Influence of Auxin and Incubation on the Relative Level of Polyribosomes in Excised Soybean Hypocotyl

R. L. TRAVIS, J. M. ANDERSON, AND JOE L. KEY
Department of Botany, University of Georgia, Athens, Georgia 30602

ABSTRACT

The influence of incubation and auxin (2,4-D) on polyribosome level in soybean hypocotyl was studied.

A marked drop in the relative level of polyribosomes in excised apical or meristematic tissue (0 to 5 millimeters below the cotyledons) occurred during incubation. The addition of auxin to the incubation medium did not affect polyribosome level. A similar decrease in polyribosome level occurred in excised elongating tissue (5 to 15 millimeters below cotyledons) during incubation, followed by a larger auxin-dependent increase in polyribosomes. While auxin is known to cause an increase in total ribosomes during incubation of the excised basal hypocotyl, the observed transformation from monoribosomes to polyribosomes was not dependent on new ribosome synthesis.

Protein synthetic activity (poly U-directed phenylalanine incorporation) of the 80S monoribosomes at low Mg++ levels increased during incubation of the excised basal hypocotyl. The increase in ribosome activity was biphasic (an initial auxin-independent phase followed by an auxin-dependent increase in activity) correlating with the biphasic increase in polyribosomes. The enhanced activity of 80S monoribosomes was related, at least in part, to an increase in the level of peptidyltRNA associated with the ribosome population. Removal of peptidyltRNA from the ribosomes reversed the auxin effect.

The hypothesis is advanced that the increase in polyribosomes in response to incubation and to auxin is preceded by and dependent upon the activation of 80S monoribosomes. This activation is in addition to a requirement for continued RNA synthesis, at least in part mRNA, for the transition from monoribosomes to polyribosomes.

While considerable work has been done relating RNA and protein synthesis, in some cases even specific enzymes, to hormonal control in plants (7, 24), much less information is available relating to the influence of hormones on the level and activity of polyribosomes. Trewavas (25) reported that the level of polyribosomes decreased during incubation of excised pea epicotyl; auxin caused the maintenance of a somewhat higher level of polyribosomes than in excised control tissue. This effect was attributed to an auxin-induced increase in ribosome formation resulting from an enhanced rate of rRNA synthesis. Evins (3) and Evins and Varner (4) reported a GA-induced polyribosome formation (a relative increase in polyribosomes in addition to an increase in total ribosome level) in barley aleurone layers. The increase in the relative level of polyribosomes was reversed by ABA. Similar observations were made by Poulson and Bevers (20) on the influence of GA and ABA on barley leaf ribosomes.

We report here studies on the influence of auxin on polyribosome level and protein synthetic activity in excised soybean hypocotyl tissue. Since the protein synthetic activity (as evidenced by polyribosome level) of the basal hypocotyl responded markedly to auxin during incubation, this tissue was used to explore further the influence of auxin on protein synthesis.

MATERIALS AND METHODS

Plant Material. Soybean seeds (Glycine max var. Hawkeye) were germinated in the dark for 72 or 96 hr as previously described (5). Three sections were cut from the hypocotyl and designated as apical (0-5 mm below the cotyledons), elongating (5-15 mm below the cotyledons), or basal (10 mm excised from the hypocotyl at least 20 mm below the cotyledons). Approximately 100 sections from the apical and elongating regions or 130 sections from the basal region were incubated in 20 ml of medium containing 2% sucrose and 50 μg/ml chloramphenicol (to inhibit growth of bacteria) (21). Tissue was incubated in 125-ml flasks at 30 C in an agitating water bath. Basal sections used in studies of auxin-induced polyribosome formation and 80S ribosome activation were excised from 96-hr plants and treated with 8 x 10⁻⁴ M 2,4-D (pH 6.4). Tissue for all other experiments were from 72-hr seedlings.

Preparation of Polyribosomes. Polyribosomes were isolated as previously described (1). Nuclease activity in the extracts was prevented by use of DEP* at pH 7.5 (1) or by increasing the concentration of tris-HCl in the homogenizing medium to 200 mM and the pH to 8.5. When polyribosomes were not purified prior to layering on the sucrose gradient (Figs. 1, 2), a special gradient was used. The gradient consisted of a 12 to 22%, w/v, linear region (19 ml) layered on top of a 35 to 45%, w/v, linear region (16 ml). The profiles obtained from such gradients show a compressed polyribosome region and expanded monomer-subunit region. Amino acid incorporation was done as previously described (1).

*Abbreviation: DEP: diethylpyrocarbonate.
Preparation of Monoribosomes. To separate monoribosomes, approximately 10 ml of the postmitochondrial supernatant were layered on a 3-ml discontinuous sucrose gradient consisting of 2 ml of 1 M sucrose in tris- K-Mg buffer (50 mM tris, pH 7.5; 15 mM KCl; and 20 mM MgCl₂) layered over 1 ml of 2 M sucrose. After centrifugation at 159,000g for 30 min (Spinco type 65 rotor) the monoribosomes were recovered from the upper layer of the gradient (1 M sucrose) and reglazed over 4 ml of 1.5 M sucrose containing tris-K-Mg²⁺ buffer. The polyribosome fraction (2 M sucrose) was discarded. Monoribosomes were pelleted at 221,000g for 85 min (Spinco type 65 rotor) and then resuspended in tris-K-Mg²⁺ buffer. All steps were carried out at 0 to 3 C.

Poly U-directed Phenylalanine Incorporation. Ribosome preparations were used for in vitro poly U-directed ³⁵S-phenylalanine incorporation following modified methods of Mans and Novelli (16, 17) and Williams and Novelli (27) as previously described (23). Supernatant factors (protein) were prepared from shoots of 48-hr dark-germinated corn seedlings as previously described (14).

Formation of H-Peptidylpuromycin. Peptidylpuromycin formation was studied by the method of Evins (3) with the following modifications. Five A₅₅₀ units monoribosomes were incubated for 30 min at 37 C in a 1-ml reaction mixture (23) containing 5 µC of ³H-puromycin (3000 mCi/mmmole) and 10 µg of carrier puromycin. The reaction was terminated by the addition of 5 ml of 10% trichloroacetic acid, filtered on a Whatman GFA glass fiber disk, washed with 50 ml of 5% trichloroacetic acid, dried, and counted in a Packard liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Polyribosome Levels. The influence of auxin on cell elongation and RNA synthesis has been studied in some detail in the excised soybean hypocotyl. There are marked differences in response between the tissues from the different regions of the hypocotyl (regions delineated as dividing, elongating, and maturing [11]). In this study on the influence of auxin and incubation on polyribosomes, there were again marked tissue differences. Excision and incubation of tissue from the apical or elongating region of the hypocotyl resulted in a rapid drop in the level of polyribosomes (Fig. 1). A similar though less dramatic decrease in polyribosomes during growth of excised tissue was noted for pea epicotyl (25). Auxin had no apparent effect on the polyribosome level of apical tissue during incubation. Auxin did, however, cause a small but reproducible stabilization of the level of polyribosomes, especially in the heavy polyribosome region (Fig. 1), in the elongating tissue, similar to that noted for pea (25). The decrease in polyribosome level occurred even though continued RNA and protein synthesis are required for endogenous and auxin-induced cell elongation of excised tissue (7, 24). The stabilization effect of auxin on polyribosomes of elongating tissue, although slight, was highly reproducible. The low level of polyribosomes remaining was apparently sufficient to maintain the level of protein synthesis required to sustain cell elongation at an auxin-induced rate approaching the rate expressed by the intact seedling. Thus, the rate of cell elongation shows no clear relationship to the protein synthetic capacity, i.e., polyribosome level of the tissue. The large drop in polyribosomes upon excision does correlate positively with the loss in net protein synthetic ability of such tissue (Key, unpublished data).

While the initial level of polyribosomes in the intact seedling was lower in basal or mature tissue than in apical or elongating tissues, excision and incubation of tissue from this region of the hypocotyl resulted in an increase in polyribosomes. Polyribosomes increased from 33 to 49% of the total ribosome population during a 6-hr incubation (Fig. 2, A, B). Auxin treatment resulted in an increase of polyribosomes to nearly 75% of the ribosome population during the same period (Fig. 2C). The accumulation of polyribosomes in the mature tissue occurred in two distinct phases (Fig. 3). The first phase was an auxin-independent rapid accumulation of polyribosomes during the 1st hr of incubation (Fig. 3). Only a slight increase in polyribosome level occurred during the remainder of the treatment period. The second phase was an auxin-dependent sustained accumulation of polyribosomes which continued for at least 8 to 10 hr (Fig. 3). Auxin increased the rate of polyribosome formation during both phases but had the greatest effect during phase 2. The delayed addition of auxin after the initial rapid accumulation resulted in a corresponding de-
the functional protein-synthesizing apparatus of the tissue. Polyribosome preparations from tissue treated for 6 hr with auxin supported an increased rate of in vitro $^{14}C$-leucine incorporation over both 6-hr incubated control and zero time control (nonincubated) tissue (Fig. 4).

In other plant tissues the transformation of cytoplasmic monoribosomes to polyribosomes involves, in addition to the requirement for mRNA synthesis (12), an increased ability of the 80S ribosomes to participate in protein synthesis (15, 23). To determine whether a similar situation exists in auxin-treated basal soybean hypocotyl, protein synthesis by 80S ribosomes was tested in an in vitro system utilizing a synthetic rRNA (polyuridylic acid). As in the case of auxin-induced polyribosome formation, the activation of 80S ribosomes occurred in two distinct phases (Fig. 5). The initial increase in activity occurred during the first 15 min of incubation and was independent of auxin treatment. A second, auxin-dependent phase, occurred only after a 1.5- to 2-hr lag period. The initial or auxin-independent increase in 80S ribosome activity correlates with the initial auxin-independent phase of polyribosome formation. The second or auxin-dependent increase in 80S ribosome activity correlates with the auxin-dependent second phase of polyribosome formation.

Mammalian (2) and higher plant (15, 23) ribosomes isolated from tissues with a relatively high level of protein synthesis are generally more active at low Mg$^{2+}$ levels than similar ribosomes prepared from tissues less active in protein synthesis. As the Mg$^{2+}$ level is increased, the situation is reversed, and ribosomes from less active tissue will incorporate more phenylalanine. This phenomenon is associated with a higher level of peptide-tRNA associated with ribosomes isolated from active tissues relative to ribosomes isolated from less active tissues (2, 15, 22, 23). Van der Jeist et al. (26) recently clarified this phenomenon in describing two types of poly U-directed phenylalanine incorporation by yeast ribosomes. At low Mg$^{2+}$ levels phenylalanine is incorporated only into pre-existing polypeptides. This type of incorporation is mediated by the interaction between ribosomes carrying endogenous peptidyl-tRNA and poly U. At high Mg$^{2+}$ levels true poly U-directed oligophenylalanine synthesis occurs. A similar situation apparently exists with soybean ribosomes isolated from control and auxin-treated basal hypocotyl sections (Fig. 6). At low Mg$^{2+}$ levels (10–12 mM) ribosomes from auxin-treated tissue were approximately twice as active at phenylalanine incorporation as ribosomes from control (freshly cut) sections. The in
The anaerobic activity of both Arabidopsis thaliana and Amaranthus杂交单位的活动在低氧环境中增加。如图5所示，将植物组织从新鲜切下后用氮气浸泡1小时可显著提高组织的蛋白合成活性。这一结果与Casteles等人的研究一致，他们发现由于放线菌素D的有效抑制，从哺乳动物细胞中剥离的tRNA能够促进肽的合成并通过哺乳-肽酰-tRNA的提前释放来抑制肽的合成。从无氧环境中从0.5 μg的poly U中提取的tRNA能显著地提高肽的合成活性。

The activity of the ribosomes from control tissue increased to a maximum at 20 mM Mg²⁺, then declined as the Mg²⁺ concentration was increased. Incubation of ribosomes from control tissue increased to a maximum at 20 mM Mg²⁺, again followed by a sharp decline as Mg²⁺ concentration was increased. Ribosomes from control tissue were approximately twice as active as ribosomes from auxin-treated tissue at all Mg²⁺ concentrations above 15 mM.

If the lower Mg²⁺ optimum and increased activity at low Mg²⁺ levels associated with ribosomes from 6-hr auxin-treated tissue are related to the presence of peptide-tRNA, the auxin response might be reduced or abolished by removal of peptide-tRNA. Castles et al. (2) have shown that incubation with puromycin effectively strips mammalian ribosomes of peptide-tRNA through the premature release of nascent protein as peptide-puromycin. Subjecting plant tissue to an anaerobic environment for 1 to 2 hr (tissue submerged in water in an air-tight container with nitrogen gas bubbled through the system) will also produce peptide-tRNA-free ribosomes by preventing further initiation of peptide synthesis and leading to the production of run-off ribosomes (13). While the mechanisms of peptide-tRNA stripping by puromycin and anaerobiosis differ, both result in a loss of peptide-tRNA from ribosomes. We have compared the effectiveness of the two treatments and found them to be equal (23). In the following experiments nitrogen was used to prepare stripped ribosomes.

A 1-hr N₂ pretreatment of the tissue prior to ribosome preparation completely reversed the auxin response (Fig. 6). The anaerobic treatment decreased phenylalanine incorporation by ribosomes from treated tissue at low Mg²⁺ levels and increased the activity at high Mg²⁺ levels, effectively equalizing the activity of both preparations at all Mg²⁺ levels tested.

Table II. Effect of Mg²⁺ Concentration on Poly U-directed 
¹⁴C-Phenylalanine Incorporation by Monoribosomes Prepared from Excised Basal Hypocotyl

<table>
<thead>
<tr>
<th>Mg²⁺ Conc (mM)</th>
<th>Control (fresh cut)</th>
<th>Without auxin</th>
<th>With auxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>4,100</td>
<td>5,350</td>
<td>5,550</td>
</tr>
<tr>
<td>12.5</td>
<td>8,450</td>
<td>9,400</td>
<td>11,050</td>
</tr>
<tr>
<td>20</td>
<td>45,500</td>
<td>22,400</td>
<td>22,650</td>
</tr>
<tr>
<td>30</td>
<td>23,600</td>
<td>12,300</td>
<td>12,300</td>
</tr>
</tbody>
</table>

¹⁴C-Phenylalanine incorporation after 30 min incubation.

Nitrogen treatment had no effect on ribosomes prepared from fresh tissue. Actual levels of peptide-tRNA can be measured by incubating ribosomes with ³H-puromycin. Ribosomes containing peptide-tRNA bind puromycin, a peptide bond is formed between puromycin and the nascent peptide, and the peptide is subsequently released as peptide-puromycin. Each ribosome can form only a single molecule of peptidylpuromycin. Levels of ³H-peptidylpuromycin formed by ribosomes from control (nonincubated) and auxin-treated tissue were 170 and 325 pmoles/mg of RNA, respectively.

Ribosomes from basal hypocotyl which had been incubated for 0.5 hr (plus or minus auxin) were more active in phenylalanine incorporation (at low Mg²⁺ levels) relative to ribosomes from control tissue (Fig. 5, Table II); however, Mg²⁺ requirements for optimal activity were similar in each system (Table II). Hence, the initial increase in activity (auxin-independent phase) does not involve a decrease in the Mg²⁺ opti-
mum as does the second, auxin-dependent, phase. The significance of this early auxin-independent phase in ribosome activation is not clear.

CONCLUSIONS

There was a dramatic decrease in polyribosomes during incubation of excised apical and elongating soybean hypocotyl, similar to that observed in pea (25). While the underlying mechanism of the loss of polyribosomes is not known, it provides a basis for understanding the loss in net protein synthetic ability of these tissues relative to those of the intact seedling. In elongating soybean hypocotyl as in pea epicotyl (25), auxin caused the maintenance of a somewhat higher level of polyribosomes than in comparable control tissue. This small effect on polyribosome level possibly relates to the protein synthesis required for auxin-induced growth and for changing patterns of protein synthesis (19) in the tissues undergoing cell elongation.

In contrast to the growing tissues of the soybean hypocotyl, there was a small increase in the relative level of polyribosomes in the basal or mature tissue during the 1st hr of incubation. Auxin caused a much larger increase in the relative level of polyribosomes after a lag period of 1 to 2 hr. Although auxin caused a significant increase in the number of ribosomes in this tissue (9), the transition from monoribosomes to polyribosomes was not dependent upon new ribosome synthesis (based on results from 5-FU experiments). These results are not in agreement with the view of Trewavas (25) that polyribosome formation in response to auxin results from auxin-enhanced rRNA synthesis and ribosome formation. It is dependent upon, and possibly causally related to, the auxin-enhanced synthesis of mRNA-like RNA in this tissue (8).

In addition to apparently depending upon mRNA synthesis, the transition from monoribosomes to polyribosomes in basal hypocotyl is associated with some change(s) in the 80S ribosome which leads to an increased ability of the ribosome to participate in protein synthesis. The increased level of peptidyl-tRNA associated with monoribosomes from auxin-treated tissue suggests that this auxin-enhanced ribosome activity is associated with an increased level of peptide chain initiation. This conclusion is warranted since an enhanced level of peptide chain initiation would obviously lead to an increase in peptidyl-tRNA associated with a ribosome population.

Possible explanations of a causal mechanism responsible for auxin-enhanced peptide chain initiation include regulation at the level of the initiation factors (activation, synthesis, or affinity for ribosomes) and possible changes in the complement of other ribosomes proteins. Experiments to test these possibilities are in progress.

LITERATURE CITED


