DNA-DEPENDENT RNA SYNTHESIS IN CHLOROPLAST PREPARATIONS

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Received December 3, 1963

It has already been shown (Kirk, 1963a; 1963b) that broad bean chloroplasts contain a type of DNA which differs from the nuclear DNA in its base ratio and in the ease with which it may be extracted by acid. Also, Chun et al.(1963) found that chloroplasts of Spinacia oleracea and Beta vulgaris contain DNA with a density different from that of the nuclear DNA. These workers further reported that Chlamydomonas reinhardi contained a minor DNA component in addition to the major, nuclear DNA: however, the intracellular location of this minor DNA was not determined. It was subsequently shown by Sager and Ishida (1963) to be associated with the chloroplasts. Leff et al.(1963) found that green cells of Euglena gracilis contained a minor DNA component which was absent from cells of a strain which had lost the ability to form chloroplasts: they suggest that this DNA is necessary for chloroplast formation.

The aim of the present work was to investigate whether the presence of DNA in chloroplasts enables these bodies to synthesize RNA. Bandurski and Maheshwari (1962), in an investigation of nucleic acid synthesis in tobacco leaf nuclei, mention that the chloroplast fraction incorporated

some radioactivity from ^{14}C-ATP into nucleic acid. However, the chloroplasts were isolated by differential centrifugation and were contaminated by nuclear material.

METHODS

30 g of broad bean leaves were ground for 4 min with a pestle and mortar, in 90 ml of 0.5 M sucrose, 0.05 M tris (pH 7.4), 1 mM EDTA, 2.1 mM cysteine.HCl. Very pure chloroplast preparations were obtained from the homogenate by high speed density gradient centrifugation as previously described (Kirk, 1963b). A nuclear fraction was also obtained as previously described. RNA formation was studied by incubating chloroplasts or nuclear material for 10 min at 30° in a medium containing 50 µmoles tris (pH 8.0), 8 µmoles $MgSO_4$, 3 µmoles cysteine.HCl, 40 mµmoles CTP, 40 mµmoles GTP, 40 mµmoles UTP and 7.4 mµmoles 8-^{14}C-ATP (2.3 µC/µmole), in a total volume of 0.8 ml. The reaction was stopped by the addition of 4 ml of 0.25 N $HClO_4$. In the case of nuclear material, a crude mixture of chloroplasts and nuclei was then added as a carrier. The tubes were left in an ice bath for 30 min: the precipitate was then centrifuged, washed twice in 0.2 N $HClO_4$, once in water and plated out for counting. DNA was estimated as already described (Kirk, 1963b).

RESULTS

Under the above conditions the purified chloroplasts incorporated radioactivity from ^{14}C-ATP into a material which was precipitated by 67% (v/v) ethanol or 0.2 N $HClO_4$. After incubation with ribonuclease (25 µg/ml) at 30° for 20 min, only 10-14% of the radioactive material could be precipitated by 0.2 N $HClO_4$. Treatment with 0.5 N $HClO_4$ for 20 min at 70° rendered the reaction product completely

soluble. Not more than 3% of the radioactive material could be precipitated by 0.2 N $HClO_4$ after incubation with 0.5 N NaOH for 18 hours at 30°. These data suggest that the radioactivity was being incorporated into a polyribonucleotide.

Incorporation was almost completely abolished by omission of CTP, GTP and UTP, or by addition of deoxyribonuclease (DNAase). Actinomycin D, which inhibits RNA synthesis *in vivo* (Kirk, J.M.,1960) and RNA polymerase activity *in vitro* (Hurwitz *et al.*,1962; Goldberg *et al.*,1962), by combining with the DNA primer, also inhibits incorporation in the chloroplast preparations. The results of typical experiments are shown in Table 1.

		μmoles ^{14}C-adenine incorporated/10 min			
Expt.	Conditions	Chloroplasts	% change	Nuclear fraction	% change
1	Complete system.	408	-	59	-
	" " minus CTP,GTP,UTP.	13	-97	69	+17
2	Complete system.	672	-	77	-
	" " plus 10 μg DNAase.	71	-89	39	-49
3	Complete system.	286	-	52	-
	" " plus 3 μg actinomycin D.	108	-62	36	-31

Table 1. Incubation conditions as described in the Methods section. In each experiment chloroplast and nuclear fractions prepared from same batch of leaves. Expt. 1: 23.3 mg dry weight chloroplasts containing 54 μg DNA. Nuclear fraction containing 67 μg DNA. Expt.2: 14.0 mg dry weight chloroplasts containing 47 μg DNA. Nuclear fraction containing 59 μg DNA. Expt.3: 22.1 mg dry weight chloroplasts containing 53 μg DNA. Nuclear fraction containing 52 μg DNA.

Incorporation was not stimulated by light, in the presence or absence of 0.1 mM phenazine methosulphate.

The specific incorporation activity(μμmoles adenine incorporated/μg DNA) obtained with the chloroplast fraction was always higher (5- to 12-fold) than that shown by the nuclear fraction. Also, as shown in Table 1., the activity of the nuclear fraction was less sensitive to actinomycin and to DNAase than the chloroplast activity, and was less dependent upon the presence of the three unlabelled nucleoside triphosphates (omission of these causing either stimulation or only relatively slight inhibition).

DISCUSSION

The fact that the chloroplasts have a much higher specific incorporation activity than the nuclear fraction, taken together with the different characteristics of the two systems (different sensitivities to actinomycin, DNAase, omission of unlabelled triphosphates) suggests that the activity in the chloroplast preparations is not due to contaminating nuclear material. The simplest and most probable hypothesis is that the activity represents a chloroplast RNA polymerase working with the chloroplast DNA as a primer. Nevertheless, it could be argued that the chloroplast preparations are contaminated by a specific fraction of the nuclear material which for some reason has a much higher activity than the rest of the nuclear material: however, this explanation seems less plausible than the one suggested above.

It is hoped to investigate in more detail the characteristics of the reaction and the nature of the reaction product.

ACKNOWLEDGEMENTS

The author is indebted to the Department of Scientific and Industrial Research for financial support.

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