COMPARATIVE STUDIES ON TRANSCRIPTION IN ISOLATED NUCLEI. EFFECT OF HOMOLOGOUS AND OF HETEROLOGOUS CYTOSOL

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SUMMARY

In-vitro transcription of cell nuclei isolated from normal rat liver and from a rat hepatoma is stimulated when the nuclei are incubated in homologous cytosol. This stimulating effect can be increased in both types of nuclei by using cytosol from regenerating liver.

Isolated nuclei preserve for some time their capability for synthesizing RNA (1,2). The extent of this RNA transcription depends on the medium in which the nuclei are incubated. A relatively high and prolonged rate of nuclear RNA synthesis can be obtained by adding cytoplasm to the medium (3). By using cytosol of different origins, such a system might be appropriate to study in-vitro interactions between nuclei and cytoplasm which apparently play an important role in controlling gene expression of eucaryotic cells.

This paper reports on experiments done with cell nuclei isolated from normal and regenerating rat liver and from a minimum deviation rat hepatoma (Morris hepatoma 9121). In comparative studies nuclei were incubated either in cytosol (= particle-free cytoplasm) derived from the same tissue or in heterologous cytosol, the nuclear RNA synthesis then being measured. The cell-free system described by McNamara et al. (4) was applied because it permits in-vitro synthesis of pre-mRNA as well as of rRNA. This

system contains no $(NH_4)_2SO_4$ or other non-physiological components in contrast to conditions prefered by other authors (5,6) who use a medium with relatively high ionic strength and ammonium sulfate in order to activate RNA polymerase II.

MATERIALS AND METHODS

Preparation of cell nuclei

In principle the method of Pogo et al. (7) was applied. Nuclei were isolated from the liver of female Wistar-rats (weight 140-160 g) and from Morris hepatomas 9121, which had been transplanted to male ACI rats (inbred animals, weight about 250 g) into both upper thighs, 4-5 weeks after transplantation the tumors had a diameter of about 4 cm. The fresh tissue was homogenized in 0.32 M sucrose, 3 mM $MgCl_2$ (Potter-Elvehjem); centrifuged for 7 min. at 800 g; the sediment was resuspended in 2.4 M sucrose, 1 mM $MgCl_2$ and centrifuged for 1 hour at 55,000 g. The sedimented nuclei were washed twice in a solution containing 0.25 M sucrose, 4 mM $MgCl_2$ and 0.01 M Tris-HCl (pH 8.3) and subsequently kept in this medium until being used for the incubation experiments. An aliquot of the nuclear suspension was measured in a Coulter Counter for estimating the number of nuclei per ml.

Cytoplasm

The various tissues (normal and regenerating rat liver, Morris hepatoma 9121) were homogenized (w/v = 1 : 1) in a solution containing 0.2 M Tris-HCl (pH 8.0), 0.05 M $MgCl_2$ and 0.03 M ß-mercapto-ethanol. The homogenate was centrifuged for 15 min. at 12,000 g, the supernatant again centrifuged for 1 hour at 105,000 g. The 105,000 g - supernatant (= cytosol) was dialyzed overnight against distilled water. Protein content was estimated according to (8). In the case of regenerating rat liver the animals were killed 24 hours after partial hepatectomy (9).

RNA synthesis

Nuclei were incubated in a shaking water bath at 30°C. Total volume per tube 0.5 ml, containing 6 - 8 x 10^7 nuclei; together with 50 mM Tris-HCl (pH 7.5), 2.5 mM $MgCl_2$, 2.0 mM dithiothreitol, 0.5 mM $CaCl_2$, 0.3 mM $MnCl_2$, 5.0 mM NaCl, 2.5 mM Na_2HPO_4, 5.0 mM spermidine, 2.5 mM ATP, 2.5 mM phosphoenolpyruvate, 0.5 mg/ml yeast-RNA, 17.5 units pyruvate kinase, dialyzed cytosol (7 mg protein/ml), 1 mM CTP, 1 mM GTP, 0.4 mM UTP, 25 µl [^{14}C]UTP (60 mCi/m mole); essentially as described by (4). At various times of incubation aliquots of 0.05 ml were taken, precipitated with 2 ml of 10 % TCA on Millipore filters and washed (5 x) with 3 ml of 5 % TCA. [^{14}C]-activity of the filters was measured in a "Low-Background GM-Counter".

The chemicals used were purchased from E. Merck, Darmstadt; α-amanitin and non-labeled nucleoside triphosphates from Boehringer, Mannheim, [^{14}C]UTP from Amersham-Buchler, Braunschweig.

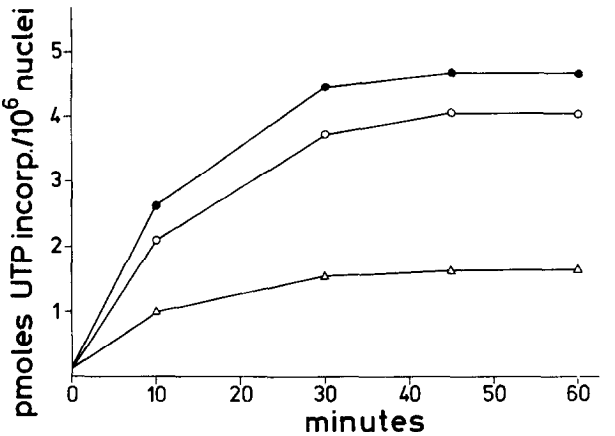

Fig. 1: Incorporation kinetics. Rat liver nuclei incubated at 30°C, Δ with rat liver cytosol and α-amanitin (1 µg/ml), O with rat liver cytosol, ● with cytosol from regenerating rat liver.

RESULTS

The described system was applied for examining the influence of soluble cytoplasm on the in-vitro transcription of isolated nuclei. Nuclei were derived from Morris hepatomas, from normal and from regenerating rat liver.

In general, cytosol leads to an increase of RNA synthesis in the incubated nuclei. Replacement of cytosol by an equivalent amount of bovine albumin causes a considerable decrease of [^{14}C]UTP-incorporation (~25 %). A still lower incorporation takes place when nuclei are incubated in a buffered salt medium containing no proteins. Apparently RNA polymerase I as well as RNA polymerase II are involved in the nuclear transcription in-vitro, because addition of α-amanitin (1 µg/ml) causes a partial block of RNA synthesis (10); inhibition about 60 %, Fig. 1.

In isolated rat liver nuclei RNA synthesis continues for 45 min. (at 30°C), while in nuclei from Morris hepatomas maximal incorporation is already obtained after 30 min. The amount of

Table I

UTP-incorporation into RNA of isolated cell nuclei: Isolated nuclei were incubated at 30° C in homologous cytosol as described in Materials and Methods. Values are given ± S.E.M. In brackets the number of experiments.

Cell Nuclei Isolated from	UTP Incorporated p moles/ 10^6 Cell Nuclei/30 min.
Morris Hepatoma 9121	3.39 ± 0.22 (17)
Rat Liver	4.32 ± 0.37 (15)
Regenerating Rat Liver	6.01 ± 0.83 (4)

Table II

Effect of cytosol from regenerating rat liver on UTP-incorporation into isolated nuclei in comparison with incorporation in homologous cytosol. Nuclei were incubated as described in Material and Methods at 30°C. Values are given ± S.E.M.

Cell Nuclei Isolated from	% Stimulation
Rat Liver	12.52 ± 0.61 (5)
Morris Hepatoma 9121	33.48 ± 11.42 (9)

[^{14}C]-labeled UTP incorporated into RNA of isolated nuclei depends on their origin and on their metabolic state. Nuclei prepared from Morris hepatomas show a fairly low value, whereby nuclei from regenerating rat liver have the highest incorporation of labeled precursors into RNA, when incubated in homologous cytosol (Table I). Cytosol derived from regenerating rat liver stimulates RNA synthesis in normal rat liver nuclei as well as in nuclei from hepatoma cells (Table II). The stimulating effect of cytosol from regenerating liver is a very constant one in

Table III

Effect of cytosol from regenerating rat liver on UTP-incorporation into isolated nuclei from Morris hepatoma compared to incorporation in homologous cytosol. Same experimental conditions as in Table II.

Hepatoma Nuclei Grouped according to Stimulation	% Stimulation
I	3.5 ± 2.5 (4)
II	37.9 ± 3.6 (3)
III	86.4 ± 0.5 (2)

transcription of nuclei from non-growing liver cells. On the other hand this effect varies when comparing RNA synthesis in tumor cell nuclei from different preparations. One can distinguish three groups of hepatoma nuclei: those which respond with a considerable increase in transcription rate; a second group with a medium increase; and a third one with almost no increase (Table III). Cytosol derived from normal rat liver does not cause measurable effects on UTP-incorporation in tumor nuclei as compared to the effect of homologous cytosol.

DISCUSSION

The presented results indicate that soluble cytoplasm stimulates transcription in isolated nuclei, apparently in a specific way. The stimulating factor in the cytosol responsible for these effects is probably a protein; it is thermolabile, at temperatures higher than 45° - 50° C its stimulating effect gets lost (11). Stein and Hausen (12) describe a cytoplasmic factor which increases the activity of RNA polymerase II at low ionic strength. Maybe these factors are similar or even identical. The effect

of cytosol can partially be replaced by proteins such as bovine albumin. Therefore in addition to real stimulation of nuclear transcription the in-vitro systems using isolated nuclei seem to be stabilized by the presence of cytoplasmic proteins.

An incorporation of nucleotides is also possible by elongation of already present RNA without transcription at a DNA template (13). Under the applied conditions this kind of incorporation of [^{14}C]UTP is negligible; it was found to be less than 2 % of the total incorporation of labeled precursors into RNA. Apparently, transcription in isolated cell nuclei is somehow correlated with the proliferating activity of the tissue origin (Table I). The Morris hepatoma 9121 is a very slowly growing tumor. On the other hand, normal liver has to fulfil so many metabolic tasks that an intensive RNA synthesis has to take place even without growth.

In contrast to the report by Dvorkin et al. (14), an inhibition of transcription in normal rat liver nuclei due to cytosol from tumor cells could not be observed. However, this result does not exclude the possibility of a qualitative effect of the heterologous cytosol on RNA synthesis. The most interesting observation is that the stimulation of transcription by cytosol derived from a normal proliferating tissue (regenerating rat liver after partial hepatectomy) is not limited to normal liver nuclei, it can also be effective in hepatoma nuclei. In this respect the hepatoma nuclei do not show a uniform behaviour. Although there is no direct proof as yet, it seems that various causal factors are involved, especially differences in growth rates of the individual tumors, tumor size and tumor age.

An important point is the question whether cytosol from rat

liver might effect gene expression in hepatoma nuclei. To clarify this extensive hybridization experiments have been begun. Preliminary results indicate that the stimulation of transcription in isolated nuclei by cytosol from regenerating liver as described in this paper is apparently due to an increased synthesis of all kinds of nuclear RNA; however preferentially ribosomal 45 S RNA is involved.

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REFERENCES

1. Weiss, S.B. (1960) Proc.Natl.Acad.Sci. USA 46, 1020-1030.
2. Widnell, C.C. and Tata, J.R. (1964) Biochem. J. 92, 313-317.
3. Wu, G.-J. and Zubay, G. (1974) Proc.Natl.Acad.Sci.USA 71, 1803-1807.
4. McNamara, D.J., Racevskis, J., Schumm, D.E. and Webb, Th.E. (1975) Biochem. J. 147, 193-197.
5. Marzluff, W.F., Jr., Murphy, E.C., Jr. and Huang R.C. (1973) Biochemistry 12, 3440-3446.
6. Soll, D.R. and Sussmann, M. (1973) Biochim.Biophys.Acta 319, 312-322.
7. Pogo, O., Alfrey, V.G. and Mirsky, A.E. (1966) Proc.Natl. Acad.Sci.USA 56, 550-557.
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J.Biol.Chem. 193, 265-275.
9. Higgins, G.M. and Anderson, R.M. (1931), A.M.A.Arch. Pathol. 12, 186-202.
10. Kedinger, C., Gniazdowski, M., Mandel, J.L., Jr., Gissinger, F. and Chambon, P. (1970) Biochem.Biophys.Res.Comm. 38, 165-171.
11. Yannarell, A., Schumm, D.E. and Webb, Th.E. (1976) Biochem.J. 154, 379-385.
12. Stein, H. and Hausen, P. (1970) Eur.J.Biochem. 14, 270-277.
13. Harbers, E. and Heidelberger, C. (1959) Biochim.Biophys. Acta 35, 381-388.
14. Dvorkin, G.A., Efimova, B.F. and Koganiskaya, L.I. (1973) Dokl.Akad.Nauk SSSR 210, 461-463.