EFFECT OF EXOGENOUS RNA ON RNA RELEASE FROM ISOLATED NUCLEI

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Cell-free systems which are models of RNA release from nuclei can be used to study the effect of various physicochemical factors (of nuclear and cytoplasmic origin) on RNA transport *in vitro*. Most frequently it is impossible to study the effect of these factors *in vivo*. The use of systems of isolated nuclei has made it possible to study the influence of factors such as pH, the ionic composition of the medium, nucleoside triphosphate, and the cytosol on DNA release [4, 6, 7, 11]. Data on the role of cytoplasmic factors in RNA transport are particularly interesting for the study of mechanisms of nucleo-cytoplasmic relations. The effect of various cytoplasmic proteins on RNA transport in a system of isolated nuclei has received the most study [8, 13]. We know much less about the influence of RNA on such transport, although it has been shown that the addition of different types of RNA to a transport system *in vitro* can modify RNA release from the nuclei [5, 10].

The aim of this investigation was to study the effect of exogenous RNA on release of different types of RNA from isolated nuclei.

EXPERIMENTAL METHOD

Cells of the striped hairy-footed hamster, transformed by SV40 [3], were labeled with ^{3}H-uridine ($0.9 \cdot 10^6$ or $3.7 \cdot 10^6$ Bq/ml) or with sodium ^{32}P-phosphate ($37 \cdot 10^6$ Bq/ml) for 15 or 60 min. After incubation the cells were sedimented by centrifugation at 1000g for 10 min. The nuclei were isolated by the method described previously [2]. All operations on isolation of the nuclei were carried out at 0°C. The cell residue was suspended in solution A (50 mM Tris-HCl buffer, pH 7.6, 25 mM KCl, 5 mM $MgCl_2$, 20 mM 2-mercaptoethanol), containing 0.25 M sucrose and 0.15% Triton X-100, and homogenized in a Dounce homogenizer. The homogenate was then applied to a layer of 1.5 M sucrose in solution A and the nuclei were sedimented at 26,000 rpm for 1 h (Spinco L2-65B ultracentrifuge, SW-27 rotor, volume of homogenate per tube 15 ml, volume of 1.5 M sucrose 20 ml). The residue of nuclei was washed twice with solution A containing the 0.25 M sucrose.

The nuclei were incubated in the system described in [9] at 30°C with constant shaking. The incubation medium in the control was solution A containing 0.25 M sucrose, 2 mM ATP, 5 mM creatine phosphate, and 0.03 mg/ml creatine phosphokinase. In the experimental series 500 µg/ml of yeast RNA (from Sigma, USA) was added to the medium. After incubation, the nuclei were sedimented at 2000g for 10 min and the supernatant was used to determine the released RNA quantitatively and qualitatively.

To study the nature of the ribonucleoprotein (RNP) particles released from the nuclei the supernatant was centrifuged by the method described in [9] for 16 h at 25,000 rpm in a sucrose gradient (10-30%), made up in 25 mM Tris-HCl buffer, pH 7.6, containing 25 mM KCl and 3 mM EDTA (Spinco L2-65B centrifuge, SW-27 rotor). Ribosomal subunits, isolated from rat liver, served as markers.

RNA was extracted by Scherrer's method [12]. The RNA was fractionated by centrifugation for 15 h at 25,000 rpm (SW-27 rotor) in a linear sucrose gradient (5-20%), made up in

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TABLE 1. Effect of Exogenous RNA on Release of Labeled RNA from Nuclei

Labeling time, min	Time interval of release, min	Radioactivity of RNA, cpm	
		control	exogenous RNA
15	0—15	51 709	33 131
	15—30	42 392	17 507
	30—90	165 396	59 507
60	0—15	61 838	39 809
	15—30	56 770	24 472
	30—90	159 859	47 824

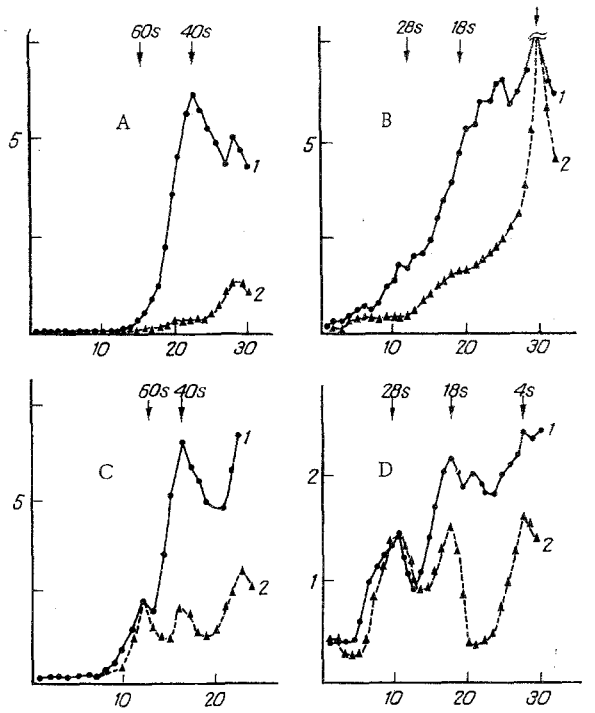

Fig. 1. Effect of exogenous RNA on sedimentation characteristics of radioactive material released from isolated nuclei. A) RNP particles released from nuclei labeled with ^{3}H-uridine ($3.7 \cdot 10^6$ Bq/ml) in the course of 15 min; B) RNA from these same particles; C) RNP particles released from nuclei labeled with ^{3}H-uridine ($0.9 \cdot 10^6$ Bq/ml) for 60 min; D) RNA from these particles; 1) control; 2) experiment. Abscissa, fraction Nos; ordinate, radioactivity$\cdot 10^{-2}$ (in cpm).

10 mM potassium acetate buffer, pH 5.1, containing 0.5% sodium dodecylsulfate, 0.1 M NaCl, and 100 µg/ml polyvinyl sulfate. RNA from whole cells of hamsters of the same species served as marker. The nucleotide composition of the RNA was determined by the method described in [1]. Radioactivity was measured with an Intertechnique SW-4000 scintillation counter (France).

EXPERIMENTAL RESULTS

Data on RNA release from the nuclei in the control and in medium containing exogenous RNA are given in Table 1. The quantity of DNA released from the nuclei was estimated from the radioactivity of acid-insoluble material liberated from the nuclei at different time intervals. Exogenous RNA was usually added to the system *in vitro* to protect RNA released from the nuclei against the action of endogenous nucleases. If the action of exogenous RNA was

TABLE 2. Nucleotide Composition of RNA released from Isolated Nuclei

Experimental conditions	Control of bases, moles%				$\frac{G+C}{A+U}$
	C	A	G	U	
Control	20.2	27,4	28,9	23,5	0,97
Exogenous RNA	17.5	28,6	23,1	30,8	0,68
Cytoplasmic RNA	26.0	23,5	26,9	23,6	1,13

Legend. C, A, G, U) Cytidylic, adenylic, guanylic, and uridylic acids respectively. Each result is mean value from three independent experiments; 10 determinations in each experiment.

confined to that, the quantity of acid-insoluble material in the supernatant would increase. However, the results show that RNA had a modifying action on the system. Addition of RNA to the incubation medium led to a decrease in the quantity of RNA released from the nuclei (Table 1). Exogenous RNA reduced the release of RNA both from cell nuclei labeled with ^{3}H-uridine for 15 min, when mainly mRNA succeeded in becoming intracellularly labeled, and also from nuclei labeled *in vivo* for 60 min, when all types of labeled RNA were present in the nuclei. To discover whether exogenous RNA changes the release of ribosomal and mRNA from the nuclei equally, sedimentation characteristics and the nucleotide composition of RNA released from the nuclei were studied.

In the first experiments RNP particles and RNA contained in them, released from cell nuclei labeled beforehand for 15 min, were analyzed (Fig. 1A, B). In this case material sedimenting in the 40S region (Fig. 1A) was released from the nuclei. This material consisted of RNP particles with sedimentation constant of 40. Most of the RNA isolated from these particles was concentrated in the 18-4S region (Fig. 1B). Release of 40S particles was strongly inhibited by the presence of exogenous RNA.

In the next experiments the period of preliminary labeling of the cells was increased to 60 min. In this case labeled 60S and 40S RNP particles containing both mRNA and ribosomal RNA were released from the nuclei (Fig. 1C, D). Addition of exogenous RNA to the incubation medium appreciably reduced the release of 40S RNP particles whereas the release of 60S particles was virtually unchanged. Sedimentation profiles of RNA released in the composition of these particles from the nuclei indicate that exogenous RNA reduced the release of RNA sedimenting in the 18-4S region but had hardly any effect on release of RNA in the 28-18S region.

Measurement of the nucleotide composition of RNA released from cell nuclei labeled with sodium phosphate showed that exogenous RNA mainly affects release of DNA-like RNA. The nucleotide composition of the RNA was shifted toward an increase in the content of GC-nucleotides, closer to the composition of cytoplasmic RNA, used for comparison, and close to the composition of ribosomal RNA (Table 2).

The results thus show that release of all types of RNA is observed in a system of isolated nuclei. Release of exogenous RNA into the incubation medium enables the RNA transport system from isolated nuclei to be modified, and as a result release mainly of ribosomal RNA can be studied. This fact is interesting because it was previously impossible to separate release of particles containing ribosomal RNA and mRNA from nuclei because of the absence of specific inhibitors of mRNA synthesis. Lengthening the labeling time does not give the desired results, for under those circumstances a fairly high background level of RNP particles containing DNA-like RNA always remains.

The causes of selective inhibition of release of mRNA from nuclei by exogenous RNA are not yet clear. This effect may perhaps be due to differences in the processes of regulation of intracellular transport of ribosomal and mRNA. The results of the present investigation also are further evidence in support of the view that cytoplasmic macromolecules may have a substantial influence on RNA transport.

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DISTRIBUTION OF MELANOCYTES IN THE DORSAL COAT OF MOUSE CHIMERAS

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The study of interaction between cells and its role in the formation of the ultimate phenotype of an organ is a difficult task in developmental biology. The value of chimeras for the study of these problems is that the experimenter can combine for the convenience of his own examination several genetically different cell populations in the same animal chimera. However, such an analysis largely depends on the presence of suitable markers. One such marker is pigment, genetically controlled variants of which are carried by melanocytes. The first genetic marker which was used when aggregation chimeras were obtained was melanocyte pigment [3, 11].

Mintz [6, 7] suggested that melanocytes of the skin arise from 17 paired clones distributed randomly along the length of the neural crest. The number of melanoblast clones arising on each side of the neural crest should correspond to the number of ancestral cells in the neural crest at the beginning of melanoblast migration, and not to their number during determination. Mouse chimeras in certain combinations of melanocyte genotypes give identical patterns of coat pigmentation; pigmented and white regions, moreover, are distributed more or less uniformly over their whole body. In chimeras, however, the distribution of pigmented skin is not uniform [12]. The ability of chimeras to form definite transverse stripes, and not a speckled pattern, of coat color varies in different combinations of lines. A striped pattern was evident for all combinations studied by Mintz [3, 4, 6, 7] and also for certain combinations described by McLaren and Bowman [2]. Meanwhile the striping in CBA—CBA T6T6 chimeras was less marked [9]. It is possible that genetic differences between components of chimeras prevent the haphazard migration of cells. This shows that the ultimate phenotypic effect of pigmentation in chimeras depends on the genotypes of the interacting cells. Variants of pigment distribution in the coat of mouse chimeras shed light on the way in which different clones of melanocytes are distributed in particular regions of skin.

The aim of the present investigation was to analyze the distributions of pigmentation in the dorsal coat of C57BL/Mib↔AKR and C57BL/Mib↔c/c mouse chimeras.

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