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Auxin and Growth Regulation of Rice Coleoptile Segments[1]

Molecular Analysis

Diego Breviario*, Silvia Giani[2], Paolo Di Vietri, and Immacolata Coraggio

Istituto Biosintesi Vegetali, Consiglio Nazionale delle Ricerche, Via Bassini 15, 20133 Milano, Italy

ABSTRACT

Auxin-stimulated elongation of apical segments of rice (*Oryza sativa* L. cv Arborio) coleoptiles occurring in the first 4 hours of treatment has been studied. Cell extension promoted in the first 2 hours by 10 micromolar indole-3-acetic acid (IAA) is specifically auxin-dependent, whereas after 4 hours, elongation also depends on endogenous production of ethylene. Similar to other systems, rice coleoptile cell elongation stimulated by auxin requires continuous synthesis of RNA and protein. Two-dimensional gel analysis of the *in vitro* translation products obtained from polyadenylated RNAs extracted from treated and untreated segments after 1 or 4 hours from the initial addition of IAA shows few transcriptional differences. At 60 minutes of treatment, the level of three mRNAs coding for proteins of 22.5, 25, and 33 kilodaltons was moderately enhanced while the disappearance of a 38 kilodalton translation product was observed. Additional repression of another mRNA coding for a 28 kilodalton product begins to show by this time, but becomes more evident after 4 hours treatment. At 4 hours, four IAA-specific mRNA enhancements coding for proteins with molecular masses ranging between 35 to 40 kilodaltons were also observed. We discuss these data in relation to the possible involvement of IAA-mediated transcriptional regulation in growth promotion of rice coleoptiles and, more widely, in control of cell elongation.

Auxin-stimulated cell elongation has been intensively studied for many years with auxin-responsive stem sections such as corn coleoptiles, soybean hypocotyls, and pea epicotyls (reviewed in refs. 2, 7, 9). The response of many types of stem and hypocotyl cells to auxin is biphasic. The initial phase of the response is similar to growth that can be induced by low pH, and the second phase has properties similar to other long-term auxin responses such as cell division, differentiation, and morphogenesis (6). The first phase is associated with cell wall loosening that is likely induced by an acidification mechanism, which, in turn, is followed by the deposition of new cell wall material (25, 29).

The molecular mechanism by which auxin causes cell extension is unknown. Different models have been proposed in which auxin could either directly activate a membrane-bound H^+ pump or cause H^+ extrusion by a mechanism requiring the synthesis of specific proteins, or regulate the amount of proteins affecting cell wall rigidity (19, 25).

The requirement of continuous protein and RNA biosynthesis for auxin-stimulated cell elongation is well documented (13, 19). Two-dimensional gel electrophoresis analysis of *in vitro* translation products encoded by mRNA extracted shortly (30–60 min) after auxin treatment of elongating etiolated segments of pea or soybean has revealed several transcripts whose amount is specifically enhanced by the hormone (25, 30). Some of the genes coding for these transcripts have been cloned recently and shown to be subject to rapid (5–30 min), auxin-dependent transcriptional regulation (4, 8, 16, 18). However, their specific function in the first phase of auxin-stimulated cell elongation has yet to be shown. In addition, and in apparent contrast with the theory of elongation based upon auxin-mediated transcriptional enhancement, recent findings show that previously described early mRNA increases are not specific for regions undergoing cell extension (5, 17).

Auxin stimulates the elongation of intact rice coleoptiles as well as segments (12, 21). IAA-induced elongation of rice coleoptiles is regulated by complementation between a direct effect of auxin and an indirect effect via ethylene produced in response to IAA (11, 12). The selective contribution of IAA and ethylene to elongation can be resolved with inhibitors of ethylene production such as AVG[3] or aminooxyacetic acid (11, 12). In this way, it was shown that the effects of IAA and ethylene on rice coleoptile elongation were additive, that the two hormones act independently of each other, and that ethylene-stimulated elongation begins a few hours after the initial addition of auxin. This positive growth response to ethylene was also observed in other aquatic plants (24).

Rice coleoptiles are of special interest from the point of view of auxin action because two binding sites for the hormone were recently identified in the plasma membrane (27) and a GTP-binding activity shown to be activated in crude membrane preparations treated with IAA (28). Furthermore, it has been proposed that the remarkable ability of rice coleoptiles to grow under water at low oxygen concentrations

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[2] Present address: Istituto Tecnologie Biomediche Avanzate, C.N.R., Via Ampère 56, 20131 Milano, Italy.

[3] Abbreviations: AVG, aminoethoxyvinylglycine; 1 or 2NAA, α- or βnaphthaleneacetic acid; AcD, actinomycin D; 5-FU, 5 fluorouracil; CH, cycloheximide; IEF, isoelectric focusing.

may be due to abnormally high levels of endogenous auxin that accumulate under these conditions (26).

In an effort to broaden our understanding of auxin-stimulated elongation of rice coleoptiles, we have begun a molecular analysis of this event. Here we show that, as in other well-characterized systems, continuous RNA and protein synthesis are required. We also identified, in accordance with other reports, two phases of the elongation response: the first (up to 2 h from the initial addition of IAA), which is solely dependent on auxin, and the second (between 2 and 4 h), in which elongation was also ethylene dependent. In relation to these two phases, we show a pattern of differential expression in the *in vitro* translation products obtained from RNA extracted at 1 or 4 h after the addition of 10 μM IAA. Transcriptional regulation involving IAA-mediated enhancement or repression of specific mRNAs is shown.

MATERIALS AND METHODS

Plant Material

The dehulled seeds of rice (*Oryza sativa* L. cv Arborio) were surface-sterilized for 2 min with 70% (v/v) ethanol and for 30 min with commercial diluted (1:5, v/v) NaClO, each treatment being followed with several rinses with sterile distilled water. Germination was carried out for 60 h on sterile wet Whatman 3MM paper in Petri dishes at 29°C in the dark.

Coleoptiles were cut 7 or 10 mm from the apices, including the tips, under dim green light and placed in Heller's incubation medium (Sigma). Microbial contamination was checked by incubating an aliquot of the medium on an appropriate agar-nutrient plate for yeast and bacterial cells (15, 23). Data from contaminated samples were discarded.

Growth of Coleoptile Sections

Excised coleoptile sections were preincubated for at least 2 h in Heller's medium to let them recover from the cut damage. For growth measurements, 25 sections in 20 mL Heller's medium were incubated in 250 mL flasks at 29°C in the dark with continuous shaking. For RNA extractions, 500 sections in 40 mL medium were used. The different concentration of rice segments in the medium did not influence the elongation response. Experiments were carried out with and without 50 μg/mL chloramphenicol, and the results obtained were identical in both conditions.

After preincubation, coleoptile segments were treated with IAA and the different compounds described in the "Results" section. At the selected time, the length of coleoptiles was measured. None of the treatments affected viability of the coleoptile sections, as ascertained by staining the tissues with tetrazolium salts. Each growth experiment was repeated at least twice. Statistical analysis was performed using an Asystant program (Asystant Software Tecnologies) based on Student's *t* distribution with a level of significance of 1%.

For RNA extractions, at the end of the incubation period the coleoptile sections were collected onto a Nalgene filter unit, rinsed, and immediately frozen in liquid nitrogen. Filtered medium was checked for microbial contamination. Sections were stored at −70°C until utilized for RNA extractions.

Isolation of RNA

The protocol of Chomczynski and Sacchi (3) for single-step isolation of total RNA from animal cells was adapted for the extraction of total RNA from plant tissues. Briefly, 5 g of frozen segments were ground to a fine powder in a prechilled mortar and subsequently transferred to a 50 mL polypropylene tube containing 10 mL solution D (4 M guanidium thiocyanate, 25 mM sodium citrate, pH 7, 0.5% sarkosyl, and 0.1 M 2-mercaptoethanol). Once the mixture was thawed, 1 mL 2 M sodium acetate, 10 mL water-saturated phenol, and 2 mL chloroform-isoamyl alcohol mixture (49:1, v/v) were sequentially added. The final suspension was shaken vigorously for 1 min and cooled on ice for 15 min. After centrifugation at 10,000*g* for 20 min at 4°C, RNA present in the aqueous phase was precipitated by the addition of an equal volume of isopropanol. Final resuspension of total RNA was in a few hundreds of a microliter of sterile distilled water.

Polyadenylated RNA was prepared by oligo(dT)-cellulose chromatography (LKB-Pharmacia, type 7). Total RNA solution and oligo-dT were equilibrated in 1 × KET buffer (0.5 M KCl, 10 mM Tris, pH 7.5, 1 mM EDTA). RNA samples were heated at 68°C for 3 min, quickly chilled, then loaded on the oligo-dT column. Samples were passed through the column two times, and the column was washed with 1 × KET buffer until poly(A)$^-$ RNA was completely eluted out. Afterward, an elution buffer (10 mM Tris, pH 7.5, 1 mM EDTA) was applied to the column and 0.5 mL fractions were collected. Absorbance measurements at 260 nm were made to locate the poly(A)$^+$ RNA, and this RNA was precipitated in the presence of 0.3 M potassium acetate (pH 5.4) and two volumes of ethanol at −70°C. For use in translation assays, polyadenylated RNA was dissolved in sterile distilled water. Some of the poly(A)$^+$ RNA preparations were obtained by passing the RNA sample two times through the oligo-dT column. *In vitro* translation results obtained from these preparations were identical to the ones obtained from a single oligo-dT step.

In Vitro Translation

One microgram of polyadenylated RNA was added to a wheat germ lysate (Promega) and reactions were carried out according to the supplier's directions, with 50 mM potassium acetate as the optimum potassium concentration. Labeling was done with [^{35}S]methionine (>1000 Ci/mmol) at a final concentration of 500 μCi/mL. To determine incorporation into polypeptides, aliquots were spotted onto Whatman No. 1 filters that were at first treated for 15 min with 5% TCA at 4°C, then boiled 15 min in 5% TCA plus 3% casamino acids, and sequentially washed with 5% TCA, ethanol, and acetone. Filters were dried and placed in scintillation fluid.

Electrophoresis

Two-dimensional PAGE with IEF in the first dimension and SDS in the second dimension was performed according to directions suggested in a Bio-Rad laboratory manual for protein electrophoresis that are based on the method of O'Farrell *et al.* (20) The IEF sample buffer contained ampholines with pH range 3.5 to 10. *In vitro* translation aliquots contain-

ing 7.5 × 10^5 TCA-precipitable cpm were loaded on the first dimension gel in any given experiment. Gels were run with 6 mM phosphoric acid as anode buffer and 100 mM NaOH as cathode buffer. IEF was carried out at 400 V for 12 h followed by 2 h at 800 V constant voltage. Extruded tube gels were then equilibrated for 30 min in SDS sample buffer (14) and either stored at −20°C or immediately loaded on the second dimension SDS-polyacrylamide gel. ^{14}C-labeled mol wt standards were also loaded. Second dimension electrophoresis was performed as described by Laemmli (14), in which 12% (w/v) polyacrylamide resolving gels were used. Electrophoresis was for 14 h at 10 mA. Radioactive proteins were visualized by fluorography using Kodak XAR-5 film after treatment of the gel with 20% (w/v) 2,5-diphenyloxazole in DMSO.

RESULTS

Effect of IAA on the Elongation of Rice Coleoptile Segments

Application of 10 μM IAA to apical segments of rice coleoptiles caused a significant growth increment after 2 h of treatment in comparison with untreated control coleoptiles (Fig. 1). Growth increments induced by exogenous application of 10 μM IAA to sections with or without tips were similar after either 2 or 4 h of incubation, suggesting that if any endogenous free auxin was still present in the sections with tips, its contribution to cell elongation was negligible (data not shown). In accordance with previous data, dose-response experiments showed that 10 μM IAA was a suboptimal concentration in the promotion of coleoptile elongation (data not shown) (21).

The elongation response was specific for auxin as a similar increase in length was obtained when coleoptile segments were treated with 50 μM of the synthetic auxin 1NAA (Fig. 2A). Furthermore, no effect on elongation was seen when the stems were treated with a precursor of IAA biosynthesis such as tryptophan or the weak anti-auxin 2NAA (Fig. 2B).

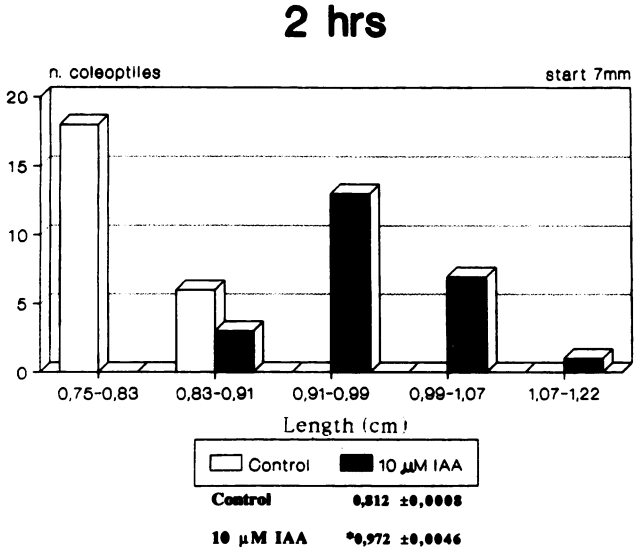

Figure 1. Effect of IAA application on length distribution of apical segments of rice coleoptiles. Length measured 2 h after addition of 10 μM IAA. The values are the means ± SE. An asterisk (*) indicates a significant difference ($P < 0.01$) compared with untreated control.

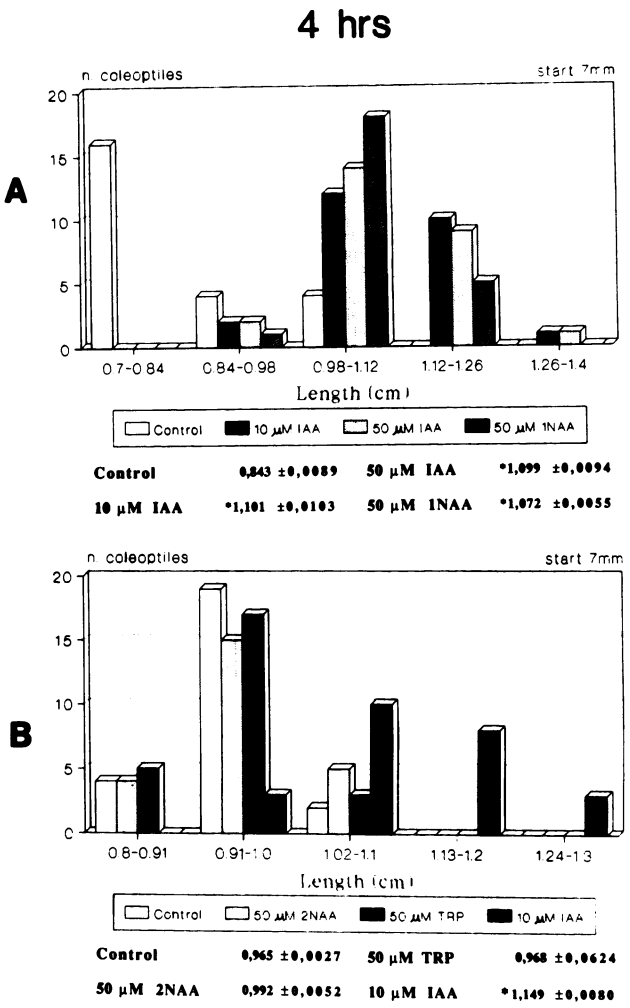

Figure 2. Effect of auxin analogs on growth of apical segments of rice coleoptiles. Length measured 4 h after addition of the different auxin-like compounds. The values are the means ± SE. An asterisk (*) indicates a significant difference ($P < 0.01$) compared with untreated control.

It has been previously reported (11, 12) that auxin-stimulated elongation of rice coleoptiles partially depends on the production of ethylene that is induced by IAA. This dependence was also shown using specific inhibitors of ethylene synthesis such as AVG.

To ascertain the possible contribution of ethylene to rice coleoptile elongation during the first hours after the addition of auxin, we measured the length of the stems after 2 and 4 h of simultaneous treatment with 10 μM IAA and 1 μM AVG.

As shown in Figure 3, the presence of AVG did not reduce elongation of IAA-treated segments after 2 h growth. At this time, the length of IAA and IAA + AVG-treated sections was similar and significantly higher than that observed in the untreated control (Fig. 3). On the contrary, AVG did affect IAA-stimulated elongation after 4 h treatment (Fig. 3). In

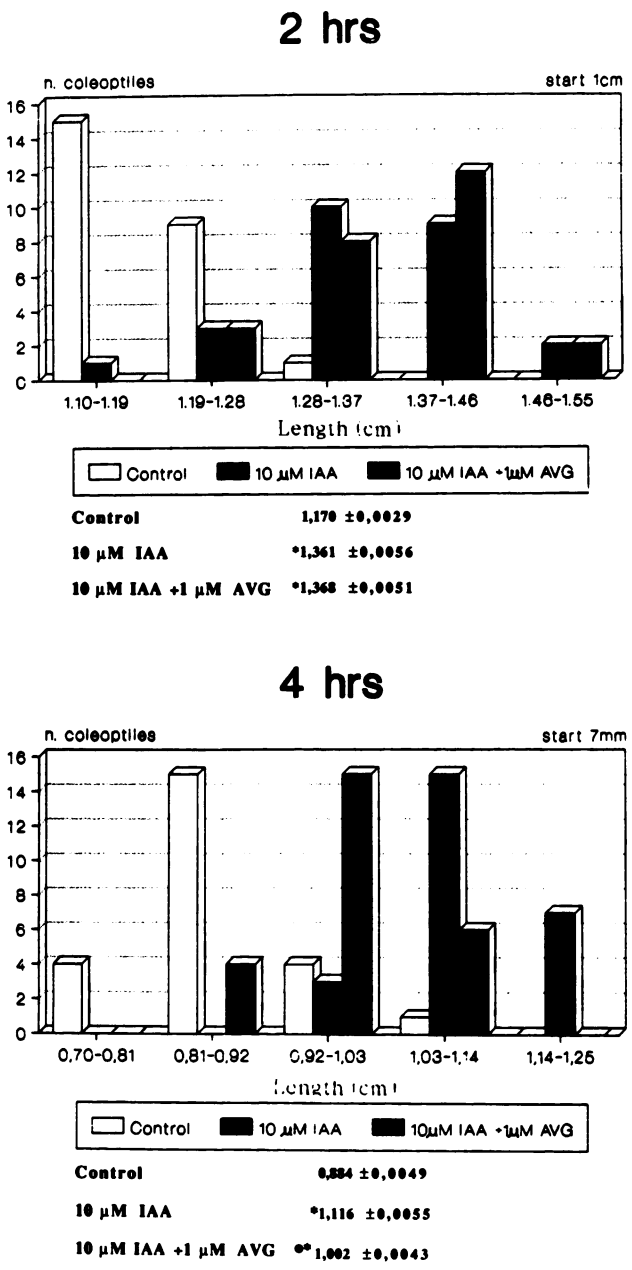

Figure 3. Effect of AVG on elongation of apical coleoptile segments. Length measurements were taken after 2 or 4 h from the initial addition of 10 μM IAA and 1 μM AVG. The values are the means ± SE. An asterisk (*) indicates a significant difference ($P < 0.01$) compared with control; ● indicates a statistically relevant difference ($P < 0.01$) compared with IAA-treated segments.

fact, a 50% decrease in the length of IAA-treated segments was observed when 1 μM AVG was also present.

Consistent with these data was the finding that 100 μM aminocyclopropane-l-carboxylic acid (a precursor of ethylene biosynthesis) promoted rice coleoptile elongation after 4 h treatment, whereas no effect was observed at shorter times (data not shown).

Altogether, these data suggest that ethylene, produced in response to exogenously applied 10 μM IAA, begins to stimulate elongation of apical segments of rice coleoptiles, but not before 2 h from the initial addition of auxin.

Requirement of RNA and Protein Biosynthesis for IAA-Induced Elongation of Apical Segments of Rice Coleoptiles

A requirement of RNA and protein synthesis for continued cell elongation of excised plant tissues treated with auxins is well documented (19). Most of these investigations have been performed with inhibitors of the synthesis of RNA and protein. Similar studies have not been performed with elongating rice coleoptiles.

To understand if and how elongation of rice coleoptiles stimulated by IAA depends on RNA and protein biosynthesis, we carried out the experiments shown in Figures 4 and 5. First, we measured the effect of inhibitors of RNA synthesis such as AcD and 5-FU on IAA-stimulated elongation of rice coleoptile segments. AcD is a potent and more wide-spectrum inhibitor compared with 5-FU, which has a weaker inhibitory effect on the synthesis of mRNA.

Increasing concentrations of AcD, added simultaneously with 10 μM IAA, progressively reduces IAA-stimulated elongation of rice coleoptiles measured after 4 h from the initial addition of auxin. The most effective concentrations of AcD, 1 and 5 μg/mL, caused 40 and 65% decreases of the length increment observed in segments treated only with IAA (Fig. 4A). When AcD was added 2 h before the addition of IAA, auxin-promoted elongation of the coleoptiles was strongly suppressed (Fig. 4C). At the higher concentration (5 μg/mL), rice coleoptiles grew even less than the untreated control. Addition of AcD after 2 h auxin treatment significantly reduced the length, measured after an additional 3 h growth, of IAA-treated segments (Fig. 4C).

On the other hand, when 5-FU was used as an inhibitor of RNA biosynthesis, a smaller inhibitory effect on IAA-stimulated elongation of coleoptile segments was observed (Fig. 4B). In particular, treatment with 1 μg/mL 5-FU had no effect, whereas a 5 μg/mL concentration reduced only 22% the length increment observed in IAA-treated coleoptiles. No relevant inhibitory effect on elongation was observed when 5 μg/mL 5-FU was added 2 h after the initial addition of 10 μM IAA and length measurements taken after 3 h additional growth (data not shown).

Altogether, these data indicate that early IAA-stimulated elongation of rice coleoptiles depends on continuous RNA biosynthesis and suggests a more strict requirement for mRNA synthesis. This conclusion is consistent with all the data so far collected on auxin-stimulated elongation in many other excised plant tissues.

In these systems, a requirement for continuous protein

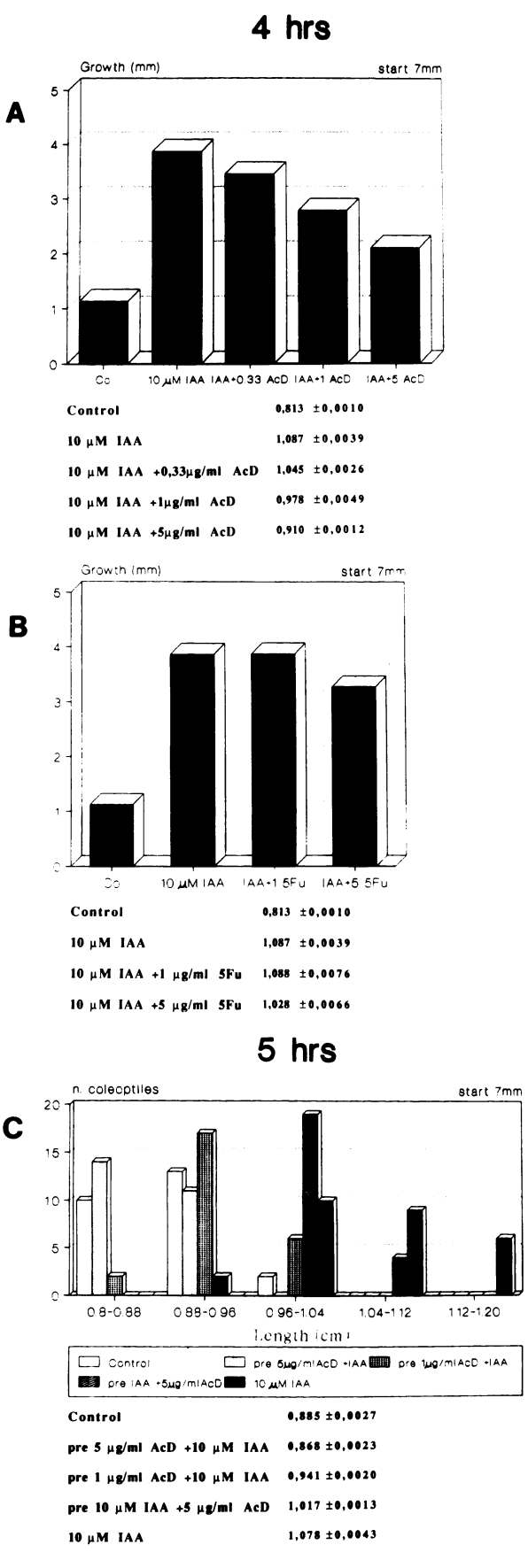

Figure 4. Effect of RNA synthesis inhibitors on IAA-stimulated growth of rice coleoptile sections. Panels A and B show the effect on the average length increment of coleoptile sections treated for 4 h with 10 µM IAA in the presence of increasing amounts of AcD (A) or 5-FU (B). Panel C shows the length distribution of coleoptile sections after 5 h of the following: untreated control; 2 h pretreatment with either 5 or 1 µg/mL AcD followed by application of 10 µM IAA and 5 h of additional growth (pre AcD); 2 h treatment with 10 µM IAA followed by 3 h additional growth in the presence of 5 µg/mL AcD (pre IAA); 5 h treatment with 10 µM IAA. The values are the means ± SE.

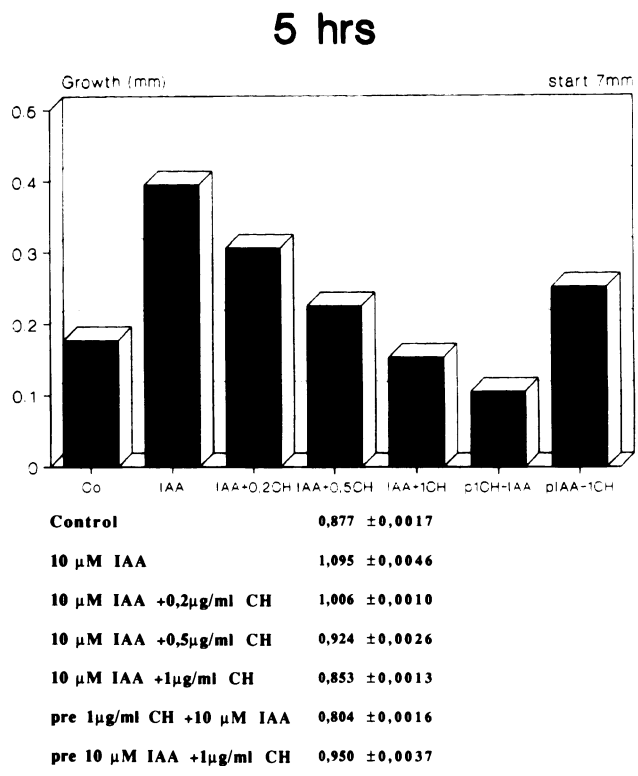

Figure 5. Inhibition by CH of auxin-induced growth in elongating rice coleoptile sections. Effect of increasing amounts of CH on average length increments measured after 5 h from the addition of 10 µM IAA is shown. Pretreatments (p) were as followed: 2 h 1 µg/mL CH followed by 5 h growth in the presence of 10 µM IAA (pre CH) or 2 h growth with IAA followed by application of 1 µg/mL CH and 3 h additional growth (pre IAA). The values are the means ± SE.

synthesis has also been shown. Typically, elongating sections treated with CH, an inhibitor of cytoplasmic protein synthesis, show a parallel inhibition of growth and protein biosynthesis (19).

Similarly, addition of various amount of CH from 0.2 to 1 µg/mL progressively decreases IAA-stimulated elongation of apical segments of rice coleoptiles (Fig. 5). A 40% inhibition was already observed with 0.2 µg/mL CH, whereas 1 µg/mL dramatically affected elongation to yield segments with a length even lower than the one observed in the untreated control. A 2 h pretreatment with 1 µg/mL CH completely blocks any effect of IAA on elongation. Furthermore, addition

of CH 2 h after the initial incubation with 10 μM IAA dramatically suppresses growth of elongating coleoptiles after 3 h additional time; their length increment is reduced by 70%.

IAA Effects on Translatable mRNAs Extracted from Rice Coleoptiles after 1 and 4 h Treatment

Because of the requirement for mRNA and protein synthesis, and similar to the work done very extensively on elongating soybean or pea stems, we looked for possible differences in the population of mRNAs extracted from rice coleoptile segments at a relatively short time (60 min) after the initial addition of IAA. This time is critical for two reasons. First, in any of the systems so far studied, full auxin-dependent induction of early-mRNAs synthesis always occurs by 60 min. Therefore, even if the latent period between IAA addition and elongation is not known for rice coleoptiles, it is likely that any early transcriptional change possibly associated with cell extension would be detected by 60 min. Second, on the basis of our data (see Fig. 3), and in agreement with previous reports on the kinetics of ethylene production induced by IAA, any transcriptional change detected at this time should be specifically ascribed to auxin.

A careful cross-examination of two-dimensional gels of the *in vitro* translation products obtained from polyadenylated RNA isolated from elongating rice coleoptiles that were treated or not treated for 60 min with 10 μM IAA reveals few differences in the level of specific transcripts. More precisely, only three translational products (O, F, and A) with molecular masses of 25, 22.5, and 33 kD, respectively, showed moderately increased levels (Fig. 6), and these differences were maintained after 4 h of IAA treatment (Fig. 7).

On the other hand, there is a strong decrease in a 38 kD protein (q) and the decrease was maintained after 4 h hormonal treatment (Fig 7). In addition, IAA reduction of another mRNA coding for a protein of 28 kD (d) began to be visible. The amount of this mRNA was further reduced after 4 h treatment (Fig. 7).

Next, we compared by two-dimensional gel analysis the pattern of the *in vitro* translational products obtained from mRNAs extracted from rice coleoptiles treated or not treated for 4 h with 10 μM IAA. At this time, an effect on elongation caused by endogenous production of ethylene was evident (Fig. 3).

As shown in Figure 7, the amount of four additional mRNAs coding for proteins G, H, T, and P with molecular masses ranging between 35 and 40 kD was significantly enhanced.

Few other differences that might be found by comparing panels A and B of Figures 6 and 7 were not confirmed in replicate experiments.

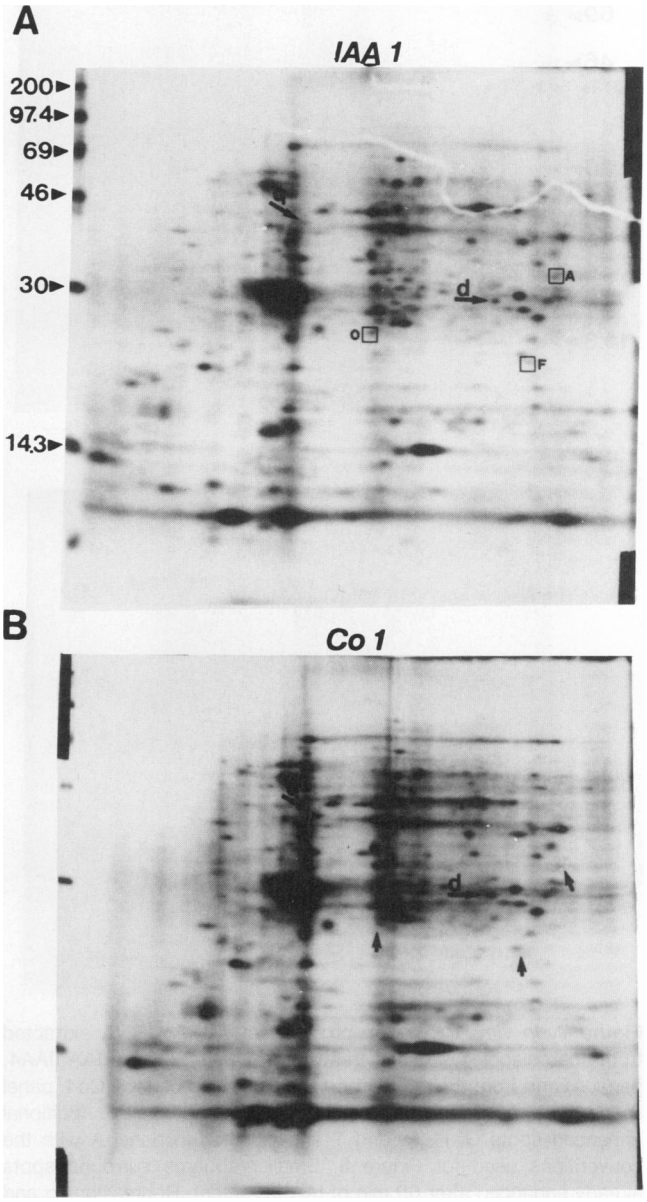

Figure 6. Fluorograph of labeled *in vitro* translation products of polyadenylated RNAs extracted from rice coleoptile sections treated for 60 min with 10 μM IAA (IAA1, panel A) and from their corresponding untreated control (Co1, panel B). pH gradient (first dimension), from acidic (pH 3.5) to basic (pH 10), runs from left to right and the mol wt gradient (second dimension) runs from bottom to top as shown by the mol wt standards run at the left of the gels. Enhanced spots (A, F, and O) are surrounded by a square and their corresponding positions in the control gel are indicated by short arrows. Repressions q and d occurring after IAA treatment are shown by longer arrows on both gels.

DISCUSSION

We have undertaken a study aimed at understanding the molecular mechanisms possibly involved in auxin-stimulated elongation of apical sections of rice coleoptiles.

First, we confirmed and extended previous physiological data. We detected a specific auxin-dependent elongation response within 2 h of treatment with 10 μM IAA. This time of

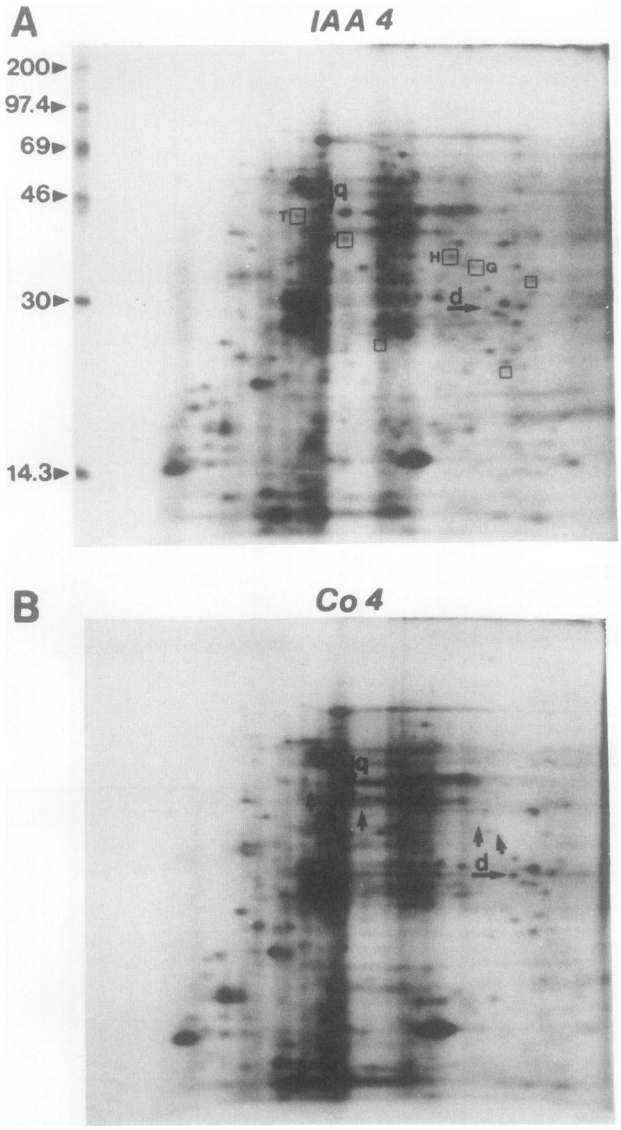

Figure 7. *In vitro* translation products of poly(A)$^+$ RNA extracted from rice coleoptile sections treated for 4 h with 10 μM IAA (IAA4, panel A) and from their corresponding untreated control (Co4, panel B). Gel features are as described in the legend of Figure 6. Additional enhanced spots G, H, P, and T are indicated in panel A with the conventions used for Figure 6. Smaller squares surround spots already enhanced after 60 min of IAA treatment. Repressions q and d are also shown.

incubation was selected as the minimal time after which we could take reliable length measurements, and 10 μM IAA was the most effective concentration promoting cell elongation. Furthermore, by using AVG (an inhibitor of ethylene biosynthesis), we were able to detect two separate phases during the first 4 h of IAA-stimulated elongation. After 2 h auxin treatment, no contribution of ethylene to elongation was observed. Ethylene's contribution to auxin-stimulated growth became evident after 4 h from the initial addition of IAA. These data are consistent with previous reports showing that the ethylene-positive effect on elongation of rice coleoptiles is delayed a few hours after the initial addition of auxin (12).

Several studies on auxin-stimulated cell elongation of excised plant tissues have shown that continued mRNA and protein synthesis are essential for elongation responses to be sustained for as long as several hours (19). Using specific inhibitors such as AcD, 5-FU, and CH, we also show that IAA-stimulated growth of rice coleoptiles is dependent upon additional or new mRNA and protein synthesis. These data indicate that enhanced growth of rice coleoptile segments must be sustained by an increased synthesis of mRNA and protein that may be associated with the deposition of new cell wall material.

On the other hand, it has been shown by two-dimensional gel electrophoretic analysis and northern-blot hybridization with pea and soybean cloned-DNA sequences that auxin can rapidly induce transcription of specific genes. It has been suggested that these genes code for proteins involved in the first phase of cell elongation, where cell wall loosening and H^+ extrusion are promoted. We have performed two-dimensional gel electrophoresis analysis of translational products obtained from poly(A)$^+$ RNA extracted from rice coleoptiles treated for 60 min with 10 μM IAA. Our data show moderate enhancements of only three different mRNAs. This situation is quite different from the one observed in elongating soybean hypocotyls or pea epycotyls, where more numerous and relevant differences in the induction of specific mRNAs are seen. However, it should be recalled that direct involvement in cell elongation of any of the products encoded by cloned genes corresponding to some of these enhanced mRNAs has not yet been shown.

Our data suggest that auxin-mediated early-transcriptional changes are less dramatic in rice compared with the other systems. One possible explanation is that IAA may increase the amount of other very low-abundance mRNAs that, even when enhanced, remain below our limit of detection. In addition, because we have used sections with tips, we cannot rule out the possibility of the presence in the control group of some free endogenous auxin that may mask the detection of other additional IAA-mediated transcriptional changes.

On the other hand, the most striking difference we were able to detect comparing the pattern of the *in vitro* translational products of IAA-treated and untreated rice coleoptiles at 60 min was a reduction of an mRNA coding for a 38 kD protein with an approximate isoelectric point of 6. Auxin-mediated transcriptional repression has been known for a long time (1). However, mRNA repression is usually not regarded as a likely basis for stimulation of growth. Nevertheless, several recent reports indicate that auxin-repressed gene expression may be a mechanism as crucial as gene induction in the mode of action of the hormone. First, IAA repression of two epidermis-specific mRNAs in elongating pea stems has been shown. Interestingly, repression of one of these mRNAs occurs within 1 h of auxin treatment of light-grown pea stems and represents the only early transcriptional effect observed (5). Second, it has been shown in rice coleoptiles that IAA decreases the level of cell wall glycoproteins that are thought to suppress coleoptile growth by decreasing cell wall extensibility (10). These are structural proteins with a high content of hydroxyproline or hexosamines. An inhibitory effect on the transport of glucosamine-containing proteins has been shown, but a more direct effect on the synthesis of both types

of proteins cannot be excluded. Third, a positive correlation between strawberry fruit growth and transcriptional repression of the auxin-regulated λ*SAR5* gene has been shown (22).

Finally, we have shown induction of four different mRNAs after 4 h of IAA treatment. They code for proteins with molecular masses ranging from 35 to 40 kD, slightly higher than the one observed for the translational products enhanced after 60 min of treatment. At the moment, we do not know if these differences are due to a late auxin effect or represent transcriptional enhancements induced by ethylene. Further investigations are required to answer this question.

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