Selective Modulation of RNA Polymerase I Activity during Growth Transitions in the Soybean Seedling

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ABSTRACT

RNA polymerase I and II activities were measured in tissues of the soybean (Glycine max, var. Wayne) hypocotyl where dramatic changes in the relative level of RNA synthesis are associated with normal and auxin-induced growth transitions. When assayed in isolated nuclei, the activity of RNA polymerase I changed much more than the activity of RNA polymerase II during these growth transitions. The activity of RNA polymerase I expressed in the nuclei generally showed a positive correlation with the relative level of RNA synthesis (i.e., accumulation) of that tissue. Following solubilization of the RNA polymerases from these isolated nuclei and fractionation of them on DEAE-cellulose, the activity of RNA polymerase I relative to that of RNA polymerase II showed smaller changes during these growth transitions than when assayed in the nucleus. Thus, these data indicate that the activity of RNA polymerase I is significantly modulated in the nucleus, up or down depending upon the growth state, during growth transitions in the soybean in addition to lesser changes which occur in the apparent level of the enzyme.

There are marked changes in RNA metabolism associated with normal and auxin-induced growth transitions in plant tissues (18, 33). During normal development in the soybean, there is a large net accumulation of RNA during division and the early stages of cell elongation (zone a, Fig. 1); net accumulation of RNA ceases by the time cells are fully elongated (base of zone b, Fig. 1). Often, there is a small net decrease in RNA content during cell maturation (zone c, Fig. 1). Treatment of the seedlings with auxin (especially 2,4-D) leads to very marked aberrations in the growth pattern and in RNA metabolism (4, 20). Cell division is inhibited in zone a, and cell elongation ceases in zones a and b (20); radial enlargement of cells is induced in zones b and c. After about 12 hr of treatment, cell division is initiated at the base of zone c and progresses through zone b by 36 hr. The accumulation of RNA which accompanies normal development in zone a is completely suppressed by auxin (20); after a short lag, RNA accumulation is induced in zones b and c and precedes by several hr the onset of DNA synthesis and tissue proliferation. There is a marked increase in the RNA-protein, RNA-DNA, and rRNA-tRNA ratios in zones b and c of auxin-treated tissue.

The incubation of excised segments of the soybean hypocotyl is also associated with changing patterns of RNA synthesis. The rate of synthesis of stable species of RNA in zones a and b (i.e., rRNA and tRNA) is so low that a net decrease in RNA content occurs (19). During the RNA synthesis-dependent (17, 29) enhancement of cell elongation in excised zones a and b by auxin, there is an enhancement of precursor incorporation into RNA (19), and thus, less RNA loss than in control tissue. There is no change in RNA content of zone c during excised incubation; after a 1- to 2-hr lag, auxin induces a marked and linear increase in RNA content (19).

The chromatin isolated from zones b and c of auxin-treated soybean seedlings has a 3- to 10-fold higher RNA synthetic capacity than comparable control chromatin (15, 26). The higher RNA synthetic activity of chromatin from the auxin-treated tissue relates mainly to a large increase in the levels of an RNA polymerase activity refractory to α-amanitin (10, 14), which has been characterized as RNA polymerase I, the ribosomal RNA polymerase (11, 12). Nuclei isolated from auxin-treated tissue have a much higher rate of RNA synthesis than control nuclei (3, 12); this greater RNA synthetic activity relates primarily to a large and preferential increase (3- to 5-fold) in RNA polymerase I activity. RNA polymerase II activity increases only a few percent if at all (3, 12).

The RNA polymerase activity referred to here as RNA polymerase I has been purified to near homogeneity (11) and shown to correspond to RNA polymerase I of other eukaryotes (23, 28, 29), based on its localization in the nucleolus of soybean nuclei (21), its chromatographic behavior and insensitivity to α-amanitin (12), and its preferential transcription of rDNA cistrons (13).

Because of the very different patterns of RNA synthesis in the intact and excised hypocotyl tissues and the differential response of the meristematic (zone a) and mature (zone c) tissues to auxin, we have made comparative studies of the RNA synthetic activities of isolated nuclei and of the RNA polymerases solubilized from them using the following tissues of the soybean hypocotyl: zones a and c of normal and auxin-treated soybean seedlings; excised tissues from zones a and c incubated with and without auxin. The results generally show: (a) that the patterns of RNA accumulation in the various tissues show a strong correlation with the level of RNA polymerase I activity expressed in nuclei isolated from those tissues; (b) that the RNA polymerase I activity is rapidly modulated during the various growth transitions; (c) that RNA polymerase I activity may be significantly repressed in nuclei during some of these growth transitions while being present in normal levels following solubilization and assay on a DNA template, and (d) that RNA polymerase II activity is relatively stable during these growth transitions compared to RNA polymerase I.

MATERIALS AND METHODS

Soybean seeds (Glycine max, var. Wayne) were germinated in moist vermiculite as described (19). About 72-hr germinations, seedlings were sprayed with auxin (500 μg/ml 2,4-D); comparable control (no auxin) and auxin-treated tissues were harvested at various times after treatment. In experiments where

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from mature (zone c) tissue associated with only a 10 to 15% decrease in RNA polymerase II during this growth transition results in a decrease in the ratio of I to II from about 1.3 in zone a to about 0.35 in zone c. When the RNA polymerases are solubilized from nuclei isolated from meristematic and mature hypocotyl and fractionated on DEAE-cellulose, the relative activities (Fig. 2, a and b) of RNA polymerases I and II are similar to those observed in the nuclei. The level of solubilized RNA polymerase II is similar between zones a and c whereas the level of RNA polymerase I is 2- to 3-fold higher in the meristematic tissue. This large decrease in RNA polymerase I activity, which occurs during the growth transition from the meristematic to mature state, generally correlates with the in vivo state of rRNA synthesis.

Following auxin treatment of intact seedlings, there is a very large decrease in the RNA polymerase activity expressed in nuclei isolated from zone a (Table I) where cell division and RNA accumulation are suppressed (20). RNA polymerase I activity decreases more than RNA polymerase II, resulting in a lower ratio of RNA polymerase activity in zone a of auxin-treated relative control tissue even though there is a significant drop in RNA polymerase II activity. When the RNA polymerases are solubilized from nuclei and fractionated on DEAE-cellulose, the ratio of RNA polymerase I to II activity is similar from control and auxin-treated tissue (zone a, Fig. 2, a and c). These data suggest that the activity of at least RNA polymerase I is significantly suppressed compared to the actual level of enzyme present in the nuclei of auxin-treated meristematic tissue.

Commensurate with the very large increase in rRNA accumulation in zone c of auxin-treated seedlings, there is a severalfold increase in RNA polymerase I activity expressed in isolated nuclei associated with little or no change in RNA polymerase II activity (Table I) as reported earlier (12), resulting in an elevated RNA polymerase I to II activity ratio (greater than 2 after 24-hr treatment with auxin compared to 0.3-0.4 in control tissue). Following solubilization of the enzymes from nuclei, the elevated level of RNA polymerase I activity from auxin-treated zone c tissue is to a large extent maintained, based on the DEAE-cellulose profiles (Fig. 2, b and d).

When the meristematic or zone a tissue is excised and incubated, there is a dramatic and rapid decrease in the activity of RNA polymerase I expressed in the isolated nuclei associated

Table 1. Activities of RNA Polymerase I and II Expressed in Isolated Nuclei

<table>
<thead>
<tr>
<th>Zone A</th>
<th>Zone C</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA Polymerses</td>
<td>1/112</td>
</tr>
<tr>
<td>Intact Seedlings</td>
<td>Control</td>
</tr>
<tr>
<td>Auxin (12 hr)</td>
<td>32</td>
</tr>
<tr>
<td>Auxin (24 hr)</td>
<td>28</td>
</tr>
<tr>
<td>Rugcell Fractionation</td>
<td>Control (12 hr)</td>
</tr>
<tr>
<td></td>
<td>Auxin* (12 hr)</td>
</tr>
</tbody>
</table>

1 RNA polymerase I activity expressed as pmol 3H-UMP incorporated/30 min - at 28 C in the presence of 2 µg/ml of a-amanitin as described previously (12). RNA polymerase II activity expressed as a-amanitin-sensitive RNA synthesis activity (Guilfoyle et al., 12).

2 Ratio of the activity of RNA polymerase I to RNA polymerase II expressed in isolated nuclei using the methods of Guilfoyle et al. (12).

3 Three-day-old seedlings were sprayed with 500 µg/ml 2,4-D. At 12 and 24 hr after treatment, zones a and c were used to prepare nuclei; comparable control or untreated tissue of zones a and c was used from treated seedlings.

4 Zones a and c were excised from 3-day-old untreated seedlings, and incubated with or without 10 µg/ml 2,4-D for 12 hr prior to isolation of nuclei.

Results

The activities of RNA polymerases I and II were measured in isolated nuclei and after solubilization from comparable nuclei and subsequent fractionation on DEAE-cellulose. Whereas practically all of the RNA polymerase I activity of soybean tissue is recovered in isolated nuclei and chromatin, much of the RNA polymerase II is apparently free as a "soluble" enzyme (22; Guilfoyle, unpublished). In these studies, we have only monitored the activities of the nuclear associated RNA polymerases. Recognizing this limitation in the analyses, the relative activities of RNA polymerases I and II were compared in tissues of the soybean hypocotyl representative of different growth states as discussed above (e.g. meristematic with respect to mature tissue; excised incubation with respect to zero time controls; auxin-treated with respect to control excised and intact seedling tissues).

During normal growth and development, there is a marked decrease in RNA polymerase I activity expressed in isolated nuclei, whereas the activity of RNA polymerase II decreases only a few percent (Table I); the 4-fold higher level of RNA polymerase I in nuclei from meristematic (zone a) than in nuclei

Fig. 1. Diagrams of soybean seedlings depicting regions of the hypocotyl which were used in these studies representing normal and auxin-induced growth transitions. a: meristematic region; b: zone of maximum cell elongation; c: mature zone. Treatment with 2,4-D results in inhibition of cell division in zone a and of cell elongation in zones a and b. Radial enlargement of cells and cell division are induced in zones b and c following 2,4-D treatment. See ref. 20 for experimental details of these responses.

Excised tissues were studied, tissues representing zones a and c were excised from the hypocotyl of 3-day-old control seedlings and incubated in 50 µg/ml chloramphenicol (to prevent bacterial growth) with or without 10 µg/ml 2,4-D (10-3 M K phosphate, pH 6, was included in some experiments).

Nuclei were isolated and their RNA polymerase activities assayed as previously described (3). The RNA polymerases were also solubilized from these nuclei, fractionated on DEAE-cellulose, and assayed according to the methods of Guilfoyle et al. (11, 12).

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with little or no change in the activity of RNA polymerase II. This very large decrease in RNA polymerase I activity expressed in the isolated nuclei associated with only a small decrease in RNA polymerase II activity results in a drop in the RNA polymerase I to II activity ratio from about 1.2 to 1.4 in the zero time control tissue to about 0.25 from 12-h incubated tissue (Table I). Auxin treatment of the excised zone a tissue did not significantly affect the activity of the RNA polymerases expressed in the isolated nuclei. This decay in RNA polymerase I activity as expressed in isolated nuclei during excised incubation of zone a tissue occurs with a relatively short half-life (in the order of 2 hr depending upon the conditions of incubation). In contrast to the very large decrease in the activity of RNA polymerase I relative to II expressed in the nuclei isolated from 12-h incubated zone a tissue, the relative activities of RNA polymerase I and II following solubilization from nuclei and fractionation on DEAE-cellulose are essentially identical between zero time control and 12-h incubated tissue (Fig. 2, a and e). These data provide a very dramatic demonstration of the preferential “suppression” of RNA polymerase I activity in nuclei.

When zone c tissue is excised and incubated, there is no significant change in the activity of RNA polymerases expressed in nuclei isolated from control tissue; also, the levels of RNA polymerase I and II activities observed after solubilization and fractionation on DEAE-cellulose are similar to those of unincubated zone c tissue (Fig. 2, b and h). When this tissue is excised and incubated in the presence of auxin, there is a large increase in RNA polymerase I activity expressed in isolated nuclei resulting in a much higher RNA polymerase I to RNA polymerase II activity ratio (Table I). Following solubilization and fractionation of the enzymes on DEAE-cellulose, the activity of RNA polymerase I is significantly increased in the auxin-treated tissue (Fig. 2f) compared to control tissue (Fig. 2h). Thus, the ratio of RNA polymerase I to II activity is increased following auxin treatment of excised mature (zone c) tissue, but the increase is not as dramatic as that observed in zone c of intact seedlings.

**DISCUSSION**

The results presented here and those from previous studies (4, 12, 19, 20) demonstrate a very strong positive correlation between the level of RNA polymerase I expressed in isolated nuclei and the in vivo level of rRNA synthesis in various control and auxin-treated tissues of soybean seedlings. These data also show that the activity of RNA polymerase I may be significantly regulated in nuclei relative to the level of activity which is observed when assayed on a free DNA template after solubilization and fractionation on DEAE-cellulose. RNA polymerase I decreases about 3- to 4-fold relative to RNA polymerase II during the growth transitions from the meristematic to the mature state; the net accumulation of rRNA per cell ceases during the elongation phase of hypocotyl growth. Auxin treatment of seedlings results in an inhibition of rRNA accumulation in meristematic cells and a suppression of RNA polymerase I activity in isolated nuclei, but apparently not in the level of the enzyme. There is a severalfold increase in RNA polymerase I activity in mature tissue expressed both in isolated nuclei and in the solubilized state in conjunction with a massive accumulation or rRNA, both in the intact seedling and in the excised tissue, following auxin treatment. When the meristematic tissue is excised and incubated, there is a net decrease in the rRNA content associated with a marked decrease in the activity of RNA polymerase I expressed in isolated nuclei. This decrease in RNA polymerase I activity may relate to a “suppression” of the enzyme activity since the level of the enzyme activity after solubilization is similar in zero time control and 12-h incubated tissue.

The activity of RNA polymerase I is also selectively modulated in several other eukaryotic systems (6, 16, 30, 34). Although there is little definitive information about the mechanism of regulation of RNA polymerase I in these systems, there is suggestive evidence that the modulation in amount (rapid turnover) or activity of a subunit or regulatory component may be involved (34).

There is also a very strong positive correlation between the level of RNA polymerase I activity expressed in these soybean tissues (and the level of rRNA synthesis) and the functional state of the protein synthetic apparatus as assessed by the level of polyribosomes (32). When zone a tissue is excised and incubated, a net loss of RNA ensues (19), RNA polymerase I activity expressed in isolated nuclei (but not after solubilization and fractionation on DEAE-cellulose) decreases severalfold, and the level of polyribosomes rapidly drops from 75% to about 10% of the total ribosome population (32). The reverse occurs following auxin treatment of zone c or mature tissue; rRNA synthesis increases severalfold (19), the level of polyribosomes rapidly increases from about 30% to 75% (32), and RNA polymerase I activity increases both as expressed in isolated nuclei and after solubilization and fractionation on DEAE-cellulose. A close coupling of rRNA synthesis and ribosome function has been subject of intense study in bacteria (5) and to a lesser extent in some eukaryotic systems (8, 9). The relaxed locus in *Escherichia coli* is the control element whose product, the stringent factor or the enzyme which catalyzes the formation of ppGpp (1), may serve to couple the synthesis of stable RNA species to protein synthesis. In this system, the accumulation of ppGpp or pppGpp which is synthesized by “idling” ribosomes (i.e. ribosomes inactive in protein synthesis) and stringent factor
(2) may serve to suppress the synthesis of stable RNA species (27, 31). A different mechanism probably is operative in eu-
karyotes to couple ribosome synthesis and function inasmuch as
ppGpp (and pppGpp) has not been detected in animal systems
(7, 24); also we have not detected these compounds in soybean
in recent preliminary experiments (Mori and Key, unpublished
observations).

Work in progress is directed to an analysis of the mechanisms
operative in the regulation of RNA polymerase I during normal
and hormone-induced growth transitions and in the coupling of
rRNA synthesis to ribosome function in the soybean.

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