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Auxin-regulated gene expression in intact soybean hypocotyl and excised hypocotyl sections

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Abstract. A library of complementary DNA (cDNA) clones has been prepared from polyadenylated RNA (poly(A)$^+$RNA) from auxin (2,4-dichlorophenoxyacetic acid)-treated soybean (*Glycine max* (L.) Merr. cv. Wayne) seedlings. Using differential hybridization, four clones were selected as auxin-responsive, and characterized. The levels of the RNA sequences homologous to the cDNA clones were examined in the hypocotyl of the intact seedling and in excised hypocotyl sections before and after auxin treatment, using RNA blot hybridization analysis. RNA levels are rapidly increased (within 0.25–0.5 h) following auxin treatment and the response in the hypocotyl of the intact seedling is transient, reaching maximum RNA levels 2–4 h after auxin application. Increases in RNA levels were also observed with the auxins indole 3-acetic acid and 2,4,5-trichlorophenoxyacetic acid, but not with the ethylene-producing compound, Ethephon (2-chloroethylphosphonic acid). Hybridization analysis of in-vitro transcription products made in nuclei isolated from untreated and auxin-treated soybean primary leaves and excised hypocotyl sections indicates that, for the two cDNA clones analyzed, the increased RNA levels in auxin-treated organs are at least partially the result of increased transcriptional activity of specific DNA sequences.

Key words: Auxin induced mRNA – cDNA clones – *Glycine* (auxin and gene expression) – Nuclear "run off" transcription – RNA blots.

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Abbreviations: cDNA = complementary DNA; 2,4-D-2,4-dichlorophenoxyacetic acid; IAA = indole-3-acetic acid; kb = kilobase; kbp = kilobase pair; poly(A)$^+$RNA = polyadenylated RNA; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid

Introduction

The mechanism by which the plant growth regulator, auxin, influences such diverse growth processes as cell elongation, cell division and cell differentiation is poorly understood. It has been well documented that long-term (12–48 h) exposure of intact plants and plant parts to an exogenous supply of auxin has dramatic effects on nucleic-acid and protein synthesis (for a review, see Baulcombe et al. 1981). There is, for example, a massive increase in RNA polymerase I activity and rRNA synthesis in auxin-treated soybean hypocotyl (Zurfluh and Guilfoyle 1981). In contrast, it has been shown by nucleic acid hybridization studies that the level of expression of the majority of the polyadenylated RNA (poly(A)$^+$RNA) sequences remains unchanged, with a limited number of sequences in the abundant class of poly(A)$^+$RNA shown to be modulated following auxin treatment (Baulcombe and Key 1980). The nucleic-acid hybridization data and a two-dimensional polyacrylamide gel analysis of in-vitro translation products show that this modulation results in both the reduction and increase in relative concentration of certain sequences within this poly(A)$^+$RNA population.

Recently, several studies have focused on the effects of short periods of exposure to auxin on gene expression. Using a two-dimensional gel analysis of in-vitro translation products, Zurfluh and Guilfoyle (1982a, b, c) have shown that within 15 min after auxin application, a specific set of translation products is induced in the soybean hypocotyl and maize coleoptile. Similar results have been obtained by Theologis and Ray (1982) using pea epicotyl. In addition, Zurfluh and Guilfoyle (1982a, b) observed that auxin induces a similar set of in-vitro translation products in both elongat-

ing and non-elongating excised soybean hypocotyl sections as well as in the hypocotyl from intact seedlings. These latter results suggest that some of the earliest auxin-specific alterations in gene expression may be common to organs that ultimately respond very differently to auxin application (e.g., cell division verses cell elongation).

The results of the in-vitro translation studies indicate that auxin may alter the amounts of specific translatable mRNAs. Using cDNA clone probes selected from a cDNA library prepared from RNA present in the elongating region of the soybean hypocotyl, Walker and Key (1982) have shown that the concentration of at least two poly(A)$^+$RNA species is increased rapidly in elongating soybean hypocotyl sections following auxin treatment. Preliminary results indicating that auxin rapidly induces specific mRNAs in pea stem tissue have also been reported (Theologis et al. 1983). In this paper, we report the isolation and characterization of four cDNA clones which were selected from a library of clones prepared from 2,4-dichlorophenoxyacetic acid (2,4-D)-treated soybean hypocotyl poly(A)$^+$RNA and which contain sequences homologous to auxin-responsive poly(A)$^+$RNAs. We show that the endogenous levels of the RNA sequences homologous to these clones and the rapid induction of RNA levels by auxin are similar in elongating and non-elongating excised soybean hypocotyl sections, and that these mRNAs are also rapidly induced by auxin in hypocotyls of intact seedlings. We also present data to demonstrate that, for two of the clones, increases in RNA levels following auxin treatment are regulated at the level of transcription in both hypocotyls and leaves (plumules). To our knowledge, our results provide the first direct evidence that applied auxins rapidly alter the transcriptional activity of specific DNA sequences transcribed by RNA polymerase II.

Material and methods

Plant material. Soybean seeds (*Glycine max* (L.) Merr., cv. Wayne; Illinois Seed, Wilken Seeds, Pontiac, Ill., USA) were germinated in a 1:1 (v/v) mixture of moistened vermiculite and Perlite (Silbrico Corp., Hodgkins, Ill., USA) in the dark at 30° C and watered daily. After 72 h, the seedlings were subjected to experimental manipulation. Procedures for spraying and isolation of hypocotyl from intact seedlings and the incubation of either elongating or basal hypocotyl sections were as described in Zurfluh and Guilfoyle (1982a, b). Intact seedlings were sprayed with solutions (pH 6) of 2,4-dichlorophenoxyacetic acid (2,4-D), indole 3-acetic acid [IAA], or 2,4,5-trichlorophenoxyacetic acid [2,4,5-T] at $2.5 \cdot 10^{-3}$ M; Ethephon solution (pH 6) was sprayed on intact seedlings at 1 mg/ml. The auxins 2,4-D and IAA, and Ethephon (2-chloroethylphosphonic acid) were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). The 2,4-D was recrystallized twice with benzene. The auxin 2,4-5-T was purchased from Pfaltz & Bauer (Stamford, Conn., USA). Following treatment of intact seedlings, the entire hypocotyl was excised (subsequently referred to as intact hypocotyl) and processed as described below for excised sections. Elongating sections were incubated in a 2,4-D solution (pH 6) at $5 \cdot 10^{-5}$ M or in an IAA solution (pH 6) at $2 \cdot 10^{-4}$ M; basal sections were incubated in 2,4-D at $2 \cdot 10^{-4}$ M. The auxin concentrations used in these studies were those used previously (Zurfluh and Guilfoyle 1982a, b) and that give the maximum cell expansion and cell division response in this soybean tissue. After treatment, tissues were rinsed in deionized H_2O, frozen in liquid N_2 and stored at $-70°$ C. For transcription studies, etiolated seedlings grown for 96 h were used to isolate basal hypocotyl sections; etiolated seedlings grown for 120 h were used to isolate primary leaves (plumules). Basal sections and leaves were incubated with or without 2,4-D ($5 \cdot 10^{-5}$ M) for 4 h. For isolation of nuclei, tissues were rinsed and processed immediately.

RNA isolation. Poly(A)$^+$RNA used to construct and screen the cDNA library was isolated as described by Zurfluh and Guilfoyle (1982a). RNA used for blotting was extracted by homogenizing frozen tissue in buffer containing 100 mM 2-amino-2(hydroxyethyl)-1,3-propanediol (Tris)-HCl (pH 8.8), 2% sodium dodecyl sulfate, 1 mM ethylenediaminetetraacetic acid. The homogenate was twice extracted with phenol chloroform *iso*-amyl alcohol (25:24:1, by vol), and isopropanol-precipitated. The nucleic-acid pellet was collected by centrifugation, resuspended and reprecipitated in ethanol prior to RNA purification through a CsCl cushion (Glisin et al. 1974). Poly(A)$^+$RNA was isolated by two rounds of oligo d(T)cellulose chromatography (Bantle et al. 1976).

cDNA library preparation and screening. Double-stranded cDNA was prepared essentially as described by Wickens et al. (1978) using RNA isolated from 2,4-D treated (2 h) intact hypocotyl as template for the synthesis. The ligation of Eco RI and Sal I linkers (Collaborative Research, Lexington, Mass., USA) to the cDNA and insertion into pBR322 was by the method of Kurtz and Nicodemus (1981). Transformation into *E. coli* strain LE392 (I. Rubenstein, University of Minnesota) was by the method of Kushner (1978). Transformants were plated onto nitrocellulose and filters prepared for in-situ colony hybridization (Grunstein and Hogness 1975). Replicas were hybridized with ^{32}P-labeled single-stranded cDNA (Baulcombe and Key 1980) or 5′-end-labeled [^{32}P]RNA (Maizels 1976) from either control (untreated) or 2,4-D-treated intact hypocotyl (differential hybridization). The cDNA library was also screened by making mini-plasmid preparations (Ish-Horowicz and Burke 1981), dot-blotting the DNA onto nitrocellulose (Kafatos et al. 1979), and subjecting the filters to differential hybridization using [^{32}P]cDNA probes.

Plasmid DNA isolation and labeling. Plasmid DNA contained in transformants selected for further analysis was isolated following chloramphenicol amplification, and purified through two rounds of CsCl-ethidium bromide centrifugation, essentially as described by Bolivar and Backman (1979). Treatment of the plasmid preparation with RNAase was omitted. Plasmid DNA was labeled by nick translation (Rigby et al. 1977) using [α-^{32}P]dCTP ($15 \cdot 10^5$ GBq $\simeq$ 400 Ci/mmol; Amersham, Arlington Heights, Ill., USA) to specific activities of $4 \cdot 10^7 - 10 \cdot 10^7$ cpm/μg DNA. To determine the size of the cDNA insert, plasmid DNA was cleaved with Eco RI and Sal I and fractionated by agarose gel electrophoresis.

RNA blot analysis. RNA was subjected to agarose gel electrophoresis in 6% formaldehyde (Rave et al. 1979) and transferred to nitrocellulose (Thomas 1980). RNA was "slot-blotted" (Schleicher & Schuell, Keene, N.H., USA) onto nitrocellulose using the denaturing conditions (formaldehyde) recommended by Schleicher & Schuell. Filters were prehybridized, hybridized, washed and exposed to X-ray film (Kodak X-omat AR; Eastman-Kodak, Rochester, N.Y., USA) for 12–36 h as described by Olszewski et al. (1982). RNA "slot-blot" filters were cut and the radioactivity associated with the hybrids on each slot was counted by liquid scintillation.

Nuclear transcription assays. Soybean nuclei were isolated from incubated leaves and basal sections according to Olszewski et al. (1982). To assay for transcription, nuclei were incubated for 1 h at 30° C in 10 mM 4-2(hydroxyethyl)-1-piperazine ethanesulfonic acid (Hepes) (pH 7.9), 12.5 mM $MgCl_2$, 200 mM $(NH_4)_2SO_4$, 2 mM dithiothreitol, 60 μM each of ATP, GTP and CTP, 10 μM [α-^{32}P]UTP ($3.75 \cdot 10^5$ or $7.5 \cdot 10^5$ GBq; Amersham), 25% glycerol and 75 units of placental ribonuclease inhibitor (Blackburn 1979). Leaf nuclear assays contained 40 μg nuclear DNA and basal section nuclear assays contained 20 μg nuclear DNA. Following incubation, the assays were processed by the method of Manley et al. (1983) and the [^{32}P]RNA hybridized to filter-bound plasmid DNA using conditions employed for the RNA blot analysis. Filters were washed, air-dried and subjected to autoradiography. Autoradiograms were scanned with a densitometer (Helena Laboratories, Quick Scan R&D, Beaumont, Tex., USA) and the area under the peaks determined using the MOP-AMO3 System (Carl Zeiss, Oberkochen, FRG). In other cases, hybridization was quantitated by liquid scintillation counting.

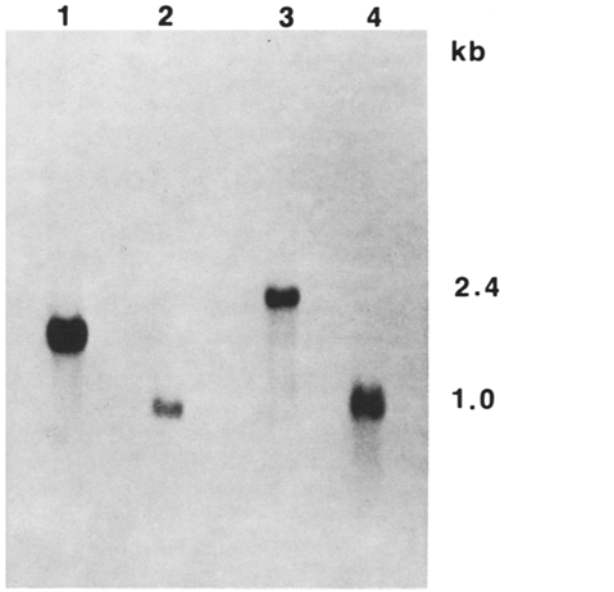

Fig. 1. Hybridization of labeled cDNA plasmids to RNA isolated from 2,4-D treated (2 h) intact soybean hypocotyl. Poly(A)$^+$RNA (5 μg for *lanes 1, 2* and *4*; 2.5 μg for *lane 3*) was fractionated by electrophoresis in a 6% formaldehyde/1.4% agarose gel, transferred to nitrocellulose, and hybridized to [^{32}P]cDNA plasmids 1) pGH1, 2) pGH2, 3) pGH3 and 4) pGH4. RNA size (*kb*) was determined by comparison of DNA and RNA markers run on the gel (pBR322 DNA digested with Alu I and Bgl I; soybean rRNA, TMV RNA and MS2 RNA) and stained with acridine orange

Results

Plasmid selection and RNA identification. A cDNA library of 2000 tetracycline-sensitive colonies was initially screened using the in-situ colony hybridization technique. This screening method proved unreliable and resulted in numerous "false positive" signals. The library was rescreened by the mini-plasmid preparation method from which four transformants were selected (designated pGH1, pGH2, pGH3, pGH4). The sizes of the cDNA inserts contained within these plasmids were 1.2 kilobase pairs (kbp) (pGH1), 0.2 kbp (pGH2), 1.3 kbp (pGH3) and 0.3 kbp (pGH4), and the size class of mRNA to which these clones hybridized was 1.7 kilobases (kb) (pGH1), 1.0 kb (pGH2), 2.4 kb (pGH3) and 1.1 kb (pGH4) (Fig. 1). As a control, a cDNA clone (pGH7) with an insert size of 0.4 kbp and which hybridized to an mRNA species (2.5 kb) that was not increased in concentration following auxin treatment was selected and used in the studies on RNA levels.

RNA levels in intact hypocotyl. Poly(A)$^+$RNA from intact soybean hypocotyl that had not been sprayed or had been sprayed for varying lengths of time with 2,4-D was "slot blotted" and hybridized to each of the labeled plasmids. The autoradiogram of the hybridization of pGH3 to RNA on the slot blot is shown in Fig. 2 (inset). To quantitate the relative amount of hybridization, the nitrocellulose slots were cut out and counted by liquid scintillation; the data for all four plasmids is plotted in Fig. 2. The concentration of the sequences in the unsprayed, intact hypocotyl (c) varies from an almost undetectable level for pGH2 and pGH3 to a relatively high concentration for pGH1. With a 2,4-D treatment for 0.5 h, the concentration of these sequences is increased for all four clones (two- to three fold for pGH1 and pGH4, six fold for pGH2, and 16-fold for pGH3). The response we observe with the clones is transient, with a maximum level of the sequences reached by 2–4 h following auxin application. RNA levels for all clones are decreased after 24 h of auxin treatment, even though the auxin-induced growth responses and increases in macromolecular synthesis (e.g., DNA, RNA protein) continue for an additional 24–48 h (Zurfluh and Guilfoyle 1981). The mRNA levels for pGH7 were unchanged during the first hour of 2,4-D treatment and declined with longer treatment.

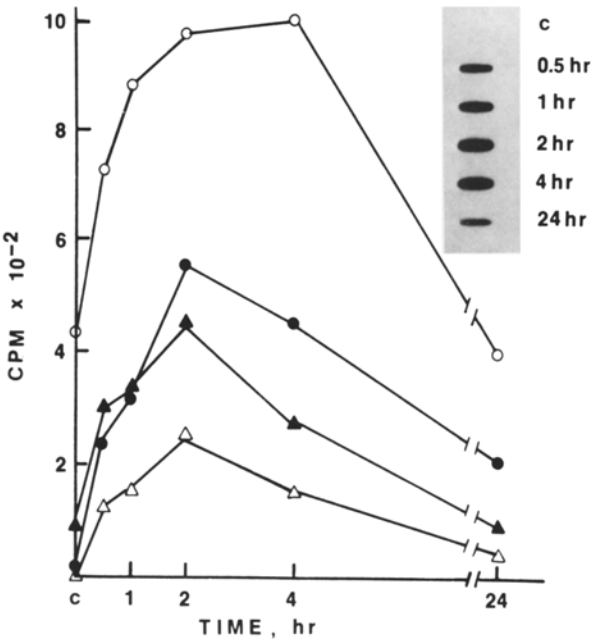

Fig. 2. Hybridization analysis of cDNA plasmids to RNA from untreated (*c*) and 2,4-D-treated intact soybean hypocotyl. Following treatment of the hypocotyl with 2,4-D (for 0.5, 1, 2, 4, 24 h), poly(A)$^+$RNA was extracted; two micrograms of RNA were denatured, "slot blotted" onto nitrocellulose and hybridized to labeled cDNA plasmids. The autoradiogram of the hybridization of pGH3 to the slot blot is shown (*inset*). Radioactivity associated with individual slots was determined by scintillation counting and the counts (the average of duplicate slots) plotted (○, pGH1; △, pGH2; ●, pGH3; ▲, pGH4)

RNA levels in excised sections. Sections (1.2 cm) from two distinct regions (elongating and basal) of the soybean hypocotyl were excised and incubated in the absence or presence of 2,4-D for varying lengths of time. Poly(A)$^+$RNA was isolated, "slot blotted" onto nitrocellulose and hybridized to labeled plasmid probes. The results for elongating sections are shown in Fig. 3A and for basal sections, Fig. 3B. The autoradiogram of the hybridization of pGH3 to the slot blot of elongating section RNA (Fig. 3A, inset) and basal section RNA (Fig. 3B, inset) is shown. The radioactivity associated with each slot was counted, and the data for all four plasmid probes are shown (Fig. 3A, B).

The endogenous level of RNA in elongating sections prior to any treatment varies from low for pGH2 and pGH3 to high for pGH1 (Fig. 3A, u). Sections were preincubated (p) for 2 h in a medium lacking 2,4-D to deplete the sections of endogenous auxin. Major changes in RNA levels were observed for pGH1 and pGH4 and minor changes were observed for pGH2 and pGH3 following the preincubation. After the addition of 2,4-D to the incubation medium for more than 0.5 h, the RNA levels for all four clones increased over preincubation levels (2–3 fold for pGH1, pGH2 and pGH4; 33 fold for pGH3). Fluctuations in RNA levels during the first 0.5 h of incubation in 2,4-D were observed for pGH1, pGH2 and pGH4. After 6 h incubation in 2,4-D, RNA levels declined for pGH1, reached a plateau for pGH2, and further increased for pGH3 and pGH4.

The RNA levels in unincubated basal sections (Fig. 3B, u) are similar to those observed in elongating sections. Preincubation of basal sections in medium lacking 2,4-D resulted in changes in RNA levels for pGH1, pGH2 and pGH4. Following preincubation, a 0.5-h incubation of basal sections in the presence of 2,4-D caused an increase in RNA levels for all four clones (two- to three fold for pGH1, pGH2 and pGH4; 98-fold for pGH3); the maximum level was reached with a 2-h incubation. The mRNA levels for pGH7 in basal and elongating sections were unchanged during the preincubation period and declined during the incubation in the presence of 2,4-D.

We have estimated the abundance of the auxin specific RNA sequences by a comparative hybridization analysis of the auxin-regulated cDNA clone pJCW1 (Walker and Key 1982) to the RNA samples used in this report. The RNA level for pJCW1 has been estimated to be 0.01% of the poly(A)$^+$RNA in unincubated, elongating hypocotyl sections (J.C. Walker, University of Georgia, Athens, USA, personal communication). Our results indicated that the amount of hybridization of pJCW1 to RNA from unincubated, elongating sections was only slightly less than that observed with RNA from elongating sections that had been preincubated (2 h) and then incubated in 2,4-D (2 h). Based on the RNA level for pJCW1 (0.01%) and the hybridization results obtained in these studies, we estimate that, at the maximum levels of hybridization after 2,4-D treatment (basal-section RNA, 2 h preincubation and 2 h 2,4-D incubation), pGH3 and pGH4 sequences each represent approx. 0.01% of the poly(A)$^+$RNA, while pGH1 and pGH2 sequences are present at three- and ten fold lower concentrations, respectively.

RNA levels with additional treatments. The levels of RNA sequences complementary to the four clones following treatment of intact hypocotyl with two additional auxins (IAA and 2,4,5-T) and one non-auxin plant growth regulator (Ethephon) were examined (Table 1). Application of IAA (4 h) caused an increase in the levels of the RNA sequences for all four clones relative to untreated

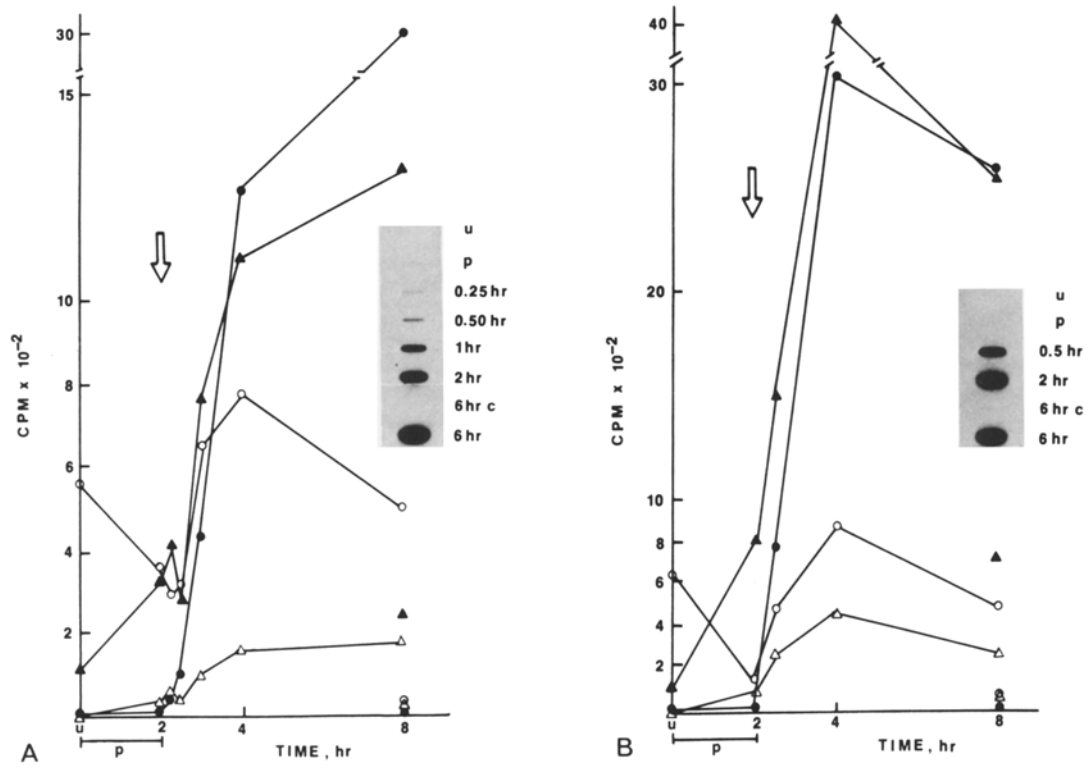

Fig. 3 A, B. Hybridization analysis of cDNA plasmids to RNA from unincubated (*u*), preincubated (*p*) and 2,4-D-treated elongating sections of soybean hypocotyl (**A**) and basal sections (**B**). All 2,4-D treated sections were preincubated (2 h) in the absence of 2,4-D prior to the start of treatment (*arrow*). Poly(A)$^+$RNA (2 μg) was blotted and hybridized as described in the legend of Fig. 2. The autoradiogram of the hybridization of pGH3 to the slot blot is shown (*inset*). Radioactivity associated with the hybrids was counted and plotted (○, pGH1; △, pGH2; ●, pGH3; ▲, pGH4). The unconnected symbols in **A** and **B** at 8 h show the level of hybridization of labeled cDNA plasmids to RNA extracted from sections that had been preincubated (2 h) and further incubated (6 h) in the absence of 2,4-D. The hybridization of pGH3 to the RNA from this control incubation is shown on the autoradiogram (*insets*, 6 h c)

Table 1. Ratio of RNA levels in treated intact hypocotyl to that of untreated hypocotyl of soybean. RNA levels were determined by hybridization of ^{32}P-labeled plasmid DNA to poly(A)$^+$RNA extracted from treated and untreated intact hypocotyl. The amount of radioactivity associated with the hybrids was determined by liquid scintillation counting

Clone	Treatment				
	2,4-D (2 h)	2,4,5-T (2 h)	2,4-D (4 h)	IAA (4 h)	Ethephon (4 h)
pGH1	2.2	2.8	2.2	1.6	0.6
pGH2	12.6	24.0	7.0	2.3	1.0
pGH3	37.0	64.0	30.0	16.0	0.8
pGH4	5.0	11.5	3.0	1.4	0.7

hypocotyl, although this increase was less than that observed after treatment with 2,4-D (4 h). Following treatment with 2,4,5-T (2 h), however, RNA levels for all clones were higher than those observed after similar treatment with 2,4-D (2 h). RNA levels decreased (pGH1, pGH3 and pGH4) or were unchanged (pGH2) relative to untreated hypocotyl following Ethephon treatment.

RNA levels of sequences complementary to the four clones were increased over control levels following incubation of elongating sections in IAA. With the exception of results obtained for pGH3, the RNA levels were lower than those observed with a similar treatment with 2,4-D.

Nuclear transcription studies. To determine whether the increase in amounts of auxin-induced mRNAs was regulated at the transcriptional level, we conducted "run-off" in-vitro transcription with isolated nuclei from untreated and 2,4-D-treated excised basal hypocotyl sections. Based on scintillation counting and densitometry, hybridization of the [^{32}P]RNA synthesized in vitro to blots of pGH3 and pGH4 DNA shows that there is several fold greater hybridization (15–20 fold for pGH3 and two- to three fold for pGH4) to the blots using transcripts from nuclei isolated from auxin treated sections than from nuclei isolated from untreated

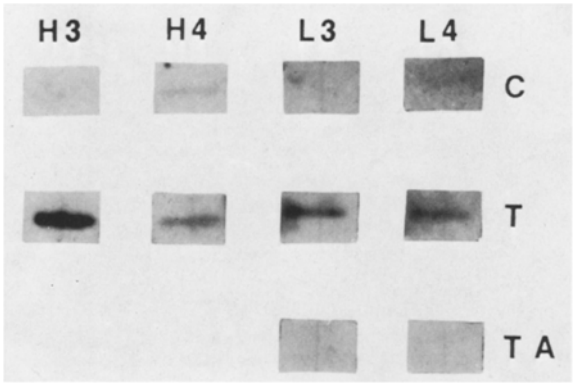

Fig. 4. Hybridization analysis of ^{32}P-labeled in-vitro "run-off" transcripts of nuclei from excised basal soybean hypocotyl sections (*H*) and leaves (*L*) to slot blots of cDNA plasmid clones pGH3 (*3*) and pGH4 (*4*). Nuclei isolated from untreated (*C*) or auxin-treated (*T*) basal hypocotyl sections or primary leaves were used in in-vitro transcription assays in the presence of [α-^{32}P]UTP. Transcripts were hybridized to 10 µg plasmid DNA (pGH3 or pGH4) which had been denatured and blotted onto nitrocellulose. Input counts for hybridization from hypocotyl nuclear assays were $2.3 \cdot 10^7$ cpm and $2.45 \cdot 10^7$ cpm for untreated and auxin-treated samples, respectively. Input counts for hybridization from leaf nuclear assays were $2.5 \cdot 10^7$ cpm and $1 \cdot 10^7$ cpm for untreated and auxin-treated samples, respectively, and $3.2 \cdot 10^6$ cpm from auxin-treated leaf nuclear assays performed in the presence of 1 µg/ml α-amanitin (*TA*)

sections (Fig. 4). Similar results were found with nuclei isolated from plumules which were incubated in the presence or absence of 2,4-D (Fig. 4). For clones pGH3 and pGH4, we conclude that the increased amounts of mRNA present in auxin treated hypocotyl and plumules are, at least in part, the result of increased transcriptional activity of these DNA sequences. RNA polymerase II is responsible for this transcription based on α-amanitin (Boehringer Mannheim, Indianapolis, Ind., USA) inhibition of the hybridization signal (Fig. 4). Results with intact soybean hypocotyl treated with 2,4-D for 2 h indicate that DNA sequences corresponding to pGH1 and pGH2 are also under transcriptional control.

Discussion

From a library of cDNA clones prepared from poly(A)$^+$RNA from auxin-treated intact soybean hypocotyls, we have selected four independent clones which contain sequences homologous to auxin-responsive RNAs. Application of 2,4-D to intact soybean hypocotyl and excised hypocotyl sections results in a rapid increase in the levels of the RNA sequences homologous to the cDNA clones. In the intact hypocotyl, the RNA levels reach a maximum within 2–4 h after auxin application and decline thereafter. Two other auxins, IAA and 2,4,5-T, cause an increase in the levels of RNA sequences homologous to the clones. Based on the finding that treatment of intact soybean hypocotyl with either auxin or ethylene results in increased amounts of a similar set of in-vitro translation products, while another set of translation products is specifically induced by auxin in all zones of the hypocotyl (Zurfluh and Guilfoyle 1982b), we examined RNA levels following treatment with Ethephon (an ethylene-producing compound). Our results indicate that Ethephon does not cause an increase in levels of the RNA sequences homologous to the clones.

The auxin-responsive cDNA clones described in this report do not share sequence homology to the clones described by Walker and Key (1982), pJCW1 and pJCW2. In addition, while the endogenous levels of the RNA sequences homologous to pJCW1 and pJCW2 were shown to be higher in elongating hypocotyl sections than in non-elongating (mature) sections (Walker and Key 1982), the endogenous levels of the RNA sequences homologous to the clones described in this paper are approximately equivalent in sections excised from different regions of the hypocotyl (slightly higher levels in basal sections). Thus, these sequences may represent a portion of the group of auxin-induced mRNAs observed by Zurfluh and Guilfoyle (1982a, b) which, by in-vitro translation analysis, appeared to be induced at equivalent levels in elongating and basal regions of the hypocotyl.

The changes in RNA levels that are observed following auxin application could be the result of regulation at one or more stages of mRNA synthesis and accumulation, including the transcription of a nuclear precursor mRNA, the posttranscriptional processing, transport of the RNA, or the stability of the mature mRNA. The results from the nuclear transcription studies presented here indicate that, for two of the clones (pGH3 and pGH4), regulation is, at least in part, at the transcriptional level. This result demonstrated that the plant hormone, auxin, can rapidly alter gene expression at the transcriptional level.

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References

Bantle, J.A., Maxwell, I.A., Hahn, W.E. (1976) Specificity of oligo (dT)-cellulose chromatography in the isolation of polyadenylated RNA. Anal. Biochem. **72**, 413–427

Baulcombe, D.C., Key, J.L. (1980) Polyadenylated RNA sequences which are reduced in concentration following auxin treatment of soybean hypocotyls. J. Biol. Chem. **255**, 8907–8913

Baulcombe, D.C., Kroner, P.A., Key, J.L. (1981) Auxin and gene regulation. In: Levels of genetic control in development, pp. 83–97, Subtelny, S., Abbott, U.K., eds. Liss, New York

Blackburn, P. (1979) Ribonuclease inhibitor from human placenta: rapid purification and assay. J. Biol. Chem. **254**, 12,484–12,487

Bolivar, F., Backman, K. (1979) Plasmids of *Escherichia coli* as cloning vectors. Methods Enzymol. **68**, 245–267

Glisin, V., Crkvenjakov, R., Byrus, C. (1974) Ribonucleic acid isolated by cesium chloride centrifugation. Biochemistry **13**, 2633–2637

Grunstein, M., Hogness, D. (1975) Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. USA **72**, 3961–3965

Ish-Horowicz, D., Burke, J.F. (1981) Rapid and efficient cosmid cloning. Nucl. Acids Res. **9**, 2989–2998

Kafatos, F.C., Jones, W.C., Efstratiadis, A. (1979) Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. Nucl. Acids Res. **7**, 1541–1552

Kurtz, D.T., Nicodemus, C.F. (1981) Cloning of α_{2u} globulin cDNA using a high efficiency technique for the cloning of trace messenger RNAs. Gene **13**, 145–152

Kushner, S.R. (1978) An improved method for transformation of *Escherichia coli* with ColEl derived plasmids. In: Genetic engineering, pp. 17–23, Boyer, H.W., Nicosia, S., eds. Elsevier, New York

Maizels, N. (1976) Dictyostelium 17S, 25S, and 5S rDNA lie within a 38,000 base pair repeated unit. Cell **9**, 431–438

Manley, J.L., Fire, A., Samuels, M., Sharp, P.A. (1983) In vitro transcription: whole-cell extract. Methods Enzymol. **101**, 568–582

Olszewski, N.E., Hagen, G., Guilfoyle, T.J. (1982) A transcriptionally active, covalently closed minichromosome of cauliflower mosaic virus DNA isolated from infected turnip leaves. Cell **29**, 395–402

Rave, N., Crkvenjakov, R., Boedtker, H. (1979) Identification of procollagen mRNAs transferred to diazobenzyloxymethyl paper from formaldehyde agarose gels. Nucleic Acids Res. **6**, 3559–3567

Rigby, P.W.J., Dieckmann, M., Rhodes, C., Berg, P. (1977) Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**, 237–251

Theologis, A., Ray, P.M. (1982) Early auxin-regulated polyadenylated mRNA sequences in pea stem tissue. Proc. Natl. Acad. Sci. USA **79**, 418–421

Theologis, A., Huynh, T., Davis, R.W. (1983) Isolation of DNA sequences complementary to early auxin-regulated mRNAs by differential plaque filter hybridization. (Abstr.) Plant Physiol. **72**, Suppl., 3

Thomas, P.S. (1980) Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**, 5201–5205

Walker, J.C., Key, J.L. (1982) Isolation of cloned cDNAs to auxin-responsive poly(A)$^+$RNAs of elongating soybean hypocotyl. Proc. Natl. Acad. Sci. USA **79**, 7185–7189

Wickens, M.P., Buell, G.N., Schimke, R.T. (1978) Synthesis of double-stranded DNA complementary to lysozyme, ovomucoid, and ovalbumin mRNAs. J. Biol. Chem. **253**, 2483–2495

Zurfluh, L.L., Guilfoyle, T.J. (1981) Auxin-induced nucleic acid and protein synthesis in the soybean hypocotyl. In: Levels of genetic control in development, pp. 99–118, Subtelny, S., Abbott, U.K., eds. Liss, New York

Zurfluh, L.L., Guilfoyle, T.J. (1982a) Auxin-induced changes in the population of translatable messenger RNA in elongating sections of soybean hypocotyl. Plant Physiol. **69**, 332–337

Zurfluh, L.L., Guilfoyle, T.J. (1982b) Auxin- and ethylene-induced changes in the population of translatable messenger RNA in basal sections and intact soybean hypocotyl. Plant Physiol. **69**, 338–340

Zurfluh, L.L., Guilfoyle, T.J. (1982c) Auxin-induced changes in the population of translatable messenger RNA in elongating maize coleoptile sections. Planta **156**, 525–527

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