Chromatin- and Nuclei-Directed Ribonucleic Acid Synthesis in Sugar Beet Root

C. T. Duda and Joe H. Cherry
Horticulture Department, Purdue University, Lafayette, Indiana 47907

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ABSTRACT

The synthesis of RNA by chromatin-bound RNA polymerase prepared from sugar beet (Beta vulgaris) root tissue is completely dependent on the presence of a divalent metal (Mg2+ or Mn2+) and the presence of four ribonucleoside triphosphates. Accumulation of labeled acid-insoluble product is inhibited by the addition of RNase and actinomycin D to the reaction. When beet root slices are washed for 25 hours, chromatin-associated RNA polymerase activity increases 7-fold over that of unwashed tissue. This enzyme activity declines with further washing. DNA template availability, as measured by saturating levels of added Escherichia coli RNA polymerase, was also found to follow a pattern similar to that for RNA polymerase. Nearest neighbor frequencies of the RNA synthesized by chromatin isolated from unwashed and washed tissue are different.

Washing tissue in solutions of gibberellic acid and auxin enhances template availability of the isolated chromatin. Experiments with isolated nuclei indicate an effect of these hormones on RNA synthesis.

When intact storage tissue, such as roots or tubers, is sectioned and washed, a dramatic increase in metabolic activity ensues. This appears to be a general phenomenon in higher plants and has been regarded as a derepression phenomenon (11, 13, 14, 20, 24, 31). Potato discs incubated in aerated, distilled water undergo a time-dependent increase in respiration (11), which is dependent upon the synthesis of new RNA and protein. The synthesis of invertase and of other enzymes in washed discs of sugar beet, artichoke, carrot, and red beet is also dependent upon the synthesis of new RNA and protein (2, 8, 13, 21, 31). The synthesis of nucleic acid precedes the rise in enzyme activities. The development of invertase activity can be enhanced further by addition of exogenously applied GA and can be inhibited by added auxin (8, 12, 22).

This paper describes the changes which occur in chromatin- and nuclei-directed RNA synthesis by preparations isolated from washed sugar beet tissue. RNA polymerase (EC 2.7.7.6) activity associated with a chromatin-rich fraction was isolated and characterized. The template availability of the chromatin was also determined. The data presented here are in accord with a derepression phenomenon. Addition of GA and auxin during the washing period enhances the synthesizing capacity of isolated chromatin. Experiments with isolated nuclei also indicate that these hormones affect RNA synthesis at the level of gene transcription.

MATERIALS AND METHODS

Plant Material. Sugar beets (Beta vulgaris L.) were grown in the field and stored at 3 to 5 C for use throughout this study. In order to reduce tissue variability, beet roots were chosen for their uniform size (about 1000 gm), and comparable transverse sections were employed for differential treatments.

In experiments involving various aeration or washing periods, transverse sections, 2 mm thick, were cut into 5-mm squares and placed into autoclaved Erlenmeyer flasks. The tissue was washed several times with autoclaved distilled water, and sufficient sterile phosphate buffer, 0.005 M, pH 6.5, was added to nearly cover the tissue. The flasks were shaken rather vigorously in a water bath at 30 C for specific times. Bacterial contamination was minimized by performing all procedures, subsequent to sectioning, in a transfer hood and by adding streptomycin sulfate and penicillin G, each at 104 M, to the sterile buffer. Furthermore, the aeration medium was periodically (at least every 4 hr) changed during the aeration period, thereby also ensuring a constant pH of the suspending medium. Bacterial contamination was monitored throughout the aeration period by mixing 0.2-ml aliquots or dilutions of the aeration medium under sterile conditions with 5 ml of a 1.5% Bactonutrient agar (Difco) preparation in a Petri dish. At the termination of the experiment, aerated tissue was crushed and mixed with the agar preparation. The plates were incubated for 24 hr at 35 C and were viewed under magnification. No visible bacterial colonies could be detected in any of the plates. Parallel runs with nonsterilized medium were also monitored and found to be highly contaminated with bacteria.

Nucleic Acid Metabolism. Sugar beet discs were incubated with 3H-adenosine for 2 hr in sterile phosphate buffer (0.005 M, pH 6.5) containing 104 M penicillin G and streptomycin sulfate. The nucleic acids were extracted from the tissue as outlined previously (9) and were purified by chromatography through Sephadex G-50. A260 of the eluant was determined with a Beckman DU spectrophotometer. An aliquot of the labeled nucleic acid was added to cold 10% trichloroacetic acid, and the resultant precipitate was transferred to a cellulose nitrate membrane filter (Bac-T-Flex, type B-6) and washed with cold 5% trichloroacetic acid. After drying, the filter was placed in scintillation fluid and the radioactivity was assayed with a Packard liquid scintillation spectrometer which gave a counting efficiency of approximately 40% for tritium.

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2 Present address: MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48823.

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Respiration. Sugar beet root tissue was finely diced (2 × 2 mm, 1 g) and wetted with phosphate buffer (0.005 M, pH 6.5) in reaction vessels. The center was contained 0.2 ml of 4% KOH, and the filter paper wick. The flask was opened to the atmosphere when measurements were not being taken. Respiration rate was measured as microliters of O₂ consumed per gram of tissue per 30-min period with a Gilson differential respirometer. Washing conditions closely approximated those when larger amounts of tissue were used.

Polyribosome Isolation. Three grams of sugar beet tissue were ground in a mortar and pestle (previously cooled to −20 °C) with 6 ml of a medium containing 0.25 M sucrose, 0.05 M tris-succinate buffer, pH 7.8, 0.015 M KCl, 0.01 M MgCl₂, 0.005 M 2-mercaptoethanol, and 0.05% deoxycholate (16). The resultant soup homogenate was filtered through glass wool, and the filtrate was centrifuged at 20,000 g for 10 min. The supernatant was layered over 0.5 M sucrose which was supported by a 1.6 M sucrose cushion (both solutions contained 0.01 M tris-succinate, pH 7.8, 0.01 M MgCl₂, 0.015 M KCl, and 0.005 M 2-mercaptoethanol) and was then layered on a 25-ml linear sucrose gradient (10 to 34%) containing 0.01 M tris-succinate buffer, pH 7.8, 0.01 M MgCl₂, 0.005 M KCl, and 0.005 M 2-mercaptoethanol and was then layered on a 25-ml linear sucrose gradient (10 to 34%) containing 0.01 M tris-succinate buffer, pH 7.8, 0.01 M MgCl₂, and 0.001 M spermidine. The gradient was centrifuged in a SW-25 rotor at 23,000 rpm for 3 hr in a Spinco-50 rotor. The resultant ribosomal pellet was suspended in 1 ml of 0.01 M tris-succinate buffer, pH 7.8, containing 0.01 M MgCl₂, 0.015 M KCl, and 0.005 M 2-mercaptoethanol and was then layered on a 25-ml linear sucrose gradient (10 to 34%) containing 0.01 M tris-succinate, pH 7.8, 0.01 M MgCl₂, and 0.001 M spermidine. The gradient was centrifuged in a SW-25 rotor at 23,000 rpm for 3 hr in a Spinco model L centrifuge. All operations were performed at 0 to 4 °C. The centrifuged gradient was monitored at 254 nm by passing the gradient through the photocell of an ISCO spectrophotometer, model 180.

Chromatin Isolation. Chromatin was isolated from washed and unwashed sugar beet root tissue according to the method of Huang and Bonner (15), with minor modifications. The sectioned tissue was blotted dry and chilled to 4 °C (this temperature was maintained throughout the remainder of the isolation). The tissue was coarsely chopped and then homogenized in a Vibrit homogenizer in an equal volume (w/v) of homogenizing medium (0.125 M sucrose, 0.1 M tris-HCl, pH 8.0, 0.001 M MgCl₂, 0.02 M 2-mercaptoethanol); Dow Corning antifoam B was added at a concentration of 0.2 ml per 100 ml of homogenizing medium. Homogenization was done for 15 sec at low speed, followed by 45 sec at high speed. The homogenate was filtered through a double thickness of cheesecloth and then through Miracloth. The filtrate was centrifuged at 10,000 g for 30 min and the supernatant was discarded. The crude chromatin layer was scraped from the underlying starch and cellular debris and was suspended in 0.01 M tris-HCl buffer, pH 8.0, containing 0.25 M sucrose and 0.01 M 2-mercaptoethanol (wash buffer). The chromatin layer was recovered by centrifugation, resuspended in 7 ml of the wash buffer, and was layered over 20 ml of 2 M sucrose (containing 0.01 M tris-HCl, pH 8.0, 0.01 M 2-mercaptoethanol) in a SW-25 (Spinco) centrifuge tube. The upper one-third of the tube was stirred gently. The resultant crude gradient was centrifuged at 20,000 rpm for 3 hr in a Spinco model L centrifuge. The supernatant was syringed off, and the sucrose-purified pellet (chromatin) was suspended in 0.05 M tris-HCl, pH 8.0, containing 0.01 M 2-mercaptoethanol. Aliquots of this suspension were assayed for RNA polymerase activity.

Chromatin RNA Polymerase Assay. The standard assay tube contained 20 μmoles of tris-HCl, pH 8.0; 1.0 μmole of MgCl₂; 0.25 μmole of MnCl₂; 1.0 μmole of dithiothreitol; 0.2 μmole each of GTP, CTP, and ATP; 0.01 μmole of [3H]-UTP (specific activity, 2 c/mmole); and chromatin (corresponding to 2 to 5 μg of DNA) in a final volume of 0.4 ml. The assay was routinely run for 15 min at 30 °C, and the reaction was stopped by the addition of 4 ml of cold 10% trichloroacetic acid. The resultant precipitate was transferred to a membrane filter, and the radioactivity was determined as outlined above.

Nearest Neighbor Analysis. To determine nearest neighbor frequencies and base composition, ²³-polynucleotides from the reaction mixture were transferred to glass filter discs (Whatman glass fiber paper, GF/A, 2.1 cm) and washed as above. The filters were dried and placed into thick walled glass centrifuge tubes containing 0.5 M KOH. Hydrolysis was carried out for 18 hr at 37 °C. The hydrolysate was neutralized with perchloric acid, and the KClO₃, and filter disk were centrifuged out and washed twice with cold, distilled water. The combined extract and wash were lyophilized and then taken up in 0.1 ml of electrophoresis buffer (3.4 ml of pyridine, 34.4 ml of acetic acid, and 3.4 g of disodium EDTA per liter, pH 3.6). Six samples were spotted on a sheet (7 ½ × 18 inches) of electrophoresis paper (Schleicher and Schuell Co., grade 589 Green Ribbon-C) together with appropriate marker nucleotides. Electrophoresis was run for 4½ hr with a potential gradient of 22 volts/inch and a current of approximately 40 ma. After electrophoresis the sheet was dried and cut into individual sample strips which were further cut into 0.5-cm sections. These pieces were immersed in scintillation fluid, and radioactivity was measured as outlined above.

Isolation of Nuclei. Nuclei from sugar beet root tissue were isolated according to the method of Rho and Chipchase (27). A machine consisting of two counter-rotating aluminum rollers, 0.01 inch apart, was employed. The tissue was held between two layers of nylon marquisette, and an equal volume (w/v) of extracting medium (0.25 M sucrose, 0.1 M tris-HCl, pH 7.0, 0.002 M MgCl₂, and 1% polyvinyl pyrrolidone) was used to wash the tissue as it passed between the rollers. The resultant exudate was filtered through a double thickness of cheesecloth and then through Miracloth. This filtrate was centrifuged at 6000 g for 10 min. The upper, gelatinous nuclear portion of the pellet was scraped from the starch portion and was suspended in 0.25 M sucrose, 0.025 M tris-HCl, pH 7.0, and 0.002 M MgCl₂.

Nuclei Assay. The crude nuclear preparation was then incubated in a standard reaction mixture of 0.25 M sucrose; 0.025 M tris-HCl, pH 7.0, containing 130 μmoles of MgCl₂; 150 μg of orotate; 140 μmoles of NaH₂PO₄; 1 μmole each of uridine, cytidine, and guanosine; and 200 μc of 'H-adenosine (specific activity, 4 c/mmole) in a total volume of 15 ml. Incubation was routinely carried out for 60 min at 30 C. The reaction was stopped by addition of 15 ml of ice-cold incubation medium containing unlabeled adenosine, and the nuclei were recovered by centrifugation (7). The nuclear pellet was then suspended in 25 ml of cold, saturated sucrose and was layered over 5 ml of supersaturated sucrose in a SW-25 centrifuge tube (Spinco). The two-phase sucrose gradient was centrifuged at 22,000 rpm for 60 min in a model L centrifuge. The nuclei banded at the interface and were collected with a wide-bore needle and syringe. The nucleic acids from the sucrose-purified nuclei were extracted by the phenol method (9), except that bentonite was omitted.

The purified nucleic acids were then separated on a methylated albumin kieselguhr column according to the method of Mandell and Hershey (25). The total nucleic acids were separated into ribosomal, DNA, and soluble components, and their concentrations were determined at 260 nm with a Beckman DU spectrophotometer. The radioactivity present as RNA or DNA in the eluant was determined by coprecipitation with 100 μg of unlabeled DNA in cold 10% trichloroacetic acid. The precipitates were transferred to membrane filters, and total radioactivity was determined.
least 30 hr of additional washing (Fig. 1b). Nucleic acid synthesis increases for 24 hr and then appears to level off (Fig. 1a, 2-hr labeling period). Figure 1c presents the polyribosome content (as percentage of total extractable ribosomes) of sugar beet tissue after various periods of washing. Maximal polyribosome content is reached after about 3½ hr of washing and remains constant for at least 12 hr. Actinomycin D (at 10 μg/ml, data not shown) prevents an increase in polyribosome content from that already present in unwashed tissue. The observed increase in polyribosome content suggests that a similar increase in functional mRNA occurred during this same period (23, 30). These data indicate that the sugar beet discs are "activated" in regard to their capacity to respire, to synthesize RNA, and apparently also, to synthesize protein.

**Chromatin.** In order to establish the optimal conditions for the sugar beet chromatin, several parameters of the chromatin RNA polymerase assay were varied until maximal enzyme activity was attained. Figure 2 shows that the polymerization reaction is temperature dependent with no activity at 0 C. Maximal enzyme activity is exhibited between 32 and 35 C at 15-min incubation time and decreases at higher temperatures.

The effect of various concentrations of Mg\(^{2+}\) and Mn\(^{2+}\) on the chromatin RNA polymerase activity is presented in Figure 3. In the presence of Mg\(^{2+}\), the optimal Mn\(^{2+}\) concentration is 0.625 μmole per ml; greater concentrations are inhibitory. The effects of varying Mg\(^{2+}\) concentration in the presence of optimal and suboptimal concentrations of Mn\(^{2+}\) are shown in Figure

**RESULTS**

**Physiology of Experimental Tissue.** Washing of sugar beet tissue results in a large change in various metabolic activities. Respiration rate increases for 14 hr and remains level for at

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**Fig. 1.** Physiology of experimental tissue. a: Changes in rate of nucleic acid synthesis is shown by the incorporation of \(^{3}H\)-adenosine into nucleic acid by differentially washed sugar beet discs; b: enhancement of respiration rate (μl of O\(_2\) consumed per g per 30 min at 30 C) of sugar beet tissue as a function of washing time; c: production of polyribosomes in sugar beet tissue during washing.

**Fig. 2.** Incorporation of UTP into RNA by chromatin-bound RNA polymerase as a function of temperature. Chromatin was isolated from unwashed sugar beet tissue and was incubated as described under standard assay conditions at the indicated temperatures for 15 min.

**DNA Determination.** Samples were prepared for analysis by precipitating DNA from aliquots of the chromatin preparation in cold 0.5 N perchloric acid. The precipitate was isolated by centrifugation, suspended in 0.5 N perchloric acid, and was heated to 70 C for 1 hr. The DNA content of the hydrolysate was estimated by the diphenylamine colorimetric method of Burton (5). Highly polymerized salmon sperm DNA was used as standard.

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**Fig. 3.** Dependence of polymerization rate on Mg\(^{2+}\) and Mn\(^{2+}\) concentrations. Standard assay conditions were employed except that Mn\(^{2+}\) concentration was varied while Mg\(^{2+}\) was kept at 1.0 μmole per assay (a), and the Mg\(^{2+}\) concentration was varied while the concentration of Mn\(^{2+}\) was maintained at 0.25 μmole (●●●●) and 0.125 μmole (●●●●) per assay (b).
Table I. Nucleotide Incorporation by Chromatin-bound RNA Polymerase

The reaction mixture (0.4 ml) contained 20 µmoles of tris-HCl, pH 8.0; 1.0 µ mole of Mg++; 0.25 µ mole of Mn++; 1.0 µ mole of dithiothreitol; labeled substrate; 0.2 µ mole of NTP and chromatin. Specific radioactivity of ATP, GTP, CTP, and UTP, respectively, were: 10 µc/0.22 µ mole, 1.0 c/mmole, 1.0 c/mmole, and 10 µc/0.1 µ mole. Incubation was for 15 min at 30 C. Incorporation of labeled nucleotide calculated on the basis of 50 µg of DNA per assay, and the activities are presented as picomoles incorporated/50 µg of DNA per 15 min at 30 C.

<table>
<thead>
<tr>
<th>Nucleotide Added</th>
<th>Amount of Labeled NTP in Assay</th>
<th>NMP Incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmole</td>
<td>µmoles</td>
</tr>
<tr>
<td>&lt;sup&gt;3&lt;/sup&gt;H-ATP</td>
<td>0.02</td>
<td>52.6</td>
</tr>
<tr>
<td>&lt;sup&gt;3&lt;/sup&gt;H-GTP</td>
<td>0.01</td>
<td>25.3</td>
</tr>
<tr>
<td>&lt;sup&gt;3&lt;/sup&gt;H-CTP</td>
<td>0.01</td>
<td>51.8</td>
</tr>
<tr>
<td>&lt;sup&gt;3&lt;/sup&gt;H-UTP</td>
<td>0.10</td>
<td>90.4</td>
</tr>
</tbody>
</table>

Table II. Properties of Chromatin-bound RNA Polymerase

Incubation was performed as described for standard assay (see Table I).

<table>
<thead>
<tr>
<th>System</th>
<th>Percentage of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>100&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>- NTP</td>
<td>50</td>
</tr>
<tr>
<td>- Mg++</td>
<td>100</td>
</tr>
<tr>
<td>- Mn++</td>
<td>81</td>
</tr>
<tr>
<td>- Mg++, Mn++</td>
<td>0</td>
</tr>
<tr>
<td>+ Actinomycin D (5 µg/ml)</td>
<td>30</td>
</tr>
<tr>
<td>+ RNase (100 µg/ml)</td>
<td>20</td>
</tr>
</tbody>
</table>

<sup>1</sup> Equal to 88 pmoles of UMP incorporated per 100 µg of DNA.

3b. At optimal Mn<sup>++</sup> content, the addition of Mg<sup>++</sup> has no effect on the relative reaction rate up to 5 µmoles per ml, whereas a higher amount is inhibitory. However, when Mn<sup>++</sup> content is suboptimal, an optimal Mg<sup>++</sup> content is observed at 2.5 µmoles per ml.

Of the four nucleotides tested, all act as substrates in the polymerization reaction (Table I). The concentration of the individual nucleotides was not known to be optimal, and it is therefore difficult to compare incorporation values. Enzyme activity was dependent on the presence of the four ribonucleoside triphosphates (Table II). The data also show that the polymerizing reaction is completely dependent on the inclusion of either Mg<sup>++</sup> or Mn<sup>++</sup> in the medium. Further, the amount of product accumulated is minimal when actinomycin D or RNase (pancreatic) is present in the assay.

The reaction kinetics of the RNA polymerase associated with the chromatin are shown in Figure 4a. Chromatin isolated from washed tissue has about 3½ times more polymerase activity than chromatin from unwashed tissue; in both preparations the reaction is complete by 15 min. Figure 4b shows that the RNA polymerase activity in the assay is directly proportional to the amount of DNA added to the reaction.

The development of chromatin RNA polymerase activity during washing of sugar beet tissue is illustrated in Figure 5. Enzyme activity is low in unwashed tissue and increases with washing of tissue. Optimal activity is reached after 24 hr of washing and then declines at about the same rate as it appeared.

It was of interest to determine whether increased chromatin transcription was due to increase in RNA polymerase, or increase in template availability, or both. The total template available for transcription by RNA polymerase was determined by saturating the available template sites with added E. coli RNA polymerase. This test assumes that E. coli RNA polymerase can copy all such sites on sugar beet DNA of the chromatin preparations. Template availability does increase.

Fig. 4. Characteristics of chromatin RNA polymerase activity. Incubation was carried out as described in "Materials and Methods." Data presented in a, represent RNA synthesis as a function of time by chromatin RNA polymerase isolated from unwashed (lower curve) and 17-hr washed (upper curve) sugar beet tissue (incorporation based on 50 µg of DNA). Data given in b, show relationship between RNA synthesis and chromat-DNA concentration.

Fig. 5. Development of chromatin RNA polymerase activity during washing of sugar beet tissue. Standard assay conditions were employed with chromatin preparations isolated from tissue previously washed for the periods indicated. RNA polymerase activity associated with chromatin (picomoles of UMP incorporated per 50 µg of DNA) is denoted by □. Template availability was determined as described for Table V and is denoted by ○ (incorporation based on 1 µg of DNA).
Table III. Nearest Neighbor Analysis of RNA Produced by Chromatin-bound RNA Polymerase

The reaction system was the same as that described for the standard assay (Table I), except that ^4H-UTP was replaced by 30 μCi UMP (150 mc/mole). Incubation was for 15 min at 30°C, and the reaction was terminated by the addition of cold 10% trichloroacetic acid. The labeled precipitate was processed as described under "Materials and Methods."

<table>
<thead>
<tr>
<th>Duration of Washing (hr)</th>
<th>Distribution of Label in Each 2',3'-Mononucleotide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMP</td>
</tr>
<tr>
<td>0</td>
<td>24.6</td>
</tr>
<tr>
<td>25</td>
<td>24.5</td>
</tr>
</tbody>
</table>

Table IV. Nearest Neighbor Analysis of RNA Produced by Chromatin Saturated with E. coli RNA Polymerase

The reaction mixture contained, in a final volume of 0.4 ml, 16 μmoles of tris-HCl, pH 8.0; 1.6 μmoles of MgCl2; 0.4 μmole of MnCl2; 0.16 μmole each of cold nucleotides; 0.5 μg of DNA (as chromatin) and 5 units of E. coli polymerase (fraction IV) as units described by Biopolymers Inc., Pinebrook, N.J.). CM2^32PPP (640 mc/mole) was present at 35.8 μc per assay and UMP^32PPP (380 mc/mole) at 30 μc per assay. Procedures of product analysis were the same as that for Table III.

<table>
<thead>
<tr>
<th>Duration of Washing (hr)</th>
<th>Labeled Precursor</th>
<th>Distribution of Label in Each 2',3'-Mononucleotide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CMP</td>
</tr>
<tr>
<td>0</td>
<td>α-32P</td>
<td>29.5</td>
</tr>
<tr>
<td>25</td>
<td>CTP</td>
<td>28.9</td>
</tr>
<tr>
<td>0</td>
<td>UTP</td>
<td>10.7</td>
</tr>
<tr>
<td>25</td>
<td>UTP</td>
<td>15.9</td>
</tr>
</tbody>
</table>

and follows a pattern of change similar to that for endogenous RNA polymerase (Fig. 5). RNA polymerase activity increases by 7-fold after 25 hr of washing, but template availability is increased only 3 times, as compared to unwashed tissue.

Product of Chromatin RNA Polymerase Reaction. The nearest neighbor technique was employed to analyze the product formed by the RNA polymerase of unwashed and 25-hr washed tissue (Table III). The RNA product formed in the standard assay for chromatin RNA polymerase was labeled with α-32P-UTP and was hydrolyzed with KOH. The individual nucleotides were then separated by paper electrophoresis. It is apparent that the product synthesized by the enzyme from the unwashed system has a nearest neighbor frequency much different from that of the 25-hr washed system (Table III). The product of the E. coli polymerase-chromatin system was also examined, and the results are presented in Table IV. Endogenous RNA polymerase contributed less than 1% of the E. coli RNA polymerase product under conditions used. The nearest neighbor frequency of the E. coli RNA polymerase products indicates that the unwashed chromatin template is different from the chromatin isolated from 25-hr washed tissue. Further, the product formed by the endogenous RNA polymerase is different from that formed by the added E. coli RNA polymerase.

Chromatin Preparations from Hormone-treated Tissue. Naturally occurring plant hormones, such as gibberellic acid and auxin, have been shown to affect the metabolism of washed sugar beet tissue (8, 12). The effect of these hormones on nucleic acid synthesis in vivo was studied, but no qualitative or quantitative change over control tissue was detected (C. T. Duda, unpublished data). If hormones have a qualitative influence on the synthesis of certain nucleic acid species, it is possible that the detection of such nucleic acid is not possible by present techniques. It was therefore desirable to look at the chromatin fraction as an effective site of action for these hormones. Sugar beet tissue was washed for 10 hr under standard conditions, without and in the presence of 10^-4 M GA or 10^-8 M IAA (concentrations which have been shown to affect development of invertase). Chromatin was isolated and assayed for associated RNA polymerase and template availability. These results are presented in Table V. The chromatin RNA polymerase isolated from GA-treated tissue is 28% more active than that isolated from control tissue. However, IAA had no detectable effect on the RNA polymerase associated with the chromatin fraction. Both GA and IAA did have an effect on the template available for transcription. GA increased the template available as detected by E. coli RNA polymerase by 83% over the control chromatin. IAA had a similar effect but not as great as GA because it increased available template by 35% over control chromatin. GA and IAA had no effect on RNA polymerase when added to control chromatin in vitro.

In Vitro Effect of Hormones on Isolated Nuclei. To study further the effect of hormones on nucleic acid synthesis, a nuclei system was tested in vitro. A crude preparation of nuclei was incubated in the presence of GA (3 x 10^-4 M) or 2,4-D (7 x 10^-7 M), and RNA synthesis was monitored by 3H-adenosine incorporation (7). The hormone-treated and control nuclei were purified, the RNA extracted and separated by MAK column chromatography (Fig. 6). The labeled nucleic acids were fractionated into four distinct fractions (9): (a) a soluble RNA fraction, (b) a DNA-RNA fraction, (c) a light ribosomal RNA, and (d) a heavy ribosomal RNA plus messenger RNA fraction. The results presented in the Figures are summarized in the inserted table of Figure 6. GA and auxin treatment lead to an increased synthesis of ribosomal RNA. GA was more effective than auxin in stimulating RNA synthesis as in the chromatin system.

DISCUSSION

Aging or washing sectioned sugar beet tissue greatly increases its basal metabolic activity. Rate of respiration and of nucleic acid synthesis and polyribosome content increases

Table V. In Vivo Effect of GA and IAA on Isolated Chromatin

Sugar beet root tissue was sectioned and washed in the presence of GA or IAA for 10 hr. Chromatin was isolated from treated and control tissue, and the activity of associated RNA polymerase was determined by using standard assay conditions. Template availability of the chromatin preparation was determined with the reaction mixture described in Table IV, except that 0.1 μmole of ^4H-UTP (2 c/mole) was added as labeled substrate. Incubation was for 15 min at 37°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pmole UMP Incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endogenous^1 With added E. coli polymerase^2</td>
</tr>
<tr>
<td>Control</td>
<td>12.6</td>
</tr>
<tr>
<td>GA, 10^-4 M</td>
<td>16.1</td>
</tr>
<tr>
<td>IAA, 10^-4 M</td>
<td>12.7</td>
</tr>
</tbody>
</table>

^1 Per 50 μg of DNA.
^2 Per 5 μg of DNA.
rapidly, without a noticeable lag. The rapid increase in polyribosomes, during the initial 3 hr of aging, detectable at 30 min, is one of the earliest biochemical changes observed in this tissue. Polyribosome content reached a constant value of about 65% of the total extracted ribosomes after 3 hr of aging, much earlier than the 6-hr reports for aged carrot tissue (23).

The RNA polymerase associated with the chromatin of sugar beet tissue possesses the general properties of partially purified RNA polymerase of various bacterial, animal and plant tissue (3, 6, 28). Activity is temperature dependent, requires divalent metal ion for activity, and requires the simultaneous presence of four nucleoside triphosphates. Synthesis of labeled RNA product is inhibited by actinomycin D and RNase; the product of the reaction is a heteropolymer of the four nucleotides added as substrate. The divalent metal requirement exhibited by the RNA polymerase associated with the sugar beet chromatin is similar to that of purified E. coli RNA polymerase (6) and sugar beet polymerase (C. T. Duda, unpublished results), i.e., addition of Mg+2 does not enhance the nucleotide polymerization reaction unless the concentration of Mn+2 is suboptimal in the reaction medium.

The kinetics of nucleotide polymerization catalyzed by RNA polymerase of sugar beet chromatin is similar to that reported for other systems (17, 26).

Washing sugar beet discs results in a time-dependent rise in chromatin-associated RNA polymerase activity for the first 24 hr. Aging for an additional 24 hr brings about a drastic drop in this activity, to the level of unwashed tissue. The cause of this drop is not obvious inasmuch as other enzyme activities continue to increase for a much longer time (2, 8, 21). Furthermore, RNA synthesis is high, and respiration is unaltered. In general, the tissue is at its peak in metabolic activities when chromatin-associated RNA polymerase begins to decline. The RNA product synthesized by the endogenous RNA polymerase after 25 hr of aging is different from that synthesized by the chromatin RNA polymerase of unwashed tissue. This is also true for the products synthesized by exogenous RNA polymerase with chromat preparations from unwashed and 25-hr aged tissue.

The saturation of chromatin with exogenous RNA polymerase (E. coli) greatly enhanced RNA synthesis, indicating that insufficient RNA polymerase is available on the isolated chromatin for transcription of all available sites. In fact, endogenous RNA polymerase is capable of transcribing only 6% of the total DNA sites after 24 hr of aging. The low level of endogenous RNA polymerase activity may be due to loss of activity during purification of chromatin or may represent a true state of affairs in the cell. There is a rather large amount of soluble RNA polymerase activity which can be isolated from the chromatin-free supernatant (C. T. Duda, unpublished observations). This suggests that certain gene sites have a higher affinity for RNA polymerase than others. Another explanation is that endogenous polymerase activity may represent a complex among RNA polymerase, DNA, and an unfinished RNA molecule, since RNA polymerase is released from the DNA template after synthesizing a RNA molecule (4).

Aging of Jerusalem artichoke tuber discs reduces the amount of histone associated with the isolated chromatin (20). Because histones have been suggested as suppressors of template activity of DNA (1, 15), it would appear that washing increased template availability in artichoke discs. In addition, washing in GA enhances the loss of histones from the chromatin in artichoke tissue (20). In this connection, we found that added GA and auxin (individually) enhanced template availability of isolated sugar beet chromatin but had little effect on associated RNA polymerase.

GA and auxin also enhance RNA synthesis of isolated nuclei of sugar beet tissue. These homones appear to stimulate the synthesis of ribosomal RNA. The effect of GA on cellular activities appears to be mediated at the level of the nucleus and probably at that of the gene (see also 10, 17–19, 29). In the sugar beet root, increased RNA synthesis and metabolic activity in general appear to be related to an increased production of RNA polymerase activity and an increase in template DNA.

**LITERATURE CITED**