Alteration of Deoxyribonucleic Acid-dependent Ribonucleic Acid Polymerase Activities in Amphibian Liver Nuclei during Thyroxine-induced Metamorphosis*

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SUMMARY

DNA-dependent RNA polymerase activity was determined in isolated tadpole liver nuclei. RNA polymerase I and II activities could be differentiated in situ by the use of α-amanitin. The nuclear RNA polymerases were shown to respond to Mn²⁺ and Mg²⁺ in a manner similar to that reported for the same enzymes from other eukaryotes and revealed a biphasic activity curve in the presence of increased ammonium sulfate concentration. It was shown that the two peaks of activity were the result of different optimal activities in the presence of ammonium sulfate of RNA polymerase I (50 mm) and of RNA polymerase II (300 mm). The nearest neighbor and base composition analyses revealed a marked change in the ratio of AMP + UMP: GMP + CMP when the isolated nuclei were assayed in the presence of α-amanitin. While the rate of RNA synthesis with isolated nuclei was nonlinear, this appeared not to be the result of RNA polymerase inactivation or of degradation of the product by ribonuclease.

Thyroxine administration, either by injection or immersion of tadpoles, caused marked stimulation of both RNA polymerases I and II. The increases in activities of both enzymes were not detectable until at least 4 days after tadpole immersion in 2.0 X 10⁻⁸ M thyroxine. An increase in mitochondrial carbamyl phosphate synthetase paralleled that of the RNA polymerases. There was no significant time lag between the increase in RNA polymerase I, RNA polymerase II, and carbamyl phosphate synthetase activities. The increase in RNA polymerase activities was also observed during natural metamorphosis.

RNA polymerase, because of its nature, must play an important role in cellular differentiation. In prokaryotes it has been demonstrated that the cell responds to its environment through the use of factors which control RNA synthesis. These factors exert precise regulation of initiation (σ factor), termination (ρ factor), and specificity of transcription (φ factor) by RNA polymerase (1-3). Similar RNA polymerase control factors in eukaryotic organisms have been reported (4-6). The significance of these control elements is as yet not clear.

The present study deals with RNA polymerases in eukaryotic cells with observations on the alterations of their activities in liver nuclei during thyroxine-induced metamorphosis of Rana catesbeiana tadpoles. The previous demonstrations that thyroxine can induce synthesis of protein and RNA in tadpole liver make this system particularly suitable for a study of the role of the different polymerases during modulation of the transcription and translation processes (7). The alterations in RNA polymerase activities have been correlated with the activity of carbamyl phosphate synthetase, which has been shown, following thyroxine exposure, to be increased in tadpole liver as a result of a de novo protein synthesis in preparation for the change from ammonotelism to ureotelism (7). This correlation suggests a relationship between nuclear RNA polymerase activity and the induction of synthesis of a mitochondrial enzyme.

MATERIALS AND METHODS

Tadpoles and Thyroxine Administration—Rana catesbeiana tadpoles were obtained from the Lemberger Company, Oshkosh, Wisconsin. The tadpoles were fed a diet of canned spinach and maintained for 2 weeks in running, dechlorinated tap water at 25° prior to use. For thyroxine administration, tadpoles were exposed to thyroxine either by intraperitoneal injection (through the tail) or by immersion in solutions at the indicated concentrations.

Isolation of Nuclei and Mitochondria—Tadpoles were cooled in an ice bath and the livers were removed by dissection, freed of the gall bladders, and pooled in ice-cold Buffer A (Tris-HCl, 0.05 M; KCl, 70 mm; MgCl₂, 10 mm; dithiothreitol, 10 mm, pH 7.4, at 25°). All subsequent procedures were performed at 0 to 2°. The livers were homogenized in a Teflon-glass homogenizer in 5 volumes of Buffer B (made up of Buffer A to which glycerol was added to give a 25% solution (v/v) of glycerol). The homogenate was then filtered through 2 layers of cheesecloth and centrifuged at 150 × g for 10 min. After one wash in Buffer B the nuclei were suspended in a 2.4 M sucrose in Buffer

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A and centrifuged at 50,000 x g for 30 min. The resulting nuclear pellet was resuspended in a small volume of the original homogenizing medium and assayed.

Crude mitochondria for the carbamyl phosphate synthetase assay were obtained by centrifugation (10,000 x g for 20 min) of the supernatant fraction resulting from the initial centrifugation at 150 x g employed during the isolation of the nuclei.

**Determination of RNA Polymerase Activities**—The incubation mixture for the polymerase reaction, in a final volume of 0.2 ml, was as follows: 20 μmoles of Tris-HCl, pH 8.0, at 25°; 14 μmoles of KCl; 0.8 μmole of MnCl₂; 4 μmoles of dithiothreitol; 0.06 μmole each of GTP, CTP, and ATP; and 5 μCi of [3H]UTP (15.9 or 22 Ci per mmole) and nuclei in suspension representing 100 to 500 μg of DNA. Ammonium sulfate was added when assaying RNA polymerase II. The reaction was terminated after incubation at 25° by addition of 100 μl of 0.10 M tetrasodium pyrophosphate and 2 mg of yeast RNA carrier. The RNA was precipitated by addition of 5 volumes of cold 5% trichloroacetic acid and centrifuged at 1200 x g for 10 min. The RNA pellet was washed twice in 5% trichloroacetic acid and once in ethanol-ether, 3:1.

Nuclear RNA polymerase I and II activities were determined in isolated nuclei by the addition of 1 μg of α-amanitin, which selectively inhibits RNA polymerase II (8), to the incubation system. The designation of RNA polymerase I and II is based on previous work indicating RNA polymerase I is nucleolar and RNA polymerase II is nucleoplasmic (9, 10).

**Determination of Base Composition and Nearest Neighbor Base Frequencies**—The labeled RNA obtained from the polymerase assay system was dissolved in 10% NaCl, pH 8.0, and placed in a boiling water bath for 30 min. After cooling, the solution was centrifuged at 150 x g for 10 min. Two volumes of ice-cold ethanol were added to an aliquot of the supernatant and the resulting precipitate was allowed to stand overnight at -20°.

After collection of this precipitate by low speed centrifugation the precipitate was washed and redissolved in 20 μl of 0.33 M KOH and allowed to hydrolyze for 16 hours at 37°. The resulting RNA mononucleotides were separated by electrophoresis on Whatman No. 3MM paper for 5 kv-hours in pyridine-acetate buffer, pH 3.5. The paper was sprayed with concentrated ammonium hydroxide, dried, and the nucleotides were visualized under ultraviolet light; or, if labeled with 32P, the paper was subjected to autoradiography for 1 hour and visualization was achieved from the exposed film.

**Measurement of Radioactivity**—In order to determine the radioactivity from the tritium-labeled RNA formed during the polymerase assay, the washed RNA pellet was dissolved in 0.3 ml of NCS solubilizer (Nuclear-Chicago) by heating to 50° for 1 hour. Scintillation fluid, consisting of 0.4% of 2,5-diphenyloxazole (PPO) and 0.02% of 1,4-bis[2-(4-methyl-5-phenyloxazoyl)]-benzene (POPOP) in toluene, was then added and the radioactivity in the dissolved RNA was measured in a Packard Tri-Carb scintillation spectrometer at 45% efficiency.

To determine the level of radioactivity of the mononucleotides after electrophoretic separation, tritium-labeled areas were cut out and heated at 50° for 1 hour with NCS solution and counted as described above. The 32P-labeled nucleotides were counted by placing the appropriate area of the paper in a vial with toluene scintillation fluid and by counting directly.

**Determination of Carbamyl Phosphate Synthetase Activity**—The measurement of carbamyl phosphate synthetase was done according to the procedure of Brown and Cohen (11).

**Table I**

**Characteristics of incorporation of [3H]UTP into RNA in isolated nuclei**

The RNA polymerase reaction was allowed to proceed for 5 min at 25°, and the incorporation of tritium was determined as described. These data were obtained from duplicate assays of nuclei prepared from 1.7 g of liver from 10 tadpoles at Taylor-Kollros stages XI to XII. Duplicate assays varied less than ±1% in all cases.

<table>
<thead>
<tr>
<th>Assay constituents</th>
<th>[3H]UMP incorporated (fmoles/mg DNA)</th>
<th>Percentage of complete system (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>9.0</td>
<td>100</td>
</tr>
<tr>
<td>Minus KCl</td>
<td>2.6</td>
<td>29</td>
</tr>
<tr>
<td>Minus (NH₄)₂SO₄</td>
<td>5.2</td>
<td>58</td>
</tr>
<tr>
<td>Minus GTP</td>
<td>0.8</td>
<td>9</td>
</tr>
<tr>
<td>Minus ATP</td>
<td>5.3</td>
<td>59</td>
</tr>
<tr>
<td>Minus ATP</td>
<td>2.1</td>
<td>23</td>
</tr>
<tr>
<td>Plus calf thymus DNA (100 μg)</td>
<td>9.0</td>
<td>100</td>
</tr>
<tr>
<td>Plus RNase A (100 μg)</td>
<td>2.2</td>
<td>24</td>
</tr>
<tr>
<td>Plus DNase (500 μg)</td>
<td>3.5</td>
<td>39</td>
</tr>
<tr>
<td>Plus actinomycin D (50 μg)</td>
<td>1.2</td>
<td>13</td>
</tr>
<tr>
<td>Minus Mn²⁺ plus Mg²⁺ (1.2 μmoles)</td>
<td>3.2</td>
<td>36</td>
</tr>
<tr>
<td>Complete-rat liver nuclei</td>
<td>174.0</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1.** Inhibition of RNA polymerase II in isolated nuclei by α-amanitin. Incubation was for 5 min at 25° in the presence of 2 mM MnCl₂ and 0.29 M (NH₄)₂SO₄. The nuclei were prepared from 2 g of liver and the assay was performed on an aliquot of the suspension of purified nuclei representing 326 μg of DNA per assay point. The specific activity of the [3H]UTP was 15.9 Ci per mmole.
Chemical Determinations—DNA was determined by the method of Burton (12), with calf thymus DNA as a standard.

Materials—ATP, GTP, CTP, UTP, calf thymus DNA, yeast RNA, and ribonuclease A were obtained from Sigma. Deoxyribonuclease was obtained from Worthington. NCS solubilizer was obtained from New England Nuclear. [3H]GTP was from International Chemical and Nuclear Corporation. α-Amanitin was purchased from Henley and Co., New York. All 32P-labeled nucleotides were a generous gift of Dr. James E. Dahlberg of this department.

Fig. 2. Response of nuclear RNA polymerases to MnCl₂ and MgCl₂. RNA polymerase I (B) was assayed in the absence of ammonium sulfate, whereas polymerase II activity (A) was determined in the presence of 0.29 M ammonium sulfate. RNA polymerase II was determined by subtracting the values for RNA polymerase I in the presence of α-amanitin and 0.29 M ammonium sulfate from the values obtained in the absence of α-amanitin. All incubations were for 5 min at 25°. O—O, Mn²⁺; ●—●, Mg²⁺.

Fig. 3. Effect of ionic strength on RNA polymerase activity in liver nuclei. Incubations were at 25° for 5 min. ●—●, complete systems; o—o, RNA polymerase I, complete system plus α-amanitin; △—△, RNA polymerase II obtained by the subtraction of the activity of RNA polymerase I from the activity of complete system.

Fig. 4. Time course of [3H]UMP incorporation into RNA of isolated liver nuclei. Thyroxine treatment was an intraperitoneal injection of 2.0 × 10⁻⁷ moles of thyroxine per tadpole. Nuclei were incubated at the time indicated at 25° with 10 μCi of [3H]UTP (15.9 Ci per mmole). RNA polymerase I (A) was a direct determination in the presence of α-amanitin, whereas RNA polymerase II (B) was obtained by the subtraction method described earlier. ●—●, control; ○—○, 1 day of thyroxine treatment, △—△, 4 days of thyroxine treatment.
other sources (9, 10) but solubilization and chromatography of the inhibition of RNA polymerase activities in tadpole nuclei by amanitin is the activity due to enzyme I and generally represents 28 to 32% of the total activity. The activity of polymerase II seems to be inherent in tadpole liver nuclei.

Fig. 1 illustrates, prior incubation of the nuclei results in loss of about 10% of the polymerase II activity. Also shown in Fig. 5 is the result of the addition of α-amanitin after 10 min of reaction time, and continuation of the incubation for 10 min thereafter. It is apparent that although synthesis of RNA due to polymerase II is stopped by α-amanitin, the total acid-precipitable RNA remains relatively unchanged for at least the following 10 min. This observation indicates no significant effect of endogenous ribonuclease activity on the RNA synthesized in situ during RNA polymerase assay in tadpole liver nuclei. Fig. 4 also illustrates the time course for RNA synthesis by RNA polymerases I and II after the tadpoles were subjected to thyroxine treatment. These time studies reveal that the activities of both enzymes increase and that the increase is proportional throughout the incubation period.

Tata (16) has reported that attempts to determine RNA polymerase activity in tadpole liver nuclei gave erratic results, probably because of a high ribonuclease activity. The results of Fig. 5 and electrophoretic analysis of the product RNA from tadpole liver nuclei indicate only a negligible effect of ribonuclease during the polymerase assays. The reproducibility of identical assay points was generally ±1% but a great deal of variation occurred when values from tadpoles of different sizes and different known ecological and nutritional backgrounds were compared. For these reasons, thyroxine experiments were always performed on the same batch of tadpoles as was used for the untreated controls. Tata also indicated significant homopolymer formation when using labeled ATP as substrate (16). The nearest neighbor analysis data in Table II do not reveal any homopolymer formation. If the RNA polymerase system is incubated for a short time period in the absence of excess unlabeled nucleoside triphosphates and then terminated by the addition of 5% trichloroacetic acid, the precipitated labeled nucleoside triphosphates are bound to an as yet unidentified factor. This effect, which results in an apparent incorporation in the absence of only one nucleoside triphosphate, could be interpreted to represent homopolymer formation. This binding phenomenon seems to be accentuated by the presence of NaF in the reaction mixture, but is eliminated by pyrophosphate addition.

Fig. 5. Stability of RNA polymerase II during prior incubation and resistance of product to degradation. All incubations were at 25° for the times indicated. Addition of α-amanitin after 10 min of incubation inhibited RNA polymerase II and the continued incubation of the mixture allowed determination of any possible decrease in acid-precipitable radioactivity attributable to product degradation. Duplicate assays containing α-amanitin for the entire time period were performed at all of the incubation times shown. These values, representing RNA polymerase I, were then subtracted from the total activity in the absence of α-amanitin, thus giving values of RNA polymerase II activity. -- , RNA polymerase II; O—O, RNA polymerase II with nuclei added to the reaction mixture at zero time in the absence of any nucleoside triphosphates; then after a 10-min incubation at 25° unlabeled GTP, CTP, ATP and [H]UTP were added to start the reaction.

The specific activity (pmoles of [H]UMP incorporated per mg of DNA) of tadpole liver nuclei is very low when compared to that of rat liver nuclei assayed in an identical manner. This lower activity of tadpole liver nuclei could not be enhanced by a variety of treatments such as addition of RNA, ribonuclease inhibitors, EDTA, or glycerol to the reaction medium. It is unlikely that this low activity is due to the presence of some inhibitor of the incorporation of [H]UMP because the total activities of the RNA polymerases from a nuclear preparation are recoverable as soluble enzymes from a DEAE-Sephadex column and the activities of these partially purified forms are not significantly altered. This low level of RNA polymerase activity seems to be inherent in tadpole liver nuclei.

Use of α-Amanitin to Determine Cation and Ionic Strength Characteristics of RNA Polymerases I and II—α-Amanitin has been shown to be a specific inhibitor of one of the DNA-dependent RNA polymerases from eukaryotic nuclei (12). Fig. 1 illustrates the inhibition of RNA polymerase activities in tadpole nuclei by α-amanitin. The activity remaining after addition of 0.5 μg of α-amanitin is the activity due to enzyme I and generally represented 28 to 32% of the total activity. The activity of polymerase II was determined by subtracting the activity for polymerase I from the activity determined in the absence of α-amanitin (13). A third RNA polymerase has been reported in nuclei from other sources (9, 10) but solubilization and chromatography of tadpole RNA polymerase reveals only trace amounts of RNA polymerase III.¹ It is assumed that any such RNA polymerase activity will be included in the assay for RNA polymerase I. The results in Fig. 2 illustrate the in situ responses of polymerases I and II to varying concentrations of Mn2+ and Mg2+. RNA polymerase II exhibits an optimal activity ratio of Mn2+:Mg2+ of 2.5, whereas polymerase I has a Mn2+:Mg2+ activity ratio of 1.0.

The alteration of activities of the polymerases as a result of an increase in ionic strength is shown in Fig. 3. A biphasic curve of [H]UMP incorporation into nuclear RNA under varying salt concentrations is obtained (14, 15). The results with α-amanitin indicate very clearly that the peak of activity at about 300 mM ammonium sulfate is due to RNA polymerase II and that the smaller peak at 50 to 60 mM ammonium sulfate is due to RNA polymerase I.

Time Course of [H]UTP Incorporation into RNA—Fig. 4 illustrates that the time course of the RNA polymerase reaction deviates from linearity. The nonlinearity is unaffected by the addition of large quantities of RNA, DNA, or variations in the temperature of incubation. It is probably not a result of the destruction of the RNA polymerase enzymes because, as Fig. 5 illustrates, prior incubation of the nuclei results in loss of about 10% of the polymerase II activity. Also shown in Fig. 5 is the result of the addition of α-amanitin after 10 min of reaction time, and continuation of the incubation for 10 min thereafter. It is apparent that although synthesis of RNA due to polymerase II is stopped by α-amanitin, the total acid-precipitable RNA remains relatively unchanged for at least the following 10 min. This observation indicates no significant effect of endogenous ribonuclease activity on the RNA synthesized in situ during RNA polymerase assay in tadpole liver nuclei. Fig. 4 also illustrates the time course for RNA synthesis by RNA polymerases I and II after the tadpoles were subjected to thyroxine treatment. These time studies reveal that the activities of both enzymes increase and that the increase is proportional throughout the incubation period.

¹ M. D. Griswold and P. P. Cohen, manuscript in preparation.
TABLE II

Nearest neighbor base frequency and base composition of RNA synthesized in isolated nuclei

For the determination of base composition, 5 μCi each of [3H]ATP (7.93 Ci per mM), [3H]GTP (11.8 Ci per mM), [3H]UTP (15.9 Ci per mM), and [3H]CTP (27.5 Ci per mM) were added to the usual reaction mixture in place of the unlabeled nucleotides; the results were corrected for the variance in specific activities of the bases. For the nearest neighbor base analysis, 10 μCi of the appropriate labeled nucleotide was added. All incubations were at 25°C for 5 min and contained ammonium sulfate except for the RNA polymerase I assays which were incubated in the presence of α-amanitin and in the absence of ammonium sulfate. The values for RNA polymerase II were determined by subtracting the RNA polymerase I activity; the value used for RNA polymerase I was 30% of the total RNA polymerase activity. This value corresponded to the average percentage of total activity due to RNA polymerase I. The thyroxine-treated tadpoles were killed 4 days after injection. The incubation conditions of the nuclei previous to extraction of RNA are shown in the first column by the type of RNA polymerase assay and the treatment of tadpoles previous to preparation of liver nuclei. All data represent the average of duplicate determinations which had a variance of less than ±0.03 in all cases.

Incubation conditions of nuclei preceding RNA extraction | Labeled nucleotide | UMP | GMP | AMP | CMP  | AMP + UMP + GMP + CMP |
---|---|---|---|---|---|---|
Control; total activity | [3H]-nucleotides | 0.25 | 0.38 | 0.21 | 0.15 | 0.9 |
Control; polymerase I | [3H]-nucleotides | 0.16 | 0.30 | 0.26 | 0.08 | 0.7 |
Control; polymerase II | [3H]-nucleotides | 0.20 | 0.27 | 0.19 | 0.19 | 0.0 |
Thyroxine-treated; total activity | [3H]-nucleotides | 0.26 | 0.54 | 0.18 | 0.08 | 0.6 |
Thyroxine-treated; polymerase I | [3H]-nucleotides | 0.20 | 0.30 | 0.21 | 0.19 | 1.0 |
Thyroxine treated; polymerase II | [3H]-nucleotides | 0.20 | 0.30 | 0.21 | 0.19 | 1.0 |

Nearest neighbor base frequency

% Base composition

Control; total activity | [α-32P]UTP | 0.29 | 0.18 | 0.28 | 0.24 | 1.4 |
Control; polymerase I | [α-32P]UTP | 0.30 | 0.19 | 0.28 | 0.23 | 1.4 |
Thyroxine-treated | [α-32P]UTP | 0.31 | 0.21 | 0.26 | 0.22 | 1.3 |
Thyroxine-treated; polymerase I | [α-32P]UTP | 0.31 | 0.18 | 0.25 | 0.29 | 1.2 |
Control; total activity | [α-32P]GTP | 0.27 | 0.38 | 0.26 | 0.08 | 1.2 |
Control; polymerase I | [α-32P]GTP | 0.23 | 0.37 | 0.21 | 0.19 | 0.8 |
Thyroxine-treated; total activity | [α-32P]GTP | 0.22 | 0.38 | 0.26 | 0.09 | 1.0 |
Thyroxine-treated; polymerase I | [α-32P]GTP | 0.23 | 0.44 | 0.21 | 0.12 | 0.8 |
Control; total activity | [α-32P]CTP | 0.25 | 0.31 | 0.25 | 0.26 | 1.0 |
Control; polymerase I | [α-32P]CTP | 0.19 | 0.31 | 0.23 | 0.26 | 0.7 |
Thyroxine-treated; total activity | [α-32P]CTP | 0.19 | 0.31 | 0.23 | 0.26 | 0.7 |
Thyroxine-treated; polymerase I | [α-32P]CTP | 0.21 | 0.27 | 0.20 | 0.32 | 0.7 |

FIG. 6. Increase in the RNA polymerase of tadpole liver nuclei as a result of thyroxine treatment. Tadpoles received in a single shipment were used in each experiment and thyroxine treatment was staggered such that sacrifice of animals and RNA polymerase assays were all done in a single day. Values are given with standard deviations and each point is a result of an average obtained from five separate experiments each utilizing 10 tadpoles. Each point then represents the results from 50 tadpoles in increases in RNA polymerase I (A) and RNA polymerase II (B). ●●●●●, injection of 2.0 × 10⁷ moles of thyroxine per tadpole; ○○○○○, immersion in 2.0 × 10⁻⁸ M thyroxine solution.
Analysis of Base Composition and Nearest Neighbor Base Frequency of Product of RNA Polymerases I and II—The results shown in Table II reveal the base composition and nearest neighbor base frequencies of the RNA formed in nuclei in the presence and absence of α-amanitin and before and after thyroxine treatment of the tadpoles. The ratio of AMP + UMP:GMP + CMP was consistently found to be smaller for the product formed in the presence of α-amanitin, suggesting the synthesis of a G-rich type of RNA. The nearest neighbor analysis reveals a similar shift in base composition. The base analysis and the nearest neighbor base frequency values were unchanged in the RNA isolated from tadpoles treated with thyroxine.

Alteration of RNA Polymerase Activities During Induced Metamorphosis and Relationship of this Alteration to Increases in Carbamyl Phosphate Synthetase Activity—The increases in RNA polymerase activities during thyroxine administration are shown in Figs. 6 and 7. The administration of thyroxine by injection leads to a very quick response, whereas immersion in a lower concentration of thyroxine reveals a longer lag period. Fig. 7 illustrates the close relationship between increases in RNA polymerase II and the rise in the activity of carbamyl phosphate synthetase. There is no perceptible lag between the increase in the RNA polymerase activity (a transcriptional change) and the induction of mitochondrial carbamyl phosphate synthetase. Thyroxine added to isolated nuclei had no effect on the RNA polymerase activity.

Alteration of RNA Polymerase Activities During Natural Metamorphosis—Fig. 8 illustrates the values of the nuclear RNA polymerase activities obtained from tadpoles at different stages of metamorphosis characterized by the leg to tail ratios (17). These results parallel for the most part the results observed with preparations from tadpoles during metamorphosis induced by thyroxine.

Discussion

Aside from the much lower level of activity, the RNA polymerases of amphibian liver nuclei have the same general properties as those separated from other eukaryotic cells (9, 14, 15, 18). Further, the present study reveals that these enzymes can be assayed and their general properties studied with whole nuclei. The validity of using nuclei for in situ determinations of RNA polymerase activity has been discussed elsewhere (19). The activity ratio of Mn²⁺:Mg²⁺ and the nearest neighbor analyses illustrate the validity of in situ determinations of both RNA polymerase I and II. Solubilized sea urchin RNA polymerase II has been shown to have a higher Mn²⁺:Mg²⁺ ratio than RNA polymerase I (9). Since RNA polymerase I is nucleolar it is expected that it will have a guanine-cytosine-rich RNA product (19).

Hormone-stimulated alterations of RNA polymerase activities have been demonstrated in other eukaryotic organisms. The total RNA polymerase activity in liver nuclei from thyroidectomized rats increases on thyroxine administration (20); androgen administration affects the total RNA polymerase activity in mouse kidney nuclei after castration (21); and estrogens increase the RNA polymerase activity in rat uterus (22). By the use of α-amanitin to differentiate between RNA polymerase I and II, Blatti et al. (10) reported a greater stimulation of RNA polymerase I than polymerase II from the nuclei of the uterus of estrogen-treated rats and from liver nuclei of glucocorticoid-stimulated rats (10). The observations presented in this report indicate that during induced amphibian metamorphosis RNA polymerases I and II increase proportionately to the same extent and there is thus very little change in the ratio of I to II throughout the hormone treatment. During natural metamorphosis RNA polymerase I activity seems to increase at an earlier developmental stage than does RNA polymerase II and therefore
the regulation of this nuclear enzyme may be independent of the regulation of the nucleoplasmic enzyme. Since sequential development of the tadpole appears to be dependent on a steadily rising titer of thyroxine within the tissues, the regulatory system for RNA polymerase I may be more sensitive to the hormone than is the system for RNA polymerase II.

The apparent relationship of carbamyl phosphate synthetase induction and the increase in RNA polymerase activities is of considerable significance. Anuran metamorphosis involves many morphological, physiological, and biochemical changes in preparation for a terrestrial life. One of the biochemical changes which has been most clearly documented is the induction in the liver of the ornithine-urea cycle enzymes in response to thyroxine administration (7). Specifically carbamyl phosphate synthetase I (mitochondrial), a rate-limiting ornithine-urea cycle enzyme, is induced de novo after immersion in 2.6 × 10⁻⁸ M thyroxine (7). This increase begins before gross morphological changes can be noted and the enzymes of the ornithine-urea cycle, in general, are induced ahead of most other enzymes in the liver (7). It is assumed that the induction of the ornithine-urea cycle enzymes must involve a transcriptional change such as increased mRNA synthesis and a change in the translational mechanisms of the cell such as increased numbers of ribosomes or ribosomes with an altered activity. Changes such as these and many others would be necessary in order to allow the synthesis of large amounts of the induced proteins. It was anticipated that comparison of the time of alteration of transcription as determined by the RNA polymerase activities to the time period for changes in translation as determined by carbamyl phosphate synthetase activity would reveal information on the mechanism of thyroxine action. It can be seen from Fig. 7, which illustrates the comparative increase in RNA polymerase activities and in carbamyl phosphate synthetase activity, that there is no detectable time lag between the two. At approximately 24 hours after immersion, the first perceptible increases in both RNA polymerase I and RNA polymerase II are accompanied by a slight increase in carbamyl phosphate synthetase. All changes which are required for the increased synthesis of RNA and induction of carbamyl phosphate synthetase must thus be taking place concurrently. While this does not directly establish a causal relationship between the transcriptional event and the induced synthesis of carbamyl phosphate synthetase, the concurrent changes in activities suggest that such a relationship exists. The nearest neighbor and total base analysis data shown in Table II further indicate that the thyroxine-induced stimulation of nuclear RNA synthesis is a general stimulation of RNA of similar base composition to that of the control.

The relatively long time lag between thyroxine administration and stimulated RNA polymerase activity and the fact that thyroxine has no effect on the RNA polymerase if it is added directly to isolated nuclei suggest two possibilities. First, it is likely that thyroxine has an effect at some other level of cellular function before RNA polymerase activity is stimulated; and second, that it is a consequence of this initial action, and not of thyroxine directly, which enhances RNA synthesis. It is unknown at this time whether the increase in RNA polymerase activity is a result of the synthesis of new enzyme, of enzyme effectors, or of a change in template characteristics. Kim and Cohen (23) reported an increase in template efficiency of chromatin prepared from thyroxine-treated tadpoles as determined by the activity of added bacterial RNA polymerase. Other attempts to reveal template alterations such as histone changes or increases in histone acetylation have shown that chromatin from thyroxine-treated tadpoles is unchanged when compared with that of the controls (24).

REFERENCES

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