Ribosomal and Informational Ribonucleoprotein Complexes of Animal Cells

STUDY ON RAT LIVER RIBONUCLEIC ACIDS AS CONSTITUENTS OF RIBONUCLEOPROTEIN COMPLEXES BY CHROMATOGRAPHY ON NUCLEOPROTEIN–CELITE COLUMNS

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(Received 18 September 1974)

A novel method of RNA fractionation has been developed. Nuclear and cytoplasmic rat liver RNA species were fractionated as constituents of corresponding ribonucleoprotein particles, which were previously adsorbed on a Celite column by their protein component. The fractionation is based on a dissociation of the particles (linear concentration gradient of LiCl and urea with subsequent temperature gradient), which results in a gradual release of the RNA molecules from ribonucleoprotein complexes. Thus the fractionation is in accordance with the tightness of the RNA–protein bonds. A gradient elution of RNA from a nucleoprotein–Celite column permitted fractionation of both ribosomal and rapidly labelled non-ribosomal RNA. The latter, both nuclear and cytoplasmic, could be separated by chromatography on nucleoprotein–Celite columns into two main fractions (components I and V). In cytoplasmic RNA components I and V presumably correspond to mRNA (messenger-like RNA of free cytoplasmic particles) and rRNA (template RNA associated with ribosomes) respectively.

A thorough study on the transfer of genetic information from the nucleus, where RNA is synthesized, to the cytoplasm, where the information is utilized, led to the discovery that in both nucleus and cytoplasm RNA was necessarily associated with the proteins (Spirin *et al.*, 1964; Samarina *et al.*, 1968; Perry & Kelley, 1968; Henshaw, 1968; Burny *et al.*, 1969; Parsons & McCarty, 1968; Pogo, 1968; Cartouzou *et al.*, 1969; Kafatos, 1968).

Newly formed nuclear RNA, non-ribosomal rapidly labelled RNA of free RNA–protein particles of cytosol, and lastly polyribosomal mRNA,* are known to exist only as RNA–protein complexes. It is widely held that all three types of non-ribosomal RNA–protein complexes are successive steps of the functional cycle of mRNA: those of nuclear RNA–protein particles which enter the cytoplasm find themselves as a part of the mRNA–protein pool and then, after proper activation, acquire the capacity to associate with the ribosomes (see Spirin, 1972).

Certain aspects of the structural organization and processing of cellular RNA–protein particles can be studied by means of RNA fractionation on a nucleoprotein–Celite column (Lichtenstein *et al.*, 1972). This chromatographic method enables one to reveal in some instances a heterogeneity of the particles which appeared homogeneous when analysed by other conventional methods.

* Abbreviations: mRNA, messenger RNA associated with ribosomes; mRNA, messenger-like RNA of cytoplasmic free particles; cRNA, cytoplasmic RNA.

The RNA–protein particles adsorbed on to the Celite (a variation of kieselguhr widely used for methylated albumin–kieselguhr chromatography) through their protein moiety (purified RNA does not bind to the column at all) are then subjected to dissociation by using a linear LiCl–urea gradient and a subsequently temperature gradient. As the dissociation proceeds various types of cellular RNA are released successively from the column and are found in distinct fractions, provided that the original RNA–protein particles differ from each other in terms of RNA–protein interactions.

In the present paper data obtained by the nucleoprotein–Celite chromatography of rat liver cellular RNA–protein particles are presented. Previous papers on this subject have been published (Lichtenstein *et al.*, 1972; Shapot & Lichtenstein, 1973).

Experimental

Animals

Male albino rats (150–200g), starved for 24h, were used.

Labelling procedure

The non-ribosomal RNA was labelled with [¹⁴C]orotic acid (100 μ Ci/rat, specific radioactivity 45 mCi/mmol; All-Union Association 'Isotope', Leningrad, U.S.S.R.) injected intraperitoneally

60min after injection of actinomycin D (40 µg/rat) and 60min before death unless otherwise mentioned.

Cell fractionation

All the ensuing procedures for isolation of the cellular particles to be studied were performed at 2–4°C.

Nuclear RNA–protein particles were extracted from purified rat liver nuclei with STM solution (0.1 M-NaCl–0.001 M-MgCl₂–0.02 M-Tris–HCl, pH 8) in the presence of ribonuclease inhibitor (Roth, 1958), as described by Samarina *et al.* (1968).

The rat liver cytoplasmic RNA–protein particles were isolated by the method of Leytin & Lerman (1969). Triton X-100 was added to postmitochondrial supernatant to a final concentration of 2% (v/v), the mixture was left in the cold for 20min and RNA–protein particles were precipitated with MgCl₂ (final concentration 0.05 M) for 60min. The RNA–protein particles were sedimented for 50min at 15000g (r_{av} , 8.5cm) and 4°C and then dissolved either by a short-term (60min) dialysis against 0.001 M-MgCl₂–0.01 M-phosphate buffer, pH 7.6 (prepared from 0.5 M-KH₂PO₄ adjusted to pH 7.6 with 3 M-NaOH) or by repeated rinsing with cold water and subsequent resuspension in the same buffer.

In some instances the total cytoplasmic RNA–protein particles were obtained directly from the post-mitochondrial supernatant by centrifugation at 269000g and 4°C for 3h (Spinco L2 centrifuge, type 65 rotor, r_{av} , 5.7cm).

Free and membrane-bound polyribosomes were isolated as described by Bloemendal *et al.* (1967).

The ribosomal subunits were obtained as described by Abakumova *et al.* (1973).

Centrifugation in sucrose and CsCl density gradients

Cytoplasmic RNA–protein particles were fractionated by centrifugation in a linear (10–35%, w/w) sucrose gradient containing 0.001 M-MgCl₂–0.01 M-phosphate buffer, pH 7.6, in a Spinco L2 centrifuge, SW 25.2 rotor, at 70000g (r_{av} , 10.8cm) and 4°C for 3.5h. In certain instances the post-mitochondrial supernatant was immediately layered on to the gradients after its treatment with Triton X-100. Samples were taken from the fractions (1.7ml) collected from the bottom of the tubes, for measurements of E_{260} and radioactivity, and the remainder were pooled for further analysis.

CsCl-band centrifugation of RNA–protein particles was performed as described by Spirin *et al.* (1965). RNA–protein particles were fixed with formaldehyde for 24h (final concn. 4%, v/v) before centrifugation on a pre-formed (1.33–1.60 g/cm³) CsCl gradient (vol. 4.6ml) containing 0.003 M-MgCl₂–0.001 M-phosphate buffer, pH 7.6, 4% and (v/v) form-

aldehyde in a Spinco model L2 centrifuge, rotor SW 50 at 160000g (r_{av} , 7.3cm) and 4°C for 18–24h.

Nucleoprotein–Celite chromatography of cellular RNA

For the method proposed, Celite, a variation of kieselguhr, was chosen as a well-known adsorbent with a high affinity for proteins but not for nucleic acids. Before use, the Celite 545 (60–80 mesh; L.P.C. Chemicals and Dyes, London W.C.1, U.K.) was boiled for 5min in TM buffer (0.005 M-MgCl₂–0.02 M-Tris–HCl, pH 7.6) to remove some u.v.-absorbing material and, after cooling, was washed several times to remove fines. The cold solution of the RNA–protein particles (about 25 E_{260} units) in TM buffer was loaded on to the Celite column (0.7cm × 7.0cm) at room temperature (20°C) and then unadsorbed material was washed out with TM buffer.

The RNA molecules to be fractionated were released from the ribonucleoprotein–Celite complexes by means of a LiCl–urea gradient ranging from TM buffer to 2 M-LiCl–4 M-urea in TM buffer at 37°C, which dissociates the RNA–protein bonds. As soon as the LiCl–urea gradient was completed, a linear temperature gradient from 37° to 98°C (approx. 1.3°C/min) was started, the maximal concentration of the eluting solution being kept constant. The E_{254} was registered by a Uvicord instrument (LKB, Stockholm, Sweden).

The fractionation procedure in the salt and temperature gradients analogous to that mentioned above was elaborated previously for RNA separation on a methylated albumin–kieselguhr column (Lichtenstein, 1970; Lichtenstein & Shapot, 1971).

Spectrophotometric assay (Kalckar, 1957) of the material eluted has shown that it represents nucleic acids practically free of protein contamination.

General methods

The extraction of RNA from RNA–protein particles, fractionation of RNA in sucrose density gradients and nucleotide composition analysis were performed as described previously (Lichtenstein *et al.*, 1972; Lichtenstein & Shapot, 1971). For the determination of radioactivity an RNA carrier (rat liver cytoplasmic RNA, 100 µg/ml) and trichloroacetic acid to a final concentration of 5% (w/v) were added. The precipitates of [¹⁴C]orotic acid-labelled RNA were collected on Millipore filters and after drying were measured in scintillation cocktail, consisting of 4g of 2,5-diphenyloxazole and 0.2g of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene/litre of toluene solvent, in a liquid-scintillation counter (Nuclear–Chicago Mark 2) with a counting efficiency of about 70%, as determined by the channels-ratio method.

Results

Cytoplasmic RNA-protein complexes

The study of the cytoplasmic RNA-protein complexes poses some problems regarding their representativeness and possible contamination with RNA-protein complexes of nuclear origin. The cytoplasm is known to contain two basic types of RNA-protein particles (ribosomal and mRNA-containing ones), and each of them may be in a free state or associated in polyribosomes.

To reveal mRNA-containing RNA-protein complexes in the preparations, the bulk of which consists of ribosomal RNA-proteins, we took advantage of low-dose actinomycin D injections, which specifically inhibit rRNA synthesis (Georgiev *et al.*, 1963; Perry, 1963). When 40 µg of actinomycin D was injected into the rat, a heterogeneous population of newly formed RNA with the maximum at about 14S, determined as described by Martin & Ames (1961) with 28S and 18S cytoplasmic rRNA as markers, was demonstrated in the cytoplasm. Under the conditions used, no significant incorporation of the label into 18S and 28S rRNA could be detected.

Fig. 1. Sedimentation profile of cytoplasmic RNA-protein particles

Rats were injected with actinomycin D (40 µg/rat) 1 h before [^{14}C]orotic acid was administered (100 µCi/rat). After labelling for 90 min the rats were killed and cytoplasmic RNA-protein particles were isolated by Mg^{2+} precipitation as indicated in the Experimental section. The solution of RNA-protein particles in 0.001 M- MgCl_2 –0.01 M-potassium phosphate buffer, pH 7.6, was layered on a 10–35% (w/w) sucrose gradient in the same buffer and centrifuged at 70000g (r_{av} , 10.8 cm) and 4°C for 3.5 h in a Spinco L2 centrifuge with a SW 25.2 rotor. Fractions (1.7 ml) were collected from the bottom of the tube for measurements of E_{260} (—) and radioactivity (----).

Cytoplasmic RNA-protein particles obtained by Mg^{2+} precipitation include a full set of components: polyribosomes, monoribosomes and ribosomal subunits (Fig. 1). The label of non-ribosomal rapidly labelled RNA is distributed mainly in the polyribosome zone, although significant amounts of it were found in the ribosomal and post-ribosomal zones as well. This sedimentation pattern shows that both 'true' mRNA and free mRNA occur in rat liver cytoplasm.

The CsCl-band centrifugation also indicates the occurrence of two kinds of mRNA-containing particles in rat liver cytoplasm (Fig. 2a). As shown previously (Quirin-Stricker & Mandel, 1969; Henshaw & Loebenstein, 1970; Sugano *et al.*, 1971) and confirmed here, this method enables distinction between free mRNA-protein particles and those bound in polyribosomes. The latter could be released from their association with ribosomes if RNA-protein complexes were treated with EDTA (Fig. 2b).

Fig. 2. Density profiles of cytoplasmic RNA-protein particles

(a) The conditions for labelling and isolation of RNA-protein particles were as described in the legend to Fig. 1. The RNA-protein particles fixed with formaldehyde were centrifuged on a pre-formed CsCl density gradient at 160000g (r_{av} , 7.3 cm) and 4°C for 18 h in a Spinco SW 50 rotor as indicated in the Experimental section. (b) The preparation of cytoplasmic RNA-protein particles was incubated at 2°C with 0.02 M-EDTA for 60 min before fixation with formaldehyde. —, E_{260} ; ----, radioactivity.

Fig. 3. Elution of RNA from the nucleoprotein-Celite column

RNA-protein particles (about 20–30 E_{260} units), obtained as described in the legend to Fig. 1, were adsorbed on the Celite column in TM buffer at 20°C. A linear salt-urea gradient (total volume 60 ml) from the original TM buffer to 2M-LiCl-4M-urea in TM buffer was run through the column at 37°C. When the salt-urea gradient was completed the maximal concentration of solution was kept constant and the temperature gradient (total vol. 60–80 ml) was started from 37° to 98°C (1.3°C/min). —, E_{260} ; ----, radioactivity; ○, LiCl-urea gradient; □, temperature gradient. The arrow indicates the start of the temperature gradient.

Free mRNA-protein particles seem to exist in the cytoplasm as normal components, rather than being due to nuclear contamination. This view is substantiated by several lines of evidence. First, nuclear RNA isolated in the presence of cytoplasmic ribonuclease inhibitor (Roth, 1958) has a higher average molecular weight than cytoplasmic non-ribosomal RNA (compare Figs. 4 and 7). Secondly, the kinetics of labelling of rat liver cRNA reveals a 15–20 min lag before labelled material appears in the cytoplasm (A. V. Lichtenstein & V. S. Shapot, unpublished work). Finally, a high EDTA-sensitivity of our polyribosomal preparations (results not shown here), a feature characteristic of cytoplasmic polyribosomes, but not of nuclear particles (Penman *et al.*, 1968), supports this point of view.

Nucleoprotein-Celite chromatography of cytoplasmic RNA-protein complexes

Nucleoprotein-Celite chromatography enables separation of cRNA into several fractions (Fig. 3). rRNA (shown by the E_{260} curve), which represents the bulk of cRNA, consists of several components: the first two (II and III) are eluted by the salt-urea gradient, and the next one (IV) is eluted by the temperature gradient. Component I, as reported previously (Lichtenstein *et al.*, 1972), is eluted at the

very beginning of the salt-urea gradient. However, it was not always seen separated from component II and could be detected often only as a peak of radioactivity on the left side of component II.

The above RNA fractions are eluted under the conditions approximately as follows: component I, 0.25M-LiCl-0.5M-urea to 0.5M-LiCl-1.0M-urea, 37°C; component II, 0.45M-LiCl-0.9M-urea to 0.6M-LiCl-1.2M-urea, 37°C; component III, 1M-LiCl-2M-urea to 1.2M-LiCl-2.4M-urea, 37°C; component IV, 2M-LiCl-4M-urea, 60°C. The labelled non-ribosomal RNA species are eluted at both the very beginning (component I) and the end (component V) of the chromatogram, the latter coming out at 2M-LiCl-4M-urea, 70°C.

Thus rRNA detected by absorbance constitutes the three components II (18S), III (28S) and IV (unidentified fraction of tenaciously bound 18S and 28S rRNA), whereas non-ribosomal RNA species (radioactivity curve) are found in the two other components, I and V, with a few minor components in between (Lichtenstein *et al.*, 1972; Shapot & Lichtenstein, 1973).

There are some reasons to believe that the chromatographic patterns obtained do not result from some artifact, such as from a random complexing of RNA and protein molecules previously not bound to each other. It has been shown

Fig. 4. Sedimentation profile of RNA species before and after nucleoprotein-Celite chromatography

The RNA-protein particles were labelled and isolated as described in the legend to Fig. 1. The RNA of native ribonucleoproteins (a) and the RNA eluted in the salt-urea (b) and temperature (c) gradients were treated with phenol-chloroform (1:1, v/v) in the presence of 0.5% sodium dodecyl sulphate (to the RNA eluted by the temperature gradient, cytoplasmic non-labelled RNA was added as a carrier). After repeated ethanol precipitations, RNA samples were analysed by sucrose-density-gradient (10–35%, w/w) centrifugation (Spinco L2 instrument, SW 25.2 rotor, 70000g, r_{av} 10.8cm, 4°C, 16h) and fractions (1.7ml) were collected for measurements of E_{260} (—) and radioactivity (----).

previously that no redistribution of RNA, with the formation of artificial secondary complexes, occurs in the course of the chromatographic procedure (Lichtenstein *et al.*, 1972). Also, we have not seen any signs of RNA breakdown during the separation procedure, by comparing sedimentation patterns of RNA before and after the nucleoprotein-Celite chromatography (Fig. 4).

In the preliminary experiments it was shown that purified nucleic acids (both double-stranded and single-stranded DNA and RNA) have no affinity for the Celite column, in contrast with proteins of various origins [bovine serum albumin, both intact and methylated; rat liver cytoplasmic post-ribosomal proteins, precipitated with $(NH_4)_2SO_4$ (60% satd.); proteins obtained from rat liver chromatin by 2M-NaCl dissociation] which are adsorbed on the Celite column almost quantitatively. On varying the conditions under which adsorption of proteins was performed (high and low ionic strength, presence of urea and EDTA, different temperatures) no changes in the extent of the binding to Celite were observed. Once adsorbed on the Celite column, most of the proteins could be released only by treatment with sodium dodecyl sulphate (0.1–1.0%), but not by the elution procedure used as a routine, i.e. salt-urea and temperature gradients (A. V. Lichtenstein & V. S. Shapot, unpublished work).

Not only Celite, but also other adsorbents with high affinity for proteins (not for nucleic acids), such as kieselguhr and silica gel, could be used for RNA-releasing chromatography with basically similar results (A. V. Lichtenstein & V. S. Shapot, unpublished work).

Moreover, the chromatographic pattern appears very reproducible and independent of the means by which RNA-protein particles were isolated. The preparations to be compared were isolated from Triton X-100-treated post-mitochondrial supernatant in different ways: Mg^{2+} precipitation; sedimentation at 269000g (r_{av} 5.7cm) for 3h; sucrose-density-gradient centrifugation (for details see the Experimental section). In addition, free and membrane-bound polyribosomes were obtained. In all these cases the chromatographic patterns were found to be essentially similar to that shown in Fig. 3.

Finally, the nucleoprotein-Celite chromatography permits separation of RNA molecules with different intracellular functions (see below); this substantiates the validity of the method.

Identification of cytoplasmic messenger and messenger-like RNA

The question arises as to whether the fractions obtained by nucleoprotein-Celite chromatography can be identified with any of the kinds of cytoplasmic RNA already known. It is to be emphasized here

that the low-molecular-weight cytoplasmic RNA species (tRNA, 5S RNA) were not taken into consideration in the present study.

The present work deals with mRNA and mlRNA only, since the behaviour of rRNA has been published previously (Lichtenstein *et al.*, 1972) and then confirmed with nucleoprotein–Celite chromatography of purified ribosomal subunits (A. V. Lichtenstein & V. S. Shapot, unpublished work).

The location of non-ribosomal RNA on the chromatogram was ascertained by using a specific inhibition of rRNA synthesis by low doses of

actinomycin D (Penman *et al.*, 1968; Spohr *et al.*, 1970). As shown above, the non-ribosomal cytoplasmic RNA labelled under these conditions could be divided into two sorts, 'true' mRNA and free mlRNA, in accordance with earlier reports (Spirin, 1969; Spohr *et al.*, 1970). The non-ribosomal rapidly labelled RNA species were found at the very beginning (component I) and the end (component V) of the nucleoprotein–Celite chromatogram (Fig. 3), thus indicating that they originate from particles with different resistances to the dissociating action of the salt–urea treatment.

Fig. 5. Nucleoprotein–Celite chromatography of polyribosomal mRNA–protein particles and free mlRNA–protein particles

Cytoplasmic RNA–protein particles were labelled as described in the legend to Fig. 1 and then subjected to sucrose-gradient centrifugation to separate the polyribosomes and post-ribosomal particles as indicated in Fig. 1. Polyribosomes (a) and post-ribosomal fraction (b) were adsorbed on the Celite column and RNA was eluted as usual. —, E_{260} ; ----, radioactivity; ○, LiCl–urea gradient; □, temperature gradient.

Fig. 6. Nucleoprotein-Celite chromatography of RNA-protein particles treated with EDTA

Labelled cytoplasmic RNA-protein particles (see the legend to Fig. 1), before adsorption on the Celite column, were incubated with 0.02 M-EDTA at 4°C for 15 min. The elution of RNA from the nucleoprotein-Celite column was performed as usual, i.e. in the presence of 0.005 M-MgCl₂. The first arrow indicates the start of the salt-urea gradient and the second one the start of the temperature gradient. —, E_{260} ; ----, radioactivity; ○, LiCl-urea gradient; □, temperature gradient.

The separation of non-ribosomal rapidly labelled RNA into two fractions by means of nucleoprotein-Celite chromatography seems to reflect the occurrence in the cytoplasm of two types of mRNA-containing particles, mRNA-protein and mlRNA-protein. The experiment shown in Fig. 5 corroborates this supposition. Polyribosomes and light particles were separated as demonstrated in Fig. 1 and each of them was then subjected to nucleoprotein-Celite chromatography. The bulk of radioactivity was detected in either component I or V, depending on whether 'light particles' or polyribosomes were analysed respectively.

It is noteworthy that the two types of mRNA-containing particles, drastically differing from each other in terms of resistance to dissociating factors, are characterized by similar RNA/protein ratios as established by CsCl-band centrifugation (see Spirin, 1972).

The question arises whether distinct chromatographic locations of mRNA and mlRNA are due to the fact that one of them is ribosome-associated, whereas the other is not. However, this was proved not to be the case, as shown in the experiment depicted in Fig. 6. The difference in the location of mRNA and mlRNA on the chromatogram is due to the features of the inner structure of corresponding RNA-protein complexes themselves rather than to the fact of association of one of them with ribosomes. The dissociation of polyribosomes with EDTA accompanied by a release of mRNA-protein particles (not shown here) did not change the distribution of radioactivity on the chromatogram,

provided that the fractionation was performed under the usual conditions, i.e. in the presence of Mg²⁺ (Fig. 6).

Moreover, both the non-ribosomal rapidly labelled fractions have approximately similar ability (about 30% of input radioactivity) to be adsorbed on the Millipore filters from solutions of a high ionic strength (Lee *et al.*, 1971), thus indicating that the poly(A) sequences are not responsible for different locations of component I and V RNA (A. V. Lichtenstein & V. S. Shapot, unpublished work).

Nucleoprotein-Celite chromatography of nuclear RNA-protein particles

The question arises whether there are nuclear precursors of the cytoplasmic components I and V. To answer the question, nuclear particles isolated as described by Samarina *et al.* (1968) were subjected to nucleoprotein-Celite chromatography. Non-ribosomal RNA was pulse labelled and nuclei were isolated and extracted with dilute salt solutions containing ribonuclease inhibitor.

Characterization of the material extracted from purified nuclei allows us to define it as a nuclear RNA-protein complex. In CsCl-gradient centrifugation RNA-protein particles pre-fixed with formaldehyde (see the Experimental section) band at 1.39 g/cm³ (not shown here), characteristic of non-ribosomal particles of informosomal type (Spirin, 1969). The presence of cytoplasmic ribonuclease inhibitor (Roth, 1958) during the isolation procedure

Fig. 7. Sedimentation pattern of RNA extracted from nuclear RNA-protein particles

The nuclear RNA was labelled with [^{14}C]orotic acid (100 $\mu\text{Ci}/\text{rat}$) for 90 min, with rRNA synthesis arrested (see the Experimental section). RNA-protein particles extracted from purified nuclei in the presence of ribonuclease inhibitor were deproteinized with phenol-chloroform (1:1, v/v). RNA obtained was centrifuged in a sucrose density gradient (10–35%, w/w) in a Spinco L2 centrifuge, SW 25.2 rotor, at 70000g, r_{av} , 10.8 cm, and 4°C for 16 h. Fractions (1.7 ml) were collected for measurements of E_{260} (—) and radioactivity (----).

preserves to some extent the structural integrity of the polynucleotide chain of nuclear RNA-protein particles, as shown by the sedimentation pattern of deproteinized RNA (Fig. 7). Size heterogeneity and the presence of a high-molecular-weight material support this view, although some degree of degradation cannot be completely ruled out.

As seen from Fig. 8, nucleoprotein-Celite chromatography has separated RNA of nuclear RNA-protein complexes into three fractions (shown by the E_{260} curve), two of them being eluted in the salt-urea and the third in the temperature gradients. The rapidly labelled non-ribosomal RNA is divided into two fractions, similar, by their location on the chromatogram, to cytoplasmic fractions I and V. This observation may indicate the occurrence in the cell nucleus of only such types of non-ribosomal rapidly labelled RNA-protein particles (as judged by the chromatographic test) as can be detected in the cytoplasm.

The most plausible interpretation of these results would be that each of the two cytoplasmic non-ribosomal particles originates from its own nuclear precursor rather than from a common one, although this notion is regarded as suggestive but in no way conclusive. It should be noted, however, that the kinetics of labelling and decay of both

nuclear and cytoplasmic non-ribosomal RNA, separated by this technique, supports our point of view.

Discussion

The primary goal of the present study was to disclose the relationship between the diversity of the tightness of RNA-protein interactions in ribonucleoprotein particles, as revealed by nucleoprotein-Celite chromatography, and their intracellular functions. The chromatographic method described separates cellular RNA species in accordance with the tightness of RNA-protein bonds within RNA-protein complexes and thus gives certain information about the heterogeneity of the RNA-protein complexes themselves.

Proceeding from this assumption, we analysed nuclear and cytoplasmic RNA-protein complexes by using nucleoprotein-Celite chromatography and identified released RNA species with already known ones. In the following sections the experimental data obtained are discussed and some conclusions drawn.

RNA-protein interactions in ribonucleoprotein complexes

The results mentioned above raise the question as to what determines different locations of various types of cellular RNA species on the chromatogram.

The data presented show clearly that RNA fractionation by nucleoprotein-Celite chromatography is based on principles different from those underlying the conventional methods. Neither molecular weight nor nucleotide composition of RNA species influences the chromatographic behaviour of the RNA species studied. Indeed, the two non-ribosomal RNA species of approximately equal molecular weights (see Fig. 4) and apparently of similar nucleotide compositions do appear on the opposite sides of a chromatogram with both heavier (28S) and similar (18S) rRNA species in between.

Thus no correlation could be revealed between chromatographic location and molecular weight and nucleotide composition of RNA species given. This could be foreseen, since mechanisms of RNA-protein interactions within the cell seem to involve features of RNA structure much more specific than merely overall nucleotide composition and/or molecular weight.

We can also state at present that neither the association of components I and V with ribosomes nor the presence of poly(A) sequences is the factor determining the difference in their location on the chromatogram. So the conclusion could be drawn that the structural organization of free RNA-protein particles and those involved in protein

Fig. 8. Nucleoprotein-Celite chromatography of nuclear RNA-protein particles

The incorporation of [^{14}C]orotic acid proceeded for 45 min under conditions of blocked rRNA synthesis (see the Experimental section). Nuclear RNA-protein particles were isolated as described by Samarina *et al.* (1968) and subjected to nucleoprotein-Celite chromatography as usual. —, E_{260} ; ---, radioactivity; ○, LiCl-urea gradient; □, temperature gradient.

synthesis as constituents of polyribosomes is different.

Ribonucleoprotein complexes and the transfer of genetic information

It is firmly established that newly synthesized RNA species are not ready to participate immediately in protein synthesis, and, being the constituents of RNA-protein complexes, first have to undergo certain steps of processing. There are at least three main types of RNA-protein particles involved in the transfer of genetic information, namely nuclear RNA-protein, free mRNA-protein and polyribosomal mRNA-protein particles (see Spirin, 1972).

The data obtained show that nuclear non-ribosomal RNA species are parts of RNA-protein particles very similar to cytoplasmic ones judging from the nucleoprotein-Celite chromatography. If so, it would pose the question concerning the interrelationship between nuclear and cytoplasmic non-ribosomal particles involved in the transfer of structural information from nucleus to cytoplasm. The revelation of at least two kinds of nuclear non-ribosomal RNA-protein complexes seems to make the situation much more complicated than has been thought so far.

Thus the particular advantage of the method proposed compared with others seems to be

the ability not only to separate RNA molecules in an unusual way but also to obtain some information about the heterogeneity and structural features of the corresponding RNA-protein complexes; this could hardly be revealed by conventional methods. As a result it was shown that both nucleus and cytoplasm contain two types of non-ribosomal particles which differ very much from each other in terms of tightness of RNA-protein bonds.

We are grateful to Professor G. P. Georgiev and Professor A. S. Spirin for critically reading the manuscript and Dr. M. I. Lerman for providing us with preparations of ribosomal subunits. The technical assistance of Mr. M. Zaboykin is gratefully acknowledged.

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