Effects of Growth Regulators on Ribonucleic Acid Metabolism of Barley Leaf Segments

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ABSTRACT

Illumination or gibberellic acid treatment of etiolated barley leaf segments stimulates unrolling and results in an increased level of RNA. In contrast, segments treated with abscisic acid do not unroll and have a lower content of RNA. Gibberellic acid treatment enhanced the capacity of segments to incorporate radioactivity from $^{32}$P-orthophosphate into all the RNA components detected by gel electrophoresis; abscisic acid greatly restricted the incorporation of precursors into all the RNA fractions. In conjunction with a changed capacity for RNA synthesis it was observed that abscisic acid-treated segments had a lowered soluble DNA-dependent RNA polymerase level in comparison to gibberellic acid-treated or illuminated segments. However, the influence of growth regulators on RNA polymerase content of the segments was associated with general effects on protein level rather than a specific effect on the synthesis of polymerase enzyme.

Ribosomal preparations from gibberellic acid-treated segments had a greater percentage of polyosomes and a greater capacity for amino acid incorporation in vitro into proteins than similar preparations from abscisic acid-treated segments.

We have previously demonstrated that etiolated barley leaf segments unroll when illuminated (9). GA enhances the unrolling of illuminated segments and can also induce unrolling of segments in the dark. In contrast, ABA prevents photoinduced unrolling. Inhibitor studies (1, 9) have indicated that RNA and protein synthesis must accompany the photoinduced unrolling process. In view of the light-induced changes in RNA metabolism of barley leaf segments (10), it was of interest to determine if the hormonal treatments which influenced unrolling also affected RNA metabolism in the leaf segments.

MATERIALS AND METHODS

Sections, 7 mm in length, were prepared from the primary leaf of dark-grown barley seedlings and incubated as described previously (9). Chlorophyll content of the leaf segments was determined on an 80% (v/v) ethanol extract of the segments. Protein and total RNA contents of the ethanol-insoluble residue were determined according to the method of Osborne (7).

In investigations of RNA synthesis, segments were incubated for 6 hr in the presence of $^{32}$P-orthophosphate prior to extraction of RNA (10). The extracted radioactive RNA was then separated by polyacrylamide gel electrophoresis (4), and the distribution of radioactivity in the gels was determined by slicing the gels and counting the slices in a Nuclear-Chicago gas flow counter (10).

RNA polymerase activity was determined in extracts from the leaf segments according to the method of Stout and Mans (11). Ribosomal preparations were extracted from the leaf segments as described previously, and the distribution of monosomes and polysomes was determined following sucrose density gradient centrifugation (10). The amino acid-incorporating capacity of the ribosomal preparation was determined according to the method of Mans and Novelli (6, 7), with a 150,000g supernatant prepared from germinating pea seeds serving as a source of activating enzymes and soluble RNA.

RESULTS

The observed increase in chlorophyll, protein, and RNA in illuminated barley leaf segments and the associated increase in leaf width are characteristic features of photomorphogenesis in the cereal leaf (Table I). Part of these light-induced changes can be initiated in the dark by treatment with GA. Segments treated with GA in either the light or dark have a greater leaf width, protein, and RNA content in comparison to control segments. In contrast, ABA-treated segments have a reduced chlorophyll, protein, and RNA content in comparison to control segments. ABA prevented light-stimulated unrolling and also inhibited the small amount of unrolling which occurred in segments in the dark.

Growth Regulators and RNA Synthesis. Gel electrophoresis of RNA extracted from segments incubated in the dark for 6 hr in the presence of $^{32}$P-orthophosphate showed that radioactivity

Table I. Influence of Illumination, GA, and ABA on Leaf Width, Chlorophyll Content, Protein Content, and RNA Content of Barley Leaf Segments

<table>
<thead>
<tr>
<th></th>
<th>Illuminated</th>
<th>Nonilluminated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>GA</td>
</tr>
<tr>
<td>Leaf width (1.01 mm)$^1$</td>
<td>4.45</td>
<td>5.05</td>
</tr>
<tr>
<td>Chlorophyll (0.014 OD/segment)</td>
<td>0.145</td>
<td>0.143</td>
</tr>
<tr>
<td>Protein (225 μg/segment)</td>
<td>387</td>
<td>437</td>
</tr>
<tr>
<td>RNA (16.9 μg/segment)</td>
<td>18.7</td>
<td>19.0</td>
</tr>
</tbody>
</table>

$^1$ Figures refer to initial measurements.
FIG. 1. The distribution of radioactivity following polyacrylamide gel electrophoresis of RNA isolated from barley leaf segments incubated with $^{32}$P-orthophosphate in the presence of $1.5 \times 10^{-6}$ M GA or $4 \times 10^{-6}$ M ABA. A: RNA isolated after a 6-hr incubation; B: RNA isolated after a 16-hr dark incubation during which isotope was included for the terminal 6 hr.

FIG. 2. Gel electrophoresis of $^{32}$P-labeled RNA extracted from illuminated barley leaf segments which had been treated with $1.5 \times 10^{-6}$ M GA or $4 \times 10^{-6}$ M ABA. A: RNA isolated after 6-hr illumination in the presence of isotope and growth regulators. B: RNA isolated after 16-hr illumination in the presence of growth regulators. Isotope was included during the terminal 6 hr.
was associated with soluble RNA and with the regions of the gel which have been previously characterized as having molecular weights of $1.3 \times 10^6$ and $0.7 \times 10^6$ daltons, corresponding to the heavy and light cytoplasmic rRNAs (4, 10). More radioactivity was associated with RNA extracted from segments treated for 6 hr with GA than from control segments (Fig. 1A). The increased radioactivity was apparent in the ribosomal regions, and there appeared to be a greater amount of radioactivity heterogeneously dispersed in the gel of RNA prepared from GA-treated segments. It has been suggested that this polydispersed radioactivity is associated with mRNA. Segments treated with GA for 16 hr with $^{32}$P-orthophosphate present during the terminal 6-hr period showed an even greater enhancement of $^{32}$P accumulation into RNA components in comparison to the control segments (Fig. 1B). ABA-treated segments had a reduced capacity to incorporate $^{32}$P into RNA.

The GA and ABA effects on incorporation of $^{32}$P into RNA were equally apparent in illuminated segments (Fig. 2A). RNA extracted from segments receiving extended illumination in the presence or absence of GA showed that appreciable radioactivity was associated with RNA species of molecular weight of $1.1 \times 10^6$, $0.56 \times 10^6$, and $0.40 \times 10^6$ daltons, indicating that following prolonged illumination there was synthesis of chloroplast rRNAs (Fig. 2B). Segments treated with ABA during a 16-hr illumination period showed no evidence of incorporation of radioactivity into the chloroplast rRNA regions (Fig. 2B).

RNA Polymerase. In crude preparations, the RNA polymerase activity from ABA-treated segments was consistently lower than from control segments. ABA treatment resulted in a decrease in polymerase activity, with a maximum reduction of 70% observed in ABA-treated segments. The reduction in polymerase activity was not accompanied by a corresponding decrease in mRNA levels, suggesting that the decrease in polymerase activity may be due to post-transcriptional modification or stabilization of mRNA.

Fig. 3. RNA polymerase activity in crude and partially purified extracts from barley leaf segments treated with $1.5 \times 10^{-5}$ M GA or $4 \times 10^{-4}$ M ABA. I: Crude preparation; II: partially purified preparation. A: Results expressed on activity per 0.4 ml extract; B: polymerase activity on a specific activity basis.

Fig. 4. Sucrose density gradient profiles of ribosome preparations from barley leaf segments incubated for 6 hr in the light or dark in the presence of $1.5 \times 10^{-4}$ M GA or $4.0 \times 10^{-4}$ M ABA.
from control or GA-treated segments (Fig. 3A). A consistent increase in polymerase activity was observed in partially purified preparations of illuminated segments treated with GA, whereas partially purified extracts from ABA-treated segments had a lower RNA polymerase activity. The failure to detect an increase in RNA polymerase activity in crude extracts from GA-treated segments may be due to the presence of RNAase which would lead to an underestimation of polymerase activity.

Since both illumination and growth regulator treatment altered the content of protein in the barley leaf segments, it was necessary to determine whether the observed changes in polymerase activity represented specific treatment effects or merely reflected over-all changes in protein synthesis. It was observed that ABA-treated segments had a higher polymerase activity per unit of protein than GA-treated segments (Fig. 3B). It seems, therefore, that the reduced polymerase activity of extracts from ABA-treated segments is associated with a generally lower protein level; conversely, the enhanced RNA polymerase activity of extracts from GA-treated segments may be related to an increased protein content of the segments.

**Polysome Levels.** Growth regulators had marked effects on the relative distribution of monosomes and polysomes in the ribosomal preparations. Thus, in etiolated segments, 45% of the 254 nm absorbing material is associated with the polysome region of the gradient (Fig. 4, upper). Treatment with ABA reduced this percentage to 34%, whereas GA treatment enhanced polysome formation such that the polysomes constituted 66% of the ribosome preparation.

Illumination of barley leaf segments caused an enhanced formation of polysomes (Fig. 4, lower). ABA prevented the light-stimulatory effect. GA treatment during illumination of segments further enhanced polysome formation.

Ribosomal preparations from GA-treated segments had an enhanced capacity to incorporate $^{3}C$-leucine (Table II). This GA effect was evident in both illuminated and nonilluminated segments. Ribosomal preparations from illuminated segments treated with ABA showed an amino acid-incorporating capacity comparable to preparations from nonilluminated control segments.

### Table II. Amino Acid-incorporating Capacity In Vitro of Ribosomal Preparations Extracted from Barley Leaf Segments Treated with GA or ABA

<table>
<thead>
<tr>
<th>Concentration of GA, $1.5 \times 10^{-5}$ M; ABA, $4 \times 10^{-5}$ M.</th>
<th>14C-$\alpha$-Leucine Incorporated into Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>6 hr</td>
</tr>
<tr>
<td>Nonilluminated Control</td>
<td>7,136</td>
</tr>
<tr>
<td>GA</td>
<td>10,220</td>
</tr>
<tr>
<td>ABA</td>
<td>7,528</td>
</tr>
<tr>
<td>Illuminated Control</td>
<td>14,523</td>
</tr>
<tr>
<td>GA</td>
<td>17,436</td>
</tr>
<tr>
<td>ABA</td>
<td>8,201</td>
</tr>
</tbody>
</table>

Protein synthesis and accumulation could be controlled by the availability of mRNA. Although there are no specific criteria for identifying this component, it is considered that an indication of mRNA abundance can be obtained by determining the relative distribution of monosomes to polysomes in ribosomal preparations. In this respect, it is apparent that growth regulators had a marked effect on polysome to monosome ratios. GA-treated segments produced ribosomal preparations with a high percentage of polysomes; in contrast, ABA treatment produced preparations with an abundance of monosomes. Insofar as polysome level is an index of mRNA availability, it appears that GA treatment enhances mRNA availability, and this could indicate an increased amount of DNA available for transcription following GA treatment. A similar role for GA has been suggested by Jarvis et al. (3) in studies on seed dormancy. Likewise, the decrease in polysomal level of segments treated with ABA may be due to a reduction in mRNA availability which may be indicative of a reduction in RNA synthesis via a repression of transcription.

### DISCUSSION

Both illumination and GA treatment increased the capacity for RNA synthesis in leaf segments, whereas treatment reduced RNA synthesis. Thus, the leaf system differs from barley aleurone cells where ABA and GA have no influence on RNA synthesis (2). Part of the increased capacity for RNA synthesis in illuminated or GA-treated segments could be associated with an increased RNA polymerase level in comparison to ABA-treated segments. A similar enhancement of polymerase activity following illumination has been previously reported (12). Also, Jarvis et al. (3) have suggested that an increased capacity for RNA synthesis in GA-treated hazel seeds is associated with an increased level of chromatin-bound RNA polymerase. Furthermore, Pearson and Wareing (8) observed that chromatin extracted in the presence of ABA has a reduced polymerase activity, suggesting that the hormone could regulate RNA synthesis in this manner. In the present study, however, although GA or ABA treatment of leaf segments alters the RNA polymerase level, the specific activity data suggest that the changes in this enzyme level are associated with changes in net protein level and do not necessarily indicate specific effects of the growth regulators on the synthesis of RNA polymerase.

### LITERATURE CITED