EWA GRĄBCZEWSKA, BOZENNA OLSZANSKA and ZOFIA LASSOTA

PRECURSORS OF CYTOPLASMIC RNA IN AVIAN CELL NUCLEUS

Institute of Biochemistry and Biophysics, Polish Academy of Sciences,
ul. Rakowiecka 36; 02-532, Warszawa, Poland

In nucleus of chick embryo four pre-rRNA of molecular weight of 3,43, 2,93, 2,36
and $1.87 \times 10^6$ were found in addition to the heterogeneous population of "giant"
RNA molecules larger than $4.5 \times 10^6$ daltons, partially polyadenylated, with few
methyl groups and numerous double-stranded regions.

As we have reported previously (Olszańska et al., 1974), the heavy rapidly labelled
nuclear RNA from chick embryo separates in gel electrophoresis into five distinct
species, less mobile than 28S rRNA. The supposition has been put forward that
these species may represent precursors of rRNA and mRNA. In this paper we
present the results of further investigations which suggest that four heavy RNA
species are involved in the process of rRNA maturation while the fifth, the least
mobile one, shows some features expected for presumable pre-mRNA.

MATERIALS AND METHODS

Material. Chick embryos of Leghorn breed were used on the 6th day of develop-
ment.

and [G-3H]uridine were products of the Radiochemical Centre (Amersham, England);
[2-14C]uridine was from UVVVR (Prague, Czechoslovakia).

Chemicals: N,N,N',N'-tetramethylenediamine (TEMED) and Triton X-100 were
from B.D.H. Chemicals Ltd. (Poole, England); actinomycin D, pronase, B grade,
and Tris, A grade, from Calbiochem (San Diego, U.S.A.); acrylamide and N,N'-
methylene-bisacrylamide (Eastman Kodak, Rochester, U.S.A.) were recrystallized

* This work was supported by the Polish Academy of Sciences within the Project 09.3.1.

[261]
from chloroform and acetone, respectively. Formamide, puriss., was purchased from Fluka A.G. (Buchs S.G., Switzerland), and sodium dodecyl sulphate (SDS)\(^1\) was from Koch-Light Lab. Ltd. (Colnbrook, England). Poly(U)-Sepharose 4B was a commercial product of Pharmacia Fine Biochemicals (Uppsala, Sweden), and sucrose, extra pure, was from Schwartz-Mann (Orangeburg, N.Y., U.S.A.).

Ribonuclease A, I-A type, from bovine pancreas, protease-free, salt-free, and ribonuclease T1, from Aspergillus oryzae, grade II, were products of Sigma Chemicals Co. (St. Louis, Mo., U.S.A.), and deoxyribonuclease I, from bovine pancreas, RNAase-free, was from Worthington Biochemicals Co. (Freehold, N.J., U.S.A.). 4S tRNA from Escherichia coli was a product of Calbiochem (San Diego, U.S.A.).

METHODS

RNA labelling in vivo and RNA extraction from nuclei were performed as already reported (Olszańska et al., 1974): 6-day-old embryos were incubated in ovo with 10-20 µCi of radioactive precursor, and RNA was extracted by three different procedures: 1, temperature fractionation (treatment with phenol successively at 10°, 55° and 65°C); 2, pH fractionation (treatment with phenol at pH 7.6 and thereafter at pH 8.3), and 3, SDS fractionation. This included the previously applied treatment twice with phenol at 10°C (fractions 1 and 2) and twice with phenol-SDS at room temperature (fractions 3 and 4), supplemented by the fifth step of phenol-SDS treatment at 65°C (fraction 5).

Cytosplasmic RNA was obtained from the post-nuclear supernatant, supplemented with NaCl to 0.1 M and with EDTA to 0.001 M, as described by Lassota et al. (1973).

Polyacrylamide-gel electrophoresis of RNA, scanning of the stained gels (presented in arbitrary units) and radioactivity determination in gels were also previously described (Olszańska et al., 1974).

Molecular weight of RNA was determined from the electrophoretic mobility according to Bishop et al. (1967) and Loening (1969).

For density-gradient centrifugation, a 10-30% linear sucrose gradient was prepared in 0.05 M-sodium acetate buffer, pH 5.1, containing 0.1 M-NaCl and 0.001 M-EDTA (Fantoni & Bordin, 1971). About 1 mg of RNA dissolved in 0.2 ml of the same buffer was layered over 4 ml of the gradient and centrifuged in a Spinco-Beckman L2 centrifuge provided with 39SW rotor, at 35 000 rev./min for 6 h at 4°C. Fractions of about 1 ml were collected from the bottom of the tube.

Binding of the labelled RNA on poly(U)-Sepharose was performed exactly as described by Lindberg & Persson (1972). Radioactivity of the fractions emerging from the column was measured on glass filters, then appropriate fractions were pooled and RNA was precipitated with 2 vol. of ethanol in the presence of 0.1 M-NaCl, with 4S RNA from E. coli added as a carrier. Before electrophoretic analysis the RNA fractions were dissolved in 0.1 M-sodium acetate buffer, pH 5, containing

---

\(^1\) Abbreviations used: SDS, sodium dodecyl sulphate; RNAase A, ribonuclease A, type I-A, from bovine pancreas; RNAase T1, ribonuclease T1, from Aspergillus oryzae.
0.1 M-NaCl and 0.001 M-EDTA, and reprecipitated as above. The oligo(U) content was determined by binding RNA preincubated with poly(A) on millipore filters according to Burdon & Shenkin (1972).

For pronase digestion, 1 mg of RNA dissolved in 1 ml of 0.01 M-Tris buffer, pH 7.2, containing 0.15 M-NaCl and 0.01 M-MgCl₂ was supplemented with 100 µg of self-digested enzyme and incubated for 1 h at 37°C. Pronase was removed by phenol-cresol treatment and RNA was precipitated with 2 vol. of ethanol in the presence of 0.5 M-NaCl.

Digestion with RNAase A and RNAase T₁ was performed as described by Jelinek & Darnell (1972). RNAase A (10 - 20 µg) and RNAase T₁ (1 µg) were added per 1 mg of RNA dissolved in 1 ml of 0.01 M-Tris buffer, pH 7.4, containing 0.1 M-NaCl and 0.01 M-EDTA, and the mixture was incubated for 30 min at 37°C. The enzymes were removed as indicated above.

The RNA aggregation test was performed after McKnight & Schimke (1974): RNA dissolved (1 mg/ml) in 0.01 M-Tris buffer, pH 7.5, containing 1% SDS and 0.005 M-EDTA was heated at 65°C for 10 min, then immediately cooled to 0°C and precipitated with 2 vol. of ethanol in the presence of 0.5 M-NaCl.

Denaturation of RNA in 90% formamide (Pinder et al., 1974) was performed by dissolving 1 mg of RNA in 1 ml of 0.01 M-phosphate buffer, pH 7.5, containing 0.01 M-EDTA, 0.2% SDS and 90% formamide. RNA was precipitated as indicated above.

RESULTS

_Determination of molecular weight._ Heavy RNAs were isolated from the nuclei by pH fractionation and SDS fractionation (see Methods). The molecular weight of the RNA species separated by electrophoresis in polyacrylamide gel was determined as shown in Fig. 1. The results of calculations based on the electrophoretic mobilities of the RNAs isolated at pH 8.3 are presented in Table 1. The mol.wt. values for the RNA species forming bands c, d and e (Fig. 1) did not depend on the procedure used for RNA extraction. The respective data in Table 1 are, therefore, valid also for the samples obtained by SDS-phenol treatment; slightly different values (given in brackets) were only obtained for the RNA forming band b (Fig. 1).

The results of our estimations are compared in Table 1 with the values obtained in a similar way by Stephenson & Dimmock (1974) for nuclear RNAs of chick embryo fibroblasts. The similarity of our data for the presumable precursors of rRNAs seems to be quite satisfactory.

The electrophoretic method used could not be, however, applied for determination of mol. wt. of the least mobile "giant" nuclear RNA (band a, Fig. 1) because of its poor penetration into the gel. One may only assume that this fraction consists of molecules larger than 4 x 10^6.

_Integrity of the "giant" RNA molecules._ The integrity of molecules constituting "giant" nuclear RNA was examined by procedures usually recommended for this
Fig. 1. Molecular weight and electrophoretic mobility in 2.4% polyacrylamide gel of the heavy nuclear RNA species of chick embryo. A. Electrophoretic profile of nuclear RNA extracted at pH 8.3 by the pH fractionation procedure. For details see Methods. a—e, heavy nuclear RNA species. B. Logarithmic calibration of molecular weights of the b—e RNA species versus distance migrated in the gel. 18S and 28S rRNA of chick embryo (mol.wt. $1.55 \times 10^6$ and $0.65 \times 10^6$, respectively; Stephenson & Dinmok, 1974) were used as reference markers.

Fig. 2. Effect of formamide on the electrophoretic profile in 2.4% polyacrylamide gel of nuclear RNA from chick embryo. The RNA fraction 65°C obtained by the temperature fractionation procedure, was used. Samples: untreated (— — —); dissolved in 100% formamide (— — —).

Purpose. Digestion with pronase of the nuclear RNA fraction rich in “giant” RNA, applied in order to remove the residual traces of protein, did not affect the electrophoretic profile. This indicates that the “giant” molecules were neither native nor artificial phenol-resistant nucleoprotein complexes.

Heating of the sample in 1% SDS solution at pH 7.5 for 10 min at 65°C, as recommended by McKnight & Schimke (1974) or treatment with 90% formamide, after Pinder et al. (1974), did not result in disintegration of “giant” RNA. Dissolution of the sample in 100% formamide resulted in alteration of the electrophoretic profile as shown in Fig. 2. The absence of bands in the region between the start and the position of 18S rRNA pointed to disintegration of large RNA molecules under the conditions tested. Hidden breaks, due to RNAase action, might
Table 1

Molecular weight of heavy nuclear RNA species of 6-day-old chick embryo

Heavy nuclear RNAs of pH 8.3 fraction obtained from purified nuclei by the pH fractionation procedure, were analysed. For details see Methods. Molecular weights were calculated from the electrophoretic mobility in 2.4% polyacrylamide gel (cf Fig. 1). The presented results are mean values of 12 determinations ± S.E., except values in brackets which are means of 7 determinations.

<table>
<thead>
<tr>
<th>RNA band (Fig. 1A)</th>
<th>Molecular weight (×10^-6) of pre-rRNA</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>chick embryo</td>
<td>chick fibroblasts*</td>
</tr>
<tr>
<td>b</td>
<td>3.43 ± 0.18</td>
<td>3.55 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>(3.79 ± 0.21)‡‡</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>2.93 ± 0.12</td>
<td>2.9 ± 0.01</td>
</tr>
<tr>
<td>d</td>
<td>2.36 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>1.87 ± 0.08</td>
<td>1.75 ± 0.05</td>
</tr>
</tbody>
</table>

* Stephenson & Dimmock (1974).
‡‡ In brackets, the molecular weight of band b calculated from the electrophoretic profile of nuclear RNA (fraction 3) obtained at room temperature by the SDS-fractionation procedure.

be responsible for the formamide effect seen in the "giant" RNA similarly as in the case of degradation of 28S rRNA molecule (cf Fig. 2).

Heterogeneity of "giant" RNA. The population of RNA molecules larger than 4 × 10^6, tested in sucrose gradient, was found to be heterogeneous in size. The labelled RNA extracted from nuclei at 65°C in the last step of the SDS-fractionation procedure (see Methods) and showing the usual electrophoretic profile (Fig. 3A) was centrifuged in a 10-30% linear sucrose-density gradient at high ionic strength (Fig. 3B). Taking the position of 28S rRNA as a marker, the labelled heavy RNA accounted in the gel for 70% and in the gradient for 62% of the total radioactivity of the sample. Comparison of the two distribution patterns showed that the single peak of labelled "giant" RNA seen in the electrophoretic profile corresponds to polydisperse radioactivity in the respective part of the sedimentation profile.

Actinomycin D chase. In an attempt to follow the fate of heavy rapidly labelled nuclear RNAs, the actinomycin chase was applied in vivo. Actinomycin D in a dose of 50 μg per embryo, injected simultaneously with [%H]uridine, was found to inhibit RNA synthesis in the 6-day-old chick embryo in 60% on 2-h incubation in ovo. Taking into account the observation of Bleyman & Woese (1969) that in vivo actinomycin D affects preferentially transcription of large molecules, this dose of the drug was used to chase for 2.5 h the label incorporated into embryonic RNA during 1 h pretreatment with [%H]uridine.

As shown in Table 2, the radioactive RNA shifted visibly during the chase from nucleus to cytoplasm, and the contribution of particular RNA species to this shift seemed to be different. The threefold increase of radioactivity in cytoplasmic RNA and doubling of radioactivity in nuclear RNA extractable by the first phenol treatment at 10°C were due to the appearance of the newly labelled molecules of
Fig. 3. Comparison of the electrophoretic profile (A) and sucrose-density-gradient profile (B) of nuclear RNA from chick embryo. Six-day-old chick embryos were incubated in vivo for 2 h with [G-\(^3\)H]uridine (spec. act. 2.5 Ci/m mole, 20 \(\mu\)Ci/embryo). The RNA fraction separated at 65°C by the temperature fractionation procedure, was used. Electrophoresis in 2.4% polyacrylamide gel and centrifugation in 10 - 30% linear sucrose gradient were performed as described in Methods.

--- O.D.; - - - radioactivity.

Table 2

Incorporation in vivo of \(^3\)H]uridine into nuclear RNA of chick embryo after actinomycin D chase

After 1 h incubation in vivo with [\(^3\)H]uridine (spec. act. 2.5 Ci/m mole, 20 \(\mu\)Ci/embryo), a half of the embryos were taken out from the eggs for RNA extraction. Actinomycin D (50 \(\mu\)g/100 \(\mu\)l of water per embryo) was introduced into the remaining eggs and the incubation was continued for a subsequent 2.5 h.

Extraction of cytoplasmic and nuclear RNAs and SDS fractionation of nuclear RNA were performed as described in Methods. Numbering refers to the fractions separated by the SDS-fractionation procedure (see Methods).

<table>
<thead>
<tr>
<th>RNA fraction</th>
<th>Specific activity (counts/min/mg)</th>
<th>(A) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>after</td>
</tr>
<tr>
<td>actinomycin chase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11 400</td>
<td>24 800</td>
</tr>
<tr>
<td>2</td>
<td>23 600</td>
<td>30 680</td>
</tr>
<tr>
<td>3</td>
<td>45 040</td>
<td>55 600</td>
</tr>
<tr>
<td>4</td>
<td>34 640</td>
<td>40 800</td>
</tr>
<tr>
<td>5</td>
<td>90 960</td>
<td>188 000</td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>6 960</td>
<td>20 800</td>
</tr>
</tbody>
</table>
Fig. 4. Electrophoretic profiles of the nuclear RNA fractions of chick embryo obtained by the SDS-fractionation procedure, before (A) and after (B) actinomycin chase. Chick embryos were incubated for 1 h with $[^3H]$uridine (spec. act. 2.5 Ci/mmol, 20 μCi/embryo), followed by 2.5-h incubation with actinomycin D (50 μg in 100 μl of water per embryo). For details see Table 2 and Methods. ——, Optical density scanning; ---, radioactivity.
28S and 18S rRNA. In the three subsequent fractions of nuclear RNA obtained by the SDS-fractionation procedure, no considerable increase of radioactivity was noted after the chase and the label shifted from 32S RNA to 28S RNA (Fig. 4, fraction 2) and from 45S RNA towards 28S and 18S rRNAs (Fig. 4, fraction 3). Doubling of radioactivity in the RNA firmly bound in the nucleus and solubilized with phenol at 65°C, apparently did not concern the “giant” RNA and was due to the unusually high content of labelled RNA in the 45S-18S region (Fig. 4, fraction 5).

These results point to participation of four heavy nuclear RNA species, denoted as 45S, 41S, 36S and 32S, in the process of synthesis and maturation of rRNA. No such relation could be found for the “giant” RNA.

*Methylation pattern.* Distinct methylation of the primary transcript and conservation of the methylated sequences throughout the processing within the nucleus, are characteristic for pre-rRNA (Maden, 1971). Therefore, embryos were incubated in *ovo* with $[^{14}C] luridine and $[^3H]methyl-methionine applied simultaneously. After 2-h incubation, the incorporation of both isotopes was measured in the RNA fractions isolated from nuclei by SDS fractionation (Table 3). The $^{14}C: ^3H$ ratio increased in the successive fractions indicating, as expected, a relatively poor methylation of the heavy newly-transcribed RNA molecules. The characteristic differences in the methylation pattern became still more evident on electrophoretic separation of particular RNA species. The lowest value of 0.5 for the $^{14}C: ^3H$ ratio was found in 28S rRNA and 18S rRNA which points to substantial methylation of these species. The $^{14}C: ^3H$ ratio was increased to 0.6 for 32S RNA and to 0.7 for 45S RNA, indicating that the presumable precursors of rRNA indeed contained fewer methyl groups than the mature forms. The “giant” nuclear RNA clearly differed from the other heavy species by its very low content of methyl group and a high $^{14}C: ^3H$ ratio of 2.4.

**Table 3**

*Double-labelling of chick embryo nuclear RNA with $[^{14}C] luridine and $[^3H]methyl-methionine*

Six-day-old chick embryos were labelled for 2 h *in vivo* with 50 μl of aqueous solution of $[^{14}C] luridine (spec. act. 5.3 mCi/mmol, 2 μCi/embryo) and $[^3H]methyl-methionine (spec. act. 6.4 Ci/mmol, 20 μCi/embryo).

<table>
<thead>
<tr>
<th>RNA fraction in SDS procedure</th>
<th>Incorporation (counts/min/mg)</th>
<th>$^{14}C: ^3H$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$[^{14}C] luridine$</td>
<td>$[^3H]methyl-methionine$</td>
</tr>
<tr>
<td>1</td>
<td>9 400</td>
<td>62 700</td>
</tr>
<tr>
<td>2</td>
<td>15 300</td>
<td>63 160</td>
</tr>
<tr>
<td>3</td>
<td>32 500</td>
<td>107 570</td>
</tr>
<tr>
<td>4</td>
<td>17 740</td>
<td>59 530</td>
</tr>
<tr>
<td>5</td>
<td>75 080</td>
<td>179 300</td>
</tr>
</tbody>
</table>
Poly(A), poly(U) and double-stranded structures. The features differentiating the population of very large rapidly labelled molecules, denoted as “giant” RNA, from precursors of rRNA lead us to believe that the former may correspond, at least in part, to the giant primary transcript carrying messenger sequences, postulated by Scherrer (1974). To explore this possibility, the nuclear RNA fraction rich in “giant” RNA was tested for the presence of the structures expected in presumable pre-mRNA. For comparison tests for cytoplasmic RNA and nuclear RNA rich in pre-rRNA and rRNA are presented. The content of polyadenylated RNA was determined by affinity chromatography on poly(U)-Sepharose in the RNA from embryos labelled extensively (16-18h) with [3H]uridine or [3H]adenine, and after shorter labelling (4 h) with [3H]adenosine (Table 4). In

Table 4

Affinity chromatography of chick embryo nuclear RNA on poly(U)-Sepharose 4B

Chick embryos were incubated in vivo for 18 h with [G-3H]uridine (spec. act. 2.5 Ci/mmmole, 20 μCi/embryo). Cytoplasmic RNA was obtained as described in Methods. For details of the temperature fractionation procedure of nuclear RNA and chromatography on poly(U)-Sepharose 4B, see Methods.

<table>
<thead>
<tr>
<th>RNA fraction: temperature fractionation</th>
<th>Spec. act. (c.p.m./mg)</th>
<th>Radioactivity</th>
<th>Unbound</th>
<th>Bound</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>c.p.m.</td>
<td>c.p.m.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10°C</td>
<td>116 000</td>
<td>16 000</td>
<td>380</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>55°C</td>
<td>80 000</td>
<td>28 500</td>
<td>1440</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>65°C</td>
<td>120 000</td>
<td>35 500</td>
<td>8200</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>65°C*</td>
<td>90 000</td>
<td>4 850</td>
<td>1135</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>65°C**</td>
<td>45 000</td>
<td>3 570</td>
<td>1350</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>100 000</td>
<td>16 050</td>
<td>1950</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

* The embryos were incubated for 16 h with [8-3H]adenine (spec. act. 17 Ci/mmmole, 10 μCi/embryo).

** The embryos were incubated for 4 h with [G-3H]adenosine (spec. act. 6.6 Ci/mmmole, 10 μCi/embryo).

cytoplasm, the radioactivity of RNA bound to poly(U)-Sepharose accounted for 10% of the total radioactivity applied to the column. In the nuclear RNA fractions containing mainly rRNA and its precursors, this value did not exceed 5%, while in the RNA extractable from the nucleus at 65°C it amounted to 17 and 19% after prolonged incubation with [3H]uridine and [3H]adenine, respectively. After relatively short labelling with [3H]adenosine, as much as 28% of the RNA radioactivity was retained on the poly(U)-Sepharose column, pointing to the preferential synthesis of poly(A) fragments.
Electrophoretic analysis of polyadenylated and non-polyadenylated RNA separated on poly(U)-Sepharose column (Fig. 5A) revealed that the slowly penetrating "giant" RNA is present in both samples (Fig. 5B). This indicates that only a part of the nuclear population of presumable pre-mRNA was polyadenylated.

![Affinity chromatography of chick embryo nuclear RNA on poly(U)-Sepharose 4B column.](image)

**Fig. 5.** Affinity chromatography of chick embryo nuclear RNA on poly(U)-Sepharose 4B column. Separation (A) of unbound (a) and bound (b) RNA, and electrophoretic profile (B) of fraction (b). Six-day-old embryos were incubated in vivo for 18 h with [G-3H]uridine (spec. act. 2.5 Ci/mmol, 20 μCi/embryo). RNA was obtained at 65°C by the temperature fractionation procedure. Chromatography was performed according to Lindberg & Persson (1972). RNA (0.5 mg, 60 000 c.p.m.) was applied to the column; 0.5 ml fractions of the eluate were collected and the radioactivity was measured as described in Methods. ---, Radioactivity: ---, optical density scanning.

**Table 5**

**Oligo(U) content in nuclear RNA of chick embryo**

Six-day-old chick embryos were incubated in vivo for 2 h with [G-3H]uridine (spec. act. 8.4 Ci/mmol, 20 μCi/embryo). The RNA fractions were obtained at 55° and 65°C by the temperature fractionation procedure, described in Methods. Binding on millipore filters after preincubation with exogenous poly(A) was performed according to Burdon & Shenkin (1972).

<table>
<thead>
<tr>
<th>RNA fraction: temperature fractionation</th>
<th>Spec. act. (c.p.m./mg)</th>
<th>RNA</th>
<th>applied</th>
<th>retained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>c.p.m.</td>
<td>without poly(A)</td>
<td>with poly(A)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.p.m.</td>
<td>%</td>
</tr>
<tr>
<td>65°C</td>
<td>144 000</td>
<td>7000</td>
<td>1100</td>
<td>16</td>
</tr>
<tr>
<td>55°C</td>
<td>78 000</td>
<td>7800</td>
<td>110</td>
<td>1</td>
</tr>
</tbody>
</table>
The oligo(U) fracts were also found in nuclear RNA (Table 5). The content of oligo(U) in the RNA fraction extractable from the nucleus at 65°C and rich in “giant” RNA was twice as high as in the RNA fraction in which pre-rRNA and rRNA prevailed. Furthermore, it was found that nuclear RNA enriched in “giant” RNA contained double-stranded fragments. The digestion of the labelled sample of this RNA with pronase and thereafter with RNAase A and RNAase T₁ left about 5% of RNA in the form of ethanol-precipitable short fragments showing the electrophoretic mobility similar to that of 4S RNA from E. coli. Affinity chromatography on poly(U)-Sepharose of the RNAase-resistant fragments proved that only 7% of the label was retained, representing the amount of poly(A) fragments. The unbound portion consisting of double-stranded fragments was tested electrophoretically (Fig. 6). Three peaks were found, the bulk of the material being present in the fraction slightly more mobile than 4S RNA from E. coli.

![Fig. 6. Electrophoretic profile of double-stranded fragments from nuclear RNA of chick embryo in 2.4% polyacrylamide gel. Embryos were labelled in vivo for 16 h with [8-³H]adenine (spec. act. 17 Ci/mmol, 10 μCi/embryo). RNA was obtained at 65°C by the temperature fractionation procedure. Digestion with RNAase A, RNAase T₁, and subsequent resolution of the RNAase-resistant fragments on poly(U)-Sepharose 4B column were performed as described in Methods. 4S RNA from E. coli was used as a carrier. ---, Radioactivity; ——, optical density scanning.]

DISCUSSION

Precursors of rRNA were identified among the heavy nuclear RNAs of chick embryo by the size of their molecules, the behaviour during actinomycin chase and the methylation level.

Our results concerning the molecular weight of pre-rRNA species are consistent with the values found by Stephenson & Dimmock (1974) in chick embryo fibroblasts.
The difference between the two values calculated from our experiments for the largest pre-rRNA is not significant and is due to a lower accuracy of the method when applied to very large molecules. In spite of this limitation, our results corroborate the opinion of Perry et al. (1970) that the size of the avian rRNA transcription unit is intermediate between those of its mammalian and amphibian counterparts, and reflects the evolutionary changes at the molecular level. Stephenson & Dimmock (1974) reported on four pre-rRNA species in avian fibroblasts, three of them being larger (3.55, 2.9 and 1.75×10⁶) and one smaller (1.1×10⁶) than 28S rRNA. This would suggest a maturation pattern analogous to that in HeLa cells (Wellauer & Dawid, 1973) with two intermediates between the primary transcript and 28S rRNA. Our observations point, however, to a maturation pattern similar to that in mouse L cells (Wellauer & Dawid, 1974), as three intermediary steps between 45S pre-rRNA and 28S rRNA, and four precursor RNA species larger than 28S rRNA were found in chick embryonic nuclei. One may presume that both patterns of rRNA maturation coexist in avian tissues, similarly as it was reported from Hadjiolov's laboratory (Dabeva et al., 1976) for rat liver.

The experiments with actinomycin D are open to criticism because of the pleiotropic effect of the inhibitor which impairs not only transcription but also processing of pre-rRNA and transfer of mature rRNA to cytoplasm (cf Scheer et al., 1975). The increased radioactivity in the 45S-18S region of the RNA extracted at 65°C from the nuclei of actinomycin-treated embryos (Fig. 4, fraction 5) substantiates the observations that the release of the arrested, nascent RNA chains from the template is inhibited (Egyházi, 1974) or drastically retarded (Scheer et al., 1975) during the chase with actinomycin. The transfer of mature rRNAs to the cytoplasm was apparently also retarded in the presence of actinomycin, as indicated by the still considerable content of these species in nuclear RNA extracted by the first phenol treatment at 10°C (Table 2). However, despite the complexity of the actinomycin effect in the chase experiments, the shift of the label within nucleus and out to cytoplasm seems to warrant the conclusion on participation of 45S, 41S, 36S and 32S nuclear RNA species in the synthesis and maturation of avian embryonic rRNA. This conclusion is confirmed by the fact that the content of methyl groups in the RNA species discussed, increased inversely to their molecular weight, just as it was reported for mammals (Weinberg & Penman, 1970).

The nativeness of “giant” RNA was the principal question. The formation of artificial “giant” molecules by phenol-induced thermal aggregation of avian rRNA has been thoroughly investigated (Lassota et al., 1975), and the results did not invalidate the arguments pointing to the nativeness of the “giant” nuclear RNA obtained previously (Olszańska et al., 1974) and in this work.

The preliminary communication from our laboratory on the limited but distinct methylation of “giant” RNA (Grąbczewska & Olszańska, 1974) was almost simultaneous with the reports on the presence of few methyl groups in mRNA and hnRNA (Furuichi et al., 1975; Adams & Cory, 1975; Perry et al., 1975). Therefore we suggest that the giant RNA revealed in avian nucleus might represent the “giant” mRNA postulated by Scherrer (1974) for eucaryotic organisms. None of the properties of
“giant” RNA described hitherto contradict this supposition: the giant molecules were resistant to the denaturing conditions applied; the population of molecules constituting the “giant” RNA was heterogeneous in size, as expected for a population of embryonic pre-mRNAs; poly(A) sequences characteristic of mRNA and hnRNA were found in “giant” RNA; the nuclear fractions rich in “giant” RNA contained numerous double-stranded fragments, pointing to the presence of hair-pin loops.

The content of polyadenylated RNA amounting to 20% of the RNA extractable with phenol at 65°C from chick embryonic nuclei (Table 4) is somewhat lower than that of the polyadenylated RNA (30%) reported by Hemminki (1974) for hnRNA from chick embryonic liver. However, this author observed also binding as high as 20% on the oligo(dT) column of the nucleolar RNA, in which the non-adenylated pre-rRNA and rRNA constituted the bulk of the sample. We believe that our values may be overestimated if short intramolecular oligo(A) tracts are present in avian embryonic nuclear RNA, similarly as it was reported for hnRNA from the sea urchin embryo (Dubroff & Nemer, 1975). It is noteworthy in this connection that poly(A) sequences account only for 7% of the RNAase-resistant fragments (left after digestion of nuclear RNA extractable at 65°C) while Montcon & Naora (1974) found that 13% of the RNAase-resistant residue of rat liver hnRNA binds to oligo(dT).

The double-stranded fragments from avian nuclear RNA extractable at 65°C are of similar size as the analogous fragments in hnRNA from HeLa cells (Jelinek & Darnell, 1972) and in nuclear RNA from mouse Ehrlich ascites cells (Ryskov et al., 1973), indicating a similar size of the hair-pin loops.

Although our data might be insufficient to presume the precursory role of “giant” RNA in the mRNA maturation process, we believe that this fraction is native and may be a suitable material for investigation of sequences which are transcribed from DNA but do not leave the nucleus.

The skillful technical assistance of Mrs. Barbara Kłudkiewicz is greatly appreciated.

REFERENCES

Furui Chi, Y., Morgan, M., Shatkin, J., Jelinek, W., Salidtt-Georgieff, M. & Darnell, J. E. (1975) 


**PREKURSORY CYTOPLAZMATYCZNEGO RNA W JÄDRZE KOMÓREK PTASICH**

**Streszczenie**

W jądrach komórkowych zarodków kurzych znaleziono cztery pre-rRNA o masie cząsteczkowej 3.43, 2.92, 2.36 i 1.87×10^6 oraz heterogeniczną populację „obrzymnych” cząstek RNA większych niż 4.5×10^6 daltonów, zawierającą niewielkie grupy metylowe i liczne obszary dwuściwowe; część tej populacji stanowiły cząsteczki spoladienylowane.

Received 15 April, 1977.