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Increased Ribonucleic Acid Polymerase Activity Associated with Chromatin from Internodes of Dwarf Pea Plants Treated with Gibberellic Acid[1]

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ABSTRACT

Gibberellic acid increases the level of RNA polymerase associated with chromatin isolated from expanding internodes of light-grown, dwarf pea plants (*Pisum sativum L.*), without a detectable increase in the amount of DNA template available.

Gibberellic acid brings about an increase in the RNA content of expanding internodes from intact, dwarf pea plants (1, 2) and an increase in the rate at which RNA is formed by nuclei isolated from treated pea tissue (6). The following work was done to determine if the increase observed in internodes might be attributed to an increase in the level of RNA polymerase associated with chromatin or to an increase in the amount of template DNA available for transcription by the enzyme, or to both.

MATERIALS AND METHODS

Growth of Plant Material. Seeds of the dwarf pea (*Pisum sativum* L.) cultivar Progress No. 9 (Ferry Morse Seed Co.) were imbibed in shallow water at 24 C for 18 hr and sown in plastic containers of vermiculite. The plants, 25 per container, were raised in growth cabinets (temperature 20 C, constant light of about 4000 ft-c provided by General Electric cool white reflector fluorescent tubes, and incandescent lamps) and watered daily with half-strength Hoagland's solution. The containers were randomized from time to time. Fifteen days after the commencement of soaking, the fifth internodes (counting the node of the cotyledons as No. 1) were approximately 6 mm long, and this was taken as 0 time for the experimental period. Some of the plants were then sprayed with an aqueous solution of 10^{-3} M gibberellic acid (GA_3, Merck and Co.), and fifth internodes were excised from both treated and control plants at different times after treatment. Internodes of intact plants were measured with dividers to the nearest millimeter.

Extraction of Chromatin. The methods were essentially those of Huang and Bonner (4). Internodes were excised and placed in ice-cold beakers, weighed, and homogenized in a grinding medium (tris, 0.1 M; $MgCl_2$, 0.001 M; sucrose, 0.25 M; β-mercaptoethanol, 0.02 M) in the ratio of 2 ml of medium to 1 g of tissue. Where 15 to 30 g of tissue were available, a Virtis homogenizer was used (10 sec at 40 v, 15 sec at 90 v), but where small amounts were used (2–10 g), higher polymerase activity was retained by grinding with a chilled pestle and mortar. The homogenate was filtered through four layers of cheesecloth and one of Miracloth (Chicopee Mills, N. Y.), and centrifuged at 10,000*g* for 30 min. The pellet, excluding starch, was suspended in a washing medium (tris, 0.01 M; sucrose, 0.25 M; β-mercaptoethanol, 0.01 M) and again centrifuged; the washing was then repeated. The pellet was suspended in 5 ml of washing medium, layered over 20 ml of 1.8 M sucrose containing 0.01 M tris and 0.01 M β-mercaptoethanol, and centrifuged at 40,000*g* for 3 hr. The chromatin-rich pellet was suspended in 0.01 M tris. All solutions were at pH 8.0 and 0 C.

Table I. *Effects of Changes in the Reaction Mixture on the Synthesis of Labeled, Trichloroacetic Acid-precipitable Products from Labeled UTP*

Data bulked from different experiments, in which incorporation for complete reaction mixture (see "Materials and Methods") was approximately 60 μμmoles of UMP incorporated per 100 μg of DNA.

Reaction Mixture	Incorporation
	%
Complete	100
$-Mn^{2+}$, $-Mg^{2+}$	1
−GTP, −ATP, −CTP	8
+Actinomycin D, 0.02 μg	22
+Actinomycin D, 0.2 μg	3
+Gibberellic acid, 10^{-8} M	96
+Gibberellic acid, 10^{-6} M	96
+Gibberellic acid, 10^{-4} M	86

Endogenous Polymerase Assay. Aliquots of chromatin, 0.1 ml containing about 20 μg of DNA, were added to 0.3 ml of a cold reaction mixture containing (in μmoles) tris-HCl (pH 8.0 at 35 C), 20; $MnCl_2$, 0.25; $MgCl_2$, 1.0; β-mercaptoethanol, 1.0; ATP, 0.2; GTP, 0.2; CTP, 0.2; and UTP, 0.0025 containing 10 μc of ^{3}H-UTP. Reaction tubes were transferred to a water bath at 35 C and shaken for 20 min, except where stated otherwise. There was no reaction at 0 C. Most reactions were carried out in duplicate. Each reaction was stopped by the addition of 3 ml of cold 10%

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Table II. *Effects of Additions to the Reaction Mixture on the Synthesis of Labeled, Trichloroacetic Acid-precipitable Products from Labeled UTP*

Incorporation for chromatin from untreated and treated plants was 28.7 and 32.3 $\mu\mu$moles of UMP incorporated per 100 μg of DNA, respectively.

Addition	Incorporation	
	Chromatin from untreated plants	Chromatin from GA_3-treated plants
	%	%
None	100	100
Phosphate (5 μmoles)	93	103
Pyrophosphate (5 μmoles)	5	2
RNase (10 μg)	9	3
RNase (100 μg)	6	3

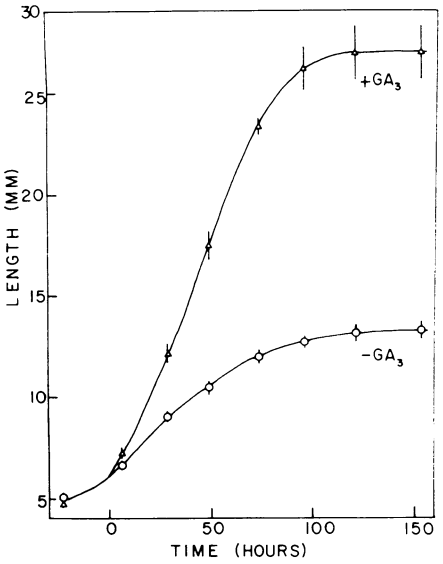

FIG. 1. Expansion of fifth internodes of intact pea plants. Each point is the mean of 24 internodes, and vertical bars indicate standard errors. Plants designated "$+GA_3$" were treated with gibberellic acid at time 0, which is 15 days after soaking of the seeds.

trichloroacetic acid containing 0.9% sodium pyrophosphate. A zero time reaction was performed by adding trichloroacetic acid immediately after chromatin to a cold reaction mixture. Trichloroacetic acid-insoluble material was recovered on membrane filters (Bac-T-Flex, B-6; Carl Schleicher and Schuell, Keene, N. H.), and washed with cold 5% trichloroacetic acid containing 0.9% sodium pyrophosphate. After drying, the filters were placed in Liquifluor scintillation fluid (New England Nuclear Corp.), and radioactivity was assayed with a Beckman liquid scintillation counter. Incorporation was measured by subtracting the counts for zero time reactions from those for the experimental samples. The DNA contents of aliquots of chromatin were measured by the technique of Burton (3).

As seen in Tables I and II, incorporation of label from UTP into a trichloroacetic acid-precipitable product was dependent on the presence of a divalent cation, and the presence of the other triphosphates. Incorporation was inhibited by pyrophosphate but not phosphate, by actinomycin D, and by RNase (Worthington Biochemical Corp.). These observations indicate that the product is RNA. Maximal incorporation was obtained at pH 8.0, in the

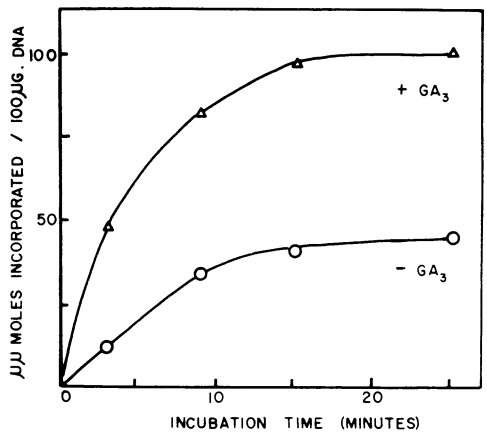

FIG. 2. Incorporation of label into RNA by chromatin isolated from fifth internodes 48 hr after GA_3 treatment, compared with chromatin from control internodes of the same age (*cf.* Fig. 1.)

Table III. *Synthesis of RNA by Chromatin Isolated from Pea Internodes at Different Times after Treatment with Gibberellic Acid*

Time	Incorporation	
	Chromatin from untreated plants	Chromatin from GA_3-treated plants
hr	*$\mu\mu$moles UMP incorporated/100 μg DNA*	
0	22.7	—
6	28.0	30.1
12	28.9	38.0
24	21.2	34.3
48	23.8	32.1
96	13.9	16.8

presence of Mn^{2+} and Mg^{2+}, either alone or in combination, at the concentrations given above.

Alterations in the ratio of grinding medium and amount of plant material, and in the method of grinding, resulted in variable losses of enzyme activity, and for comparable results the methods were standardized. In general, high activities were retained when larger masses of plant material were extracted; for time course work, where amounts of plant material had to be kept to a minimum, lower activity was recovered. With standardized techniques, good reproducibility was achieved; for example, two samples of 250 and 300 internodes harvested from untreated plants at the same time but processed separately gave incorporations of 27.0 and 28.2 $\mu\mu$moles of UMP incorporated per 100 μg of DNA.

Estimation of Changes in Template Availability. In order to detect possible changes in amount of available template, RNA polymerase isolated from *Escherichia coli* (Biopolymers Inc., Pinebrook, N. J.) was added to reaction mixtures containing chromatin. The reaction mixture was as described above, but the amount of chromatin DNA added was reduced to the region of 0.4 μg, so that the level of *E. coli* RNA polymerase required to saturate the available template could be kept low. The reaction time was 15 min. GA_3 at 10^{-8} M did not affect incorporation. In order to match closely the amount of DNA in reaction tubes containing chromatin from control and GA_3-treated plants, aliquots of chromatin were examined at 258 nm with a Cary spectrophotometer. Appropriate dilutions were made and reac-

tions were carried out; subsequently, the levels of DNA were measured more precisely with the Burton technique.

RESULTS AND DISCUSSION

Expansion of the fifth internodes of Progress No. 9 seedlings is shown in Figure 1. It can be seen that GA_3 brought about a marked increase in growth, the effect being just significant 6 hr after treatment (*t* test).

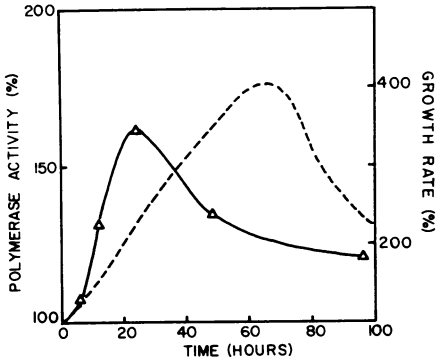

FIG. 3. The percentage increase in polymerase activity brought about by GA_3 treatment, compared with the percentage increase in growth rate (– –). Data are derived from these given in Table III and Figure 1, which were recorded during the same experiment.

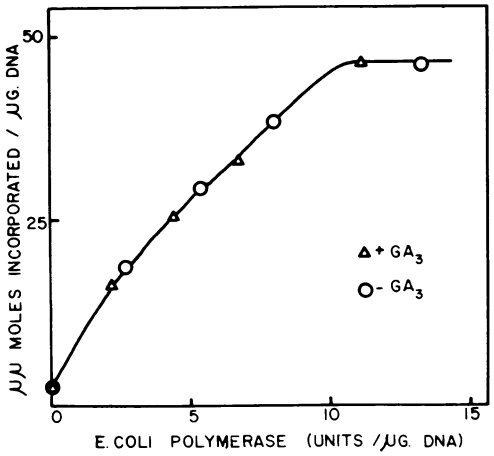

FIG. 4. Incorporation of label into RNA following addition of different levels of *E. coli* polymerase to reaction mixtures containing chromatin from internodes 12 hr after GA_3 treatment ($+GA_3$), and comparable control internodes ($-GA_3$). Reaction mixtures for chromatin from GA_3-treated plants contained 0.45 μg of DNA, controls 0.38 μg of DNA. Units of enzyme are as described by Biopolymers Inc.

Chromatin isolated from GA_3-treated plants 48 hr after treatment showed a marked increase in the level of associated RNA polymerase activity when compared with controls (Fig. 2). GA_3 had no stimulatory effect on RNA synthesis *in vitro* (Table I). Results of a time course experiment, in which chromatin was isolated at different times after GA_3 treatment, are given in Table III. Maximal activity occurred for both treated and control internodes during early expansion (*cf.* Fig. 1). The maximal increase resulting from GA_3 treatment was at 24 hr (Fig. 3); this was also found in two other experiments. Growth rates (mm/hr) were calculated for different points along the curves presented in Figure 1, and the growth rates of GA_3-treated internodes are expressed as percentages of the corresponding data for the controls. These percentages are included in Figure 3. The maximal increase in polymerase activity brought about by GA_3 precedes the maximal increase in growth rate, and both polymerase activity and growth have increased by 6 hr.

Figure 4 shows the effect of adding increasing levels of *E. coli* polymerase to chromatin isolated either from internodes 12 hr after GA_3 treatment, or from comparable controls. There is no evidence that the amount of available template is increased by GA_3 treatment. No evidence was obtained for increases at 6 and 24 hr either, though a small increase (some 10%) was observed at 48 hr. This situation is in contrast with that observed when seed dormancy is broken by gibberellin. Jarvis *et al.* (5) have shown that dramatic increases in template availability occur in hazel seeds during dormancy breaking by gibberellin, and the increase is followed by increased polymerase activity. The situation in pea internodes, which are already growing at the time of GA_3 treatment, is more reminiscent of that seen in hypocotyls treated with 2,4-dichlorophenoxyacetic acid, where an increase in polymerase occurs without detectable increase in available template (7). It remains possible that in the pea internodes there are small changes in template which cannot be detected by these techniques. Changes in RNA released by pea nuclei isolated after GA_3 treatment (6) are consistent with this possibility.

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