Regulated Transport of Ribosomal Subunits from Regenerating Rat Liver Nuclei in a Cell-free System

Ling C. Yu, Janis Racevskis, and Thomas E. Webb

Department of Physiological Chemistry, Ohio State University College of Medicine, Columbus, Ohio 43210

SUMMARY

A cell-free system is described which permits the study of the control of ribosomal RNA processing in and transport from isolated rat liver nuclei. The transport of the RNA in the form of 60 S and 40 S ribosomal subunits was energy and temperature dependent. It was also dependent on the presence of macromolecules in the cytosol but was independent of ongoing RNA synthesis.

INTRODUCTION

The regulation of ribosome formation in eukaryotic cells still remains an intractable problem despite the extensive knowledge available concerning the details of the nucleolar synthesis and processing of the rRNA. Within the nucleolus, which is the site of ribosome formation (2, 15, 20), a 45 S precursor is methylated and combined with ribosomal proteins during transcription (33); the 45 S precursor RNA is then processed to 32 S and 20 S preribosomal species which are subsequently cleaved to yield the 28 S and 18 S species. During this processing further ribosomal proteins are added, so that the 28 S and 18 S RNA species are actually transported to the cytoplasm in the form of 60 S and 40 S ribosomal subunits (31). Approximately one-half of each 45 S precursor molecule is lost during the nonconservative processing (16). The 60 S ribosomal subunit also contains a 5 S and a 7 S species of rRNA (1, 19). The mechanism regulating the rate of flow of equal numbers of the 2 ribosomal subunits to the cytoplasm is of particular importance since ribosome formation precedes and must be integrated with DNA synthesis and cell division.

Previous in vivo studies with the rat liver system suggest (23–25) that, in addition to transcriptional control, ribosome formation is controlled at the level of nuclear processing and nucleocytoplasmic transport. Similar conclusions have been drawn from the lymphocyte system (5). Accordingly, it appears that much of the potential rRNA is ordinarily degraded in the nucleus of resting cells but is conserved in ribosomal subunits, which are transported to the cytoplasm when the cells are stimulated to proliferate or are treated with the appropriate anabolic hormones. The earlier report (26) of the release in homogenates of detectable amounts of RNA from nuclei that are prelabeled in vivo suggested an approach that would permit a more definitive analysis of the posttranscriptional control of ribosome formation. The present communication describes a cell-free system, which for the first time permits the study of the control of ribosome formation in vitro, under conditions approximating the in vivo situation.

MATERIALS AND METHODS

Animals. Male rats (250 g) of the Sprague-Dawley strain were maintained on Purina chow until approximately 19 hr before use, at which time they were fasted in wire-bottomed cages. The lighting was mechanically controlled from 6 a.m. to 6 p.m. Partial hepatectomies were performed (23) under light ether anesthesia and all injections were i.p.

Cell-free System. The prelabeled nuclei were isolated (17, 25) from the 19-hr regenerating liver following the 2-hr labeling with orotic acid-6-14C, 200 ¿iCi/kg, (New England Nuclear, Boston, Mass.). The freshly purified nuclei were incubated (4 X 106 nuclei/ml) at 0° (control) or at 36° in 5 ml of a “complete medium” containing 0.60 volume of postmicrosomal supernatant (cytosol) prepared from a 1:3 homogenate of rat liver, 50 mM Tris buffer (pH 7.8), 250 mM sucrose, 2.5 mM MgCl2, 25 mM KCl, 0.5 mM CaCl2, 0.3 mM MnCl2, 2.5 mM Na2HPO4, 5 mM NaCl, 5.0 mM spermidine, 0.5 mg of low-molecular-weight yeast RNA per ml (General Biochemicals Corp., New York, N. Y.), 50 ¿g of ribosomal proteins per ml, 2.5 mM dithiothreitol, 2.0 mM ATP, 2.5 mM phosphoenolpyruvate and 6.4 units of pyruvate kinase per ml (Sigma Chemical Co., St. Louis, Mo.). The total volume of the reaction mixture was 5 ml. Prior to use the cytosol was dialyzed overnight against 2 changes of distilled water at 4°.

The ribosomal proteins, released from the purified rat liver ribosomes by LiCl (8), were also extensively dialyzed prior to use. In experiments designed to confirm the energy dependence of the RNA release, the o-g,methylene and the pyrophosphate linkage is replaced by a methylene group. Repetitive nuclear counts were made in a Neubauer counting chamber under a light microscope; the nuclei were counted both before and after incubation to confirm the presence or absence of nuclear lysis. Very slight lysis (less than 4%) may not have been detected due to a sampling error. Otherwise, triplicate nuclear counts agreed to within 10%.

Received May 2, 1972; accepted July 24, 1972.

1 This work was supported by Grant CA 12411-01 from the National Cancer Institute, NIH, Bethesda, Md.

2 To whom requests for reprints should be sent.
Detection of Released RNA. After the incubation of the nuclei in the complete or modified medium for 12 to 15 min on a shaker bath and chilling of those samples incubated at 36°, the suspensions were centrifuged at 1000 × g for 10 min to remove the nuclei. A 0.5-ml aliquot was treated with cold 5% TCA to give the acid-insoluble fractions. Aliquots of the total reaction mixture, the mixture after removal of the nuclei, the nuclei and acid-insoluble fractions were assayed for their radioactivities. All analyses were based on duplicate samples.

Characterization of Transported RNA. The RNA was purified from 5 ml of the incubation medium (freed of nuclei), from the nuclei removed from 5 ml of the medium, or from the postmitochondrial supernatant (9000 × g for 10 min) by use of hot phenol (17, 23). After isolation the RNA was precipitated with 95% ethanol containing 2% potassium acetate. The purified RNA in 0.02 M sodium acetate, pH 5.0, was layered over linear (10 to 30%) sucrose gradients containing 100 mM NaCl, 20 mM sodium acetate, and 1.0 mM EDTA, pH 5.0. The gradients were centrifuged for 16 hr at 49,000 × g average in a SW 25.1 rotor of a Beckman centrifuge. Following separation of the RNA species the effluent from the bottom of the gradient tube was monitored at 260 nm in a flow cell. A drop counter activated an event marker on the recorder to register each ml of effluent. Each 1-ml fraction was collected in scintillation vials and assayed for radioactivity.

Competition-Hybridization. Competition-hybridization experiments were performed according to the procedure of Gillespie and Spiegelman (7) except that the incubations were carried out for 18 hr at 65° on B-6 filters (Schleicher and Schuell Co., Keene, N. H.) in 4 × 0.15 M NaCl-0.01 M Na citrate, pH 7.0). Competitor unlabeled ribosomal was prepared by phenol extraction of the purified ribosomes of rat liver. The RNA was subsequently treated with DNase, then freed of the latter by phenol treatment. The DNA was purified from rat liver by the method of Ono et al. (18).

Size of the Nucleoproteins Transported. A 1.2-ml aliquot of the incubation medium, freed of nuclei, adjusted to 50 mM EDTA, fortified with 0.1 ml of ribosomal subunits [prepared by EDTA treatment of ribosomes from 0.1 g of unlabeled rat liver (24)] and adjusted to pH 7.6, was layered directly onto a linear 15 to 30% sucrose gradient; the latter contained 30 mM Tris HCl (pH 7.6), and 25 mM NaCl. The gradients were centrifuged at 49,000 × g for 15 hr in a SW 25.1 rotor of a Beckman centrifuge. The gradients were monitored at 260 nm and 1.0-ml fractions were collected for radioassay.

Buoyant Density Centrifugation of Nucleoprotein. The released ribonucleoprotein particles were recovered from the nuclei-free medium after incubation at 36° for 15 min by centrifugation at 105,000 × g for 3 hr. The ribonucleoprotein particles were resuspended, fixed with 6% formaldehyde, and then exhaustively dialyzed against 1% formaldehyde and against buffer (28). The dialyzed suspension was adjusted to 1.4 g of CsCl per 3 ml, then layered over 3 ml of buffer containing 3.3 g of CsCl. The CsCl step gradient (3) was centrifuged in a type 40 rotor of a Beckman centrifuge at 37,000 rpm for 18 hr. Twenty-drop fractions were collected from the bottom of the gradient tube and the density of every 4th fraction was estimated from the refractive index as measured with a Bausch and Lomb refractometer.

Assay of Radioactivity. The samples from the incubation mixture or the fractions from the sucrose gradients were solubilized in 0.5 ml Hyamine 10X (Packard Instrument Co., Chicago, Ill.) and counted in liquid scintillant (Aquasol, New England Nuclear) at an efficiency of 80%.

RESULTS AND DISCUSSION

RNA Release from Isolated Nuclei. Preliminary experiments indicated that detectable amounts of labeled RNA were released from prelabeled, purified nuclei when the latter were incubated in the appropriate medium. The nuclei were purified (23, 25) from the 19-hr regenerating liver of 250-g Sprague-Dawley rats following the 2-hr prelabeling in vivo with the RNA precursor, orotic acid-6-14C. The purified nuclei appeared well preserved and free of significant cytoplasmic contamination after purification when examined in the electron microscope (Zeiss 9A) after fixation in 2.5% glutaraldehyde and staining with lead citrate-uranyl acetate. The 19-hr regenerating liver was selected as the source of the nuclei since the nRNA in regenerating liver appears exceptionally stable during the purification procedure (25) and the incorporation of labeled precursors into rRNA is maximal during this period of regeneration (23, 24).

The complete incubation medium contained unlabeled, dialyzed postmicrosomal supernatant (cytosol) from normal rat liver (diluted 1:5), fortified with ATP, an energy regenerating system, ribosomal proteins, cations, dithiothreitol, spermidine, and low-molecular-weight yeast RNA. The dialysis of the supernatant was found to be essential to prevent the clumping of the nuclei during incubation. Repetitive nuclear counts under a light microscope confirmed that no detectable lysis of the nuclei occurred during incubation in the complete medium, i.e., the nuclear counts from the incubated samples were identical with the corresponding values of the controls incubated at 0°. However, the sampling error precluded the detection of less than 5% lysis with any degree of certainty.

As indicated by the data in Table 1, the release of RNA from the isolated nuclei is temperature dependent; however, it is independent of ongoing RNA synthesis, since it was insensitive to as high as a 10-μg/ml concentration of actinomycin D, which should inhibit all DNA-dependent RNA synthesis (21). Low-molecular-weight RNA from yeast, shown to be an effective inhibitor of RNase activity (12), appears to be necessary to suppress RNase activity not already sequestered by the natural RNase inhibitor (27) present in the diluted cytosol. Thus, although some RNA release is observed in the absence of yeast RNA, there is a significant loss of both the released radioactivity and of radioactivity normally retained within the nucleus, to the acid-soluble fraction. The addition of polyvinyl sulfonic acid, which is also a RNase inhibitor (13), could not substitute for the yeast RNA, when the recovery of acid-insoluble radioactivity was used as a criterion. Furthermore, the polyvinyl sulfonic acid caused significant lysis of the nuclei. Thus 500 μg of yeast RNA per ml appears to inhibit effectively all RNase activity, thereby

---

3The abbreviation used is: TCA, trichloroacetic acid.
permitting the maximum recovery of released radioactivity in the form of intact RNA. Increasing the concentration of RNA to 1 mg/ml (results not shown) did not increase this recovery. At least part of TCA-soluble radioactivity, which amounts to approximately 30% of the label released during the 15-min incubation, may be a by-product of the processing of the nuclear RNA precursors.

**Energy Requirement.** The time course studies in Chart 1 indicate that a significant amount of RNA is released by prelabeled nuclei to the complete medium (containing 2.0 mM ATP + 2.5 mM phosphoenolpyruvate) within 5 min of the start of the incubation at 36°C. The rate of release then drops sharply and eventually stops within 30 min of the start of the incubation. In contrast, there is very little RNA release when the ATP and phosphoenolpyruvate are omitted. Furthermore, the RNA release is an active catalytic process, since it is minimal when the incubation in the complete medium is carried out at 0°C. The fall-off and eventually cessation of transport beyond 5 min of incubation at 36°C is due to the exhaustion of the energy supply, the RNA transport being reactivated when 2.5 mM phosphoenolpyruvate is added after 15 min of incubation. Furthermore, a maximum rate of RNA release is achieved by adding 2.5 mM phosphoenolpyruvate to the medium after each 5-min interval of incubation. The time course curve is clearly biphasic; approximately 32% of the nuclear label was released in 45 min, and 70% of that is acid insoluble. The nonlinear nature of the release is not unexpected, since, following release of those ribosome subunits which were essentially completed in vivo, subsequent transport will be completely dependent upon the rate of processing of the rRNA precursors. The time required in vivo for the processing of the 45 S precursor (including combination with proteins) to the ribosomal subunits, is approximately 0.5 hr in the regenerating rat liver (23).

The results shown in Table 2 strongly suggest that most of the RNA transport is dependent upon the hydrolysis of the high-energy β,γ-pyrophosphate bond of ATP, i.e., that the process is energy dependent rather than merely polyanion dependent. At 36°C the ATP supply is exhausted within 2.5 min whether the initial concentration is 2 mM or 3 mM; thus transport is reduced by 50% when phosphoenolpyruvate, which maintains an effective supply of ATP for up to 10 min, is omitted from the complete medium. The energy dependence of the transport was tested in the absence of phosphoenolpyruvate since there are sufficient endogenous mono- or dinucleotides in the system to give 80% maximal transport in its presence. Both GTP and α,β-methylene-ATP are equally as efficient as ATP. However, transport is reduced to 15% of the complete system if the only potential energy source is α,β-methylene ATP. Since the β,γ-bond in this compound is resistant to hydrolysis, this would suggest that approximately 85% of the transport is energy dependent. The 15% transport observed in the presence of the analog may be the result of nondetectable (<4%) lysis, or to a physical effect on the nuclear pores, since a similar level of release is observed when phosphoenolpyruvate and ATP are replaced by AMP as the potential energy source.

In conclusion, the results indicate that the RNA transport is an active, energy-dependent process. Either a more rapid transport of the completed ribosome subunits or a longer processing time in vitro, as compared to in vivo, could account for the biphasic nature of the time course of the release of RNA from the isolated nuclei.

**Nature of Material Transported.** That a large fraction of the transported RNA is ribosomal was confirmed by the density gradient profiles of the RNA purified from the incubation medium by use of phenol (17, 23). The recovery of labeled RNA following phenol extraction was consistently 80%. Typical radioactivity profiles, which indicate the size distribution of the RNA released to the medium, as well as the size distribution of the RNA present in the nuclei before and after incubation in vitro, are shown in Charts 2, a and b. (The UV absorbance profiles are not shown, since the amount of RNA transported was below the level of detection at 260 nm.

---

**Table 1: Effect of actinomycin D and RNase inhibitors on RNA release**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Total Radioactivity (cpm/ml or 4 x 10⁶ nuclei)</th>
<th>% Nuclear Radioactivity (cpm/ml or 4 x 10⁶ nuclei)</th>
<th>Acid Insoluble Radioactivity (cpm/ml or 4 x 10⁶ nuclei)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete medium at 36°C</td>
<td>4,320</td>
<td>3.7</td>
<td>2,880</td>
</tr>
<tr>
<td>At 0°C rather than 36°C</td>
<td>2,165</td>
<td>1.7</td>
<td>1,240</td>
</tr>
<tr>
<td>Plus actinomycin D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 μg/ml</td>
<td>4,250</td>
<td>3.8</td>
<td>2,950</td>
</tr>
<tr>
<td>10 μg/ml</td>
<td>4,300</td>
<td>3.4</td>
<td>2,680</td>
</tr>
<tr>
<td>Minus yeast RNA</td>
<td>12,365</td>
<td>10.2</td>
<td>2,670</td>
</tr>
<tr>
<td>Minus yeast RNA plus polyvinyl sulfonic acid</td>
<td>15,650</td>
<td>12.9</td>
<td>3,950</td>
</tr>
</tbody>
</table>

---

on October 22, 2017. © 1972 American Association for Cancer Research.
Ribosome Transport from Isolated Nuclei

25

a. o

o

4 ÜJ

20

IO

bJK

DURATION OF INCUBATION (MIN.)

Chart 1. Kinetics of the release of labeled RNA from prelabeled nuclei in the complete medium at 0° or 36°. The data represent the percentage of nuclear radioactivity (zero time) released in an acid-precipitable form to the complete medium (•); the complete medium, with the further addition of 2.5 mM phosphoenolpyruvate at 15 min (○); the complete medium with the further addition of 2.5 mM phosphoenolpyruvate at each 5-min (cf. arrows) interval (□); the complete medium incubated at 0° (●); and the complete medium minus ATP and phosphoenolpyruvate (△). Note that the release at 0°, or at 36° without an added energy source does not increase with time, indicating that this radioactive RNA is present as a contaminant in the nuclear preparation.

following separation in the density gradient.) The sedimentation constants shown in Chart 2 were established by separating UV detectable amounts of RNA isolated directly from the cytoplasmic ribosomes or from the nuclei in density gradients of identical composition. These values are also in line with those established previously (24). A small release of 18 S and 28 S rRNA and, therefore, presumably of 40 S and 60 S ribosomal subunits, occurs, in addition to the release of 4 to 7 S labeled components, when the nuclei were incubated for 15 min at 0° in the complete medium. This release was increased 3-fold when the temperature was raised to 36°. Quantitative estimates of the various species of RNA released, based on the areas under the radioactivity profiles shown in Chart 2a, indicate that approximately 65% of the released counts are present in the 18 S and 28 S rRNA fractions. The ratio of the 28 S to 18 S RNA (Chart 2a), after correcting for the 0° control, and the ratio of the 60 S:40 S subunits (Chart 3) are both approximately 3.0 instead of the predicted 2.0, due to differences in the pool size of these components in the nucleus (5). In other experiments in this laboratory (D. E. Schumm, unpublished observations) it was observed that putative mRNA, released in vitro from nuclei prelabeled in vivo for 30 min or less, then purified by phenol extraction, sediments predominantly in the lighter (10 S to 14 S) regions of the gradient when the present isolation and assay conditions are used. Recent in vivo studies (32) also show that mRNA appearing in the cytoplasm of rat liver within 1 hr of the administration of labeled precursor sediments predominantly in the 12 S region of the gradient, with only small amounts in the 18 and 28 S regions.

Hybridization competition experiments also confirmed that over one-half of the transported labeled RNA was ribosomal in nature. For example, 60% of the RNA (purified from the medium following 15 min of incubation) that hybridized with rat liver DNA on filters (65 µg of DNA per B-6 filter) failed to bind in the presence of 5 or 10 µg of competitor rRNA. This fraction increased as the incubation proceeded. Under similar test conditions only 10% of the labeled RNA competed if the nuclei were prelabeled in vivo for only 30 min with orotic acid-6-14C (D. E. Schumm, unpublished observations).

The estimate, based on total released counts in RNA, that 60 to 65% of the total RNA released within 5 min is ribosomal is probably a lower limit since the mRNA (which appears in the lighter region of the gradient) is more rapidly labeled than the rRNA (9). In addition to mRNA and rRNA, an undetermined amount of 5 S and 7 S rRNA will be present in the transported RNA and will appear in the 4 S to 7 S region of the gradient.

In Chart 1, it is of interest that, while density gradient analysis (as for Chart 2a) of the RNA released at 30 min indicates that the amount of rRNA released to the complete medium is approximately 60%, the latter species increased to at least 70% of the total RNA released, when further phosphoenolpyruvate was added after 15 min of incubation. This suggests that the transport of nonribosomal RNA

Table 2

<table>
<thead>
<tr>
<th>Energy source</th>
<th>Acid-insoluble radioactivity</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 mM phosphoenolpyruvate</td>
<td>1062</td>
<td>100</td>
</tr>
<tr>
<td>+ 2 mM ATP</td>
<td>1252</td>
<td>52</td>
</tr>
<tr>
<td>2.0 mM ATP</td>
<td>568</td>
<td>55</td>
</tr>
<tr>
<td>3.0 mM ATP</td>
<td>541</td>
<td>51</td>
</tr>
<tr>
<td>2.0 mM AMP</td>
<td>170</td>
<td>16</td>
</tr>
<tr>
<td>2.0 mM α,β-methylene ATP</td>
<td>520</td>
<td>49</td>
</tr>
<tr>
<td>2.0 mM β,γ-methylene ATP</td>
<td>158</td>
<td>15</td>
</tr>
<tr>
<td>No potential energy source</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Nuclei, isolated from the 19-hr regenerating liver following a 2-hr prelabeling in vivo with orotic acid-6-14C (200 µCi/kg) were incubated in the cell-free system for 5 min at 36°. Aliquots of the nuclei-freed medium (1000 x g supernatant) were assayed for acid-insoluble radioactivity; the latter is corrected for the radioactive RNA released at 0°. The complete incubation medium was modified as indicated.

NOVEMBER 1972
Chart 2. Radioactivity profiles of (a) the RNA released to the complete medium from nuclei prelabeled 2 hr in vivo and (b) the distribution of radioactivity in the residual RNA in these nuclei following incubation for 15 min at 36° or 0°. The RNA purified from the medium following removal of the nuclei, or from the nuclei, was separated on 10 to 30% linear gradients (cf. “Materials and Methods”).

decreased more rapidly than did the transport of rRNA with the duration of incubation, a probable consequence of the significant reserve of rRNA precursors in the nucleus.

The nRNA patterns in Chart 2b confirm that during the 15 min of incubation at 36° there is a decrease in the nuclear rRNA precursors. A comparison of the nRNA patterns in the incubated and unincubated nuclei further shows that the residual nRNA is exceedingly stable during the in vitro incubation in the complete medium. Although the 45 S/28 S ratio decreased from 0.52 at zero time to 0.46 after 15 min of incubation at 36°, the apparent processing was insufficient to maintain the 28 S pool which also decreased. However, evidence that processing of rRNA does occur in vitro was obtained from other experiments (D. E. Schumm, manuscript in preparation) where nuclei, prelabeled in vivo for only 20 min, did not release significant amounts of labeled rRNA, as determined by hybridization competition or prelabeling with methionine-methyl-14C, prior to 30 min of incubation in vitro; the release of labeled rRNA was linear subsequent to 30 min of incubation when the phosphoenolpyruvate in the medium was continuously replenished. These results serve as a functional test for the preservation of the nuclei and nucleoli during the incubation and indicate that the processing of rRNA does occur in vitro.

Transport of Ribosomal Subunits. In order to demonstrate that the 18 S and 28 S species of rRNA are released as 40 S and 60 S subunits, respectively, the incubation medium (freed of nuclei by low-speed centrifugation), was layered directly on density gradients. As indicated in Chart 3, the radioactivity profile of the released components coincided with the UV absorbance pattern of added (unlabeled) ribosomal subunits; the latter were obtained by treating the purified cytoplasmic ribosomes with EDTA (24). (If the nuclei were prelabeled for only 30 min in vivo, the labeled ribonucleoproteins sedimented exclusively in the 40 S region, i.e., in the informosome region of the gradient.) The results confirm that the 18 S and 28 S rRNA is transported to the medium as ribosomal subunits. The RNA sedimenting in the 60 S and 40 S regions of the gradient, the latter including some mRNA in the form of informosomes, accounted for 70% of the acid-precipitable RNA released after a 15-min incubation at 36°.

The labeled ribonucleoprotein particles released during a 15-min incubation of prelabeled nuclei were also centrifuged to equilibrium in a CsCl gradient (3) after formaldehyde fixation (28). Approximately 70% of the radioactive RNA sedimented at buoyant densities of 1.61, 1.57, and 1.54 g/cm³ mm. These buoyant densities correspond to the values reported (29) for the 60 S (1.61) and 40 S (1.54) ribosomal subunits and for the ribosomal monomers (1.58). The remaining 30% of the RNA sedimented as nucleoproteins with a density less than 1.51 g/cm³ mm, i.e., the density characteristic of informosomes (28). These data are consistent with the release of rRNA as 40 S and 60 S ribosomal subunits.

Requirements for Nondialyzable Factors and Polyamines. The omission of dialyzed liver cytosol from the incubation medium (Table 3) resulted in a decrease in the release of RNA from prelabeled nuclei of over 50%. The residual release of labeled RNA which occurs in the absence of cytosol is probably due to nuclear lysis since a 9% decrease in the number of nuclei was observed in the light microscope. The cytosol requirement is specific since bovine serum albumin
Chart 3. Radioactivity and absorbance (260 nm) profiles of nucleoprotein components released to the complete medium from 2-hr prelabeled nuclei during a 12-min incubation at 36°. Further details are given in “Materials and Methods.”

Ribosome Transport from Isolated Nuclei

does not prevent the lysis of the nuclei. In agreement with earlier observations (14) a marked lysis of the nuclei also occurred in the absence of spermine or spermidine. Spermidine is, however, superior to spermine in the in vitro system since 5 mM spermine inhibits both nuclear lysis and the release of nRNA. Other data (not shown) indicated that extensive lysis occurred (up to 50%) if extra phosphoenolpyruvate was added to the medium (as for Chart 1) unless both spermidine and cytosol were present, in which case no lysis occurred. As indicated by Experiments 9 through 12, in the absence of an energy source there is slight lysis of the nuclei when cytosol is present; however, nuclear lysis and the consequent release of RNA in the absence of cytosol is prevented by the omission of the energy supply. Similarly, lysis caused by the omission of spermidine is less severe when the energy source is omitted.

In conclusion, the results indicate that both spermidine and cytosol are essential for the preservation of the nuclei while both energy and cytosol are essential for the release of the RNA from intact nuclei. The lysis that occurs in the absence of cytosol appears to be promoted by the presence of an energy source (i.e., ATP and phosphoenolpyruvate). That the apparent transport in the absence of cytosol is actually due to lysis is further supported by the observation that a plot of the percentage of lysis versus the cpm/ml for Experiments 2, 3, 9, 11, and 12 approximates a straight line which passes through the origin. These results provide strong support for the conclusion that the requirement of the cytosol for RNA release is obligatory.

Contribution of Extranuclear Ribosomes. A small, but variable number of ribosomes may be bound to cytoplasmic membrane fragments which remain attached to the outer nuclear membrane (4). It was, therefore, necessary to rule out

Table 3

Requirements for nondialyzable factors in the cytosol and polyamines

The acid-insoluble cpm released from the 2-hr prelabeled nuclei incubated for 12 min at 36° have been corrected for the cpm present in the controls similarly incubated at 0°. Bovine serum albumin and spermine were each added to the medium in a final concentration of 10 mg/ml and 5 mM, respectively. In those experiments where energy (i.e., 2.0 mM ATP and 2.5 mM phosphoenolpyruvate) was omitted from the incubation medium, UDP was added to a final concentration of 5 mM. The nuclear counts were reproducible to within 10%. The standard errors are recorded for the acid-insoluble cpm released per ml of incubation mixture (based on 3 experiments).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Incubation medium</th>
<th>cpm/ml or 4 X 10^4 nuclei</th>
<th>% of control</th>
<th>% nuclear lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Complete (control)</td>
<td>1,383 ± 171</td>
<td>100</td>
<td>N.D.</td>
</tr>
<tr>
<td>2</td>
<td>1 minus cytosol</td>
<td>663 ± 48</td>
<td>48</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>2 plus albumin</td>
<td>1,569 ± 199</td>
<td>113</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>1 minus spermidine</td>
<td>3,758 ± 213</td>
<td>272</td>
<td>b</td>
</tr>
<tr>
<td>5</td>
<td>2 minus spermidine</td>
<td>4,147 ± 150</td>
<td>300</td>
<td>72</td>
</tr>
<tr>
<td>6</td>
<td>4 plus spermine</td>
<td>327 ± 20</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5 plus spermine</td>
<td>128 ± 25</td>
<td>9</td>
<td>N.D.</td>
</tr>
<tr>
<td>8</td>
<td>1 plus spermine</td>
<td>216 ± 5</td>
<td>16</td>
<td>b</td>
</tr>
<tr>
<td>9</td>
<td>1 minus energy, plus UDP</td>
<td>430 ± 15</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>9 minus supernatant</td>
<td>38 ± 8</td>
<td>3</td>
<td>N.D.</td>
</tr>
<tr>
<td>11</td>
<td>10 minus spermidine</td>
<td>2,196 ± 200</td>
<td>157</td>
<td>28</td>
</tr>
<tr>
<td>12</td>
<td>9 minus spermidine</td>
<td>2,690 ± 210</td>
<td>193</td>
<td>23</td>
</tr>
</tbody>
</table>

a N.D., not detectable.
b Nuclear counts were precluded since the nuclei aggregated under these conditions.
Table 4
Specific activities of rRNA isolated from the cytoplasm or released from nuclei of labeled liver cells

<table>
<thead>
<tr>
<th>Duration of labeling in vivo</th>
<th>Cytoplasmic ribosomes</th>
<th>Released from nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18 S</td>
<td>28 S</td>
</tr>
<tr>
<td>2 hr</td>
<td>15,300</td>
<td>17,980</td>
</tr>
<tr>
<td>5 days</td>
<td>13,070</td>
<td>12,600</td>
</tr>
</tbody>
</table>

Ling C. Yu, Janis Racevskis, and Thomas E. Webb

the possibility that radioactivity in these components contributes to the apparent release of ribosomal subunits by isolated nuclei. As indicated in Table 4, following a 2-hr labeling in vivo with orotic acid-6-14C, the specific radioactivity of the 18 S and 28 S RNA released from the nuclei during incubation was 10 times higher than that of the ribosomes present in the cytoplasm of the cells from which the nuclei were isolated. On the other hand, the nuclear pool of labeled rRNA precursors in the nuclei obtained from the regenerating liver of rats 5 days after the administration 17 hr postoperative of 100 μCi/250 g of orotic acid-6-14C should be diminishingly small, if not completely depleted; hence any radioactivity associated with these nuclei theoretically would be contributed by cytoplasmic ribosomes of relatively high specific radioactivity, both attached to cytoplasmic membranes still associated with the nucleus (cytoplasmic tags) and as a free contaminant of the nuclei which the purification procedure failed to eliminate. The presence of free ribosomal contaminants was demonstrated in the 0° controls, and at 36° without an added energy source (Chart 1). The data in Table 4 indicate that, as predicted, the RNA's prepared from cytoplasmic ribosomes following a 5-day labeling have a high specific radioactivity; however, when nuclei prepared from these cells are incubated in the complete medium at 36° for 15 min, there is no significant release of rRNA over that of the control incubated at 0°. Also, as anticipated, the specific radioactivity of the small amount of rRNA released is the same as that of the cytoplasmic RNA. Since the 60 S ribosomal subunits of the membrane-bound ribosomes are known to be very tightly bound to the cytoplasmic membranes (22), the results suggest that much of the labeled rRNA recovered from the medium following incubation of the prelabeled nuclei at 0° is derived from cytoplasmic ribosomes that contaminate the nuclei but are not attached to the nuclear membrane. It is concluded that labeled 28 S and 18 S rRNA recovered from the complete medium, following the incubation at 36° of nuclei prelabeled for 2 hr in vivo, is not derived from ribosomes of cytoplasmic origin.

Implications of the Data. The data from the present study suggest that the posttranscriptional control of ribosome formation involves the active (energy-dependent) transport of ribosomal subunits and possibly the energy-dependent processing of the ribosomal subunit precursors in the nucleolus. The results also strongly suggest that certain macromolecules present in the cytosol are also involved in this control. These macromolecules may be enzymic in nature, or, as seems more probable, may be regulatory proteins involved in positive feedback control. In previous studies (24, 25), a negative correlation was observed between the size of the inactive ribosomal monomer pool and the rate of ribosome formation. Furthermore, it was shown that the concentration of the ribosomal subunits in the cytoplasm remains constant under a number of in vivo conditions where the concentration of the monomers varies widely (25, 30). Therefore, the regulatory macromolecules may be depleted under conditions where fewer monomeric ribosomes are incorporated into polyribosomes. Recent evidence from the effects of inhibitors of protein biosynthesis on ribosome formation in lymphocytes suggests (6) that the transport of ribosomes depends upon the formation of a regulatory protein(s).

A cell-free system has recently been described (10, 11) for studying the release of mRNA from isolated nuclei. The latter system does not utilize cytosol or spermidine which we have observed and others have reported (14) to be essential for the prevention of nuclear lysis. The amount of RNA released from the nuclei is exceedingly high in this system; i.e., up to 20% of the total radioactivity of nuclei prelabeled in vivo for 40 min was released to the medium at 20° and this increased to approximately 30% at 30°. Since no data were given on the degree of nuclear lysis under these conditions, a comparison of this system with that described in the present communication is precluded.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Dorothy E. Schumm in this laboratory for performing the competition hybridization experiments.

REFERENCES

Ribosome Transport from Isolated Nuclei


NOVEMBER 1972

2321
Regulated Transport of Ribosomal Subunits from Regenerating Rat Liver Nuclei in a Cell-free System

Ling C. Yu, Janis Racevskis and Thomas E. Webb

_Cancer Res_ 1972;32:2314-2321.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/32/11/2314

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.