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Abscission: The Role of RNA Synthesis

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Summary. Ethylene stimulated the incorporation of ^{32}P into RNA in the abscission zone of bean explants (*Phaseolus vulgaris* L. var. Red Kidney). The enhancement was observed in all fractions separated by methylated albumin kieselguhr column chromatography, although the magnitude of the increase was not the same for each fraction. Differential extraction of the nucleic acids indicated that the ethylene stimulation was confined to the fraction extracted with sodium lauryl sulfate, with the increase mainly in Fraction III (Ribosomal RNA) and Fraction IV (Messenger RNA). Actinomycin D, which blocks ethylene-stimulated abscission, inhibited ^{32}P incorporation into all column fractions. 5-Fluorouracil, which blocked 50 % of the ethylene-enhanced ^{32}P incorporation, did not inhibit ethylene-enhanced abscission. The results indicate that ethylene may regulate abscission through control of specific RNA's.

In an earlier paper (2), we presented evidence that RNA synthesis was necessary for the cell separation process during abscission. However, Valdovinos and Muir (22) do not hold this viewpoint. With the increasing knowledge of the functions of different classes of RNA, it was of interest to study their synthesis using ^{32}P labeling during control and ethylene-stimulated abscission.

In this paper, we show that all fractions of radioactive RNA separated on methylated albumin kieselguhr (MAK) columns are enhanced by ethylene, but only Fraction III (Ribosomal RNA) and Fraction IV (Messenger RNA) appear critical in regulating abscission. Other plant hormonal systems appear to exert their influence through nucleic acid regulation. These results allow further comparisons of control mechanisms in abscission with those of other hormonally controlled plant processes.

Materials and Methods

Methods used to grow and prepare explants and to measure ethylene in the surrounding gas phase have been described (1, 2, 3, 20).

Preparation of Total RNA. The perchloric acid method of RNA extraction was modified after Key and Shannon (15) as described previously (2). The phenol method of nucleic acid extraction was modified after Ingle, Key, and Holm (12). Sixty explants (6 gm) were homogenized in 1 ml of bentonite solution (100 mg/ml), 3.5 ml of 10 % sodium lauryl sulfate (SLS), 7 ml of tris buffer (0.01 M, pH 7.4), and 17.5 ml of phenol (washed and saturated with the tris buffer) at high speed for 2 minutes with a VirTis 23 homogenizer, followed by stirring at low speed for 3 minutes. After centrifugation at 10,000 × *g* for 10 minutes, the aqueous phase was removed and further deproteinized by two 2-minute extractions with equal volumes of phenol. The final aqueous phase was made 0.15 M with respect to potassium acetate, and the nucleic acid was precipitated by adding ethanol to a final concentration of 70 %. After standing at −15° for 2 or more hours, the nucleic acid was recovered by centrifugation at 20,000 × *g* for 10 minutes and dissolved in 3 ml of 0.05 M sodium phosphate buffer (pH 6.7). This solution was dialyzed against 10 liters of 0.05 M phosphate buffer, pH 6.7 (2 changes of 5 liters each) for 3 days. The nucleic acids were fractionated on MAK columns prepared as described by Mandell and Hershey (17). The sample of nucleic acid (usually 0.3 mg) was added to 1 mg of carrier RNA extracted from petiole tissue. The combined sample was added to the MAK column in 50 ml of 0.4 M NaCl and washed with an additional 50 ml of NaCl. The nucleic acids were eluted with a linear gradient of NaCl from 0.4 M to 1.2 M (400 ml each). The column was run under a pressure of 3 psi with a flow rate of 2 and one-half ml per minute. The OD of each 6-ml fraction was determined at 260 nm and the ^{32}P ($^{32}PO_4^{3-}$) incorporation was checked by plating a 1-ml sample from each tube. All solutions and operations before the MAK chromatography were carried on at 0 to 4°.

Preparation of DNA. DNA from explants labeled with ^{14}C-thymidine was extracted by the perchloric acid method. After hydrolysis in 0.3 N KOH for 18 hours at 37°, subsequent acidification to 0.3 N $HClO_4$, and centrifugation to remove the RNA nucleotides, the DNA in the pellet was removed

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according to the method of Tuan and Bonner (21). The DNA was extracted from the $KClO_4$ precipitate by hydrolysis at 70° for 40 minutes in 0.5 N perchloric acid. The resulting hydrolysate was then cooled, neutralized with KOH, the potassium perchlorate was precipitated by centrifugation, and the DNA content of the supernatant fraction was determined by measuring the OD at 260 nm. A sample was plated to determine isotope incorporation. The DNA was also extracted by the phenol method described above and separated by MAK column chromatography.

Incubation of Explants. After excision, the explants (4.5 mm of pulvinal tissue and 9.5 mm of petiole tissue) were aged by placing them in petri plates, petiole end down, in 3 mm of 1.5 % agar for 22 or 24 hours. The plates were vented after 7 hours to reduce the accumulation of ethylene. We have shown earlier (2) that only aged explants respond to ethylene. In all experiments except one (table I), the top 2 mm of pulvinal tissue were cut from senescent explants before placing the agar block containing radioactive isotopes on the pulvinal stump. When the top 2 mm were cut from the explant there was no effect on subsequent control or ethylene-stimulated abscission. Biggs (4) has also shown that shortening the pulvinus does not alter abscission rates. All tissue samples were frozen immediately after the conclusion of the experiment and thawed just before homogenization.

Application of Inhibitors of Nucleic Acid Synthesis. Inhibitors of nucleic acid synthesis used in these experiments [5-fluorouracil (5-FU), 5-fluorodesoxyuracil (5-FDU), phenethyl alcohol (PEA), mitomycin C, and methotrexate] were added to the explants by injection with a microliter syringe (1), by a 1.5 % agar droplet on the pulvinus, or by placing explants, pulvinal or petiole end down, in the agar containing the inhibitors. In the experiment reported in figure 4, the explants were cut and stored in plain agar in petri dishes. At the various times indicated after excision, the explants were placed, pulvinal or petiole end down, in 10 mM 5-FU. At 24 hours all explants were taken out of the 5-FU and placed, petiole end down, in gas-collection bottles containing plain agar with or without 2 ppm ethylene. Abscission was measured 6 hours later or 30 hours after the start of the experiment. 5-Fluorouracil had no effect on the endogenous ethylene production of the tissue.

Treatment of Bananas. Bananas (*Musa acuminata*) were purchased from a local wholesaler before they were gassed with ethylene to induce ripening and were either kept green or induced to ripen by exposure to 25 to 50 ppm ethylene. Green (untreated) or yellow (ethylene-treated) peels (7 gm of 1-cm squares) were incubated for 7 hours in sealed 125-ml Erlenmeyer flasks containing 10 ml of 5 mM NaH_2PO_4 (pH 6), 80 mM sucrose, 20 μg/ml streptomycin, and 300 μc ^{32}P, with or without 30 ppm ethylene. The RNA was extracted by the perchloric acid method.

Inorganic Phosphate Determination. Ten abscission zones (2-mm pulvinal and 3-mm petiole tissue) were homogenized in 10 ml of ice-cold deionized water for 2 minutes. The homogenate was filtered through Mira-Cloth and an 8-ml aliquot was added to 2 ml of 50 % trichloroacetic acid. After shaking, the tubes were centrifuged at 2000 × *g* for 10 minutes at 0 to 4°. Three ml of the supernatant were analyzed for P_i by the method of Fiske and Subbarow (9).

Differential Extraction of Nucleic Acid. The tissue was homogenized as described for total nucleic acid except that the SLS was omitted. After centrifugation and removal of the aqueous layer, which contained the bulk (76 %) of the tissue nucleic acid and was designated tris-NA, the phenol residue was re-extracted by shaking for 3 minutes with 16 ml of tris buffer (0.01 M, pH 7.4) containing 4 ml of 10 % SLS. The aqueous layer after centrifugation contained the fraction of nucleic acid designated SLS-NA. Both fractions of nucleic acid were deproteinized and purified as described above for total RNA.

Base Composition of the RNA. The tubes from the peak on the MAK column to be analyzed were pooled and 1 to 2 mg of carrier RNA was added. The RNA was precipitated by addition of $HClO_4$ and centrifugation at 4000 × *g* for 10 minutes in the cold. The supernatant was poured off and the resulting pellet was hydrolyzed in 0.3 N KOH for 18 hours at 37°. After chilling, 2.4 N $HClO_4$ was added to pH 1 and the DNA and $KClO_4$ were precipitated by centrifugation at 2000 × *g* for 10 minutes. The supernatant fraction was adjusted to pH 6.0 with 1 N KOH and recentrifuged. The nucleotides were separated on a Dowex 1 × 8 formate column by stepwise elution with formic acid (CMP, 100 ml of 0.15 N; AMP, 100 ml of 1.0 N; GMP, 180 ml of 3.0 N; UMP, 100 ml of 4.0 N containing 0.1 N ammonium formate). The peaks were combined and mixed, and samples were plated. Composition is expressed as ^{32}P distributed among the 4 nucleotide peaks. The ratio of GMP to AMP has been used as a convenient parameter for comparison of RNA composition (12).

Results

Enhancement of RNA Synthesis in the Abscission Zone by Ethylene. Abeles and Holm (1) had shown earlier that ethylene stimulated ^{14}C-L-leucine incorporation into protein in the abscission zones of beans and cotton (*Gossypium hirsutum* L. var. Acala 4-42), but not in the surrounding tissues. Experiments were performed to see whether ethylene had the same effect on RNA synthesis.

In this experiment, agar blocks of ^{32}P were placed on the pulvinal and petiole ends of explants lying horizontally. The explants were incubated for 6 hours in the presence or absence of 2 ppm ethylene. They were divided into 3 sections (the top 2 mm, the next 4 mm that contained the abscission zone, and the basal 9 mm), and the RNA was extracted. The

Table I. *Ethylene Stimulation of* RNA *Synthesis in the Abscission Zone of Bean Explants*

Bean explants were aged for 24 hours before being placed on their sides, and agar blocks containing 2.5 μc ^{32}P were placed on each end. After 6 hours in the presence or absence of 2 ppm ethylene, the agar blocks were removed and the tissue was cut into 3 sections. The RNA was extracted by the perchloric acid method. Abscission after 30 hours: control 24 %, ethylene 91 %.

Section	Control	Ethylene	Control	Ethylene	% Change
	μg RNA		CPM/mg RNA		
Pulvinus (top 2 mm)	116 ± 5	89 ± 3	592,309	659,863	+11.4
Abscission zone (next 4 mm)	203 ± 3	204 ± 6	25,301	39,420	+55.8
Petiole (bottom 9 mm)	234 ± 5	221 ± 4	1,216,536	1,080,216	—11.2

Table II. *Effect of Ethylene and Inhibitors on* ^{32}P *Incorporated into Nucleic Acid Fractionated on* MAK *Columns*

Explants aged 18 hours at 400 ft-c and 25°. Top 2 mm of pulvinus were cut off and the inhibitors were added as described in Materials and Methods (5-FU by agar droplet, actinomycin D by injection). At 22 hours after excision (i.e., 4 hours after inhibitor treatment), ^{32}P agar blocks (8 μc/agar block) were placed on the pulvinal surface and incubated with or without 2 ppm ethylene for 3 and 6 hours. The nucleic acids were extracted by the phenol method and separated on MAK columns with a linear gradient. % Abscission: 3 hour control-0, 3 hour ethylene-17, 6 hour control-17, 6 hour ethylene-80, 6 hour ethylene + 5-FU-80, and 6 hour ethylene + actinomycin D-10.

MAK column fraction	C_2H_4 Increased or decreased over control		Inhibition of C_2H_4 stimulation	
	3 Hr	6 Hr	6 Hr C_2H_4 + 10 mM 5-FU	6 Hr C_2H_4 + 1 μg act D
	%	%	%	%
I (s-RNA)	—2	58	49	87
II (DNA-RNA)	62	62	46	95
III (r-RNA)	72	84	53	94
IV (m-RNA)	45	75	35	83
Total Nucleic acids	42	77	51	94

Table III. *Effect of Ethylene on the* ^{32}P-RNA *Base Composition of the Messenger*-RNA *and Total* RNA

Total RNA prepared by the perchloric acid method as described in Materials and Methods. The m-RNA was precipitated from the MAK column fraction as described in Materials and Methods.

Treatment	^{32}P Mole fraction CMP	AMP	GMP	UMP	GMP/AMP	CPM
Total RNA						
3 Hr control	21.9	25.7	30.7	21.7	1.19	96,545
3 Hr C_2H_4	22.8	25.2	30.1	21.9	1.19	136,749
m-RNA Fraction						
3 Hr control	18.8	28.4	31.8	21.0	1.12	25,232
3 Hr C_2H_4	20.7	27.1	32.2	20.0	1.19	36,582
6 Hr Control	20.7	27.6	32.9	18.8	1.19	148,236
6 Hr C_2H_4	22.1	26.1	32.0	19.8	1.23	251,467
6 Hr C_2H_4 + 5-FU	20.9	29.1	31.8	18.2	1.09	194,131
Total explant RNA	23.6	23.8	31.5	21.1	1.32	

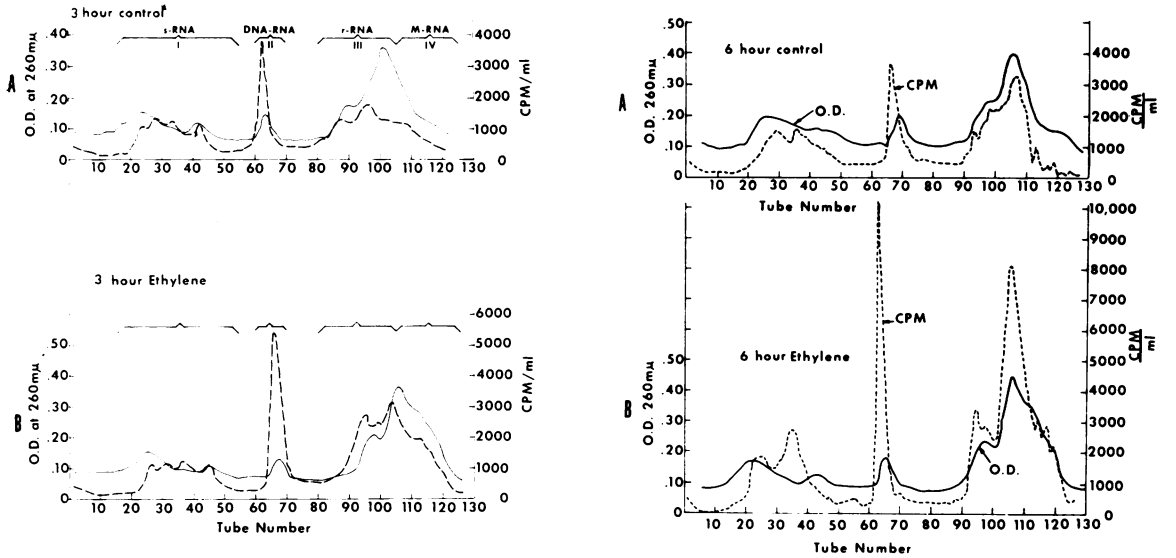


FIG. 1. (left) A) Distribution of radioactivity in various nucleic acid fractions after 3 hours' incubation of aged explants in air. In this and succeeding figures the solid line represents the OD at 260 nm and the dashed line represents CPM. B) Distribution of radioactivity in various nucleic acid fractions after incubation of aged explants in 2 ppm ethylene for 3 hours.

FIG. 2. (right) A) Distribution of radioactivity in various nucleic acid fractions after incubation of aged explants in air for 6 hours. See table II for details. B) Distribution of radioactivity in various nucleic acid fractions after incubation of aged explants in 2 ppm ethylene for 6 hours. See table II for details.

ethylene enhancement occurred mainly in the abscission zone (56 %) and to a lesser extent in the top section (table I). The lower rate of synthesis of ^{32}P-RNA in the abscission zone appears to result from less ^{32}P diffusing into that area rather than from a slower rate of synthesis. Subsequent experiments, in which the top 2 mm of tissue were removed, showed that the abscission zone incorporates ^{32}P at rates similar to those in the other 2 sections. The rest of the experiments reported in this paper were performed using explants with the top 2 mm removed after an aging period of about 22 hours. Removing the top 2 mm of pulvinus before placing the ^{32}P agar block shortened the diffusion path for ^{32}P increasing the rate of ^{32}P incorporation into abscission zone RNA.

Other possible explanations for the enhancement of RNA synthesis are that ethylene either caused increased uptake of ^{32}P from the agar blocks or altered the P_i pool in the tissue, resulting in more isotope incorporation. Neither of these explanations can account for the ethylene-stimulated increase in ^{32}P. Ethylene had no effect on the uptake of ^{32}P from the agar blocks or on the amount of the isotope in the tissue. The soluble P_i pool size did not seem to be altered by ethylene.

Separation of Nucleic Acids by MAK Chromatography. Figures 1 and 2 show the separation of ^{32}P-labeled nucleic acids from bean explants after 3- and 6-hour exposures to atmospheres with or without 2 ppm ethylene. The first 2 peaks consisting of tubes 15 to 55 are designated as fraction I, the third peak, tubes 60 to 70, as fraction II, the next 2 peaks, tubes 80 to 105 as fraction III, and the trailing shoulder, tubes 105 to 125 as fraction IV. Even though there is no unequivocal evidence as to the nature of these peaks, it will facilitate subsequent discussion of the results if we assume that they are similar to the ones described for other plant material and designate fraction I as s-RNA, II as DNA-RNA, III as r-RNA, and IV as m-RNA. These separations were obtained by a single NaCl gradient from 0.4 M to 1.2 M. The separations (figs 5, 6) were obtained by 2 linear gradients (0.4 M to 0.85 M and 0.85 M to 1.2 M) that caused the various peaks to be shifted to the left.

At 3 hours, ethylene caused increases in all fractions of nucleic acids except the soluble RNA (fig 1 and table II). The largest increase was in the ribosomal fraction, but the isotope peaks did not coincide with the OD peaks either in the control or in the ethylene-treated explants. After 6 hours (fig 2 and table II) ethylene-treated explants showed increases in all fractions, with more complete coincidence of label and optical density.

Bacterial contamination of explant tissue would contribute to the labeling patterns observed. We have found that there are about 10^4 bacteria per gram fresh weight of explant tissue though values as high as 10^5 and as low as 10^3 have been observed. On the assumption that an average bacteria weighs 2×10^{-12} grams (23) our contamination is about 2 parts in 10^8 or less than that shown by Lonberg-Holm (16) to alter the labeling of seedling nucleic acids.

Inhibitor Treatment. The pyrimidine analogue 5-FU showed different effects on abscission rates, depending on the time of application (fig 4). 5-Fluorouracil (10 mM) had less of an effect on control or ethylene-stimulated abscission in aged explants (those excised 18–20 hrs) than it did on freshly excised tissue. This seems to occur because 5-FU interferes with the aging process (unpublished results). The greater inhibition with the pulvinal application of 5-FU appears to come from the shorter diffusion path to the abscission zone (4 and one-half mm vs 9 and one-half mm).

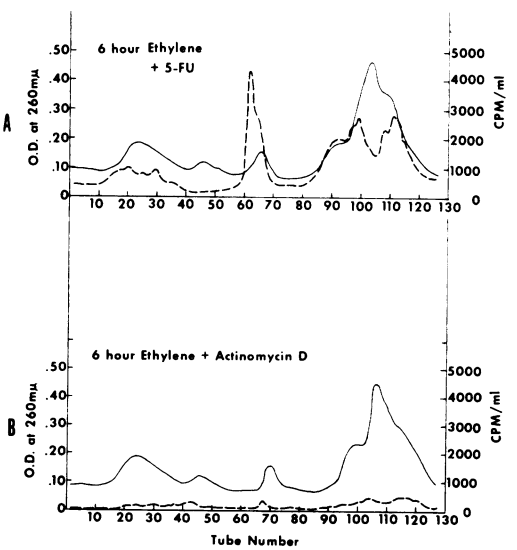
The results for 5-FU shown in figure 3a and table II were obtained from explants treated with 5-FU 18 hours after excision and 4 hours before ethylene treatment. This treatment did not alter the subsequent ethylene-stimulated abscission, but it d'd inhibit the ^{32}P nucleic acid stimulation of ethylene by 51 % (table II). The greatest inhibition occurred in the soluble and ribosomal RNA fractions, indicating that not all of the nucleic acid synthesis enhanced by ethylene was needed for abscission to occur.

Actinomycin D (1 μg) injected into explants 4 hours before ethylene treatment blocks ethylene-stimulated abscission (1) and causes a 94 % inhibition of the ethylene-enhanced ^{32}P incorporation (fig 3b and table II). The inhibition is similar in all fractions and supports the concept that actinomycin D inhibits all DNA-dependent RNA synthesis.

Base Ratio Analysis of Explant ^{32}P-RNA. Because ethylene caused a large stimulation of ^{32}P nucleic acid synthesis (70–80 %) by 6 hours, a preliminary study was undertaken to determine whether ethylene changed the base composition of the RNA synthesized. Experiments extracting total ^{32}P-RNA from control and ethylene-treated explants showed slight or no differences (table III). The m-RNA fraction is the one that should exhibit the largest differences in base composition if newly synthesized messenger is important in the abscission process. The m-RNA fraction was examined for base composition differences at 3 and 6 hours. Table III shows some differences in the base ratios of the control and ethylene m-RNA fractions. The ratio of GMP to AMP in this fraction indicates contamination by ribosomal RNA if one assumes that plant messengers have a high AMP content (12). 5-Fluorouracil, which inhibited ribosomal RNA synthesis by 53 % (table II), lowered the ratio of GMP to AMP of the ethylene-treated explants although the total counts were still greater than the controls.

Differential Extraction of Nucleic Acids. An attempt was made to further characterize the nucleic acids produced under ethylene stimulation by differential extraction techniques that have been shown in other plant tissues to separate the m-RNA, DNA, and DNA-associated RNA from the bulk of the tissue RNA (7, 12). This technique involves homogenizing the tissue with buffered phenol and bentonite, centrifuging, and drawing off the aqueous phase (tris-extracted nucleic acid). The interphase and phenol residue are then re-extracted with buffer and SLS (called SLS-extracted nucleic acid).

Table IV shows that the tris buffer extracted 76 % of the explant nucleic acids although it did not show an ethylene enhancement. The SLS-extracted nucleic acids, however, contained the ethylene stimulated fraction and contained the highest specific activity ^{32}P-RNA. Separation of the nucleic acids on



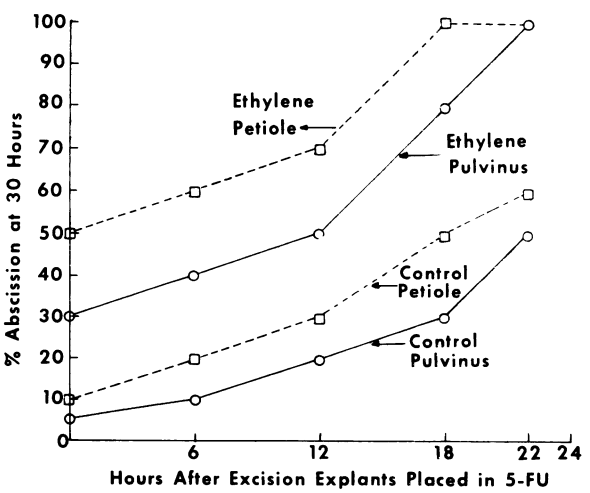


FIG. 3. (left) A) Distribution of radioactivity in various nucleic acid fractions after incubation of aged explants treated with 5-FU in 2 ppm ethylene for 6 hours. See table II for details. B) Distribution of radioactivity in various nucleic acid fractions after incubation of aged explants treated with actinomycin D in 2 ppm ethylene for 6 hours. See table II for details.

FIG. 4. (right) Effect of time and site of 5-FU application on abscission.

Table IV. *Differential Extraction of Nucleic Acid* (NA) *With Tris and Sodium Lauryl Sulfate* (SLS)

Conditions for the experiment were the same as those described in table VI. The total counts from the column peaks were determined from figures 5 and 6. The mg nucleic acid came from the amount extracted by each technique and before 1 mg of carrier nucleic acid was added for column separation.

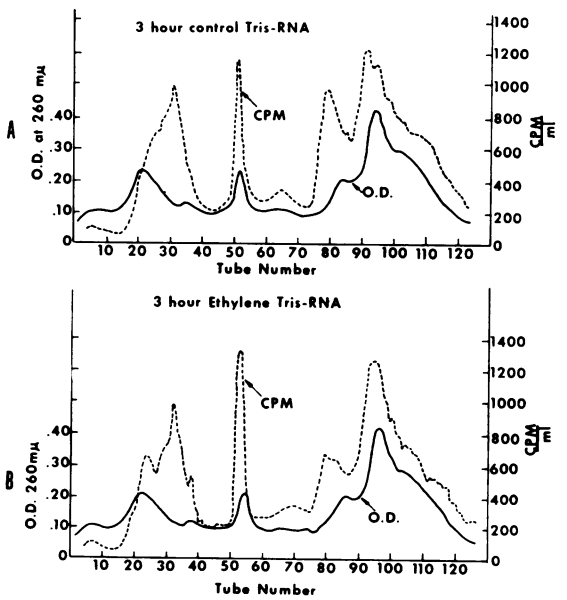
Treatment	Extraction medium	Radioactivity from column peaks	Nucleic acid extracted	Specific Activity	Increase
		CPM	mg	CPM/mg Nucleic Acid	%
3 Hr control	SLS	117,456	0.122	962,758	
3 Hr C_2H_4	SLS	253,950	0.116	1,497,429	+56
3 Hr control	Tris	320,136	0.488	656,016	
3 Hr C_2H_4	Tris	306,648	0.459	668,078	+2

MAK columns (figs 5, 6) shows that there is little difference in the labeling pattern of control and ethylene treatments obtained by tris extraction (fig 5), but that ethylene enhancement is observed in the SLS-extracted RNA fractions (fig 6). Table V indicates that the major ethylene enhancement occurs in the messenger and ribosomal regions.

Examination of DNA Synthesis During Abscission. Data in tables II and V show that ethylene enhanced ^{32}P incorporation into the DNA-RNA fraction. This fraction contains RNA, probably as a natural hybrid with DNA (8). The use of ^{32}P suggests the possibility of ethylene-enhanced DNA synthesis. Experiments were first performed to see if inhibitors (mitomycin C, PEA, and methotrexate) reported to block DNA synthesis in other systems would inhibit abscission. Nitsan and Lang (18) have shown that these compounds inhibit DNA synthesis and cell elongation in peas and lentils. The results shown in table VI indicate that only PEA at the highest concentration used inhibited ethylene-stimulated abscission. It is known that PEA also inhibits RNA synthesis to some extent (19), so this result can not be taken as completely valid because we have shown that some inhibitors of RNA synthesis also inhibit abscission (2). 5-Fluorodesoxyuracil also was tried at various times after excision (either by agar droplet or by injection method) and it had no effect on abscission.

Results indicate that although there is a considerable amount of thymidine-^{14}C incorporation into the DNA, ethylene does not enhance the amount incorporated (table VII). Similar results were obtained with perchloric acid and phenol extraction methods.

Enhancement of RNA Synthesis in Banana Peel. Since ethylene is thought to be the compound respon-



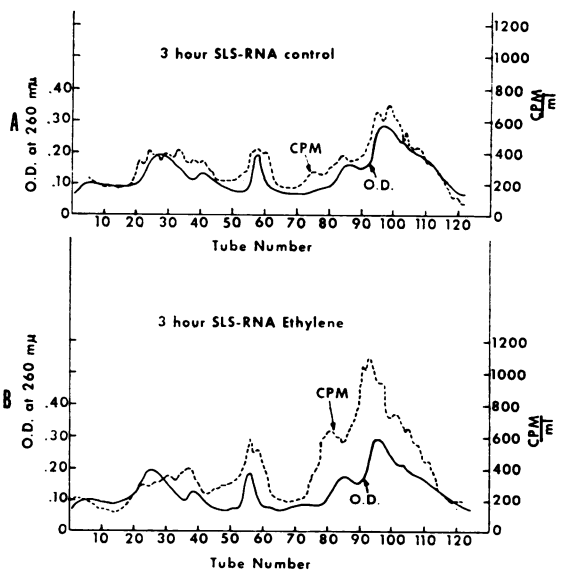


FIG. 5. (left) A) Distribution of radioactivity in various nucleic acid fractions extracted by the tris buffer method from aged explants incubated in air for 3 hours. B) Distribution of radioactivity in various nucleic acid fractions extracted by the tris buffer method from aged explants incubated in 2 ppm ethylene for 3 hours.

FIG. 6. (right) A) Distribution of radioactivity in various nucleic acid fractions extracted by the SLS method from aged explants incubated in air for 3 hours. B) Distribution of radioactivity in various nucleic acid fractions extracted by the SLS method from aged explants incubated in ethylene for 3 hours.

Table V. *Effect of Differential Extraction on Ethylene-Stimulated ^{32}P Nucleic Acid*

Explants were aged for 22 hours before the top 2 mm of pulvinal tissue were removed and an agar block (10 μc/agar block) was placed on top. The explants were treated, with or without 2 ppm ethylene, for 3 hours and the nucleic acids were differentially extracted as outlined in Materials and Methods. The nucleic acids were separated on MAK columns using linear NaCl gradients. The percentage increase or decrease by ethylene was calculatetd from the total counts in each of the peaks described.

MAK column fraction	C_2H_4 Increase or decrease over control	
	SLS-extracted nucleic-acid	Tris-extracted nucleic acid
	%	%
I (s-RNA)	20	4
II (DNA-RNA)	24	12
III (r-RNA)	55	—12
IV (m-RNA)	62	—6
Total NA	48	—4

sible for the respiratory climacteric in banana fruit (5), it could cause an induction of RNA synthesis in this system.

Green bananas were divided into 2 groups, one of which was given a 2-day ethylene pretreatment. The peels were then removed and incubated in a buffered medium containing ^{32}P in the presence or absence of 30 ppm ethylene. The green peels that had not been previously treated with ethylene showed an enhancement of ^{32}P-RNA synthesis. The previously induced peels that were yellowing showed a small enhancement (table VIII).

Discussion

In earlier papers we presented evidence that ethylene stimulated ^{32}P-RNA synthesis in explant tissue (2), and that ethylene enhanced ^{14}C-L-leucine protein formation in the separation layer and not in the surrounding tissue (1). As reported here, the ethylene enhancement of ^{32}P-RNA was confined to the 2-mm area on each side of the abscission zone (table I). The ethylene enhancement is not due to

Table VI. *Effect of DNA Synthesis Inhibitors on Ethylene-Stimulated Abscission*

Explants were cut and aged for 20 hours at 25°. At that time 1 μl of the various inhibitors were injected through the petiole. At 22 hours (i.e., 2 hrs after injection) the explants were given 2 ppm ethylene. Abscission was measured at 28 hours.

Treatment	Abscission at given molar concentration of inhibitor					
	5×10^{-3}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
	%	%	%	%	%	%
Control			97			
PEA	57	80	97	100		
Mitomycin C		100	100	100		
Methotrexate			100	100	100	100

Table VII. *Effect of Ethylene on ^{14}C-Thymidine Incorporation into Bean Explant* DNA

Senescent explants had the top 2 mm of pulvinal tissue removed and agar blocks containing 0.78 μc ^{14}C-thymidine (2×10^{-4} M) were placed on the pulvinal stumps. The explants were kept in atmospheres, with and without ethylene, for 7 hours. The DNA was extracted as described in Materials and Methods.

Treatment	% Abscission at 29 hrs	μg DNA per 20 explants	CPM/mg DNA
Control	27	36.3 ± 1.0	22,000 ± 200
2 ppm C_2H_4	87	37.2 ± 0.8	20,900 ± 300

Table VIII. *Effect of Ethylene on ^{32}P Incorporation into RNA of Yellow and Green Banana Peels*

Green bananas were stored either in air or in ethylene to stimulate ripening. 7 Grams of either green or yellow peel were incubated for 7 hours at 30° in 10 ml of 5×10^{-3} M phosphate buffer, pH 6; 0.08 M sucrose; 20 μg/ml streptomycin; and 300 μc ^{32}P in sealed 125-ml Erlenmeyer flasks with or without 30 ppm ethylene. The RNA was extracted by the perchloric acid method.

Peel	Atmosphere	μg RNA	CPM/mg RNA	% Increase
Green	control	602 ± 6	72,475 ± 1,111	
Green	C_2H_4	576 ± 5	95,676 ± 1,151	+32
Yellow	control	232 ± 3	68,346 ± 3,688	
Yellow	C_2H_4	249 ± 4	76,658 ± 5,978	+12

increased isotope uptake into the tissue or to decreased inorganic phosphate pool size. However, the gross measurement of pool sizes may be misleading because of unknown effects of cell compartmentalization and the possibility of more than one phosphate pool.

The 3- and 6-hour labeling periods were chosen for studying 2 different time intervals during ethylene-stimulated abscission. At 3 hours, the enhancement of ^{32}P is 30 to 40 % greater than that of the controls, but abscission has not started. At 6 hours, the enhancement has reached 70 to 80 % and the ethylene-treated explants have abscised 70 to 90 %, the controls 5 to 10 %. The differential effect of ethylene after 3 hours' treatment on the stimulation of ^{32}P into the nucleic acid fractions indicates that ethylene may be rather specific in regulating the production of certain RNA's that in turn produce the protein (enzymes) necessary for abscission.

After 3 or 6 hours of ethylene treatment, the enhancement of ^{32}P incorporation is greatest in the ribosomal RNA fraction (figs 1, 2). At 3 hours there was no effect on soluble RNA synthesis but at 6 hours there was an increase. Since the ethylene-treated explants have almost completely abscised at 6 hours, the increase in s-RNA could result from the degradation of other classes of RNA, or it could be a net synthesis. At 6 hours actinomycin D inhibits 94 % of the nucleic acid synthesis (table II) and blocks abscission (2) stimulation by ethylene. The inhibition appears to be similar in all nucleic acid fractions. On the other hand, 5-fluorouracil added at 18 hours after excision inhibits the ethylene enhancement of nucleic acid synthesis 50 %, yet it does not alter the abscission rate. The greater inhibition of soluble and ribosomal RNA with 5-FU compared with that of messenger RNA is analogous to other plant systems where 5-FU has been tested (8, 13, 14). This suggests that not all fractions of RNA be newly synthesized for abscission, but that newly synthesized m-RNA is essential.

In an attempt to determine whether ethylene was altering the nucleotide composition of the RNA that was synthesized, base ratio analyses were performed on the newly synthesized ^{32}P-RNA. The total ^{32}P-RNA showed no difference between control and ethylene-treated explants. Since 5-FU showed greater inhibition of soluble and ribosomal RNA synthesis without affecting abscission, the m-RNA fraction was examined. Although differences are noted (table III) at 3 and 6 hours, the effect was slight. The higher ratio of GMP to AMP for the ethylene treatments probably resulted from ribosomal RNA contamination of the m-RNA fraction, because the greatest ethylene stimulation was in the ribosomal fraction. The ethylene and 5-FU combination showed a decreased ratio of GMP to AMP, which also indicated ribosomal contamination. The total counts for the m-RNA for ethylene and 5-FU were still higher than those of the controls.

Assuming that explant m-RNA had a GMP to AMP ratio of 0.60 as the D-RNA in soybean hypocotyl tissue (11, 12), then the m-RNA fraction in our separation contains only 18 to 20 % m-RNA after 3 hours of ethylene, comprising only 4 to 5 % of the total ^{32}P-RNA synthesized. This is apparently different from other plant tissues (8, 11) where excision results in greatly reduced ribosomal RNA synthesis, thereby magnifying the m-RNA production. Cherry and van Huystee (8) found that excising *Xanthium* buds caused 50 % more ^{32}P-m-RNA than in intact buds. They likened excision of plant parts to bacterial step-down cultures (10) that preferentially synthesize informational RNA's.

Differential extraction of the nucleic acids from explants at 3 hours showed the enhancement by ethylene to be in the SLS fraction (table IV). This is further support for the idea that the action of ethylene is to promote synthesis of specific RNA's. Other investigators (7, 12) have shown that tris extraction with phenol removes the bulk of the soluble and ribosomal RNA. Re-extraction of the interphase and phenol residue with SLS and tris removes the DNA and rapidly labeled RNA (i.e., m-RNA). The separation of explant nucleic acids by MAK chromatography, however, showed that the tris extraction yielded some DNA as well as ribosomal and soluble RNA (fig 5). The specific activities of the individual fractions could not be determined accurately because the amounts extracted (0.1–0.5 mg) could not be analyzed without added carrier nucleic acid. The specific activities (table IV) were determined from the total counts from the MAK column peaks and the total amount of nucleic acid extracted before addition of the carrier. The ethylene enhancement in the SLS fraction was again a differential effect (table V) on the fractions, as it had been in the total extraction (table II), although the greatest stimulation in the SLS extraction was in the m-RNA.

DNA synthesis does not appear necessary for abscission. Compounds shown to be inhibitors of DNA synthesis in other systems had no effect on ethylene-stimulated abscission (table VI), and ethylene showed no stimulation of ^{14}C-thymidine incorporation into DNA (table VII). However, since there was incorporation of ^{14}C-thymidine, there was some turnover or synthesis of DNA. Cherry (6) has shown that the DNA peak from peanut cotyledons on MAK columns contains 24 % RNA. Ingle et al. (12), however, reported that the DNA peak from soybean tissue contained only 5 % RNA. In our experiments, the amount of ^{32}P-RNA in the DNA peak ranged from 5 % for controls to 20 % for ethylene-treated. This was determined by KOH hydrolysis, followed by subsequent perchloric acid hydrolysis of the DNA peak.

That ethylene may act through RNA regulation in other systems where it serves as a hormone is shown in the experiment with green and yellow (ripening) banana peels (table VIII). The ethylene enhancement of ^{32}P-RNA was greatest in the peels that had not been previously induced to ripen by

ethylene, indicating possible nucleic acid control and limited additional response once the ripening process is initiated.

The addition of ethylene to explant tissue seems only to speed the natural abscission process that appears to occur with endogenous ethylene levels. Stimulation of explant abscission by ethylene appears analogous to banana ripening as reported by Burg and Burg (5), in which added ethylene only shifts the normal respiratory climacteric, which is caused by endogenous ethylene production, to an earlier time. During our experiments, the gas-collection bottles were sealed the same way for both treatments except that 2 ppm of ethylene were injected into 1 set. The control explants produce enough ethylene to allow an accumulation of 0.05 to 0.10 ppm in the atmosphere, which is enough to cause abscission. There appears to be a 3- to 4-hour lag between the control and ethylene-treated explants. As shown in figures 1 and 2, the distribution of ^{32}P on the MAK columns for the 6-hour control and the 3-hour ethylene appears similar. The specific activities for the total nucleic acids are also similar. Table III also shows that the base composition of the m-RNA fraction for the 6-hour control and the 3-hour ethylene is similar, as are the abscission rates (table II).

It is impossible at this time to show an absolute requirement for ethylene in the abscission process because there is no way known to inhibit ethylene production. It appears from our work that ethylene controls abscission at the RNA level.

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