RAPIDLY LABELLED DNA IN PLANTS

K. Šebesta, J. Bauerová, and Z. Šormová
Institute of Organic Chemistry and Biochemistry
Czechoslovak Academy of Science
Prague, Czechoslovakia

Received January 28, 1965

Evidence has accumulated in the past years that the metabolic stability of DNA or rather the dependence of DNA synthesis on cell division is not as complete as previously assumed. In animal and plant tissues a certain degree of synthesis and turnover of DNA can be observed even without any connection with the mitotic cycle (1-4). Demonstrations of DNA outside cell nucleus have also accrued (3, 5-10). In plant tissue the so-called satellite DNA has been studied more extensively, this DNA differing from the main part of DNA in its buoyant density and composition as well as in its preferential localization in the chloroplasts (11-18). This DNA, the synthesis of which is light-stimulated, displays a considerable turnover rate and represents thus the metabolically active DNA of plants (19-21).

In a previous report on the utilization of nucleic acid precursors in the light and in dark the stimulation of DNA synthesis in seedlings placed in the light was described (22). In view of the above papers on satellite DNA in chloroplasts and since DNA with rapid turnover was demonstrated even in nongreen parts of plants (23) it was considered to be of interest to find out whether and to

what extent the DNA with light-stimulated synthesis is identical with rapidly labelled DNA and to establish the localization of this DNA in the green and nongreen parts of plants and the dependence of its formation on the photosynthetic apparatus.

For this investigation we used cucumber seedlings which were infiltrated with uracil-2-^{14}C at the age of 7 days and incubated for 8 h in the light. The localization and properties of newly synthesized DNA were then estimated on the basis of determining ^{14}C-thymine. In order to determine the character of the newly formed DNA we used fractionation on columns with methylated albumin (24). Nucleic acids for chromatography were prepared (25) by crushing plants frozen to -40°C and extracting with ethanol at 0°C. The dry powder thus obtained was extracted with standard sodium chloride-sodium citrate, to which sodium dodecylsulphate (5%) was added. The extract was deproteinized with phenol and chloroform-isoamylalcohol and further fractionated with isopropanol (basically according to Marmur (26)). The fraction precipitated by adding isopropanol to 38.8% (v/v) was resolved on a methylated albumin column (Fig.1). In view of the fact that the precipitation with isopropanol removes sRNA the fraction contains only DNA (demonstrated according to Burton (27)) and highmolecular RNA. The fraction corresponding to the radioactivity peak placed asymmetrically with respect to the DNA peak of the absorbancy elution curve contains both RNA and DNA and is apparently identical with a similar fraction obtained from the peanut by Cherry (28, 29). The DNA and RNA activities were determined by measuring the radioactivity of thymine and uracil respectively after acid

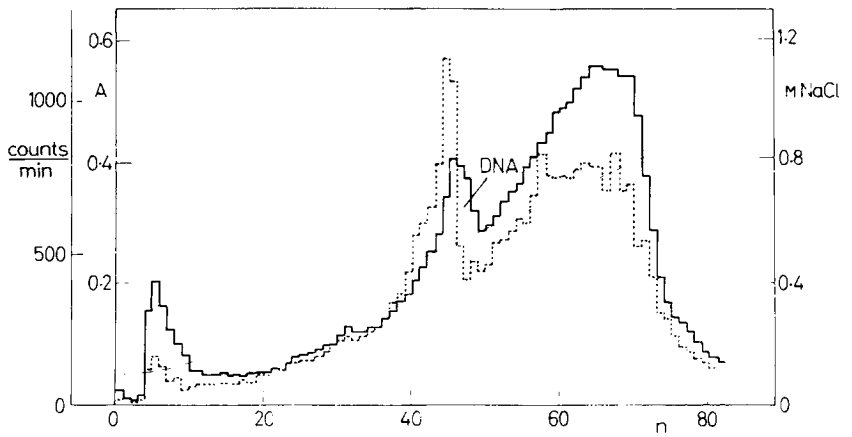

Fig. 1. Separation of Nucleic Acids on a Methylated Albumin Column.

Column 2 x 11 cm in size, gradient 0.1-0.9M-NaCl, 550 ml, 7 ml fractions. 150 A units of nucleic acids applied to the column. Radioactivity estimation: 1 ml of each fraction mixed in the presence of 100 µg cold RNA with TCA to the final concentration of 5% and filtered through a membrane filter which was used for measurement in a flow counter. n number of fractions, —— absorbancy, ····· radioactivity, ——— NaCl concentration in the elution solution.

hydrolysis of this fraction with perchloric acid and chromatography of bases in isopropanol-HCl in the presence of cold uracil, thymine, and cytosine as carriers. The ratio of radioactivity T/U in this fraction was 1.8 : 1. The radioactivity peak thus contains rapidly labelled DNA which is distinct from the main fraction of DNA. In contrast with the preliminary results obtained with an analogous fraction from the peanut (28) the nucleic acids in our preparation are hydrolyzed by RNase or by DNase.

In the attempt to achieve better separation of the rapidly labelled DNA from the less labelled main fraction of DNA we tried to make use of fractional precipitation

with isopropanol. It was found that this procedure precipitates the rapidly labelled DNA mostly at higher saturation while a part of the main fraction of less labelled DNA is precipitated at lower saturations (Table I).

TABLE I

Fractional Precipitation of Nucleic Acids with Isopropanol

% Isopropanol (V/v)	Data for the fraction precipitated at the given isopropanol concentration				
	A	µg DNA	counts/min[x] in fraction	µg DNA per absorbancy unit	counts/min in thymine/ 100 µg DNA
32.7	6	200	13,700	33.5	1,033
35.2	14,4	224	1,390	15.6	994
38.8	57.6	200	1.360	3.5	5,210

[x] sum of radioactivity of NA-pyrimidines

Fractions obtained by isopropanol precipitation were again subjected to fractionation on a methylated albumin column (Fig. 2). It follows from the results that the sharp peak of rapidly labelled DNA precipitated at higher isopropanol concentrations is very homogeneous and differs in its position in the column quite distinctly from the main DNA fraction which is practically unlabelled. The ratio of radioactivity T/U at this peak is 1.48 : 1. Radioactive DNA which is precipitated at lower isopropanol concentrations does not display this striking homogeneity but the T/U radioactivity ratio has the same value. The T/U ratio cannot be considered as a dependable measure of the

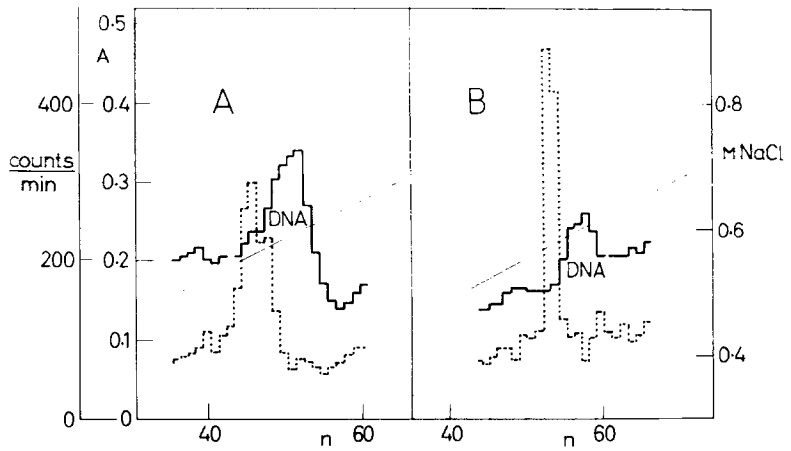

Fig. 2. Separation of Fractions after Isopropanol Precipitation on a Methylated Albumin Column.
A Fraction precipitated in 34.7% (v/v) isopropanol, B fraction precipitated between 34.7% and 38.8% (v/v) isopropanol. Methylated albumin column 2 x 9.5 cm in size, gradient 0.3M-0.9M-NaCl, 800 ml, 7 ml fractions. Only the region of DNA separation is shown. Radioactivity determination as in Fig.1. n number of fractions, —— absorbancy, ····· radioactivity, —— NaCl concentration in the elution solution.

ratio of DNA and RNA in the fraction since the rate of labelling of the two bases can be different. The finding of a possible bond between both nucleic acids is being investigated at present. It follows from the fractionation results that the relationships previously determined for the formation of DNA from its precursors under illumination and in the dark (21) will hold only for the rapidly labelled fraction of plant DNA.

Further experiments had to do with the localization of the formation of rapidly labelled DNA in the plant especially because heretofore it had been thought of in a close association with the photosynthetic apparatus. After

infiltration of ^{14}C-uracil and incubation of plants in the light the plants were divided into root, hypocotyl and cotyledons. At this stage of development the latter organs are the main site of assimilation. The nucleic acid fraction was then extracted basically according to Schneider. To the fraction thus obtained cold carriers (uracil, thymine and cytosine) have been added and the mixture hydrolyzed with perchloric acid. The hydrolyzate was separated by chromatography using isopropanol-HCl as solvent. After elution of the corresponding zones the radioactivity of the pyrimidine bases was determined. In order to make sure that the results are not affected by transport, either of complete nucleic acids or of their intermediates during incubation the experiment was repeated in such a way that both the infiltration and incubation were carried out with separate plant parts (Table II). The results in this case were somewhat less consistent than with complete plants. Both experimental series document that the rapidly labelled DNA is formed independently both in the roots and in the cotyledons. The values for isolated hypocotyl where no cell division occurs indicate that the synthesis of rapidly labelled DNA does not depend on the meristematic tissue of the root tip and the growth apex. As to the specific activity it is highest in the roots but the absolute amount of rapidly labelled DNA is found in cotyledons.

It follows from the above results that the rapidly labelled DNA is synthesized in all parts of the plant and is thus not necessarily connected with the function of the photosynthetic apparatus. We are further dealing with DNA which differs from the bulk DNA with slow turnover not only in its role in the cell (as characterized by the turnover

TABLE II

Radioactivity of Pyrimidines in the Nucleic Acid (NA) Fraction after Incubation of Plants with Uracil-2-^{14}C

Plant organ	Uracil		Thymine	
	counts/min per 1 mg NA	counts/min per 1 g plant weight	counts/min per 1 mg NA	counts/min per 1 g plant weight
	Incubation of whole plant			
Root	27,400	12,150	4,370	1,080
Hypocotyl	12,800	1,470	2,380	1,140
Cotyledons	16,100	13,850	2,250	3,805
	Incubation of separate organs			
Root	11,300	6,750	1,900	845
Hypocotyl	9,050	3,120	1,710	1,220
Cotyledons	10,800	9,450	778	1,030

rate) but also in its structure. In order better to understand the function of the rapidly labelled DNA it will be of importance to establish the nature of its possible association with accompanying RNA and its localization, particularly in the individual components of the root cell with a view to the analogies between chloroplasts and mitochondria as inheritable systems (30).

REFERENCES

1. Pelc, S.R., J. Cell Biol. 22, 21 (1964).
2. Pelc, S.R., Exptl. Cell Res. 29, 194 (1963).
3. Mandel, P., Wintzerith, M., Borkowska, I., Mandel, L., Compt. rend. 258, 1914 (1964).
4. Giles, K.W., Myers, A., Biochim. Biophys. Acta 87, 460 (1964).

5. Rabinowitch, M., Plaut, W., J. Cell Biol. 15, 525 (1962).
6. Wolstenholme, D.R., Plant, W., J. Cell Biol. 22, 505 (1964).
7. Bach, M.K., Proc. Natl. Acad. Sci. U.S. 48, 1013 (1962).
8. Luck, D.J.L., Reich, E., Proc. Natl. Acad. Sci. U.S. 52, 931 (1964).
9. Guttes, E., Guttes, S., Science 145, 1057 (1964).
10. Schatz, G., Haslbrunner, E., Tuppy, H., Biochem. Biophys. Res. Commun. 15, 127 (1964).
11. Chun, E.H.L., Vaughan, Jr.,M.H., Rich, A., J. Mol. Biol. 7, 130 (1963).
12. Sager, R., Ishida, M.R., Proc. Natl. Acad. Sci. 50, 725 (1963).
13. Kirk, J.T.O., Biochim. Biophys. Acta 76, 417 (1963).
14. Baltus, E., Brachet, E., Biochim. Biophys. Acta 76, 490 (1963).
15. Wollgiehn, R., Mothes, K., Naturwiss. 50, 95 (1963).
16. Pollard, C.J., Arch. Biochem. Biophys. 105, 114 (1964).
17. Ray, D.S., Hanawalt, P.C., J. Mol. Biol. 9, 812 (1964).
18. Gibor, A., Izawa, M., Proc. Natl. Acad. Sci. U.S. 50, 1164 (1963).
19. Iwamura, T., Biochim. Biophys. Acta 61, 472 (1962).
20. Iwamura, T., Kuwashima, S., Biochim. Biophys. Acta 82, 678 (1964).
21. Richter, G., Senger, H., Biochim. Biophys. Acta 87, 502 (1964).
22. Šebesta, K., Bauerová, J., Šormová, Z., Collection Czechoslov. Chem. Commun. 29, 843 (1964).
23. Sampson, M., Katoh, A., Hotta, Y., Stern, H., Proc. Natl. Acad. Sci. U.S. 50, 459 (1963).
24. Mandel, J.D., Hershey, A.D., Anal. Biochem. 1, 66 (1960).
25. Doskočil, J., Horská, K., to be published.
26. Marmur, J., J. Mol. Biol. 3, 208 (1961).
27. Burton, K., Biochem. J. 62, 315 (1956).
28. Cherry, J.H., Plant Physiol. 39, Suppl. lv. (1964).
29. Cherry, J.H., Science 146, 1067 (1964).
30. Gibor, A., Granick, S., Science 145, 890 (1964).