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AGE DIFFERENCES IN RNA TRANSPORT THROUGH THE NUCLEAR MEMBRANE

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A controlled stage in the expression of genetic information in eukaryote cells is the process of RNA translocation through the nuclear membrane. RNA transport from nucleus into cytoplasm is controlled by interaction of two systems. The first, an energy-transforming system, unites specific sites of interaction of pore complexes with mRNA, nucleotide triphosphatase, and several other enzymes involved in the active transport of mRNA through the nuclear pores. The second, a system of cytoplasmic factors, regulates selective transport [3, 10].

To study nucleo-cytoplasmic RNA transport a simplified model of the outflow of prelabeled RNA from nuclei into a cell-free system is used [12]. An important fact established with the aid of this model is that the velocity of RNA transport depends on ATP [7]. During aging, the production and concentration of ATP are reduced, especially in liver cells [1, 2], and this may play an important role in the course of energy-dependent processes, including the expression of genetic information.

To study age differences in nucleo-cytoplasmic RNA transport the investigation described below was undertaken.

EXPERIMENTAL METHOD

Wistar albino rats of two age groups were used: 6-8 months (adult) and 26-28 months (old). The animals were given an intraperitoneal injection of 2[^{14}C]-orotic acid (molar activity 25.2 × 10^4 MBq/mmole) at the rate of 3.7 MBq/kg body weight, and were killed by decapitation 30 min later. Liver nuclei were isolated by the method in [5] and the cytosol by the method in [12]. The DNA content of the nuclei was determined spectrophotometrically and the protein content of the cytosol by a modified Lowry's method [8]. The nuclei were washed with 0.25 M sucrose solution in 50 mM Tris-HCl, pH 7.5, containing 25 mM KCl and 5 mM $MgCl_2$ (medium A), after which the suspension of nuclei was diluted with medium A to a final concentration of 200 μg/ml DNA and incubated at 30°C with continuous shaking. At definite time intervals aliquots of the nuclear suspension were taken and immediately centrifuged in a refrigeration centrifuge at 3000 rpm. Aliquots of nuclear suspension and supernatant were applied to Whatman 3MM or Filtrak FN-18 filter paper, washed 3 times with cold 5% TCA, once with 95% ethanol, and once with a mixture of ethanol and ether, dried, and transferred to flasks containing ZhS-106 scintillation mixture. Radioactivity was determined in a Mark 3 scintillation spectrometer. The yield of RNA was expressed as the ratio of radioactivity of acid-insoluble material of the supernatant to radioactivity of the acid-insoluble material of the nuclear suspension, in per cent. In

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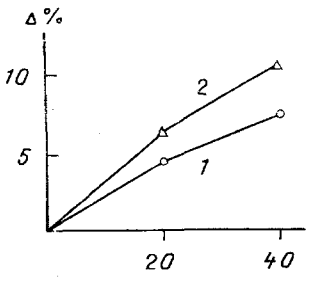
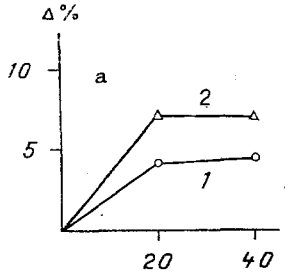


Fig. 1



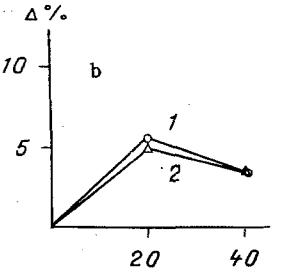


Fig. 2

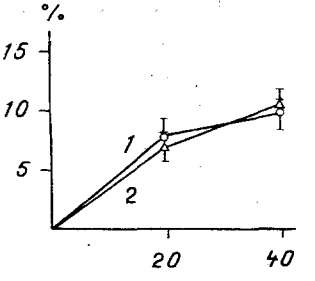


Fig. 3

Fig. 1. Outflow of RNA from liver nuclei of adult and old rats into incubation medium. Abscissa, incubation time (in min); ordinate, outflow of acid-insoluble label into incubation medium (in % of total). 1) Liver nuclei of adult rats; 2) liver nuclei of old rats.

Fig. 2. Effect of cytosol on outflow of RNA from liver nuclei of adult and old rats. Abscissa, incubation time (in min); ordinate, difference (in %) between outflow of acid-insoluble label from nuclei into incubation medium containing and not containing cytosol. a) Liver nuclei of adult rats; b) liver nuclei of old rats; 1) liver cytosol of adult rats; 2) liver cytosol of old rats.

Fig. 3. Effect of ATP-regenerating system on outflow of RNA from liver nuclei of adult and old rats. Abscissa, incubation time (in min); ordinate, difference (in %) between outflow of acid-insoluble label from nuclei into incubation medium, containing and not containing ATP-regenerating system: 1) liver nuclei of adult rats; 2) liver nuclei of old rats.

different series of experiments the incubation medium contained: 1) an ATP-regenerating system (2.5 mM ETP, 5 mM creatine phosphate, 25 U creatine phosphokinase); 2) cytosol (final protein concentration 4 mg/ml incubation medium).

The operation of partial hepatectomy was performed by the method in [6]. Methyl-[^{3}H]-thymidine triphosphate, with molar activity of 62.9-92.5 × 10^4 MBq/mmole was added in a dose of 14.8 MBq/kg body weight 1 h before decapitation.

EXPERIMENTAL RESULTS

Data on the outflow of RNA from the liver nuclei of adult and old rats depending on the incubation time in medium A are given in Fig. 1. The RNA outflow under these conditions depends on intranuclear factors and can be used as the control for studying the effect of composition of medium on transport process. Analysis of the curves did not reveal any statistically significant age differences. It will be noted that the addition of 2-mercaptoethanol to the incubation medium, and also a change in molarity of the buffer (10 mM Tris-HCL instead of 50 mM) had virtually no effect on the outflow of acid-insoluble material. The microscopic control revealed no disturbance of integrity of the nuclei. This was shown by the results of a special series of experiments in which nuclei of the regenerating liver, labeled with ^{3}H-thymidine, were incubated: throughout the period of incubation in medium A no increase was observed in the content of labeled DNA fragments, evidence that destruction of nuclei did not take place in the system used.

Addition of the cytosol fraction obtained from liver cells of rats of different ages to the incubation medium led to marked stimulation of the outflow of acid-insoluble label from the nuclei (Fig. 2). Analysis of this stimulating effect indicates that there are no statistically significant differences between the effects of the "adult" and "old" cytosol, regardless of whether it was added to liver nuclei from adult or old rats. However, under these circumstances a tendency was noted toward a stronger action as regards both magnitude and duration, of the action of stimulation of RNA outflow from liver nuclei of adult rats under the influence of liver cytosol of old rats. It can be concluded from these results that during aging no substantial qualitative or quantitative changes take place in at least those factors of the liver cell cytoplasm that participate in regulation of RNA transport on the nucleus into cytoplasm.

Considering the energy-dependence of the process of RNA transport through the nuclear membrane, an ATP-regenerating system was added to the incubation medium. This led to an increase in the outflow of RNA from the cells of the liver nuclei in both adult and old rats (Fig. 3). Under these circumstances the effect of the ATP-regenerating system on the nuclei of old rats was stronger than its effect on adult nuclei (the linear regression coefficient for adult and old rats is 0.278 and 0.240, respectively; $p < 0.001$).

Age differences thus discovered may be due to a number of factors. One stage of RNA transport from nucleus into cytoplasm is poly(A^+)-mRNA-dependent activation of specific sites of nuclear pores [3, 9], and also ATP-dependent release of adult mRNA from the nuclear matrix [11]. Under conditions of ATP deficiency, mRNA, smaller in size than the poly A-segment [4], and adult mRNA, bound with the template [11], may accumulate in the liver cells of old animals [2]. Addition of an ATP-regenerating system may therefore lead to stronger stimulation of RNA transport from nuclei obtained from liver cells of old animals. The results of this investigation thus support the view that age changes in the energy-transforming system regulating RNA transport are more marked.

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