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DIFFERENCES IN THE FRACTIONAL COMPOSITION OF CYTOPLASMIC RNP PARTICLES IN ZAJDELA ASCITES HEPATOMA CELLS AND THE LIVER CELLS OF ANIMALS WITH TUMORS

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The relative content of poly(A)-RNA in the cytoplasm is higher in cells of Zajdela's ascites hepatoma and of the liver of rats with tumors than in normal rat liver cells. Another distinguishing feature of the tumor cells (and also, to a lesser degree, of the liver cells of animals with tumors) is a change in the ordinary ratio between polyribosomes and monoribosomes (and, correspondingly, between mRNP particles and informosomes) for normal liver cells in favor of the latter, indicating the occurrence of definite changes in their protein-synthesizing apparatus. According to some of the indices investigated, cells of tumor-bearing animals occupy an intermediate position between normal and tumor cells.

KEY WORDS: *Cytoplasmic mRNA; cytoplasmic RNP particles; hepatoma; liver of tumor-bearing animals.*

Selective transport of template RNA (mRNA) is known to take place in the cells of eukaryotes, as a result of which only a very small proportion of the molecules synthesized in the cell nucleus enters the cytoplasm. There is reason to suppose that the spectrum of mRNA transported into the cytoplasm in tumor cells is broader than in normal cells [5, 8-10] and that this decrease in selectivity of transport may have some relation to the different manifestations of tumor growth. These considerations have motivated investigations of the further fate of cytoplasmic messenger RNAs. Another aspect of the problem of malignant growth is the explanation of the molecular mechanisms of the harmful effect of tumors on the host organism, which necessitates the investigation of various tissues of tumor-bearing animals [5].

The object of this investigation was a comparative analysis of the fractional composition of cytoplasmic ribonucleoprotein (RNP) particles of cells from normal rat liver, from Zajdela's ascites hepatoma, and from the liver of animals with primary tumors (Zajdela's ascites hepatoma).

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 150-200 g, deprived of food for 24 h before the experiments, were used. Rat liver mRNA was labeled with ^{14}C-orotic acid and of Zajdela's ascites hepatoma with ^{14}C-uridine (100 μCi per rat in each case) under conditions of selective suppression of ribosomal RNA synthesis (1 h before radioactive labeling the animals were given an injection of 80 μg actinomycin D). Cytoplasmic poly(A)-containing RNAs were selectively adsorbed on a poly(U)-sepharose column [11]. Cytoplasmic RNP particles were iso-

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TABLE 1. Content of RNAs Adsorbed on Poly(U)-sepharose Column (in % of total cytoplasmic RNA) in Cells of Normal Rat Liver, Zajdela's Hepatoma, and Liver of Tumor-Bearing Animal before and after Complete Block of RNA Synthesis by Actinomycin D (mean of two determinations)

Duration of actinomycin block, h	Normal liver	Ascites hepatoma	Liver of tumor-bearing animal
0	0,92	1,07	1,03
2	0,72	—	1,05
4	0,55	1,17	1,08

lated from the postmitochondrial supernatant by the magnesium precipitation method [2]. Sedimentation of the RNP particles in a 10-35% sucrose gradient was carried out in the Spinco L2-65 centrifuge with SW 25-2 rotor for 3.5 h at 23,000 rpm at 4°C. The distribution of RNPT particles by buoyant density was analyzed by the method of Spirin et al. [4] after preliminary fixation of the particles in 4% formaldehyde followed by centrifugation in a preformed cesium chloride gradient (Spinco L2-65 centrifuge, SW 50 rotor, 18-24 h at 44,000 rpm and 4°C). Radioactivity was counted in a Nuclear Chicago Mark 2 scintillation counter, using an Olivetti Programma 101 computer to correct extinction by the channel ratio method.

EXPERIMENTAL RESULTS AND DISCUSSION

The relative content of cytoplasmic RNA (conjecturally, mRNA) adsorbed on the poly(U)-sepharose column in cells of the normal liver, hepatoma, and liver of tumor-bearing animals is shown in Table 1. Clearly the relative concentration of poly(A)-containing RNAs in the cytoplasm of normal liver cells was rather lower than in hepatoma cells or cells of the tumor-bearing animal.

Differences between these tissues also were found when the rate of breakdown of cytoplasmic mRNAs was investigated after the synthesis of nuclear RNAs had been completely blocked by actinomycin D. Whereas in normal liver cells 4 h after such treatment the mRNA content fell from 0.92 to 0.55% (of the total cytoplasmic RNA), in cells of the hepatoma and liver of the tumor-bearing animals the mRNA content remained substantially unchanged. This observation could indicate either the greater stability of the mRNA in cells of these types or the existence of a relatively larger reserve of nuclear precursors of cytoplasmic mRNAs in them, capable of maintaining nucleo-cytoplasmic transport at a constant level for the duration of the experiment. This second hypothesis is confirmed by the results of an investigation of the kinetics of incorporation of radioactive precursors into mRNAs of the comparable objects during selective inhibition of ribosomal RNA synthesis by actinomycin D [5].

The cytoplasm of animal cells is known to contain mRNA both in a form associated with ribosomes (and active in translation) and in the free state, as informosomes (the inactive form). Consequently, the fact that the relative mRNA content was higher in the cytoplasm of the hepatoma and liver of the tumor-bearing animals than in normal cells by itself is insufficient to allow conclusions to be drawn regarding the number of templates involved in protein synthesis in the polysomes. It was accordingly necessary to fractionate the cytoplasmic RNP particles in a sucrose concentration gradient (Fig. 1) and in a cesium chloride density gradient (Fig. 2); the mRNAs in the composition of these particles were selectively labeled by radioactive precursors when ribosomal RNA synthesis was blocked by actinomycin D.

The relative content of monoribosomes in the hepatoma cells was found to be sharply increased at the expense of a decrease in the number of polysomes, a fact observed during the investigation of the ribosome spectrum of several other tumors [1]. Meanwhile the relative content of polyribosomal mRNAs in the tumor cells also was sharply reduced, so far as could be judged from the distribution of the radioactive label: By far the greater part of the nonribosomal rapidly labeled RNAs was concentrated in the zone of postribosomal particles. The sedimentation profile of the RNP particles of liver cells from tumor-bearing animals resembled that of the original normal tissue, but differed from it in having a higher relative mRNA content in the postribosomal zone.

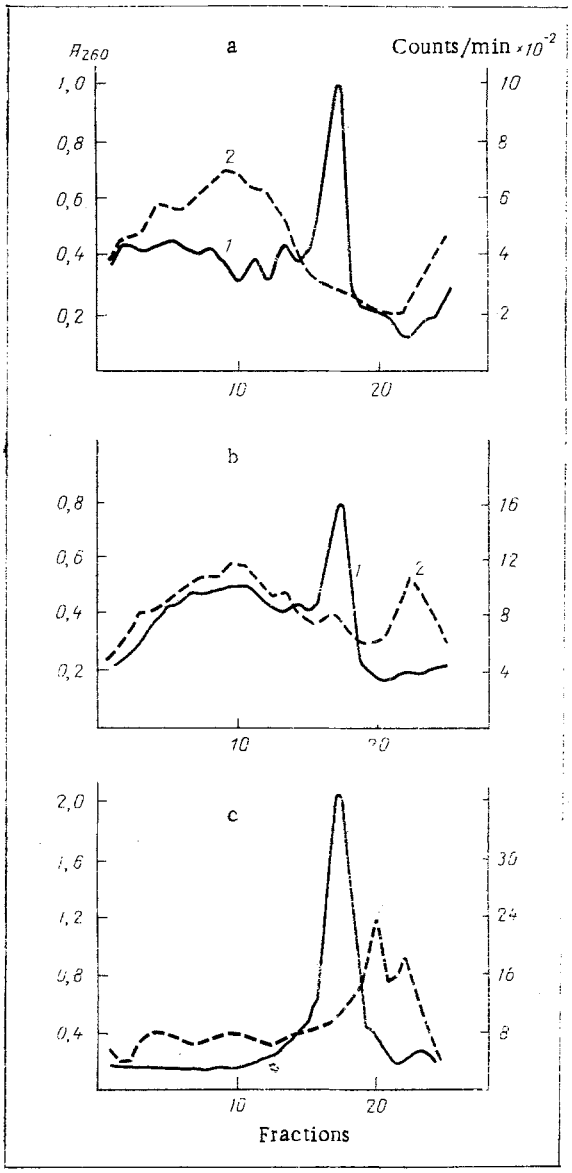

Fig. 1

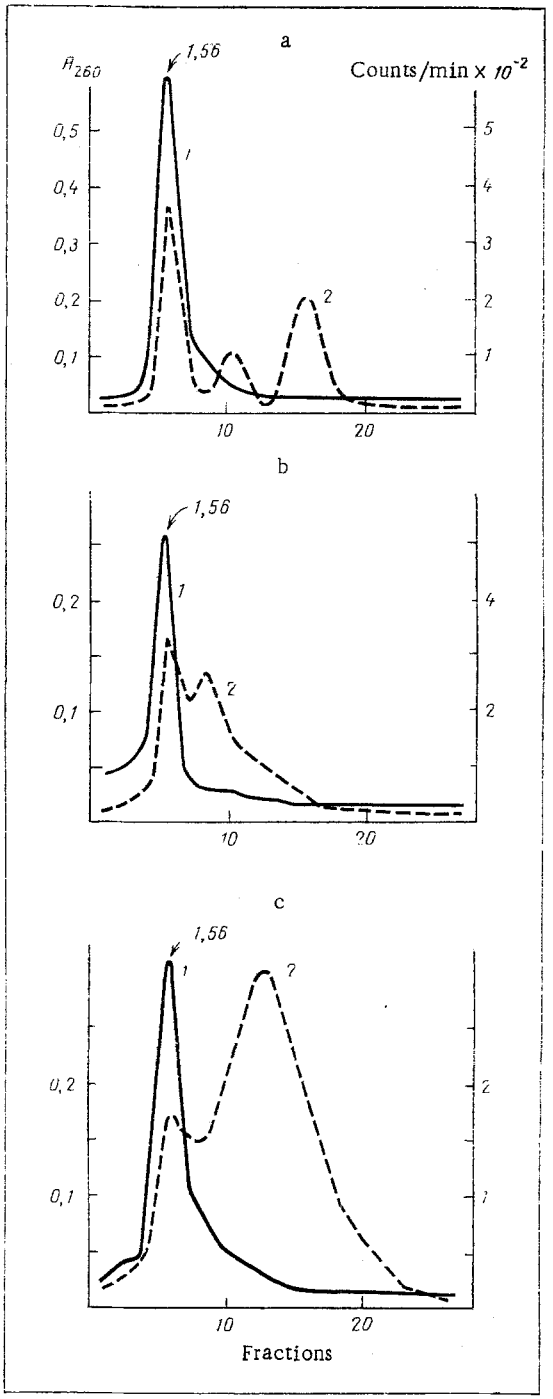

Fig. 2

Fig. 1. Sedimentation profile of cytoplasmic RNP particles labeled for 1.5 h with ^{14}C-orotic acid (a, b) and ^{14}C-uridine (c) during partial actinomycin block in a sucrose density (10-35%) gradient: a) normal liver; b) liver of tumor-bearing rat; c) Zajdela's ascites hepatoma. 1) Optical density at 260 nm; 2) radioactivity.

Fig. 2. Density distribution of cytoplasmic RNP particles labeled for 1.5 h with ^{14}C-orotic acid (a, b) and with ^{14}C-uridine (c) during partial actinomycin block in a cesium chloride gradient. Arrows indicate buoyant density of ribosomes. Remainder of legend as in Fig. 1.

The increase in the fraction of informosomes in the hepatoma cells and liver cells of the tumor-bearing rats compared with normal liver cells was confirmed by the results of equilibrium centrifugation of the corresponding RNP particles in a cesium chloride density gradient (Fig. 2). Here, just as in the preceding experiment, mRNA was selectively labeled by radioactive precursors during partial actinomycin block. By this method it was possible to separate mRNAs associated with ribosomes (buoyant density 1.56 g/cm^3) from mRNAs of informosomes, the buoyant density of which was significantly lower (1.38-1.45 g/cm^3). Clearly in both tissues (hepatoma and liver of the tumor-bearing rat) the fraction of radioactive material outside the ribosomal zone, i.e., outside the principal peak of optical density, was much greater than that in the normal liver cells.

These results thus indicate considerable differences in the relative proportions of polysomes and monosomes (and, correspondingly, of mRNP particles and informosomes) in the hepatoma cells and, to a lesser degree, in the liver cells of tumor-bearing animals compared with normal liver cells. It is obvious that the increase in the relative content of monosomes and of postribosomal particles, which is evidently a feature of many tumor cells [1], cannot be regarded as evidence of their lower rate of protein synthesis. For example, it may be due, perhaps only partially, to active synthesis of ribosomes in the tumor cell which has not yet become involved in protein synthesis. In fact the number of ribosomes calculated per unit wet weight of tissue is significantly higher in tumors than in normal tissues [1]. Furthermore, the reduced rate of elongation and termination which occurs in mitosis of some animals cells [6, 7] can also be responsible for the phenomenon described: an increase in the reserves of free informosomes and a decrease in the number and size of the polysomes in actively proliferating Zajdela's hematoma cells. Finally, the possibility also cannot be ruled out that a deficiency of certain biologically active compounds, including specific tRNAs [3], may be felt in the cells of actively metabolizing tissue.

It is interesting to note that with respect to some of the indices studied the liver cells of the tumor-bearing animals occupied an intermediate position between normal and tumor cells.

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