INHIBITION OF GREENING OF ETIOLATED LEAVES BY ACTINOMYCIN D.*

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Chloroplasts in green leaves of higher plants contain large amounts of chlorophylls a and b and a highly organized system of lamellae and grana (tightly packed lamellae). Proplastids in leaves of dark-grown (etiolated) plants such as bean or maize contain small amounts of protochlorophyllide and protochlorophyll; few, if any, lamellae are present but there may be one or more regularly arranged crystal lattice-like bodies (prolamellar bodies). Upon illumination of etiolated leaves the protochlorophyllide present in the proplastids is quickly converted to chlorophyllide. Then, after a lag of several hours, additional chlorophyll is formed; the prolamellar body dissociates almost immediately while lamellae and grana form later. Except for the photoconversion of protochlorophyllide to chlorophyllide and probably some of the rearrangements of the prolamellar body, the changes which occur in plastids upon illumination appear to involve the synthesis of structural protein and enzymes. For example, Marguilies (1962) has demonstrated that chlorophyll formation during the illumination of etiolated bean leaves is partially inhibited by chloramphenicol.

Ribonucleic acid particles about 170 Å in diameter are present in both proplastids and chloroplasts of maize (Jacobson et al., 1963)

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and presumably of other plants. Lyttleton (1962) has isolated ribosomes from green spinach chloroplasts; they differ from cytoplasmic ribosomes in base composition and sedimentability. There is also increasing evidence that chloroplasts contain deoxyribonucleic acid (e.g. chemical determinations of DNA in isolated chloroplasts: spinach -- Biggins and Park, 1961; tobacco and spinach -- Chiba and Sugahara, 1957; cytological evidence: Chlamydomonas -- Ris and Plaut, 1962; Swiss chard -- Kislev, Swift, and Bogorad, unpublished.)

In the present investigation the possible functional relationship between proplastid DNA and RNA was studied. The production of chlorophyll in illuminated etiolated leaves of bean and maize has been found to be inhibited by actinomycin D*. In view of the mode of action of this antibiotic (e.g. Reich et al., 1962; Hurwitz et al., 1962), it appears that DNA-dependent RNA synthesis is required for the full development of chloroplasts from proplastids. Light seems, in some manner, to induce RNA synthesis in this system. That light may play an inductive role in RNA synthesis and the formation of plastids in Euglena (a somewhat different system than the development of chloroplasts from "etiolated" proplastids in higher plants) was suggested by the experiments of Brawerman, et al. (1961). Furthermore, Smillie (1963) has reported that 5-fluorouracil inhibits the development of chlorophyll, and presumably of plastids, in illuminated Euglena; supporting the suggestion that RNA synthesis is involved in the greening of this organism.

Experimental

Leaf segments from 14 to 18 day-old dark-grown bean (Phaseolus vulgaris var. Red Kidney) or 8 to 10 day-old maize (Zea mays Wf9 x 1337) were incubated with various levels of actinomycin D, or actinomycin-free solution in the case of the control treatments, in 35 x 10 mm petri

*The actinomycin D used in these experiments was kindly provided through the courtesy of Dr. George E. Boxer as a gift of Merck, Sharp and Dohme, Rahway, N. J.

dishes containing a sheet of filter paper and 0.4 ml. of solution for 24 hours. During the ensuing 12 hours some control tissues were left in darkness while all the other groups of leaf material were illuminated. "Dark" manipulations were conducted under a dim green safelight. Chlorophyll was determined spectrophotometrically in 80% aqueous acetone extracts of the leaf tissue (Arnon, 1949). Results of four experiments are tabulated in Tables I and II. The apparent differences in sensitivity of maize and bean to actinomycin D may be real or may merely reflect species-related variability in penetration.

TABLE I

EFFECT OF ACTINOMYCIN ON
CHLOROPHYLL FORMATION BY ETIOLATED MAIZE LEAVES

	mμgm. Chl / mg. F.W.	% of chl. formed by Lt. Control.
Dark Controls	94	--
Light Controls	343	--
Actinomycin:		
9 μgm/ml.	274	72%
45 μgm/ml.	176	33%
90 μgm/ml.	150	22%

TABLE II

EFFECT OF ACTINOMYCIN ON
CHLOROPHYLL FORMATION BY ETIOLATED BEAN LEAVES

	EXP. 1		EXP. 2		EXP. 3	
	Chl*	% of Control+	Chl*	% of Control+	Chl*	% of Control+
Dk Controls	41	--	11	--	42	--
Lt Controls	332	--	173	--	216	--
10 μgm/ml.	317	95%	193	112%	163	81%
30 μgm/ml.	--	--	56	28%	97	31%
60 μgm/ml.	25	-5%	31	12%	26	-7%

*Chl. = mμgm chlorophyll/mg. fresh weight.
+% of Control = % of chlorophyll formed by light control.

In the experiments performed with maize, six small sections, each approximately 2 x 4 mm. were cut from the middle sector of each leaf

and were distributed randomly among the treatments. Such sections responded to actinomycin D only if infiltrated with a solution of the antibiotic at the initiation of the preincubation in darkness, after infiltration the leaf segments were placed in petri dishes as described above. The greening of etiolated whole detached maize leaves upon illumination was inhibited by actinomycin D if they were placed, with their cut bases immersed in an inhibitor solution, in front of a fan to stimulate uptake of the solution during the dark preincubation. At least at higher concentrations of actinomycin D, 12 hours of dark preincubation was adequate to elicit strong inhibition of greening of leaf segments.

In accord with previous observations (Eilam and Klein, 1962), sections of leaf from etiolated bean were found to green better on 0.2 M sucrose than on distilled water. Consequently sugar at this concentration was supplied in the present experiments. Circles of bean leaf tissue cut with a 0.6 mm. diameter cork borer were used in experiments 1 and 2 (Table II); two circles were cut from each of the two primary leaves and the discs were randomly distributed among the treatments to be tested. Leaf halves were used in the third experiment of Table II. Bean leaf tissue responded to actinomycin D without infiltration.

Little if any effect of blue light upon inhibition by actinomycin D could be observed. Illumination in experiment 1 was by unfiltered fluorescent light while in experiments 2 and 3 two layers of red cellophane were used to remove blue light.

Discussion:

The present experiments indicate that the light-induced development of chlorophyll in proplastids in bean and maize requires DNA-dependent RNA synthesis. The precise site and nature of the block introduced by actinomycin D is not certain ---- it would be most attractive to assume that there is a DNA-dependent production of messenger RNA in chloroplasts which is stimulated by light but can be inhibited by actinomycin D. This may well be the case but the data presented here

could equally easily be taken to show that actinomycin D inhibits a light-induced production of some plastid transfer RNA. The unavailability of a single type of transfer RNA would be the equivalent, for protein synthesis, of an amino acid deficiency. It seems unlikely, in view of the abundance of RNA particles in proplastids that actinomycin D inhibits greening by blocking ribosomal RNA synthesis. Finally the possible contribution of extra-plastidic nucleic acid and protein metabolism to the greening process is not understood and some part of the observed actinomycin D effect might be exercised through the nucleus and cytoplasm. The action of actinomycin D in this system is being used as a tool for further studies of nucleic acid metabolism in plastids.

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