Inhibition of Auxin-induced Deoxyribonucleic Acid Synthesis and Chromatin Activity by 5-Fluorodeoxyuridine in Soybean Hypocotyl[1]

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ABSTRACT

Rootless soybean (*Glycine max*) seedlings were used as a test system to examine the action of auxin on chromatin-directed RNA synthesis. Chromatin from the basal tissue of rootless seedlings (both control and auxin-treated) had RNA synthetic capacity similar to that of chromatin from comparably treated intact seedlings. When DNA synthesis normally induced in the basal tissue by auxin was blocked in the rootless seedlings by 5-fluorodeoxyuridine, the auxin enhancement of chromatin activity was inhibited 70%. This level was still three times the control level, indicating that auxin influenced the synthetic activity of existing DNA template. Experiments with *Escherichia coli* RNA polymerase revealed that chromatin from both auxin- and auxin plus 5-fluorodeoxyuridine-treated tissue saturated at higher levels than chromatin from control tissue.

The hypocotyl of young soybean seedlings, intact and excised, has been used to study control of growth and development by auxin. Several investigators found increases in RNA, mainly ribosomal, in the hypocotyl of intact seedlings sprayed with auxin (1, 7). Auxin also promotes RNA synthesis in excised hypocotyl tissue (6).

Chromatin isolated from auxin-treated seedlings shows increased capacity for RNA synthesis relative to controls (10). O'Brien *et al.* (10) concluded that the enhanced synthesis of RNA by auxin treatment results from a larger quantity of RNA polymerase associated with the chromatin. There are several problems inherent in soybean hypocotyl tissue which make interpretation of the chromatin data difficult. Key *et al.* (7) reported that auxin enhances cell division in the differentiated, mature zone of the hypocotyl within 12 hr after treatment. Since many of the chromatin analyses were performed with chromatin isolated from tissue treated with auxin for 12 hr, it is possible that the auxin-enhanced chromatin activity results from chromatin which contains newly synthesized DNA associated with auxin-induced cell division. Furthermore, chromatin from the rapidly dividing apical section of soybean hypocotyl has twice the RNA synthetic capacity as chromatin from the nondividing basal tissue (4).

In order to determine whether DNA synthesis contributes to auxin-induced chromatin activity, we used rootless soybean seedlings as a test system (3). The conditions were regulated so that the basal hypocotyl tissue of both intact and rootless seedlings responded similarly to auxin. Under these conditions, DNA synthesis induced by auxin in the rootless seedlings was blocked by FUDR,[2] and the RNA synthesis responses were evaluated at the chromatin level.

MATERIALS AND METHODS

Soybean seeds (*Glycine max* var. Hawkeye 63) were germinated in moist vermiculite for 3 days at 30 C. Rootless seedlings, 4.5 cm long, were prepared as described previously (3). The tissue between 2 and 4 cm below the cotyledons was used in these studies and is referred to as the basal section. Key *et al.* (7) have shown the cells in this section to be fully elongated. Intact seedlings were premarked to delineate the basal section and were treated as before (4). Total tissue RNA and DNA were determined by the methods of Key and Shannon (8). Bacterial levels were monitored by previously described methods (11) and were between 4 and 5×10^4 bacteria per g fresh weight. Test solutions for all experiments contained 50 μg/ml CAP, which completely inhibited bacterial increases during the test period and did not alter the tissue response to auxin. Chromatin from 100 basal sections (12–15 g) was isolated according to the method of Huang and Bonner (5). Chromatin-directed RNA synthesis was assayed by the procedure of O'Brien *et al.* (10).

RESULTS

Treatment of intact soybean seedlings with auxin results in radial enlargement or swelling of the basal portion of the hypocotyl (7). In preliminary experiments, rootless seedlings treated with auxin showed little or no swelling, although the nucleic acid enhancement was similar to that in the intact seedlings. Addition of the synthetic cytokinin, SD 8339, to the rootless tissue caused little swelling in the control tissue but markedly promoted swelling in the auxin-treated tissue (Fig. 1). A swelling response similar to that caused in the intact plant by auxin was obtained with 5×10^{-8} M SD 8339 and auxin in the rootless seedlings. Higher concentrations of SD 8339 in combination with auxin yielded additional fresh

[2] Abbreviations: FUDR: 5-fluorodeoxyuridine; SD 8339: 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine; CAP: chloramphenicol.
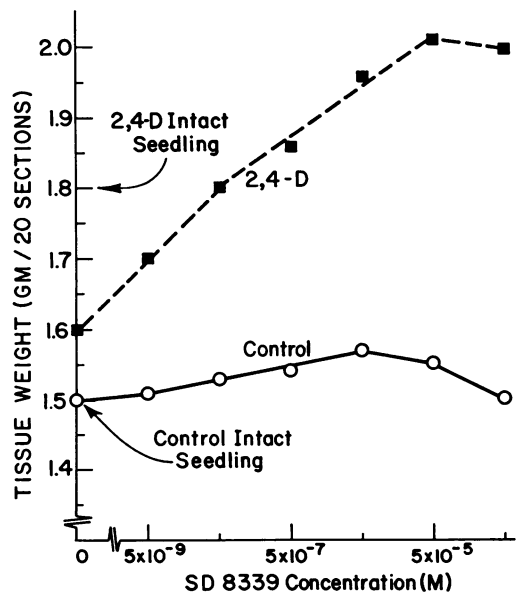
FIG. 1. Tissue swelling in rootless soybean seedlings. Rootless soybean seedlings were marked to designate the basal section. The rootless seedlings were incubated for 24 hr in distilled water containing 50 μg/ml CAP (±10⁻⁴ M 2,4-D) and various concentrations of SD 8339. After the incubation period, the basal sections were harvested for fresh weight determinations.
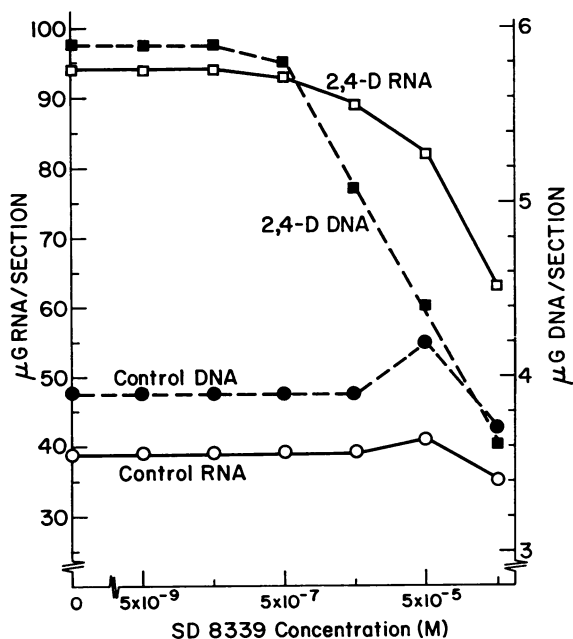

FIG. 2. Effect of SD 8339 on 2,4-D-induced RNA and DNA synthesis. Rootless soybean seedlings were marked and treated as described in Figure 1. After the incubation period, the basal sections were harvested for DNA and RNA analyses.

weight increases, but concentrations above 5×10^{-6} M drastically inhibit the auxin-induced increases in nucleic acid accumulation (Fig. 2). Subsequent rootless seedling experiments were conducted with 10^{-7} M SD 8339 because this concentration resulted in intact seedling swelling responses without influencing the auxin-induced nucleic acid changes.

The base analogue, FUDR, was used to block DNA synthesis resulting from auxin treatment in the rootless seedlings

(Table I). At 5×10^{-4} M FUDR, the auxin-enhanced accumulation of DNA was completely inhibited while the auxin-induced increase in RNA was 35% above control levels. The chromatin from the rootless tissue (both control and auxin-treated) exhibited an RNA synthetic capacity very similar to that of chromatin from intact seedlings (Table I). The auxin enhancement of chromatin activity was inhibited 70% by
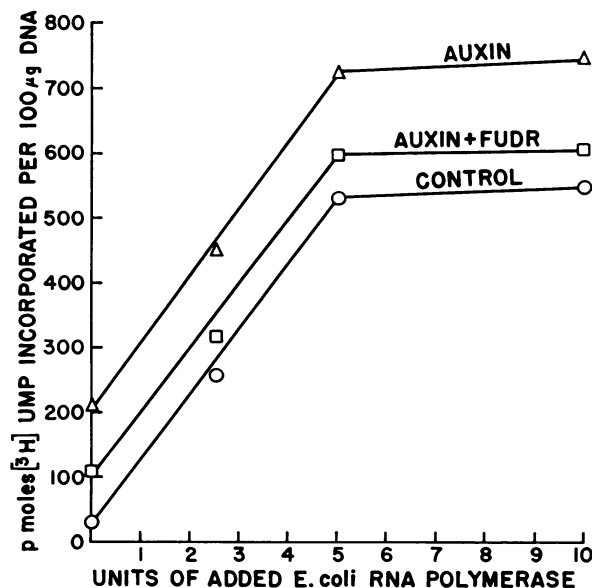

FIG. 3. Saturation of chromatin DNA from control, auxin-, and auxin plus FUDR-treated basal soybean hypocotyl with exogenous *E. coli* RNA polymerase. Rootless soybean seedlings were marked to designate the basal section and were incubated for 24 hr in 50 μg/ml CAP and 10^{-7} M SD 8339 (±10⁻⁴ M 2,4-D and ±5 × 10⁻⁴ M FUDR). After the incubation period, the basal tissue was harvested, and the chromatin was isolated from it as described in the text.

Table I. *Effect of FUDR on Nucleic Acid Accumulation and Chromatin Activity*

Rootless and intact soybean seedlings were marked to designate the basal section. The rootless seedlings were incubated for 24 hr in distilled water containing 50 μg/ml CAP (±10⁻⁴ M 2,4-D and 5 × 10⁻⁴ M FUDR) and 10⁻⁷ M SD 8339. Intact seedlings were sprayed with water (controls) or 10 ml of 110 μg/ml 2,4-D prior to the 24-hr incubation. After the incubation period, the basal sections were harvested for RNA and DNA determinations and chromatin isolation.

Treatment	Fresh wt	RNA	DNA	Chromatin Activity
	$\frac{g/20}{sections}$	μg/section		$\frac{pmoles}{{}^3H\text{-}UMP\ incorporated/100\ \mu g\ DNA}$
Initial	1.47	38	3.8	...
Control				
Intact seedlings	1.50	39	3.8	29
Rootless seedlings	1.51	37	3.7	28
Rootless seedlings + FUDR	1.49	38	3.7	26
Auxin				
Intact seedlings	1.95	87	5.8	246
Rootless seedlings	1.86	89	5.9	236
Rootless seedlings + FUDR	1.91	53	3.7	91

FUDR, but this level was still three times higher than the control level.

When chromatin preparations from control, auxin-, and auxin plus FUDR-treated rootless seedlings were saturated with *Escherichia coli* RNA polymerase, they all showed similar saturation curves with a maximal RNA production at 5 units of added enzyme (Fig. 3). The increases exhibited by both auxin and auxin plus FUDR over control in the absence of added *E. coli* RNA polymerase were still observable at the saturating levels of enzyme.

DISCUSSION

Key *et al.* (7) reported that the initial swelling induced by auxin in soybean hypocotyl is caused by radial enlargement of cortical and pith cells followed by cell divisions within 12 hr after treatment. This is accompanied by marked increases in RNA and DNA. However, the basal sections of rootless seedlings did not enlarge or swell when treated with auxin, except when SD 8339 was added, even though normal nucleic acid accumulation was induced by auxin. In other experiments not reported here, ethylene was able to cause tissue swelling with SD 8339 in the rootless seedlings, but the ethylene did not alter nucleic acid synthesis. Auxin plus SD 8339-induced swelling was not inhibited by FUDR, although FUDR completely inhibited the DNA accumulation caused by auxin (Table I). These results show that there is no connection between auxin induction of tissue swelling and the regulation of nucleic acid accumulation in soybean hypocotyl.

Auxin-induced accumulation of RNA was still 35% above the control level in the presence of a concentration of FUDR which completely inhibited DNA synthesis. Fan and Maclachlan (2) reported that FUDR blocks IAA-induced DNA synthesis in pea epicotyl without inhibiting RNA accumulation or cellulase synthesis during the first 2 days of treatment. They concluded that the action of auxin in producing these responses is on existing cells. Auxin enhances total RNA accumulation 30% in the excised basal section of soybean hypocotyl—a tissue which does not accumulate DNA in response to auxin (6). The basal section of rootless soybean seedlings, like the above tissue, did respond to auxin with increased RNA synthesis in the absence of DNA synthesis and cell division in the presence of FUDR.

The chromatin isolated from the rootless tissue exhibited endogenous RNA synthetic capacity similar to that of chromatin from intact seedlings. The results showing auxin enhancement of chromatin activity agree with those of O'Brien *et al.* (10) obtained with intact seedlings. Holm *et al.* (4) reported that chromatin isolated from the rapidly dividing apical tissue of untreated soybean hypocotyl has 2- to 3-fold higher RNA synthetic capacity than chromatin isolated from the basal section. The increased chromatin activity in the basal section of auxin-treated soybean hypocotyl is associated, in part, with the increased cell division activity in this tissue as indicated by the FUDR results. However, auxin does influence chromatin-directed RNA synthesis in the absence of DNA synthesis, as shown by the response to auxin within 4 hr (10) and the 3-fold increase in the FUDR experiments. Apparently, the

chromatin of cells formed during the auxin treatment is activated to a greater extent by auxin than chromatin of the cells present at the time of auxin treatment.

The findings with *E. coli* RNA polymerase are similar to those of O'Brien *et al.* (10) with chromatin isolated from control and auxin-treated tissue. In addition, the present investigation compares these results with those obtained with chromatin isolated from auxin plus FUDR-treated tissue. At saturating levels of *E. coli* RNA polymerase, the chromatin isolated from auxin- and auxin plus FUDR-treated tissue exhibited the same absolute increase in RNA synthetic capacity over chromatin from control tissue as occurred when no *E. coli* RNA polymerase was added. O'Brien *et al.* (10) interpreted their results as an indication of more RNA polymerase associated with the chromatin from auxin-treated tissue. It would appear, however, from the results of Figure 3 that increased template availability following auxin treatment may be involved in the response. The nearest neighbor analyses of RNA synthesized by control and auxin chromatin are also suggestive of a change in sites on the DNA which are being transcribed (4). Matthysse and Phillips (9) recently isolated and partially purified a protein which reacts with auxin to increase template activity of purified chromatin. They observed an increase in the rate of RNA synthesis with auxin in the presence of saturating amounts of *E. coli* RNA polymerase and concluded that auxin and the protein act by making more of the genome available for transcription. Clearly, the question of whether auxin causes more RNA polymerase, or increased template availability, or both, will not be resolved until RNA polymerase from control and auxin-treated tissue can be isolated separately from the chromatin and assayed with a DNA template.

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