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AUXIN-INDUCED ENHANCEMENT OF RNA POLYMERASE ACTIVITY IN SOYBEAN AS A FUNCTION OF DEVELOPMENT

PETER J. RIZZO* and JOE H. CHERRY
Horticulture Department, Purdue University, West Lafayette, IN 47907, U.S.A.

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Abstract—Studies on chromatin and solubilized RNA polymerase from control and 2,4-D treated whole hypocotyls indicate that the activity of RNA polymerase I is enhanced by the auxin treatment while the activity of polymerase II is essentially the same in control and treated tissue. However, different portions of the hypcotyl respond differently to the auxin treatment. The enhancement of solubilized polymerase I activity is greater in the lower half of the hypocotyl than it is in the upper half. In the hook region an inhibition of RNA solubilized polymerase activity is observed. Similarly, 2,4-D treatment results in an inhibition of chromatin bound RNA polymerase activity in nuclei isolated from first leaves. Chromatography on DEAE-Sephadex also reveals a selective enhancement of polymerase I in the upper and lower halves of the hypocotyl.

INTRODUCTION

Chromatin isolated from auxin treated soybean hypocotyls exhibits a higher RNA polymerase activity than that of control tissue [1,2]. The majority of this increase in RNA polymerase activity solubilized from chromatin was found to be insensitive to α-amanitin using exogenous template [3]. In addition, it has recently been shown by chromatography of solubilized soybean RNA polymerase on DEAE-cellulose that auxin increases the activity of the α-amanitin insensitive enzyme several fold, while the activity of the α-amanitin sensitive enzyme is increased only slightly [4]. This agrees with the earlier report [5] that auxin increases the activity of the α-amanitin insensitive polymerase but not that of the α-amanitin sensitive enzyme in lentil root, as judged by chromatography on DEAE-Sephadex. Since α-amanitin is known to inhibit the nucleoplasmic RNA polymerase (polymerase II) and not the nucleolar enzyme (polymerase I) in animal cell nuclei [6], as well as in the nucleus of the lower plant *Acetabularia* [7], it is very likely that the α-amanitin insensitive RNA polymerase from higher plant cell nuclei and chromatin is also of nucleolar origin. Therefore in the present paper we assume from chromatographic behavior and α-amanitin sensitivity that polymerase I is of nucleolar origin and synthesizes ribosomal RNA, and that polymerase II is nucleoplasmic and synthesizes messenger RNA.

Several workers have reported that different responses to auxin can be seen in cells of different ages in the same organ. For example, Gotô and Esashi [8] found that IAA inhibited growth in zones corresponding to the hook region of *Phaseolus* hypocotyls, but enhanced growth in older portions of the same tissue. They concluded that the inhibition was due to an IAA-induced rise in endogenous ethylene. Working with wheat coleoptiles, Likholat and Pospelov [9] showed that IAA inhibited the template activity of chromatin isolated from seedlings 36 hr after treatment but the template activity was increased by 72 hr. Template activity was measured with exogenous RNA polymerase however, and no change in the endogenous RNA polymerase level was observed. Using soybean hypocotyl tissue Holm *et al.* [2] showed that the level of chromatin-bound RNA polymerase was increased 9-fold in the elongating region of the hypocotyl, but slightly inhibited in the apical region following a 24 hr treatment with 2,4-D.

Previous experiments [10] have shown that chromatin isolated from soybean hypocotyl tissue in different stages of development (tissues of different age), contains different proportions of RNA polymerases I and II. The meristematic upper half of the hypocotyl contains a higher proportion of polymerase I relative to II than does the elongated lower half. Since auxin alters the proportions of the two major forms of nuclear RNA polymerase in the whole hypocotyl, it was of interest to determine whether the developmental stage of the cell is important in the auxin-induced enhancement. In the present report we show that the effect of auxin on RNA polymerase depends on the developmental stage of the cells at the time of treatment.

RESULTS

Crude chromatin isolated from whole hypocotyls treated with 2,4-D contain a higher level of endogenous RNA polymerase activity than that isolated from control tissue (Table 1). However, the magnitude of the increase is much lower than that reported by other workers for chromatin which was centrifuged through dense sucrose [1,2,11]. If our crude chromatin from control and treated tissue is centrifuged through 2 M sucrose as described by Huang and Bonner [12], the enhancement is

* Present address: Biology Department, Texas A & M University, College Station, TX 77843, U.S.A.

Table 1. Endogenous RNA polymerase activity of crude chromatin and 2 M sucrose purified chromatin from control and 2,4-D treated whole hypocotyls

Chromatin	Cpm/mg FW	% of Control
Crude control	19.23	100
Crude 2,4-D treated	34.00	177
Purified control	6.10	100
Purified 2,4-D treated	18.21	298

much greater (Table 1). However, we [13] had previously shown that centrifugation through dense sucrose yields chromatin enriched in polymerase I due to a selective loss of polymerase II.

When RNA polymerase is solubilized from crude chromatin of control and treated hypocotyls, the enhancement by auxin is noted mainly for the α-amanitin insensitive enzyme (Table 2, whole hypocotyl). This agrees with the chromatin data in Table 1 and suggests that the nucleolar enzyme is primarily responsible for the increase in activity.

While 2,4-D treatment increases the level of RNA polymerase I in the whole hypocotyl, the result is quite different when RNA polymerase levels in only the hook region are examined. As seen from Table 2 2,4-D treatment resulted in a lower level of activity compared with the control, in both the presence and absence of α-amanitin. This agrees with the results obtained using a chromatin-bound RNA polymerase system from apical sections of soybean hypocoty [2]. We also cut treated and control hypocotyls in half and determined the effect of 2,4-D on the RNA polymerase levels in the upper and lower portions. In both the upper and lower halves of the hypocotyl 2,4-D results in an increase in the activity of the α-amanitin insensitive enzyme only (Table 2) while the larger increase in polymerase I activity appears in the lower half.

The increase in α-amanitin insensitive activity can also be seen when polymerase from the upper and lower halves of control and treated hypocotyls is chromatographed on DEAE-Sephadex columns (Figs. 1 and 2). The elution profile of polymerase from the lower half of the control hypocotyl (Fig. 1a), shows that the activity consists entirely of a single activity peak eluting at about 0.3 M KCl (polymerase II). In the lower half of the

Table 2. RNA polymerase activity solubilized from crude chromatin from control and 2,4-D treated hypocotyl tissue

Polymerase preparation	Cpm/mg Tissue α-amanitin (5 mg/ml) − (Both erzymes)	+ (Poly-merase I)	Difference (Poly-merase II)
Whole hypocotyl control	8.80	2.29	6.52
Whole hypocotyl treated	12.53	5.57	6.96
Upper half control	16.40	7.05	9.35
Upper half treated	18.94	9.66	9.28
Lower half control	5.94	0.77	5.17
Lower half treated	8.04	3.30	4.75
Hook control	43.34	26.68	15.67
Hook treated	35.39	21.52	13.76

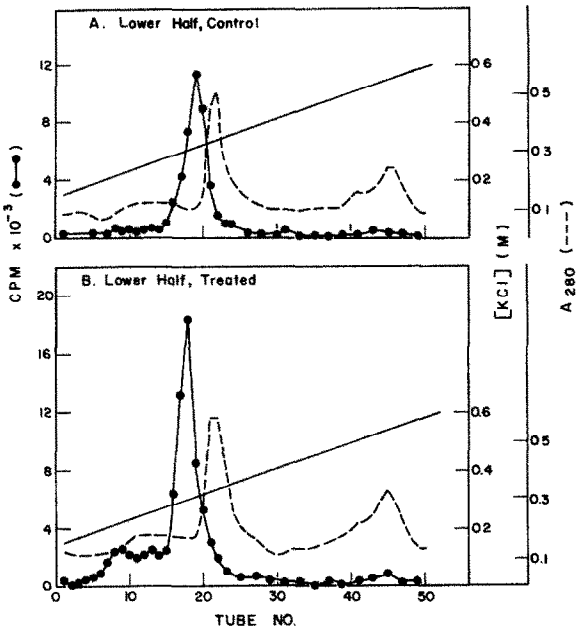

Fig. 1. DEAE-Sephadex A-25 chromatography of RNA polymerase prepared from the lower half of control (a) and treated (b) hypocotyls as described in Experimental.

treated hypocotyl, enzyme II still predominates but the two components of peak I are clearly evident (Fig. 1b). Chromatography of RNA polymerase from the upper half of the control hypocotyl shows that polymerases I and II both are present and the two major activity peaks

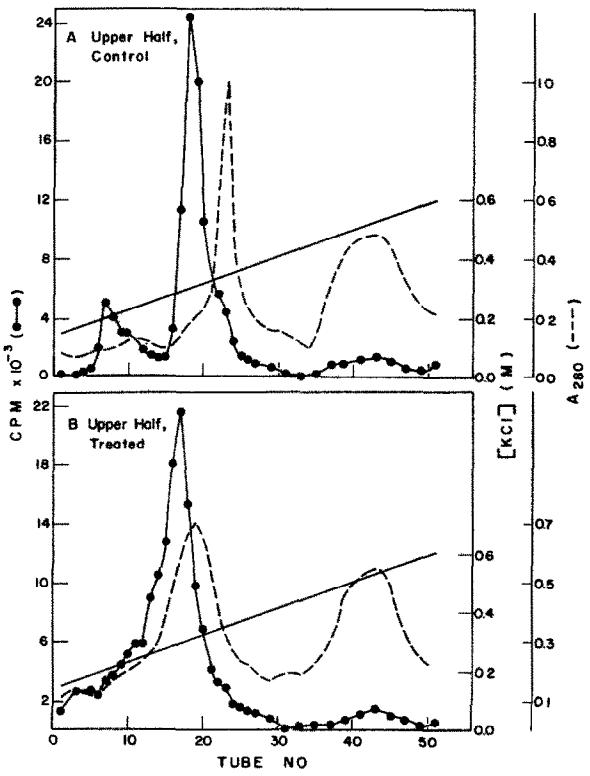

Fig. 2. DEAE-Sephadex A-25 chromatography of RNA polymerase prepared from the upper half of control (a) and treated (b) hypocotyls as described in Experimental.

Table 3. RNA polymerase activity of nuclei isolated from control and 2,4-D treated first leaves

Nuclei	Cpm/mg Tissue α-amanitin (5 μg/ml) − (Both enzymes)	+ (Polymerase I)	Difference (Polymerase II)
Control	94.15	63.08	31.07
Treated	69.95	39.17	30.78

are easily resolved (Fig. 2a). In the treated upper half, however, the increase in the amount of polymerase I precludes the resolution of the two major peaks (Fig. 2b). The merging of peaks I and II is not due to an increase in the amount of polymerase II, because the fractions from the part of the gradient where polymerase I elutes (shoulders in Fig. 2b) are insensitive to α-amanitin. It should be noted that the values given in Table 2 are averages from three to five replications. Although the response to 2,4-D in the lower half was quite reproducible, some variability was noted in the response of the upper half to 2,4-D. Thus the profiles of the particular polymerase preparations shown in Fig. 2 indicate a greater response to 2,4-D in the upper half than would be expected from the composite data given in Table 2.

The elongated part of the hypocotyl thus appears to be more responsive to auxin treatment than the meristematic parts of the hypocotyl. We also treated etiolated first leaves with 2,4-D to see if this meristematic tissue would respond to auxin. Since clean nuclei can be isolated rather easily from first leaves, we were able to use intact nuclei to assay RNA polymerase activity. Table 3 shows that 2,4-D treatment results in a decrease in the RNA polymerase I activity of nuclei isolated from etiolated first leaves. Similar results were obtained when the RNA polymerase activity of isolated nuclei from control and 2,4-D treated light-grown first leaves was compared. 2,4-D treatment results in changes in activity and levels of multiple RNA polymerase of meristematic tissue responds differently to 2,4-D treatment than that of non-meristematic tissue.

DISCUSSION

Treatment of soybean hypocotyls with the synthetic auxin, 2,4-D, results in an increase in both chromatin-bound and solubilized RNA polymerase activities. In the case of chromatin-bound RNA polymerase, the magnitude of auxin enhancement is greater if the crude chromatin is centrifuged through 2 M sucrose. Furthermore, the assay of solubilized RNA polymerase in the presence of α-amanitin shows that the insensitive enzyme (polymerase I) accounts for practically all of the enhancement. Also chromatography of RNA polymerase on DEAE-Sephadex shows an increase in the amount of polymerase I activity from treated hypocotyls relative to that of control tissues in agreement with previous findings reported by Teissere *et al.* [5] for lentil root, and Guilfoyle *et al.* [4] for soybean hypocotyl.

Since cells along the length of the hypocotyl are in different stages of development and contain different proportions of RNA polymerase I and II, it was of interest to examine the effects of 2,4-D on the levels of these enzymes in different portions of the hypocotyl. In both the upper and lower halves of the hypocotyl, 2,4-D increased the amount of RNA polymerase I relative to II as judged by assays employing α-amanitin, and from chromatography on DEAE-Sephadex. However, a larger increase was observed in the activity of polymerase I in the lower half. The response of the upper half of the hypocotyl to 2,4-D showed some variation from preparation to preparation. For instance, if the level of polymerase I was high in the control, only a slight enhancement was observed, and occasionally a decrease in activity was seen. Polymerase I activity in the lower half was always enhanced by auxin.

RNA polymerase activity in the hook region, on the other hand, was decreased by treatment with 2,4-D. Previous experiments involving chromatography on DEAE-Sephadex [10] showed that the relative amount of RNA polymerase I activity is high in crude chromatin isolated from the hook region of control tissue. Similarly, RNA polymerase activity in nuclei isolated from first leaves is inhibited rather than enhanced by 2,4-D treatment. Thus, there seems to be a correlation between the developmental state of the tissue and the response to auxin. Cells that are meristematic and characterized by a high level of polymerase I activity do not exhibit an enhancement of RNA polymerase I activity in response to 2,4-D as non-meristematic cells do. Presently we are unable to explain this differential response of meristematic and non-meristematic tissue to 2,4-D treatment.

Although the auxin-induced enhancement of polymerase I appears to result from an increased enzyme activity (not due to template availability), it is possible that changes in chromosomal components other than RNA polymerase might also be involved. For example, in the present investigation, the amount of stimulation observed with chromatin-bound RNA polymerase resulting from *in vivo* auxin treatment is greater than that observed after the enzyme has been solubilized. This effect can also be seen from the data of Guilfoyle *et al.* [4] by comparing the stimulation obtained from chromatin, nuclei and solubilized polymerase. Although the loss in auxin stimulation can be explained by a greater loss of enzyme activity from treated chromatin than from control chromatin, it seems more likely that some chromosomal components necessary for transcription are lost or altered during solubilization.

It is well documented that chromatin is a restricted template and that the absence of RNA synthesis does not necessarily imply the absence of RNA polymerase molecules [14–20]. However, we have found that solubilization results in a substantial decrease in the absolute amount of RNA polymerase activity from both control and treated chromatin. Given that chromatin is a restricted template, one would expect a greater amount of activity after solubilization than before. The present results imply that either a large loss of activity occurs during the solubilization step or that specific factors are present in native chromatin which allow efficient transcription, and are not reconstituted when the solubilized enzyme preparations are assayed with exogenous template.

It is important to note that the enhancement of RNA polymerase I activity in the present investigation as well as in other studies [4,5] is a result of a relatively long exposure to auxin. It is possible that a much shorter exposure time results in events which precede the increase in polymerase I activity, such as a stimulation

of polymerase II activity. For instance, Schmid and Sekeris [21], showed that cortisol injection stimulated the nucleoplasmic enzyme (polymerase II) earlier than the nucleolar enzyme (polymerase I) in rat liver. Similarly, Glasser *et al.* [22] observed an increase in polymerase II activity in isolated rat uterine nuclei 10–15 minutes after injection with estradiol, which precedes a later stimulation in polymerase I activity. Working with lentil root, Teissere *et al.* [5] reported that while a 14-hr treatment with IAA resulted in an increase in polymerase I activity, a 1.5 hr treatment did not affect the level of solubilized RNA polymerase, but increased the activity of chromatin-bound activity by 25%. However, a 1.5 hr treatment with 2,4-D did not increase the chromatin bound RNA polymerase activity of crude chromatin isolated from whole soybean hypocotyls or of nuclei isolated from etiolated first leaves (Rizzo, unpublished observations). We are presently investigating the role of chromatin components other than RNA polymerase on auxin-induced transcription.

EXPERIMENTAL

Plant material and chromatin isolation. Soybean seeds (*Glycine max* L. var. Wayne) were imbibed in deionized H_2O for 6 hr and sown in moist vermiculite as described earlier [3]. Auxin-treated tissue was sprayed with a solution of 10^{-3}M 2,4-D (15 ml per tray) 16–18 hr before harvest. Crude chromatin was prepared by a modification [13] of the method described by Huang and Bonner [12].

Solubilization of RNA polymerase. RNA polymerase was solubilized from crude chromatin by the method of Hardin and Cherry [3]. The post 100000 g supernatant containing the solubilized RNA polymerase was filtered through one layer of Miracloth [10]. The preparations were made 50% in glycerol (v/v) and stored at −80°.

Chemicals. Chemicals were purchased from the following companies: nucleoside triphosphates, calf thymus DNA and dithiothreitol from Sigma, ^{3}H-UTP from Schwarz/Mann; Sephadex A-25 from Pharmacia Fine Chemicals; DEAE-cellulose from Whatman; and α-amanitin from Henley and Co.

Assay of RNA polymerase. RNA polymerase activity was assayed in the presence of 121 mM tris (pH 8.0), 1.25 mM Mg^{2+}, 1.0 mM Mn^{2+}, 0.75 mM ATP, CTP and GTP, 10 μCi ^{3}H-UTP (sp. act.–14 Ci/mmol, 1.96 mM dithiothreitol, 0.017 mM EDTA,* 12.5% glycerol (v/v) and 60 μg heat-denatured calf thymus DNA. The assays were carried out in a final volume of 0.4 ml (0.1 ml enzyme) at 37° for 15 min., unless otherwise indicated. The reaction was stopped by the addition of 10 vols of cold 10% trichloroacetic acid and 0.1 M $Na_4P_2O_7$. The precipitates were allowed to stand on ice for 15 min. and then collected on glass fiber filters (Whatman GF/A), washed with 30 ml of 5% trichloroacetic acid, dried under IR lamps and counted by liquid scintillation.

Chromatography on DEAE-sephadex. Solubilized RNA polymerase preparations in 50% glycerol (v/v) were thawed and diluted 5-fold with TGMED to lower the ammonium sulfate concentration to 0.05 M. The sample was pumped onto a DEAE-Sephadex A-25 column (0.9 × 12 cm) previously equilibrated with TGMED buffer. The column was then washed with 20 ml of 0.15 M KCl in TGMED, and the enzymes were eluted with an 80 ml linear gradient ranging from 0.15 to 0.6 M KCl in TGMED (38 ml/hr), unless otherwise indicated. Fractions of 1.5 ml were collected, and aliquots of 0.1 ml were assayed for RNA polymerase activity.

Isoltaion of nuclei. Nuclei were isolated from etiolated first leaves by a modification of the method described by Hamilton *et al.* [23] for tobacco leaf nuclei. Details of the procedure will be described in a subsequent paper.

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Abbreviations used: EDTA: ethylenediaminetetracetate; TGMED: 50 mM tris (pH 8.0), 25% glycerol (v/v), 5 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM dithiothreitol; DEAE: diethylaminoethyl, 2,4-D: 2,4-dichlorophenoxyacetic acid, IAA: indoleacetic acid.

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