THE RNA SYNTHESIZING CAPACITY OF NUCLEI ISOLATED FROM CULTURED MOUSE CELLS

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Summary: Nuclei isolated from rapidly proliferating mouse L cells synthesize considerably more RNA than nuclei prepared from resting cells.

Introduction

One of the most interesting biological questions concerns the regulation of transcription in eucaryocytes. One possible first approach to study this process is the investigation of RNA synthesis in isolated nuclei (1, 2, 3, 4, 5, 6, 7). Before such a system can be reasonably used it has to be shown whether the RNA synthesis measured in isolated nuclei reflects the *in vivo* situation. In this paper, I shall present evidence that nuclei prepared from actively transcribing mouse L 929 cells produce considerably more RNA *in vitro* than nuclei prepared from cells with a reduced *in vivo* RNA synthesis rate. These data are taken to indicate that gross transcriptional control mechanisms survive the preparation and incubation of cell nuclei.

Material and Methods

Cell strain: Mouse L cells (NCTC clone 929) were obtained from Flow Laboratories and grown in Dulbeccos Modified Eagle medium (DME) supplemented with 10 % calf serum (CS) at 37° in an atmosphere with 10 % CO_2.

Preparation of cell fractions: Cells were collected and disrupted in a Dounce homogenizer in the presence of 0.1 % Triton X-100 (1). The nuclei were washed by two low speed centrifugation steps in DB buffer (30 mM Tris-HCl, pH 7.5, 120 mM KCl, 5 mM Mg-acetate, 7 mM beta-mercaptoethanol) (2). Microscopic inspection revealed less than 5 % nuclei with adherent cytoplasmic tabs.

Assay for *in vitro* RNA synthesis

Assays were performed in 0.5 ml volumes of DB, containing 6 mM phosphoenol pyruvate, 20 ug pyruvate kinase, 0.6 mM each of ATP, GTP and CTP and 0.84 uM 3-H-UTP (specific activity 12 Ci/mmole). To these mixture were added 1.0 - 1.6 x 10^6 nuclei. The samples were incubated at 30°. The reaction was stopped by the addition of 2 ml of ice-cold DB buffer followed by 2 ml of 10 % TCA. The vials were chilled for 10 min and the precipitate collected on Whatman glass fiber filters. The filters were transferred to steel grids and washed for 10 min each in 5 % TCA, followed by short rinses in water and methanol. Radioactivity on the filters was determined by standard methods.

In vivo determination of nucleic acid synthesis

Cells on 6 cm (diameter) plates were labelled for 2 hr with 25 uCi 5,6-3-H uridine (0.4 mM). The cells were then washed two times with DB buffer. Lysis was obtained in 3 ml of RB buffer (10 mM Tris HCl, pH 7.5, 10 mM KCl, 1.5 mM Mg-acetate) (2) containing 0.5 % of NP-40 and 1 % of SDS (10). The lysed samples were extracted with phenol and chloroform to remove proteins (8). The RNA was then precipitated from the aqueous phase by TCA, the precipitate collected on glass fiber filters and the radioactivity determined. Measured incorporation was normalized for 10^6 cells.

DNA synthesis was measured under identical conditions as described for RNA with 25 uCi methyl-3-H thymidine (10). After 2 hr the cells were washed and incubated over night in 2 ml of 0.5 M NaOH at 37° and the DNA precipitated with 1.5 ml of 100 % TCA. Processing was the same as for RNA.

Results

Reactions of isolated nuclei

(A) *Biochemical characterization of RNA synthesis in isolated* nuclei. The data shown in Fig. 1 demonstrate that all four ribonucleosidetriphosphates are required for optimal RNA synthesis regardless of whether freshly prepared nuclei or dialysed nuclei (see: legend to Fig. 1) were used. Optimal tempe-

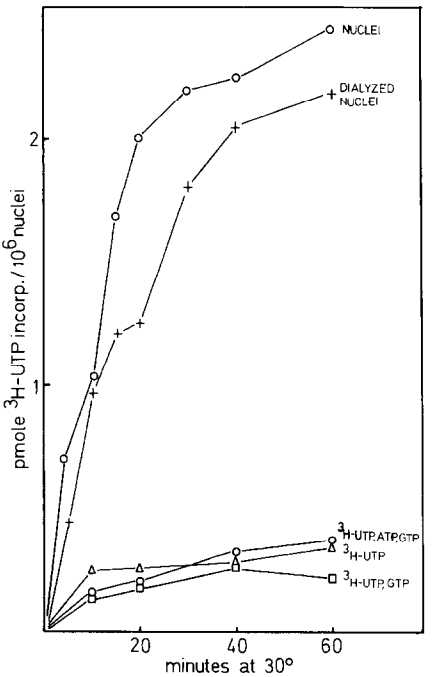
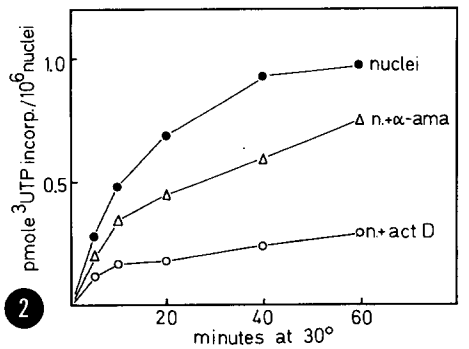


Fig. 1 Incorporation kinetics. Nuclei were prepared from exponentially growing mouse L cells. One part of the preparation was dialysed over night versus DB buffer at 0°C. Incubation was performed as described in the text except that, in some experiments, one or several of the incubated nucleosidetriphosphates have been omitted as indicated in the graph.

rature was found to be 30°C. At 37°C 60 % and at 27° 80 %, respectively, of ^{3}H-UTP was incorporated during 60 min. It is also shown in Fig. 1 that incorporation kinetics change after 20 min at 30°C when ^{3}H-UTP begins to be incorporated at a reduced rate(see also 1,5). The nuclei continue to incorporate radioactive nucleotides into RNA at this reduced rate for at least 2 hours.

Addition of 2 µg / ml α-amanitin to the reaction mixture reduced the amount of radioactivity in acid precipitable material to 60 %, actinomycin D (4 µg / Ml) to 30 % of a control value (Fig. 2). The RNA synthesized in isolated nuclei



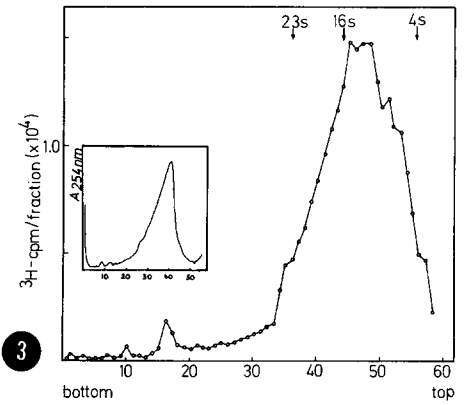


Fig. 2 Effect of α-amanitin (α-ama 2 µg / ml) and actinomycin D (act D 4 µg / ml) on RNA synthesis in isolated nuclei from exponentially growing cells.

Fig. 3 Centrifugation analysis of in vitro synthesized RNA. Nuclei from exponentially growing cells were incubated under standard conditions for 30 min. The reaction was stopped by addition of 0.1 M EDTA. Nucleic acids were extracted (1,8) with hot phenol, followed by a second extraction with chloroform. After ethanol precipitation, the RNA was sedimented through a preformed 60 % to 10 % sucrose gradient containing 0.5 % SDS, 0.1 M NaCl and buffered by 0.1 M tris-HCl, pH 7.5. Centrifugation was performed in the SW 27 rotor of the Spinco centrifuge for 19 hours at 25.000 rpm and 21°C. Bacterial ribosomal RNA was sedimented under identical conditions in a parallel tube. The arrows indicate the fraction where most of the rRNA markers were recovered in the parallel gradient.

was extracted and analyzed by sucrose gradient centrifugation (1). The sedimentation profile as shown in Fig. 3 shows a peak of radioactive RNA sedimenting with 10 - 16 S (for similar results: see ref. 9).

(B) Nuclei from proliferating and resting cells: Nuclei were prepared from logarithmically growing cells (2 - 3 days after seeding with daily medium changes) and "resting" cells (3 days after the last medium change, no further increase in cell number), respectively. It can be seen in Fig. 4 that nuclei from "resting" cells are less active in in vitro RNA synthe-

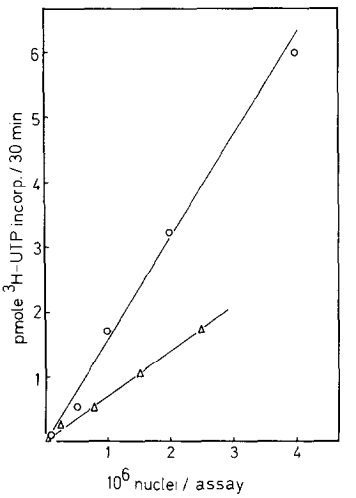


Fig. 4 The rate of RNA synthesis in nuclei from proliferating (●) and from "resting" (o) cells. "Proliferating" cells were harvested 3 days after seeding. Medium was changed daily. "Resting" cells were collected three days after the last medium change when no further increase in cell number could be detected. More than 95 % of these "resting" cells were viable as shown by the trypan blue exclusion method.

sis. It should be added that more than 95 % of the "resting" cells were viable as judged by the trypan blue exclusion method.

A systematic investigation of the correlation between the amount of RNA synthesized in intact cells and the RNA synthesizing capacity of isolated nuclei was performed. In a first series of experiments logarithmically growing cultures were transferred from a medium with 10 % calf serum to a medium with 2 % calf serum. This amount of serum is sufficient to keep the cells viable; it does, however, not permit cell proliferation. The results of Fig. 5 show that the amount of DNA and RNA synthesized during a 2 hour label period decreases with increasing time after serum reduction. It is also shown in Fig. 5 that the RNA synthesizing capacity of isolated nuclei is proportional to the amount of RNA synthesized in vivo.

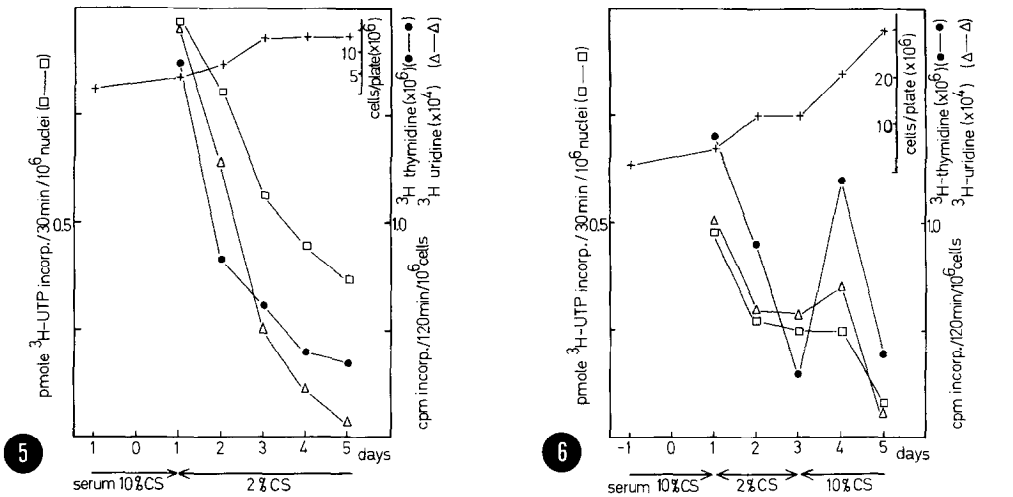


Fig. 5 Nucleic acid synthesis in serum starved cells. (Representative results from three experiments). Cells were transferred to DME medium with 2 % calf serum on day 1. Immediately before and at daily intervals after the shift to the low serum medium DNA (Δ) and RNA (●) synthesis were measured in vivo by the rate of incorporation of radioactive nucleosides. At the same time, nuclei were prepared and incubated as described (□).

Fig. 6 Reversibility of the starvation effects. (Representative results from two experiments). The experimental protocol is summarized in the graph. The estimation of DNA and RNA synthesis, respectively, was performed as described. Nuclei were prepared and incubated at the indicated times accoring to the procedures outlined above.

The effect of serum starvation on RNA synthesis in vivo and in vitro is reversible as shown by the experiment in Fig. 6. Exponentially growing cells were starved for serum for two days. During this time cellular nucleic acid synthesis as well as the RNA synthesizing capacity of isolated nuclei decreased. When the cells are refed with fresh 10% serum medium cell proliferation and DNA synthesis are stimulated, while RNA synthesis (in vivo as well as in isolated nuclei) does not change considerably (see 11). A drastic reduction occurred later when the cells had reached confluency at 3 x 10^7 cells/plate (Fig. 6).

Discussion: The amount of RNA synthesized in isolated nuclei depends on the physiological state of the cells from which the nuclei have been prepared. Optimal RNA synthesis was found in nuclei from rapidly proliferating cells. The RNA synthesizing capacity of nuclei from serum starved cells is considerably reduced. Preliminary experiments have shown that enzymes of RNA metabolisms like RNAases and RNA polymerases are present in serum starved cells in nearly the same amounts as in exponentially growing cells. This indicates that a "stringent" control mechanism blocks RNA synthesis in serum starved cells and that this regulation is also operative in isolated nuclei. This conclusion encourages a further more detailed investigation of the regulation of RNA synthesis by biochemical techniques.

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