NUCLEAR COLUMNS: RELEASE OF RIBONUCLEOPROTEINS FROM RAT LIVER NUCLEI

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SUMMARY

Isolated rat liver nuclei, prelabeled by in vivo injection of ^{3}H-orotic acid, were immobilized in columns. Release of labeled ribonucleoproteins (RNP) from these nuclei was studied by perfusion of the columns with different media. Release of RNP can be repeatedly initiated and terminated and the rate of release varied.

Data obtained with varying ATP, deoxy-GTP, $MgCl_2$ and $MnCl_2$ concentrations, indicate that (i) the rate of release depends on the ratio of nucleoside triphosphate to divalent cation concentration, and (ii) the RNP release is not coupled to an ATP-dependent energy-requiring process.

In the present experiments the release of rapidly labeled RNA from nuclei has been studied in a newly developed experimental system (1). Prelabeled rat liver nuclei were immobilized in columns (nuclear columns). Release of RNA was studied by continuous perfusion of the columns with different media and analysis of the effluent fractions. Conditions were so selected that only a small percentage of radioactive RNA, released in the form of ribonucleoprotein complexes, was degraded to acid-soluble material. It will be shown that within the course of one experiment, the influence of various experimental conditions on transport of ribonucleoproteins can be studied.

In order to ascertain whether the transport of ribonucleoproteins from nuclei is coupled to an ATP-dependent energy-requiring process, as proposed by Raskas (2) and Schumm et al. (3), the effect of nucleoside triphosphates on the reaction was investigated.

Methods. Nuclei were isolated from the liver of Sprague-Dawley rats by the method of Widnell and Tata (4). During the isolation procedures $MgCl_2$ concentrations were kept higher than 2 mmolar. Forty minutes prior to

sacrifice the rats were intraperitoneally injected with 5-(^{3}H)-orotic acid (ca. 15 Ci/mole). As previously described (1) the isolated nuclei were absorbed onto the surface of membrane filter pieces which were then transferred into micro columns. The perfusion medium contained 250 mM sucrose, 50 mM Tris.HCl (pH 7.4), 5 mM dithiothreitol, dialyzed ribonuclease inhibitor from rat liver cytosol (5), and other additions as indicated. The nuclear columns were kept in a thermostatically controlled water bath and the media were pumped through the columns at a constant rate. The effluent fractions were collected by a drop counter into liquid scintillation vials and the total radioactivity of the material released was determined in a liquid scintillation spectrometer. To control whether the radioactive material released was acid-insoluble, some fractions were collected on filter paper disks. The disks were repeatedly washed with trichloroacetic acid, ethanol and ether (5). The radioactive material was dissolved in Soluene and counted.

Results and discussion. In order to study the release of rapidly labeled RNA from nuclei, rats were injected with ^{3}H-orotic acid 40 minutes prior to sacrifice. The nuclei of the livers were isolated and immobilized in nuclear columns. The columns were perfused at such a rate that the bed volume was exchanged every 30 seconds. Fractions corresponding to about 3 times the bed volume were collected. The perfusion medium contained sucrose, buffer, and a fraction of ribonuclease inhibitor.

The labeled material found in the effluent fractions consists of ribonucleoproteins which sediment in a broad zone with a peak at about 22 S in a sucrose gradient. The greater part of the material exhibits a buoyant density of 1.45 in CsCl. There is also a small fraction with a buoyant density of 1.54. RNA isolated from the ribonucleoproteins sediments with a peak at about 12 S in a sucrose gradient.

Under the experimental conditions selected, only small amounts of the labeled material are degraded to acid-soluble products. Therefore, it

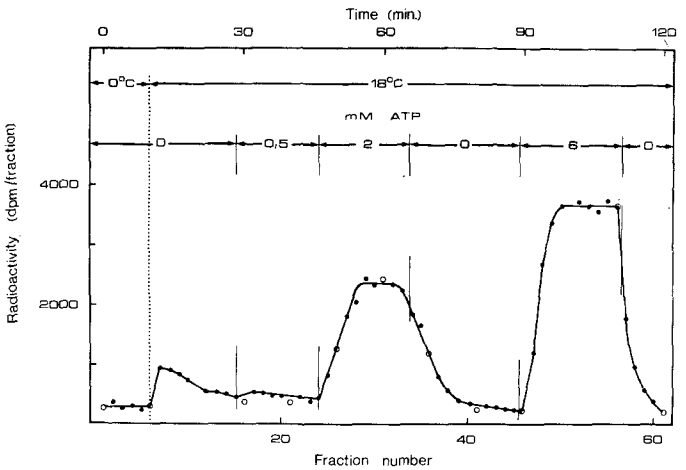

Fig. 1. Reversible induction of RNA release by ATP. Column: 30 µl volume; rate of flow: 60 µl/min. The medium contained 3 mM $MnCl_2$. The ATP concentration in the perfusion medium and the temperature of the nuclear column were varied as indicated. ●——● , total radioactivity; o——o , acid-insoluble radioactive material. In the column after the perfusion: 116 x 10^3 dpm acid-insoluble, 2.4 x 10^3 dpm acid-soluble.

sufficed to measure the total radioactivity in the effluent fractions. Some control fractions were, however, collected onto filter paper disks and analyzed for their content of acid-insoluble radioactive material. Analyses of the column contents determined after the end of the perfusion are cited in the legend to the figures.

Fig. 1 shows the effect of varying ATP concentrations on the release of RNA. Manganese chloride was included in the perfusion medium as Ishikawa et al. (6) reported in their detailed studies, that it supresses RNA release to a greater extent than magnesium chloride. Thus, with $MnCl_2$, the values of the 18°C reaction observed in the absence of ATP are close to the 0°C base line.

The column was first perfused at 0°C, then the temperature was raised to 18°C. Immediately after the temperature change there is an initial release of material in a small peak, after which release continues at a relatively low rate. Addition of 0.5 mM ATP only slightly affects the reaction. When, however, the column is perfused with 2 mM ATP, the

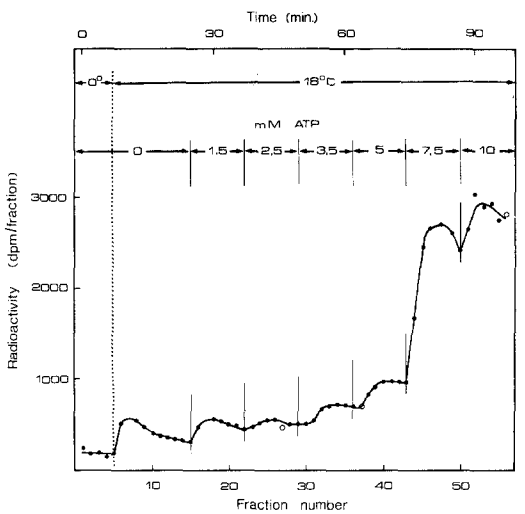

Fig. 2. Effect of ATP on RNA release at high divalent cation concentration. Conditions as in Fig. 1, except that the medium contained 6 mM $MgCl_2$ and 3 mM $MnCl_2$. In the column: 115 x 10^3 dpm acid-insoluble, 2.5 x 10^3 dpm acid-soluble.

rate of release significantly increases and finally reaches a plateau. It can further be seen that the release is almost completely inhibited when ATP is again omitted from the incubation medium. When 6 mM ATP is then pumped through the column, the rate of release reaches a plateau higher than that brought about by the 2 mM ATP solution. The release can thus reversibly be switched "on" and "off" by addition and omission of ATP, the rate depending on the nucleoside triphosphate concentration.

In the experiment of Fig. 2 the concentration of the divalent cations Mg^{++} plus Mn^{++} was maintained at 9 mmolar. Different plateaus are obtained when media with increasing concentrations of ATP are subsequently passed through the column. However, even with 5 mM ATP, the rate of RNA release is relatively low. A higher rate of release is only reached when the ATP concentration approaches the divalent metal ion concentration.

When the $MnCl_2$ concentration was 1 mmolar, significant RNA release occured at ATP concentrations above 0.5 mmolar.

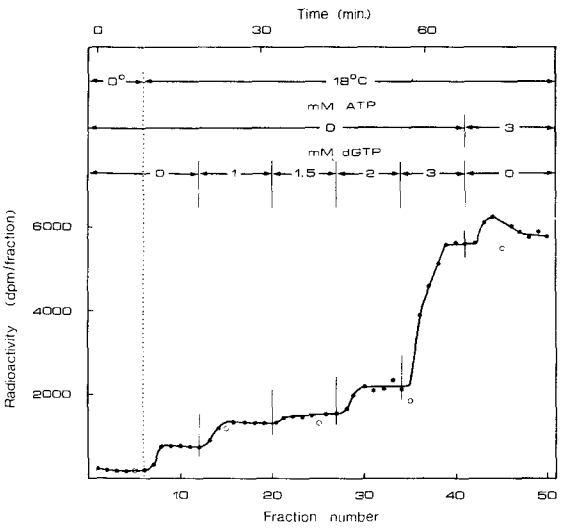

Fig. 3. Effect of dGTP and ATP on RNA release. Conditions as in Fig. 1. The medium contained 3 mM $MnCl_2$. In the column: 94 x 10^4 dpm acid-insoluble, 1.3 x 10^4 dpm acid-soluble.

Similarly, in the experiment of Fig. 3, in which the $MnCl_2$ concentration was 3 mM, the release of RNA was high when the concentration of the nucleoside triphosphate was above 2 mmolar. It thus appears that the ratio of nucleoside triphosphate to $MnCl_2$ concentration, and not the nucleoside triphosphate concentration per se is decisive for the regulation of RNA release. Significant release occurs when the nucleoside triphosphate concentration is greater than two thirds of the divalent cation concentration.

Whether ATP could be substituted by other nucleoside triphosphates was also investigated. Fig. 3 shows the results obtained with 2′-deoxy-guanosine-5′-triphosphate (dGTP). As in the experiments with ATP, high rates of release are obtained when the concentration of dGTP approaches that of $MnCl_2$. Similar results were found in experiments with 2′-deoxy-adenosine-5′-triphosphate (dATP).

When, in the course of the experiment, dGTP was substituted by an equal concentration of ATP, the rate of release only slightly increased, indicating that there are only small differences in the action caused by the two nucleotides.

It was also found that the release of RNA can be induced by the chelating agents citrate and pyrophosphate (7). Here too, the rate of the release depends on the relative concentrations of the complexing agents and the divalent metal ions.

The data suggest that the transport of ribonucleoprotein particles from nuclei is not necessarily coupled to an ATP-dependent energy-requiring process (2, 3). As originally suggested by Schneider (8) the process may be primarily affected by the divalent cations, which in turn may be influenced by chelating agents such as ATP. This view has also been expressed by Chatterjee et al. (9) in a recent publication.

The results demonstrate that the continuous flow system offers certain advantages over the conventional in vitro incubation system. In the course of one experiment, the influence of various concentrations of one agent can be tested, and the effect of different compounds can be compared.

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