Mueller and LeMahieu (1965) concluded that all classes of RNA were involved. In the fibroblast system described here, where the cell type is quite uniform and the change in rate of RNA synthesis is quite large, this change clearly affects proportionally all those classes of RNA detectable by density gradient centrifugation, i.e., the 4S, 16S and 28S classes, and the larger species described by Scherrer and Darnell (1962). Similarly when human diploid cells in a steady state of exponential growth were compared with cells whose growth had been arrested by contact inhibition, the differences in rate of synthesis of 4S, 16S and 28S RNA were also found to be proportional (Levine et al, 1965).

The effect of the serum factor on stationary phase 3T3 cells is very rapid; the rate of RNA synthesis rises to a rate characteristic of exponentially growing cells within half an hour. As this change is not prevented by cycloheximide, and therefore does not appear to depend on the prior synthesis of any protein, it may be concluded that stationary phase cells have sufficient enzymatic machinery to support the rate of RNA synthesis characteristic of growing cells. The stimulation effected by the serum

factor must therefore operate by increasing the in vivo activity of the RNA polymerase already present, perhaps by making the DNA template more available to it. The mechanism involved is, as a first approximation, non-selective with regard to the type of DNA transcribed.

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