ABSCISIC ACID INDUCED CHANGES IN NUCLEOTIDE COMPOSITION OF RAPIDLY LABELLED RNA SPECIES OF PEAR EMBRYOS*

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Summary

The plant hormone abscisic acid greatly altered the nucleotide composition of the rapidly labelled RNA species. Mechanism of abscisic acid action may be related to a change in the read out pattern of the genome.

Abscisic acid (ABA) is a widely occurring hormone of plant origin and has been shown to have a profound effect on many developmental processes (Addicott and Lyon, 1969). A selective nature of ABA action in nucleic acid and protein metabolism is indicated by a number of studies (Chrispeels and Varner, 1967; Ferrari and Varner, 1969; Villiers, 1968; Khan and Heit, 1969, etc.). It has been shown that kinetics of ABA inhibition of gibberellic acid-induced α-amylase is similar to certain base analogs (Chrispeels and Varner, 1967). Another report shows that ABA acts by increasing nuclease activity (Srivastava, 1968). It has been recently suggested that ABA influences RNA synthesis by a chromatin preparation by reacting or binding with a cytoplasmic factor (Pearson and Wareing, 1969). The present studies were initiated to further explore the mechanism of ABA action.

Results and Discussion

RNA species of excised pear embryos were separated on MAK columns and designated as 1) sRNA, 2) DNA-RNA, 3) lrRNA, 4) hrRNA, 5) mRNA and 6) tbRNA (Fig. 1) as shown in earlier communication (Khan et al, 1968). The nucleotide composition of the RNA species from ABA treated and control embryos (Fig. 1)

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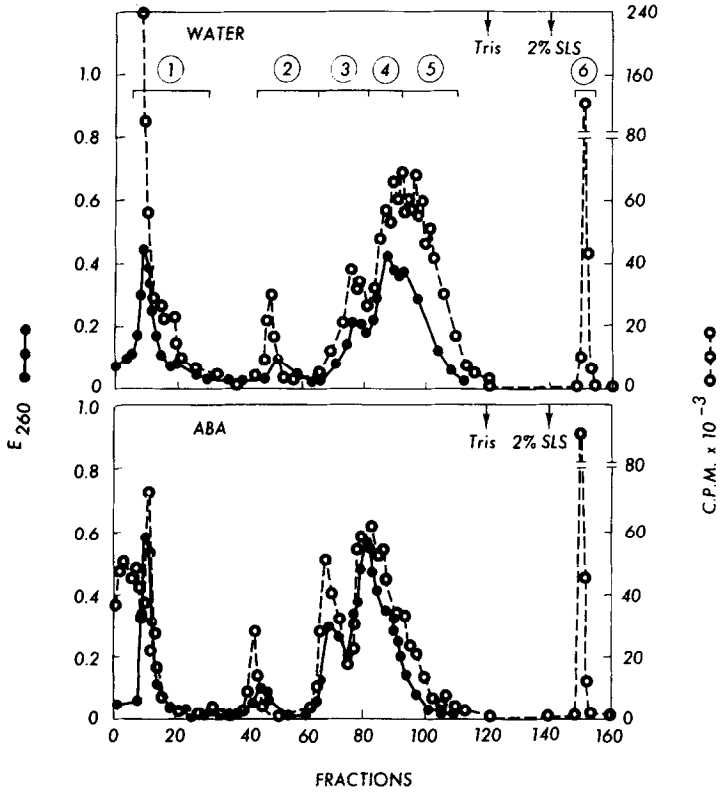

Fig. 1. MAK column fractionation of nucleic acids from excised pear embryos obtained from seeds prechilled for 34 days. 80 embryos were preincubated for 1 hour in aqueous solution of the hormone, washed thoroughly, and then reincubated for 3 hours in the heat-sterilized medium containing 0.03 M Sucrose, 0.01 M tris-HCl, pH 6.5, 0.01 M $MgCl_2$, 0.06 M KCl and 26 μc of carrier-free $H_3{}^{32}PO_4$ in 5 ml solution. Incubation was carried out at 25° on a metabolic shaker. Extraction, purification and fractionation of nucleic acids were as described (Khan et al, 1968). The column was washed following NaCl elution by 100 ml of tris-HCl, pH 7.6 and the tbRNA was eluted with 2% sodium lauryl sulfate in tris-HCl, pH 7.6 as indicated by arrows. Absorbency at 260 mμ and radioactivity were determined on aliquots of each fraction. Concentration of hormones used: ABA, 1.8×10^{-5} M; GA_3, 10^{-4} M; kinetin, 5×10^{-5} M.

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was determined on tbRNA, rRNA (lrRNA + hrRNA + mRNA) and DNA-RNA fractions all of which were found to be rapidly labelled. The RNA fraction retained on the MAK column and resisting salt elution (tbRNA) has been found to contain the highest A+U/G+C and A/U ratios of RNA fractions analyzed and has been shown to have messenger properties (Asano, 1965; Ellem, 1966). The sucrose density gradient patterns of tbRNA from ABA-treated and control pear embryos are shown

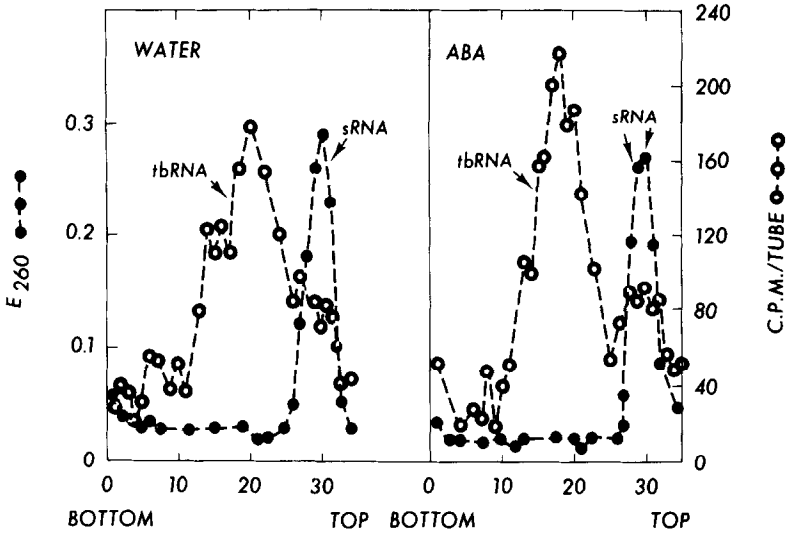

Fig. 2. Sucrose density gradient sedimentation of tbRNA (from excised embryos of seeds prechilled for 28 days). Fractions containing tbRNA (see Fig. 1, Fraction 6) were pooled, 1 mg of yeast soluble RNA was added and stirred for 5 minutes with the addition of equal volume of cold phenol in presence of bentonite (40 mg). Water layer was centrifuged off and tbRNA precipitated by addition of 2 volumes of ethanol in presence of 0.4 g of potassium acetate. Dialyzed overnight at 12° in tris-HCl, pH 7.6. Purified preparation of tbRNA was layered on 5-20% sucrose gradient in tris-HCl, pH 7.6 buffer containing 0.01 M $MgCl_2$ and centrifuged at 41,000 rev./min. for 6 hours in Spinco model SW 41 Ti rotar in L2-65B ultracentrifuge. 15 drop fractions were collected by bottom puncture, 1.5 ml water added in each fraction and absorbency at 260 mμ and radioactivity determined. Amount of tbRNA used: ABA, 41.6 embryo equivalent; water, 46.1 embryo equivalent.

in Fig. 2. It is obvious that tbRNA is a heterogenous mixture of RNA of various molecular weights.

RNA fractionation on MAK columns shows that AMP-rich RNA is obtained with increasing salt gradients with the fraction eluting last to be high in AMP content (Chroboczek and Cherry, 1966; Khan and Heit, 1969). This fraction has been shown by several workers as having messenger RNA (mRNA) properties (Key and Ingle, 1964; Ingle et al, 1965; Chroboczek and Cherry, 1966; Lin et al, 1966; Jachymczyk and Cherry, 1968). Though possessing mRNA properties this fraction has been shown to be a heterogenous mixture of several RNA species (Ingle and Key, 1968). This has often caused difficulty in our laboratory to precisely separate mRNA from rRNA from MAK columns. For the purpose of the

present studies, therefore, we have pooled the rRNA and mRNA fractions for achieving uniformity among treatments.

The fraction designated DNA-RNA as eluted from MAK columns consists of an appreciable amount of RNA associated with DNA (Cherry, 1964). This fraction has been shown to be rich in both UMP as well as AMP (Ewing and Cherry, 1967).

The nucleotide composition of tbRNA, rRNA and DNA-RNA from excised pear embryos is shown in Table 1. The tbRNA from control embryos had the highest AMP content as well as the highest A+U/C+G ratio of RNA species analyzed. The DNA-RNA fraction also showed a high A+U/C+G ratio due to high UMP content. U/G ratio was highest in the DNA-RNA fraction. In response to the hormone, ABA, there was a remarkable increase in the UMP content and a decrease in GMP content of all RNA species analyzed with little or no effect on AMP and CMP contents. Consequently, the A+U/C+G ratio increased in all RNA fractions, the effect being especially noticeable in the case of tbRNA and DNA-RNA. Still more conspicuous was the difference in U/G ratio between the controls and the ABA treatments, this ratio having increased more than twofold by ABA in the case of DNA-RNA fraction. A sum of UMP and GMP remained nearly the same in the controls and ABA treatments (Table 1).

The hormone, gibberellic acid (GA_3), at the concentrations used, did not modify the ABA effect on nucleotide composition of tbRNA and rRNA (Table 1). Kinetin, on the other hand, while not modifying the ABA effect on tbRNA, increased slightly the A+U/C+G and U/G ratios of rRNA (Table 1).

The foregoing data clearly show the specific nature of ABA action. The changed base composition is not limited to any one particular RNA species, although some species are affected more than the others. The observed change in composition of RNA species by ABA could be due to DNA template availability, selective changes at the template site, or due to increased action or inaction of available RNA polymerase. Other possibilities cannot be ruled out.

The mechanism of hormone action on RNA polymerase is not known although suggestions, based on indirect evidence, have been made. Growth promoting

Table 1. Nucleotide composition of rapidly labelled RNA species from excised Pyrus communis embryos from 34 day prechilled seeds. Data in parentheses and those corresponding with DNA-RNA are from 28 day prechilled embryos.

RNA fraction	Treatment	Percentage of total radioactivity					Nucleotide ratios	
		CMP	AMP	GMP	UMP	U+G	A+U/C+G	U/G
tbRNA	Water	19.2	31.2	24.2	25.3	49.5	1.30	1.04
		(16.6)	(37.9)	(25.3)	(20.1)	(45.4)	(1.38)	(0.79)
tbRNA	ABA	20.3	30.5	19.1	30.1	49.2	1.54	1.58
		(17.6)	(34.1)	(20.3)	(27.8)	(48.1)	(1.63)	(1.37)
tbRNA	ABA + K	19.6	30.9	19.7	29.7	48.4	1.55	1.51
tbRNA	ABA + GA_3	20.3	30.0	19.4	30.2	49.6	1.52	1.56
rRNA	water	22.9	24.5	26.4	26.1	52.5	1.02	0.98
rRNA	ABA	22.3	25.2	22.7	29.7	52.4	1.22	1.31
rRNA	ABA + K	22.0	25.5	22.9	31.0	53.9	1.30	1.48
rRNA	ABA + GA_3	22.2	25.2	22.6	30.0	52.6	1.23	1.33
DNA-RNA	water	26.9	25.5	17.4	30.1	47.5	1.25	1.73
DNA-RNA	ABA	21.6	25.0	10.6	42.6	53.2	1.73	3.99

Fractions representing DNA-RNA (2), rRNA (3+4+5) and tbRNA (6) (see Fig. 1) were pooled separately. 1 mg yeast carrier RNA was added to each fraction. Fraction (6) was deprotenized by washing once with phenol. Nucleic acid was precipitated from Fractions (2) and (3+4+5) by making them 5% with respect to trichloracetic acid (TCA) (cold). The precipitate was centrifuged off after 1 hour at 0°, washed once with 5% TCA and absolute alcohol. Fraction (6) was precipitated by absolute alcohol (2 volumes), kept for 30 minutes at -20° and then washed once each with 5% TCA and absolute alcohol. RNA from each fraction was hydrolyzed with 0.5 N KOH for 16 hours at 37°, neutralized with 2.5 M $HClO_4$ and the mononucleotides fractionated (5 ml fractions) on Dowex 1 columns. Radioactivity of each fraction was determined.

hormones, both from animals and plants, have been shown to increase RNA polymerase production. ABA, on the other hand, has been shown to decrease RNA polymerase activity in radish chromatin presumably by decreasing the DNA transcription sites (Pearson and Wareing, 1969).

A common feature of all hormones, including ABA (Pearson and Wareing, 1969), is that their addition to RNA assay systems usually has no effect. A nuclear or cytoplasamic factor appears to be essential for eliciting activity from chromatin preparations (Johri and Varner, 1968; Jarvis et al, 1968; Pearson and Wareing, 1969). A recent work shows that an increased rate of RNA synthesis by chromatin as well as nuclei is caused by a plant hormone in presence of a protein mediator even at saturating amounts of RNA polymerase (Matthysse and Phillips, 1969). Thus factors other than RNA polymerase may play an active role in controlling RNA transcription. Other enzymes may participate during RNA synthesis by hormones and chromatin preparations. ABA increases the production of nucleases in barley leaf chromatin preparations (Srivastava, 1968). Thus, nucleases or other enzymes may play a role in determining composition of RNA by selective digestion of RNA and DNA. A low molecular weight RNA has been shown in ABA treated chromatin preparations suggesting that it may represent incomplete RNA molecule synthesis (Pearson and Wareing, 1969).

By the use of E. coli RNA polymerase, it was recently shown that in soybean chromatin preparations certain DNA templates which are not normally transcribed do so in presence of the hormone 2,4-dichlorophenoxyacetic acid (O'Brien et al, 1968). This hormone also changes the composition of RNA transcribed (Holm et al). Thus template availability and the type of polymerase at the template sites may play a role in determining RNA composition. It would appear that hormones, directly or indirectly, induce changes at the transcription sites and thus cause differences in the portion of the genome being read.

Another interesting observation, though not necessarily relevant to the present discussion, is the presence of homopolymeric regions in DNA, proposed to be initiation sites of RNA synthesis (Szybalski et al, 1966). These regions are asymmetrically distributed between the two strands of DNA of many organisms (Kubinski et al, 1966; Szybalski et al, 1966). A preferential transcription or "inhibition" of such regions could account for the observed changes in base composition. However, there is no evidence that hormones take part in such a

process. On the basis of our data we propose that ABA changes the read out pattern of the genome. The mechanism by which this is achieved is not known.

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