Enhancement of Soybean RNA Polymerase I by Auxin
(chromatin/nuclei/RNA polymerase II/2,4-dichlorophenoxyacetic acid)

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ABSTRACT When etiolated soybean seedlings are treated with the synthetic auxin, 2,4-dichlorophenoxyacetic acid, cells of the mature hypocotyl become swollen and proliferate abnormally. This abnormal growth induced by auxin coincides with a 5- to 8-fold increase in the α-amanitin-insensitive RNA polymerase associated with isolated chromatin or nuclei. The α-amanitin-sensitive RNA polymerase activity of the auxin-treated hypocotyl was similar to that of control tissue. The increase in RNA polymerase I activity of chromatin and nuclei was maintained after solubilization and fractionation on DEAE-cellulose. Auxin thus appears to enhance RNA synthetic activity (i.e., ribosomal RNA) in mature soybean tissue by altering RNA polymerase I directly rather than by altering the chromatin template.

When the synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), is applied to young etiolated soybeans, cells of the mature hypocotyl enlarge radially and proliferate, while the normal growing point or apical meristem becomes quiescent. The abnormal proliferation of the mature hypocotyl is preceded by a large increase in RNA, especially ribosomal RNA (1, 2). This large accumulation of RNA in the mature tissue is associated with an enhanced chromatin-directed RNA synthetic activity (3). The auxin-enhanced chromatin activity is insensitive to α-amanitin (4), indicating that the increased RNA synthetic activity relates to a nucleolar type RNA polymerase as described in animals (e.g., ref. 5). Hybridization studies also indicate that the chromatin-bound RNA polymerase is transcribing primarily ribosomal RNA (6). Recent studies indicate that soybean chromatin isolated by conventional methodology (7) in fact possesses only RNA polymerase I activity, while nearly all RNA polymerase II is found in high-speed supernatant fractions (8).

In the present work the effects of the synthetic auxin, 2,4-D, on RNA polymerase I and II activities from both chromatin and nuclei were studied. The enzymes were solubilized and fractionated on DEAE-cellulose. RNA polymerase I activity increased several-fold after auxin treatment, while RNA polymerase II activity was at most only slightly affected.

MATERIALS AND METHODS

Plant Material. Soybean seeds (Glycine max, var. Wayne) that had been pretreated with 10% Chlorox were planted in moist vermiculite and germinated in the dark at 28°. After 72 hr of germination, the “treated” seedlings were sprayed to run-off with a 2.5 mM solution of 2,4-D (pH 6.0). Mature hypocotyl tissue from untreated and 2,4-D-treated seedlings was harvested after 96 hr of germination unless otherwise indicated.

Solutions. Buffer A contains 50 mM Tris·HCl (pH 8.0), 10 mM 2-mercaptoethanol, 1 mM MgCl₂, 250 mM sucrose, 0.1 mM EDTA, and 0.5 mM phenylmethylsulfonylfluoride. Buffer B contains 50 mM Tris·HCl (pH 8.0), 10 mM dithiothreitol, 5 mM MgCl₂, 25% glycerol, 0.1 mM EDTA, and 0.5 mM phenylmethylsulfonylfluoride.

Preparation of Soybean Chromatin. Chromatin was prepared with buffer A as described (8).

Preparation of Soybean Nuclei. Nuclei were isolated and purified by a modification of the procedure described by Stern (9). The details of this procedure will be described in a forthcoming publication.

Preparation of Soybean RNA Polymerases. RNA polymerase I was solubilized by stirring chromatin suspensions for 5 hr at 0° in the presence of buffer B containing 500 mM ammonium sulfate. The solubilized RNA polymerase I was recovered in the supernatant after centrifugation for 30 min at 40,000 rpm (Ti 65 rotor, Beckman L2-65B ultracentrifuge). RNA polymerase was precipitated by adding 0.38 g/ml of solid ammonium sulfate (Enzyme Grade, Nutritional Biochemicals). The protein precipitate was recovered by centrifugation for 30 min at 40,000 rpm. The protein pellet containing the RNA polymerase I was suspended in buffer B containing 300 mM ammonium sulfate and dialyzed overnight at 0° against the same buffer. Dialysis against buffer B containing lower concentrations of ammonium sulfate resulted in loss of enzyme activity due to precipitation. The dialyzed enzyme solution was diluted to 50 mM ammonium sulfate with buffer B just prior to fractionation on DEAE-cellulose.

RNA polymerase II was recovered from the 25,000 rpm (SW 27 rotor) supernatant of the chromatin preparation by stirring the supernatant solution with 500 mM ammonium sulfate for 2 hr followed by precipitation of the protein by addition of 0.38 g/ml of solid ammonium sulfate. The protein pellet containing RNA polymerase II was recovered by centrifugation at 27,000 × g for 30 min. The pellet was suspended in buffer B containing 50 mM ammonium sulfate and dialyzed against the same buffer overnight. The dialyzed enzyme preparation was centrifuged at 27,000 × g, and the supernatant was loaded onto DEAE-cellulose.

RNA polymerase I and II were solubilized from isolated nuclei as described for the solubilization of RNA polymerase.

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; buffer A, 50 mM Tris·HCl (pH 8.0), 10 mM 2-mercaptoethanol, 1 mM MgCl₂, 250 mM sucrose, 0.1 mM EDTA, and 0.5 mM phenylmethylsulfonylfluoride; buffer B, 50 mM Tris·HCl (pH 8.0), 10 mM dithiothreitol, 5 mM MgCl₂, 25% glycerol, 0.1 mM EDTA, and 0.5 mM phenylmethylsulfonylfluoride.

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I from chromatin. DEAE-cellulose chromatography was carried out as described by Roeder and Rutter (10) for DEAE-Sephadex, with buffer B and a linear gradient of ammonium sulfate (50–500 mM).

**RNA Polymerase Assays.** Chromatin-bound and nuclear RNA polymerases were assayed in 0.5-ml reaction mixtures containing 50 mM Tris-HCl (pH 8.0), 10 mM dithiothreitol, 10 mM MgCl₂, 20% glycerol, 0.4 mM unlabeled nucleotides, and 0.02 mM [³H]CTP or [³H]UTP (1–2 μCi).

The solubilized RNA polymerases were assayed in the presence of 50 mM Tris-HCl (pH 8.0), 10 mM dithiothreitol, 5 mM MgCl₂, 1 mM MnCl₂, 50 mM NH₄Cl, 5% glycerol, 20 μg of heat-denatured calf thymus DNA, 0.4 mM ATP, CTP, GTP, and 0.01 mM [³H]UTP (2 μCi) in 0.25 ml for 20 min.

All RNA polymerase assays were at 28°C. Reactions were terminated by addition of 2 ml of 10% trichloroacetic acid and 8 mM sodium pyrophosphate. Precipitates were collected on GF/A glass fiber discs (Whatmann), washed with 5% trichloroacetic acid and 95% ethanol, dried under heat lamps, and counted in a Packard Liquid Scintillation Spectrometer at 30% efficiency. Protein was determined by the method of Lowry et al. (11) after trichloroacetic acid precipitation.

**RESULTS**

**RNA Polymerases of Isolated Chromatin and Nuclei.** RNA synthesis by soybean chromatin isolated by the method of Huang and Bonner (7) as modified by Lin et al. (8) is almost totally α-amanitin-insensitive, a characteristic of RNA polymerase I (Table 1). The chromatin enzyme from both untreated and auxin-treated hypocotyls is optimally active at relatively low ionic strength (50 mM ammonium sulfate) and is inhibited at higher salt concentrations. Chromatin from auxin-treated hypocotyls possesses α-amanitin-insensitive RNA polymerase activity several-fold greater than chromatin from untreated hypocotyls. Only very small amounts of α-amanitin-sensitive enzyme can be detected on chromatin isolated from either untreated or auxin-treated hypocotyls.

In contrast to chromatin, soybean nuclei contain significant levels of α-amanitin-sensitive RNA synthetic activity. The α-amanitin-insensitive RNA polymerase is optimally active at 50 mM ammonium sulfate, similar to chromatin, while the α-amanitin-sensitive enzyme is optimally active at 200 mM ammonium sulfate. Like chromatin, nuclei from auxin-treated hypocotyls have several-fold greater α-amanitin-insensitive RNA synthetic activity than controls; however, the α-amanitin-sensitive enzyme activity is not significantly affected by auxin treatment.

**Time Course of Auxin-Enhanced Chromatin Activity.** Enhancement of chromatin-bound RNA polymerase activity by auxin occurs with only a short lag (2–3 hr) after application of the hormone. A greater than 2-fold increase in chromatin activity is observed within 4 hr (Fig. 1). The chromatin activity (based on fresh weight) is about 12-fold greater after a 24-hr auxin treatment; however, the specific activity (based on DNA or protein) increases only up to 12 hr, resulting in about a 5- to 7-fold increase in specific activity. Increases in total RNA polymerase activity above 5- to 7-fold presumably result from cell divisions in the mature hypocotyl (2, 12).

**RNA Polymerases Solubilized from Chromatin.** To distinguish effects of auxin on chromatin template availability from effects on RNA polymerase, equal aliquots of chromatin nucleoprotein from untreated and auxin-treated hypocotyls were added to a medium that solubilizes more than 80% of the chromatin-bound RNA polymerase activity (based on assay of the chromatin residue after solubilization). The solubilized chromatin proteins were fractionated on DEAE-cellulose (Fig. 2). Only RNA polymerase I, which is α-amanitin-insensitive, is observed as a distinct peak, and this activity is eluted by 0.11 M ammonium sulfate for both untreated and auxin-treated tissue. The total activity eluted from DEAE-cellulose is several-fold greater for the auxin-treated material.

**Table 1. RNA polymerase activities of chromatin and nuclei isolated from untreated and auxin-treated soybean hypocotyl**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Chromatin (U)</th>
<th>Chromatin (T)</th>
<th>Nuclei (U)</th>
<th>Nuclei (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Amanitin insensitive</td>
<td>115</td>
<td>1157</td>
<td>193</td>
<td>1730</td>
</tr>
<tr>
<td>α-Amanitin sensitive</td>
<td>16</td>
<td>25</td>
<td>103</td>
<td>100</td>
</tr>
</tbody>
</table>

Soybean chromatin was assayed for RNA polymerase activity with [³H]CTP and nuclei were assayed with [³H]UTP as described in Materials and Methods, with the addition of (NH₄)₂SO₄ as given in the table. The concentration of α-amanitin was 4 μg/ml.

<table>
<thead>
<tr>
<th>α-Amanitin insensitive</th>
<th>50 mM (NH₄)₂SO₄</th>
<th>200 mM (NH₄)₂SO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatin (U)</td>
<td>71</td>
<td>17</td>
</tr>
<tr>
<td>Chromatin (T)</td>
<td>662</td>
<td>27</td>
</tr>
<tr>
<td>Nuclei (U)</td>
<td>146</td>
<td>218</td>
</tr>
<tr>
<td>Nuclei (T)</td>
<td>822</td>
<td>203</td>
</tr>
</tbody>
</table>

Values are pmol of [³H]nucleotide incorporated into RNA per mg of protein.
tissue, on nearly 20-fold I; however, fold in the RNA no contained higher level RNA polymerase and levels relative auxin-treated hypocotyls. merases were yields only 0.22 M tissue were enzyme The untreated and profiles of activity. The enzyme from untreated and auxin-treated tissue, based on DEAE–cellulose profiles of equivalent amounts of supernatant protein (Fig. 3). The enzyme activities from untreated and auxin-treated tissue were totally α-amanitin-sensitive and were eluted at 0.22 M ammonium sulfate.

RNA Polymerase Solubilized from the Chromatin Supernatant. Since only a small amount of α-amanitin-sensitive enzyme is associated with the chromatin and no distinct RNA polymerase II peak is observed on DEAE–cellulose chromatography of solubilized chromatin proteins, high-speed supernatant fractions were examined for RNA polymerase II activity. The relative levels of RNA polymerase II are similar for untreated and auxin-treated tissue, based on DEAE–cellulose profiles of equivalent amounts of supernatant protein (Fig. 3). The enzyme activities from untreated and auxin-treated tissue were totally α-amanitin-sensitive and were eluted at 0.22 M ammonium sulfate.

RNA Polymerases Solubilized from Soybean Nuclei. Since chromatin yields only bound RNA polymerase I, RNA polymerases were solubilized from isolated nuclei to study the relative levels of RNA polymerase I and II in untreated and auxin-treated hypocotyls. Fig. 4 shows that while untreated and auxin-treated nuclei contain nearly equivalent levels of RNA polymerase II, auxin-treated nuclei have a 4– to 5-fold higher level of RNA polymerase I than untreated nuclei. After the nuclei were pelleted, supernatant fractions still contained significant amounts of RNA polymerase II although no RNA polymerase I was detected.

While nuclei reflect the relative levels of bound RNA polymerase I and II, chromatin and supernatant fractions reflect the total amounts of RNA polymerase I and II, respectively, in the tissue. Nuclei from untreated tissue possess about 2-fold more RNA polymerase II activity than RNA polymerase I; however, on a tissue basis, RNA polymerase II activity is nearly 20-fold greater than RNA polymerase I. Auxin-treated tissue, on the other hand, has two times more RNA poly-

RNA Polymerase I Enhanced by Auxin
II is nearly equivalent in untreated and auxin-treated tissue, which indicates that RNA polymerase I is specifically enhanced in response to auxin.

DISCUSSION

A wide variety of hormones administered in vivo have been reported to result in enhanced chromatin-bound or nuclear RNA polymerase in both plants (3, 13, 14) and animals (15–18). Both nucleolar (17, 18) and nucleoplasmic (19) RNA polymerases are subject to hormonal regulation in animals. The nucleolar RNA polymerase (RNA polymerase I) transcribes ribosomal RNA (20, 6), while the nucleoplasmic RNA polymerase (RNA polymerase I) is thought to transcribe messenger RNA (21). A third RNA polymerase (RNA polymerase III) has been found in some animal nuclei (10) and is thought to transcribe 4 and 5S RNA (22); however, no reports on hormonal regulation of this polymerase have been published.

The mechanism of the hormonal enhancement of RNA synthetic activity might be due to a modification of the chromatin template or in the activity or numbers of RNA polymerase molecules actively involved in transcription. To distinguish between alterations in template and changes in RNA polymerase molecules, the RNA polymerases can be solubilized from the chromatin and made dependent on an exogenous source of DNA template.

In the case of soybean, the enhancement of RNA polymerase I activity by the auxin (2,4-D) is maintained after solubilization from the chromatin or nuclei and its subsequent fractionation on DEAE-cellulose. The enzyme activity eluted from DEAE-cellulose is completely dependent on an exogenous source of DNA. Thus, the enzyme rather than the template appears to be regulated by auxin. RNA polymerase II in the soybean hypocotyl is only slightly affected by auxin treatment (less than a 30% enhancement) whether total tissue homogenates or isolated nuclei are examined.

While the mechanism by which RNA polymerase I activity is enhanced after auxin treatment is still in question, the increased activity appears to involve the enzyme and not the template. If the enzyme itself is altered by auxin, this could involve an increase in the number of RNA polymerase I molecules active in transcription or a change in the activity of each molecule, presumably by alteration in some regulatory factor. Guilfoyle and Hanson (23) have reported that nearly equal numbers of α-amanitin-insensitive RNA polymerase molecules were actively transcribing chromatin templates in vitro isolated from untreated and auxin-treated tissue; thus, the higher RNA synthetic activity by chromatin from auxin-treated tissue appeared to result from a greater rate of transcription by the RNA polymerase molecules. It is interesting to note that this same result has been reported for the activation of RNA polymerase I in rat uteri induced by estradiol (24) and the adrenocorticotrophic hormone-induced stimulation of adrenal RNA polymerase I in guinea pigs (25). The mechanism by which the rate of ribosomal RNA chain propagation is altered after auxin administration is not understood.

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