Ribonucleic acid synthesis in embryonic chick muscle, rates of synthesis and half-lives of transfer and ribosomal RNA species

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SUMMARY

In this study the term 'rates of synthesis' does not refer to rates of transcription but to rates of accumulation of newly synthesized RNA.

The rates of synthesis and half-lives of tRNA, 18S and 28S rRNA of 14-day and 17-day embryonic chick muscle were determined by following the accumulation of radioactive AMP in RNA after administration of \(^{3}\text{H}\)8-adenosine to chick embryos and correcting for the average specific activity of the precursor ATP pool. Transfer RNA of 14-day embryonic chick muscle is synthesized at a rate of \(2.4 \times 10^{5}\) molecules per min per 2N DNA content and decays with a half-life of 50 h. Transfer RNA of 17-day embryonic chick muscle is synthesized and decays, at similar rates. Ribosomal RNA, 18S and 28S, are synthesized at a rate of \(9.94 \times 10^{5}\) and \(8.48 \times 10^{5}\) molecules per min per 2N DNA, respectively, in 14-day embryonic chick muscle. The rates of rRNA synthesis in 17-day embryonic muscle are also similar. In both 14-day and 17-day embryonic muscle, 18S and 28S rRNA each decays with a half-life of 65 h. We conclude that the constant level of tRNA and rRNA in embryonic chick muscle from 14 to 17 days (Nwagwu & Nana, 1974) is maintained also by a constant rate of synthesis and turnover.

INTRODUCTION

The ribosome, polyribosome and transfer RNA contents of embryonic chick muscle do not change significantly during development from 11 to 17 days (Nwagwu & Nana, 1974). A number of mechanisms could regulate their amounts including the rate of RNA transcription (Emerson, 1971; Weber, 1972), the rate of degradation of primary transcriptional products during processing to tRNA, 18S and 28S rRNA (Cooper, 1973; Bowman & Emerson, 1977) and RNA turnover (Emerson, 1971; Weber, 1972; Bowman & Emerson, 1977).

Previous attempts have been made to study RNA synthesis in developing

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chick muscle, in vivo. It has been shown that rRNA synthesis is high at 11 days, low at 14 days and high once again in 18-day-old chick embryonic muscle (Herrmann, Heywood & Marchok, 1970). Nwagwu & Engemann (1979) have shown that the patterns of rRNA synthesis in muscle from the 14th to the 15th and from the 17th to the 18th day of chick development are similar, suggesting that the ribosome content during this period may be transcriptionally regulated.

In other studies of muscle cells in culture, it has been shown that ribosome accumulation ceases after cell fusion (Hosick & Strohman, 1971). Also a decrease in the incorporation of radioactive precursors after myoblast fusion has been reported (Marchock, 1966; Coleman & Coleman, 1968; Man & Cole 1972). These reports, however, have not followed any changes in the ribosome content of myofibres long after fusion has occurred. In another work, Heywood & Nwagwu (1969) showed that the incorporation of 32P into rRNA of myosin-synthesizing polyribosomes of 14-day embryonic chick muscle was considerably less than the incorporation into polysomal RNA.

Nwagwu & Onishchuk (1979) have shown that the patterns of tRNA synthesis during embryonic muscle development from the 14th day to 15th day and from the 17th day to 18th day were similar. Therefore it would seem that the tRNA content was regulated by transcriptional mechanisms.

The specific activity of the precursor pool which supplies nucleotides for RNA synthesis was not determined in any of the above studies. It has therefore been difficult to obtain a clear picture of RNA synthesis in developing chick muscle.

In this study we have followed the pattern of accumulation of AMP in rRNA and tRNA and have thereby determined the pattern of accumulation of newly synthesized RNA, after correcting for the specific activity of the precursor ATP pool. The results show that the rates of synthesis of 18S and 28S rRNA of 14-day embryonic chick muscle are similar to those of the corresponding rRNA of 17-day embryonic chick muscle; the half-life of rRNA is 65 h. Since the amounts of ribosomes of muscle of both embryonic ages are also similar (Nwagwu & Nana, 1974), we conclude that ribosome accumulation and turnover in embryonic chick muscle from the 14th day to 17th day do not change.

The results also show that the rates of tRNA synthesis in developing chick muscle of 14-day and 17-day embryos are similar and that tRNA decays with a half-life of 50 h.

**METHODS**

*Extraction of ATP*

Five 14-day chick embryos or four 17-day ones were each injected with 0.08 ml of [3H]adenosine via one of the major chorioallantoic veins and incubated at 37 °C for varying lengths of time. At the appropriate time, leg skeletal muscle (2.5 g) was dissected out quickly to minimize ATP degradation (see Mandel, 1964).
RNA synthesis in embryonic chick muscle

The muscle was homogenized in 3 vols of cold (0 °C) 70% ethanol/1% potassium citrate, pH 6.8 (v/v) (Mandel, 1964) with 20 strokes of a loose-fitting Dounce homogenizer. Prior to use, the ethanol/potassium citrate mixture was stored at −20 °C for at least 12 h. Potassium citrate was included to inhibit the conversion of ADP to ATP during the extraction steps (Seraidarian, Moinments & Wallner, 1962). The homogenate was centrifuged at 18000 rev/min in the IEC Model B20 centrifuge with a type 870 rotor for 20 min at 0 °C. The supernatant was appropriately diluted with 70% ethanol for further analysis.

Specific activity of ATP

The specific activity of ATP was determined as described by Emerson & Humphreys (1971).

Preparation of rRNA (for determination of optimal conditions for hydrolysis and for estimation of [3H] exchange during hydrolysis) and tRNA

[3H]8-adenosine was injected into 14-day-old chick embryos via one of the chorioallantoic veins and the embryos were incubated for 6 h.

Leg muscle (2-5 g) was homogenised in RSB buffer (300 mM-NaCl – 10 mM-MgCl₂ – 10 mM Tris-HCl, pH 7.0) with ten strokes of a loose-fitting Dounce homogenizer. The homogenate was centrifuged at 10000 g for 10 min at 0 °C.

The resultant S-10 supernatant fraction was further centrifuged at 120000 g for 2 h at 0 °C in a Model B 60 IEC ultracentrifuge. Ribosomal RNA was extracted from the pellet by the hot phenol–sodium dodecyl sulphate method described by Penman (1966) after resuspension of the pellet in 2–3 ml of NTES buffer (40 mM Tris – HCl (pH 7.8) – 2 mM EDTA – 100 mM-NaCl – 1% (w/v) sodium dodecyl sulphate).

Transfer RNA was prepared from the S-120 supernatant fraction by the method of Nwagwu & Nana (1974). Transfer RNA prepared in this manner, elutes as a single peak of 4S RNA following chromatography on Sephadex G-75 column (Nwagwu & Hossain, 1975). Furthermore, the tRNA peak accepts serine with high efficiency (Nwagwu & Hossain, 1975).

Hydrolysis of rRNA

Ribosomal RNA obtained as a precipitate by the above procedure, was pelleted by centrifugation at 10000 g for 10 min at 0 °C, resuspended in 3 ml of 300 mM-NaOH and alkaline-soluble radioactivity determined by the method of Humphreys (1973).

Hydrolysis of tRNA

Transfer RNA was resuspended in 1 ml of 300 mM-NaOH. A portion, 0.1 ml in duplicate, was removed for estimation of the total radioactivity of tRNA and carrier adenosine (approx. 1 A₂₆₀ unit) was added to the remaining portion (see Hecht, Zamecnik, Stephenson & Scott, 1958). Duplicate portions of the
resuspension, 0·3 ml each and equivalent to 4–4·5 $A_{260}$ units tRNA were incubated at 80 °C for 60 min. Subsequent analyses were similar to those described by Humphreys (1973).

_Determination of [³H] exchange_

The tritium of adenine exchanges with water in base (Wilt, 1969). This exchange was measured as described by Humphreys (1973).

_Preparation of radioactive 18S and 28S RNA_

Chick embryos, 14 and 17 days old were each injected with [³H]adenosine (0·08 ml) and incubated for varying lengths of time. Ribosomal RNA was prepared as described above and a portion, 0·4 ml (equivalent to 6–12 $A_{260}$ units) was layered on 27 ml or 15 % to 30 % (w/v) linear sucrose density gradient prepared in NTES buffer and centrifuged at 24000 rev/min in IEC ultracentrifuge with a swinging-bucket rotor, type SB-110, for 18 h at 24 °C. After centrifugation, fractions, 1 ml each, were collected as the absorbance ($A_{260}$ nm) was recorded with a Gilford Model 2400 spectrophotometer. Peaks corresponding to 18S and 28S rRNA were separately pooled and the RNA precipitated with 2 vol of absolute ethanol at −20 °C for 12–18 h.

_Determination of the percentage of radioactive AMP in 18S and 28S rRNA_

The RNA samples precipitated with ethanol as described above were recovered by centrifugation at 17000 g for 20 min at 0 °C. The pellets were resuspended in 1 ml of 300 ml NaOH and incubated at 80 °C for 60 min. and nucleotides obtained as described by Humphreys (1973). The nucleotides were dissolved in 0·1 ml of 100 mm-NH$_4$OH. A portion, 20 μl, was spotted on PEI thin-layer cellulose plate which has been exhaustively irrigated with distilled water. The plate was developed in 500 mm sodium formate, pH 3·4 for about 4 h, dried in a fume hood for 2–4 h at 20 °C and the spots of AMP and GMP corresponding to those of their respective standard markers were scraped into separate scintillation vials. The nucleotides were eluted with 0·5 ml of 1 m-HCl for 15 min. PCS: xylene (2:1, v/v) (10 ml) was added, the whole mixture agitated for 4–6 h and the radioactivity determined. In subsequent experiments, the total radioactivity in rRNA (18S and 28S) and tRNA was determined and multiplied by the appropriate percentage of the total radioactivity present in AMP (see Humphreys, 1973).

_Determination of radioactivity_

Radioactivity was determined in the presence of 10 ml of a scintillation mixture, PCS:xylene (2:1, v/v) with a Packard Tricarb liquid scintillation spectrometer, Model 3310, having a [³H]counting efficiency of 17 %.
Materials

Leg muscle of 14- and 17-day-old Dekalb 171 White Leghorn embryos was used in this study. [3H]8-adenosine (20 μCi/m-mole) purchased from Schwarz-Mann, Orangeburg, New York, N.Y., was rid of alcohol and finally dissolved in 0-9 % saline (v/v) to give a final specific activity of 19-3 μCi/mmole. Firefly luciferase (Sigma FLE-250) and Dowex 1-x-8 chloride from 200-400 mesh size were obtained from Sigma Chemical Co., Dekalb, No., U.S.A. Dowex 1-x-8 formate was prepared from the chloride form by extensive washing with 2 M formic acid until no more chloride ions were removed (at which point no precipitate was formed with AgNO₃). The resin was then thoroughly washed with distilled water. Polyethleneimine (PEI) thin-layer cellulose plates were purchased from Brinkmann Instruments, Toronto, Canada. Ribonuclease-free sucrose for density gradient centrifugation was purchased from Schwarz-Mann, Orangeburg, New York, N.Y. All chemicals were of analytical grade. PCS scintillant was purchased from Amersham-Searle, Toronto, Canada.

RESULTS

Incorporation of [3H]adenosine into nucleotides

Developing chick muscle was labelled with [3H]adenosine and the incorporation of the radioisotope into adenine and guanine nucleotides over a time interval of 30 min to 12 h was measured. The results presented in Table 1 show that ATP accounted for 64 % to 68 % of the total radioactivity of the ethanol-soluble fraction, while only 2-4-3 % was recovered in adenosine. The radioactivities in all adenine nucleotides represent 83 % of the radioactivity of the ethanol-soluble fraction. Thus 30 min after its introduction into muscle, a large percentage of adenosine is converted to adenine nucleotides particularly ATP. Radioactivity recovered in guanine nucleotides accounted for 17 % of the radioactivity of the ethanol-soluble fraction. Thus a significant portion of the adenosine pool is interconverted to guanine nucleotides (see Hauschka, 1973).

Amount and specific activity of ATP in 13-day and 17-day embryonic chick muscle

After correcting for the efficiency of ATP extraction and elution, the quantities of ATP in 1 g fresh weight of muscle of 14- and 17-day embryonic chick embryos were determined to be 222 and 299 nmole, respectively (Table 2). The specific activity of ATP increases rapidly up to the 60 min time point and then reaches a plateau level. The specific activity of ATP from 14-day embryonic muscle (2-5) is higher than that from 17-day embryonic muscle (2-0) by 25 % at the plateau level of the graph.
Table 1. Amount of radioactivity in the ethanol-soluble fraction and the percentage of this radioactivity recovered in ATP, AMP, ADP/dATP, adenosine, total adenine nucleotides and total guanine nucleotides

(The data are expressed as means ± S.E. The sample size is in parentheses.)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Ethanol-soluble fraction (dpm)</th>
<th>Radioactivity of ethanol-soluble fraction recovered in nucleotides (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ATP</td>
</tr>
<tr>
<td>30</td>
<td>696620 ± 70282 (4)</td>
<td>64.5 ± 1.5 (4)</td>
</tr>
<tr>
<td>60</td>
<td>774716 ± 55199 (4)</td>
<td>67.4 ± 1.2 (4)</td>
</tr>
<tr>
<td>180</td>
<td>748188 ± 30354 (4)</td>
<td>68.0 ± 0.5 (4)</td>
</tr>
<tr>
<td>360</td>
<td>758365 ± 46998 (4)</td>
<td>66.2 ± 1.8 (4)</td>
</tr>
<tr>
<td>720</td>
<td>506191 ± 42860 (4)</td>
<td>67.1 ± 1.1 (4)</td>
</tr>
</tbody>
</table>

[3H]Adenosine (0.08 ml; 1.95 × 10⁸ d.p.m.) was injected into 14-day chick embryos and, at the appropriate time, the amount of radioactivity in total muscle homogenate, the ethanol-soluble fraction, ATP, ADP/dATP adenosine and guanine nucleotides was determined as described under 'Methods' section. The amount of radioactive ATP was 12.2 nmol at the 60 min time point and remained at this level for 12 h. Results obtained with 17-day embryonic muscle were similar to those presented and are therefore omitted.

* ADP and dATP did not chromatograph distinctly from each other and were therefore pooled together.
RNA synthesis in embryonic chick muscle

Table 2. The content and specific activity of ATP of 14-day and 17-day embryonic chick leg muscle

(The data are expressed as mean ± s.e./g fresh weight of muscle. The sample size is in parentheses.)

<table>
<thead>
<tr>
<th>Day</th>
<th>ATP(nmol/g)</th>
<th>Specific activity* (dpm/pmole ATP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>222±13 (42)</td>
<td>2.5</td>
</tr>
<tr>
<td>17</td>
<td>299±10 (42)</td>
<td>2</td>
</tr>
</tbody>
</table>

Muscle (2.5 g) was homogenized in 5 ml of 70% ethanol/1% potassium citrate, pH 6.8, and ATP was separated and quantified as described under ‘Methods’.

Amount of ATP = \[\frac{100}{86.5} \times \frac{100}{70.71}\]

where \(y\) is the amount of ATP determined, without making any corrections for the efficiencies of extraction and of elution of ATP; 100/86.5 is the correction factor for the efficiency of ATP extraction (70% ethanol – 1% potassium citrate, pH 6.8, extracted 86.5% of total muscle ATP); 100/70.71 is the correction factor for the efficiency of ATP elution with 40 mM glycyl-glycine HCl buffer pH 7.41).

* The specific activity of ATP reaches a maximum after 60 min and remains at this level for 12 h.

Incorporation of [\(^3\)H]adenosine into AMP in rRNA

Approximately 51% and 20% of the radioactivity of rRNA are represented by AMP and ATP respectively, for all the time-points studied. Radioactivity in adenine nucleotides thus comprises 71% of the total radioactivity of rRNA. Of the total radioactivity in tRNA 21–27% is represented by AMP while GMP accounts for approximately 29%.

Accumulation of AMP in rRNA and tRNA

As shown in Fig. 1, AMP rapidly accumulates in both 18S and 28S rRNA of 14- and 17-day embryonic muscle within 1 h of introduction of radioactive adenosine. The time it takes for the specific activity of AMP to reach a constant level would be the time it takes to replace all old, unlabelled rRNA molecules with new, radioactive ones. However, for practical reasons, AMP accumulation in RNA was followed for only 12 h by which time only a small fraction of total RNA has been labelled. This limitation raises a major problem in working with developing systems in vivo; for if we continued labelling for much longer periods, for example 24–48 h, then we would no longer be studying 14-day embryonic muscle. Furthermore, the embryos do not survive prolonged incubation following administration of 0.08 ml of radioisotope. We have therefore made the following assumptions:

(a) the kinetics of synthesis and decay of radioactively labelled RNA are similar to those of total RNA;
Fig. 1. Time course of accumulation of 28S rRNA (●—●); tRNA (△—△) and 18S rRNA (○—○) of 14-day embryonic chick muscle. The values given represent net accumulation, that is, the difference between synthesis and degradation of RNA molecules. Developing chick embryos, 14 and 17 days old, were injected with \([^{3}H]\)adenosine (0.08 ml) and incubated for varying lengths of time. At the appropriate time, leg muscle (2.5 g) was dissected out and tRNA and rRNA were extracted and analyzed as described in the ‘Methods’ section. The 18S and 28S rRNA peaks were separately pooled and the RNA precipitated with two volumes of absolute ethanol at -20°C for 12-18 h. The amount of RNA (in A_{260} units) was determined and the RNA hydrolyzed. AMP was isolated by thin-layer cellulose chromatography and its radioactivity converted to moles of AMP, after correcting for \([^{3}H]\)exchange during hydrolysis and the average specific activity of the ATP pool. The quantity of AMP expressed in picomoles was converted to micrograms of RNA as follows:

\[
\begin{align*}
\mu g \text{ of tRNA} & = \text{pmole AMP} \times \frac{100}{18.5} \times 330 \times 10^6; \\
\mu g \text{ of 18S rRNA} & = \text{pmole AMP} \times \frac{100}{24} \times 330 \times 10^6; \\
\mu g \text{ of 28S rRNA} & = \text{pmole AMP} \times \frac{100}{18.5} \times 330 \times 10^6;
\end{align*}
\]

given that (a) 18.3% of all nucleotides in tRNA is represented by AMP (Zachau, 1972; Sodd & Doctor, 1974); (b) 24% and 18.5% of all nucleotides in 18S rRNA and 28S rRNA, respectively, are represented by AMP (Lerner, Bell & Darnell, 1963; Loening, 1968) and (c) the average molecular weight of a nucleotide is 330 daltons. The 2 n DNA contents of 14- and 17-day embryonic chick muscle are estimated as 2.1 and 2.5 mg/g fresh weight of muscle, respectively, from the data of Fig. 2 and table 3 of Herrmann et al. (1970). As the results for 17-day RNA are similar to those presented, they have been omitted.
Fig. 2. Standard decay analysis of tRNA (a) and 18S rRNA (b) of 14-day embryonic chick muscle. The logarithm of the percentage of the total RNA remaining unlabelled is plotted against time. If an equilibrium exists and the rate of RNA synthesis equals its rate of degradation, then the accumulation curves of Fig. 1 would be the inverse of the decay curve of non-radioactive RNA molecules beginning from the time when the radioisotope was administered. The percentages of the steady-state level of RNA (C°) synthesized at the different times given in Fig. 1 were calculated and the percentages of non-radioactive RNA obtained by difference. The graphs were plotted as described by Brandhorst & Humphreys (1971) and Humphreys (1973). The graphs for 28S and 18S rRNA are superposable. The graphs for 17-day RNA are also omitted as they are similar to those of 14-day RNA.

(b) radioactively labelled RNA does not represent a pool of RNA having unique properties;
(c) the first-order decay plot of RNA during the labelling period of 12 h (Fig. 2) is similar to the decay plot of RNA during the period required to reach steady-state; extrapolation of the decay graph to intersect the abscissa is a true reflection of the decay graph of RNA.

**Synthesis and accumulation of tRNA, 18S and 28S rRNA**

The steady-state levels of tRNA, 18S and 28S rRNA of 14-day embryonic muscle determined chemically are, respectively, $1.1 \times 10^8$, $5.62 \times 10^7$ and $4.79 \times 10^7$
Table 3. Steady-state amounts \( (C_w) \), rates of synthesis and decay and half-lives of tRNA, 18S and 28S rRNA of 14- and 17-day embryonic chick muscle

<table>
<thead>
<tr>
<th>Day</th>
<th>tRNA</th>
<th>18S rRNA</th>
<th>28S rRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>45.71</td>
<td>65.66</td>
<td>127.69</td>
</tr>
<tr>
<td>17</td>
<td>44.29</td>
<td>62.13</td>
<td>114.02</td>
</tr>
<tr>
<td>14</td>
<td>10.99 x 10^8</td>
<td>5.62 x 10^7</td>
<td>4.79 x 10^7</td>
</tr>
<tr>
<td>17</td>
<td>8.9 x 10^8</td>
<td>5.34 x 10^7</td>
<td>4.26 x 10^7</td>
</tr>
<tr>
<td>14</td>
<td>1.44 x 10^9</td>
<td>7.75 x 10^7</td>
<td>6.61 x 10^7</td>
</tr>
<tr>
<td>17</td>
<td>1.18 x 10^9</td>
<td>7.36 x 10^7</td>
<td>5.89 x 10^7</td>
</tr>
<tr>
<td>14</td>
<td>1.05 x 10^-6</td>
<td>1.16 x 10^-8</td>
<td>2.26 x 10^-8</td>
</tr>
<tr>
<td>17</td>
<td>0.86 x 10^-6</td>
<td>1.1 x 10^-8</td>
<td>2.20 x 10^-8</td>
</tr>
<tr>
<td>14</td>
<td>2.4 x 10^9</td>
<td>9.94 x 10^3</td>
<td>8.48 x 10^3</td>
</tr>
<tr>
<td>17</td>
<td>1.97 x 10^9</td>
<td>9.44 x 10^3</td>
<td>7.53 x 10^3</td>
</tr>
<tr>
<td>14</td>
<td>2.3 x 10^-4</td>
<td>1.77 x 10^-4</td>
<td>1.77 x 10^-4</td>
</tr>
<tr>
<td>17</td>
<td>2.3 x 10^-4</td>
<td>1.77 x 10^-4</td>
<td>1.77 x 10^-4</td>
</tr>
<tr>
<td>14</td>
<td>50 h</td>
<td>65 h</td>
<td>65 h</td>
</tr>
<tr>
<td>17</td>
<td>50 h</td>
<td>65 h</td>
<td>65 h</td>
</tr>
</tbody>
</table>

\( C_w = \) steady-state content of RNA derived either from chemical data or from incorporation of AMP into RNA.

(a) \( C_w \) is the average of 36 separate chemical determinations. The amount of tRNA was determined as described by Nwagwu & Lianga (1974). The amount of rRNA was determined by spectrophotometric analysis of each of 18S and 28S rRNA peaks after sucrose-gradient centrifugation.

(b) \( C_w \) was derived from the incorporation data of Fig. 2 by calculating the amount of total RNA synthesized at steady-state at the given rate of synthesis.

(c) The rate of synthesis \( (K_s) \) was determined from the equation,

\[
C_w = \frac{K_s}{K_d}, \quad \text{where} \quad K_d = \frac{\ln 2}{t_h} = \text{half-life},
\]

(d) The half-life, \( t_h \), was derived from Fig. 3 after extrapolating the graph to intersect the abscissa.

* The number of molecules was calculated by assuming that the molecular weights of tRNA, 18S and 28S rRNA are, respectively, 25000, 0.7 x 10^6 and 1.6 x 10^6 daltons (Loening, 1968).

molecules per DNA content (Table 3). Similar quantities are present in 17-day embryonic muscle. Thus 18S and 28S rRNA accumulate in approximately equal amounts as would be expected since they both originate from a common precursor molecule (see Perry, 1976).

In 14-day embryonic muscle the steady-state levels of tRNA, 18S and 28S rRNA derived from incorporation of AMP into RNA are, respectively, 1.44 x 10^9, 7.75 x 10^7 and 6.61 x 10^7 molecules per 2n DNA content (Table 3). Similar quantities are also present in 17-day embryonic muscle. Since the steady-state levels derived from both chemical and incorporation data are in good agree-
RNA synthesis in embryonic chick muscle

Table 4. Specific radioactivities of AMP (dpm/p-mole AMP) in tRNA, 18S rRNA and rRNA of 14- and 17-day embryonic chick muscle at their respective steady-state levels

<table>
<thead>
<tr>
<th>Day</th>
<th>pmoles AMP per A260 unit RNA (A)</th>
<th>Total radioactivity at steady state (B)</th>
<th>Specific radioactivity (B/A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Since the specific radioactivity of ATP was determined at steady state, the specific radioactivity of AMP in RNA was also estimated at steady state. The specific radioactivity of AMP in RNA given as dpm AMP/A260 unit RNA was converted to dpm/pmole AMP in RNA. We calculated (a) the number of moles of AMP in 1 A260 unit of RNA given that (i) 1 A260 unit of tRNA is equivalent to 20 μg (Nwagwu & Liang, 1974); (ii) 1 A260 unit of 18S or 28S rRNA is equivalent to 54-5 μg (Wool & Cavicchi, 1967); (iii) AMP represents 18-3% of the total nucleotides in tRNA and 24% and 18-5% of all nucleotides in 18S and 28S rRNA respectively (Lerner et al., 1963; Loening, 1968); (iv) the average molecular weight of a nucleotide is 330. (b) the radioactivity which would be present in AMP in RNA at steady state (that is, 100% labelling of AMP) given that (i) after 12 h of labelling, approximately 27% of the steady-state content of tRNA and 11% of the steady-state content of each of 18S and 28S rRNA are radioactive (calculated from Fig. 1 and Table 3). The values obtained by using time points other than 12 h do not differ significantly from those given in the table.

From the equations and parameters described by Galau, Lipson, Britten & Davidson (1977) for molecules whose decay follows first-order kinetics, we have determined the rates of accumulation of newly synthesized tRNA, 18S and 28S rRNA of 14- and 17-day embryonic chick muscle. Ribosomal RNA molecules, 18S and 28S, are synthesized at similar rates of 9-94 × 10^3 and 8-48 × 10^3 molecules/min/2N DNA content (Table 3) in 14-day embryonic muscle. Similar rates were obtained for 17-day 18S and 28S rRNA. Transfer RNA of 14-day is synthesized at a rate of 2-4 × 10^5 molecules/min/2N DNA content. The rate for 17-day tRNA is also similar. Thus, the rates of accumulation of newly synthesized tRNA and rRNA are not co-ordinately regulated. This finding is collaborated by the results of other workers (see Neidhart, 1964; Smith, 1976). Both 18S and 28S rRNA of 14- and 17-day embryonic muscle decay with a half-life of 65 h; tRNA decays with a half-life of 50 h (Table 3).
Specific radioactivity of AMP in tRNA, 18S and 28S rRNA

ATP may be compartmentalized in muscle and the ATP pool we have measured may therefore not be the real precursor pool for AMP in RNA. The results in Table 4 show that the specific radioactivity of AMP in tRNA, 18S and 28S rRNA is similar to that of the ATP pool. We therefore conclude that tRNA, 18S and 28S rRNA are all synthesized from the same precursor ATP pool (see Wiegers, Kramer, Klapproth & Hilz, 1976).

DISCUSSION

This study represents the first reported attempt to determine the rate of synthesis and the half-life of rRNA in developing chick muscle, in vivo. Its reliability, or otherwise, depends largely on the accuracy of the specific activity of the ATP precursor pool which allowed us to determine more precisely the accumulation of AMP in RNA. The specific activity of ATP reaches a constant maximum level within 1 h in general agreement with the observations of other workers (Brandhorst & Humphreys, 1971; Bowman & Emerson, 1977). Since we have not followed the synthesis of the primary transcription product, probably a 40S ribosomal RNA (Bowman & Emerson, 1977), we cannot make a definite statement about transcriptional regulation of rRNA synthesis, especially as the 40S rRNA precursor may turn over during processing, and the 18S and 28S rRNA molecules may themselves turn over before assembly into ribosomes. The rates of RNA synthesis we have determined therefore, more appropriately refer to rates of accumulation of newly synthesized RNA, following transcription and processing. What is clear, however, is that newly synthesized rRNA accumulates in ribosomes of muscle of chick embryos, 14 and 17 days old, at similar rates.

It should be informative to determine the pattern of accumulation of newly synthesized RNA in younger embryos in which the percentage of proliferating cells is higher than those of 14- and 17-day embryos (see Herrmann et al. 1970). One could then follow, in vivo, the pattern of RNA synthesis during the transition from a stage when myoblasts predominate to one in which myotubes and myofibres form. For practical reasons, we could not include younger embryos in this study. We presume that our results, at best, would apply also to 11-day embryos since from these results and those obtained earlier (Nwagwu & Nana, 1974), there is apparently a direct relationship between the quantity of RNA and the rate of accumulation of newly synthesized RNA.

Bowman & Emerson (1977) have shown that the specific activity of ATP in myoblasts is more than ten times that of ATP in fibres, in culture. In chick leg muscle at 14 and 17 days, only 22% and 18%, respectively, of the total cell population are proliferating (Herrmann et al. 1970). However, the total incorporation of ATP into this relatively small percentage would be higher than
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the total incorporation into fibres. Our ATP data therefore reflect the average incorporation of ATP into both myoblasts and fibres as well as other non-muscle cells.

Both 18S and 28S rRNA decay with a half-life of 65 h (Table 3). Emerson (1971) estimated that the half-life of rRNA of growing fibroblasts is approximately 40 h. Weber (1972) showed that rRNA is stable in growing chick fibroblast cells but begins to turn over with a half-life of 35–45 h as the cells become contact-inhibited. Bowman & Emerson (1977) found that the half-life of 18S and 28S rRNA in muscle fibres in culture is 45 h. Abelson, Johnson, Penman & Green (1974) have shown that in resting 3T3 cells, 28S and 18S rRNA decay with half-lives of 50 and 72 h respectively. The half-life of tRNA of developing chick muscle of 14- and 17-day-old embryos is 50 h (Table 3). Results obtained by other workers show that the half-life of tRNA is 35–45 h in cultured chick fibroblasts (Weber, 1972), 36 h in resting 3T3 and 3T6 cells and 60 h in growing 3T6 cells (Abelson et al. 1974).

We calculated that there are approximately 15 AMP residues in tRNA of developing chick muscle given that (1) the steady-state amount of AMP in tRNA is approximately 30 nmole per A260 unit (calculated from Fig. 1); (2) 1 mg of tRNA is equal to 20 A260 units (Nwagwu & Lianga, 1974); (3) transfer RNA has a molecular weight of 25000 daltons. From the data obtainable from other systems, it would seem that there are 12–17 AMP residues in several tRNA species (see Zachau, 1972). We are therefore confident that the methods we have used to determine the specific activity of the ATP pool and the accumulation of AMP in tRNA are reliable.

The rates of accumulation of newly synthesized rRNA which we determined agree with the corresponding rates in other systems (1·16 × 10⁻⁸ μg/min/2 N DNA for 18S rRNA at 14 days and 2·26 × 10⁻⁸ μg/min/2 N DNA for 28S rRNA). In growing fibroblasts the rate of rRNA synthesis is calculated to be 4·7 × 10⁻³ pg/min/2 N DNA content (Emerson & Humphreys, 1971). The rate calculated for rapidly dividing somatic tissues is approximately 4·5 × 10³ rRNA molecules/min/cell (see Davidson, 1968). Brandhorst & McConkey (1974) have shown that L cells synthesize rRNA at the rate of 1·8 × 10⁻² pg/min/cell. In blastula–gastrula stages of sea-urchin embryos the rate of rRNA synthesis was determined to be 2·2 × 10⁻² pg/min/2 N DNA content (Galau et al. 1977). Bowman & Emerson (1977) calculated the rate of rRNA accumulation in myoblasts and fibres as 1150 and 630 molecules/min/2 N DNA, in contrast to a rate of approximately 7600–10000 molecules/min/2 N DNA obtained in this study. Of course, direct comparisons cannot be made between physiological systems in vitro and in vivo, especially as they are both subject to different regulating factors, such as hormonal influences, rate of supply of nutrients and influence of cell–cell interactions, among others.

The rates we have determined depend principally on three parameters, the steady-state content of RNA (C_0), the half-life of RNA (from which the decay
constant $K_d$ is obtained) and the specific activity of the ATP pool. Since the half-life of rRNA determined in this study (65 h) is similar to that obtained in muscle cell cultures by Bowman & Emerson (1977), only our calculation of $C_\infty$ could affect the rate of synthesis, $K_s$. The value for $C_\infty$ is an average of 36 separate chemical determinations. Furthermore, our calculations of $C_\infty$ based on incorporation of AMP into RNA and RNA turnover agree favourably with the values of $C_\infty$ determined chemically (having regard for all the assumptions made in the calculations). There is no evidence for compartmentalization of the ATP pool of muscle cells (see Emerson & Humphreys, 1971). The results shown in Table 4 also support this, suggesting that the ATP pool we have measured supplies AMP for tRNA and rRNA synthesis. We conclude that the rates of RNA synthesis we have determined are reliable.

It has been suggested that detailed analysis of ribosome turnover should be undertaken, since ribosome turnover may result in qualitative differences between the ribosomes and in increased efficiency of the ribosomes in protein synthesis (Nwagwu & Nana, 1974). The results of this study show that ribosomes of developing chick muscle are unstable and turnover with a half-life of 65 h. Analysis of the efficiency of ribosomes in protein synthesis would elucidate the role of translational mechanisms in regulating protein synthesis in developing chick muscle.

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REFERENCES


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