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Short Communication

Effect of chloramphenicol and lincomycin on chloroplast DNA amplification in greening pea leaves

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Abstract

The light-induced increase in chloroplast DNA was not inhibited by two inhibitors of protein synthesis on 70S polysomes, chloramphenicol and lincomycin, in greening pea leaves. The changes in chloroplast DNA were observed by fluorescence microscopy and measured by hybridization to specific cloned probes. The results suggest that the light-induced increase in chloroplast DNA proceeds without *de novo* protein synthesis in the chloroplast, in agreement with those with mutants and cultured leaf tissue.

Introduction

Chloroplast DNA amplifies and amounts to 10 to 300 copies per chloroplast. The DNA replication is probably controlled by nucleo-chloroplast interaction, but is not well understood. In wheat that lacks substantial portions of chloroplast genome, the chloroplast DNA still replicates [3]. Amplification of chloroplast DNA occurs in chloroplast ribosome-deficient mutants of maize [14], rye [4] and barley [11]. In cultured spinach leaf tissue, a single cycle of chloroplast division and chloroplast DNA synthesis is insensitive to chloramphenicol [7]. These results suggest that chloroplast gene products are not needed for chloroplast DNA replication. Light stimulates chloroplast DNA amplification [13, 10, 1], but it is not known whether this stimulation requires *de novo* synthesis of chloroplast-encoded protein. We examined the effects of chloramphenicol or lincomycin, potent inhibitors of protein synthesis on 70S polysomes, on chloroplast DNA level in greening pea leaves. The inhibitors were directly supplied to intact plant. The DNA level was measured by hybridization to specific cloned probes, and observed by fluorescence microscopy.

Materials and methods

Growth of plants

Etiolated pea seedlings (*Pisum sativum* cv. Alaska) (7 days old) were illuminated with white fluorescent lamps of about 130 μE m^{-2} s^{-1} for 1–2 days. Starting 17 h or 6 h before the illumination began, either chloramphenicol (100 μg/ml) or lincomycin (100 μg/ml) was continuously supplied by transpiration *via* cotton threads sewn through the stalk at a flow rate of about 0.02 ml/h. In these conditions, the protein synthesis in chloroplast was almost com-

pletely (>95%) inhibited 6 h after administration (data not shown). Manipulations in the dark were done under a green safety light. As the control, water was used instead of the inhibitor solution. The apical buds were used for extraction of total DNA.

Fluorescence microscopic analysis

Specimens for fluorescence microscopy were prepared basically by a squashing procedure [6, 5] using a solution of 0.3 μg/ml 4,6-diamidino-2-phenyindole (DAPI) and 0.3% glutaraldehyde dissolved in buffer containing 0.6 mM spermidine, 0.25 M sucrose, 0.05% mercaptoethanol, 1 mM EDTA, and 10 mM Tris-HCl (pH 7.6). Specimens were observed under an epifluorescence microscope (Olympus BHK RFK type) equipped with phase contrast optics.

Measurement of chloroplast DNA levels

Total DNA was extracted from 3–10 apical buds, dot-blotted, and hybridized as described previously [10]. The probe used to detect chloroplast DNA was the large subunit gene of ribulose bisphosphate carboxylase (*rbc*L), the 1.2 kb *Bam* HI fragment from tobacco chloroplast [12], and that for nuclear DNA was chlorophyll a/b-binding protein gene (*Cab*), the 0.6 kb *Bam* HI fragment from pea cDNA [2].

Results and discussion

Etiolated pea seedlings were exposed to light for 2 days in the presence or absence of chloramphenicol, and the chloroplast DNA of the apical buds was stained wiht DAPI and observed by fluorescence microscopy [6, 5] (Fig. 1a). In etiolated leaves (A and B), a plastid nucleoid containing a

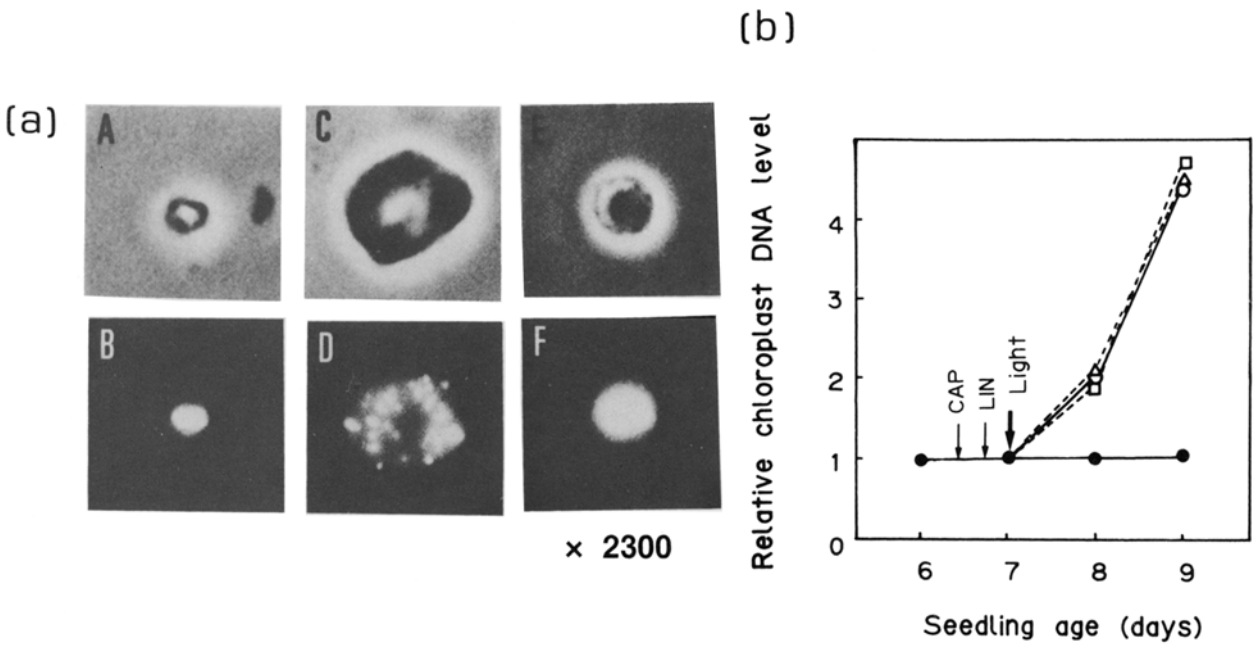

Fig. 1. Effects of inhibitors on the chloroplast DNA. (a): Pea seedlings grown in the darkness for 7 days were kept for 2 days more in the dark (A, B) or were exposed to white fluorescent light for 2 days with (E, F) or without (C, D) chloramphenicol (100 μg/ml). Phase contrast (A, C, E) and fluorescent micrographs (B, D, F) show plastids (A, C, E) and their plastid nucleoids (B, D, F) after DAPI staining. Photomicrographs (A, B), (C, D) and (E, F) are each of the same field. (b): Total DNA from pea buds treated with chloramphenicol (CAP) or lincomycin (LIN) was hybridized as described [10]. The *rbc*L dot was scanned by densitometry and plotted, with the value for etiolated seedlings (7 days old) taken as 1. Dark: (●), light: in the presence of 100 μg/ml chloramphenicol (□), 100 μg/ml lincomycin (△), or neither (○).

small amount of plastid DNA (chloroplast DNA) was found. Upon exposure to light, the chloroplast DNA increased in the presence (E and F) and absence (C and D) of chloramphenicol and thus plastid nucleoids in these conditions increased in size and emitted strong fluorescence. In the absence of chloramphenicol (C and D), the plastid nucleoid divided to form many small plastid nucleoids, which were dispersed throughout the entire plastid. The results suggest that light induction of chloroplast DNA accumulation proceeds without *de novo* protein synthesis in plastids, but that growth of the plastid and dispersion of newly synthesized chloroplast DNA within the plastid does require *de novo* protein synthesis in plastids.

To measure the chloroplast DNA level, a fixed amount of total DNA was dot-blotted and hybridized with *Cab* and *rbc*L probes. *Cab* gene dosages were almost invariable in the different DNA samples (data not shown), which indicated that a constant number of *Cab* gene copies was present in the DNA. The *rbc*L gene dosage which indicates the relative change in chloroplast DNA per cell [9] changed with illumination (Fig. 1b). The DNA levels of plants illuminated for 2 days increased about 4-fold compared to those of etiolated seedlings. This increase was not inhibited by chloramphenicol or lincoymcin. The results obtained with chloramphenicol and lincomycin being identical implies that the effects of chloramphenicol are not due to a side effect of this drug unrelated to its action as a protein synthesis inhibitor.

The present results, in agreement with earlier work with spinach leaf disk [7] and those with mutants [3, 4, 11, 14], suggest that chloroplast genes are not important for light-induced chloroplast DNA amplification. In the complete sequences of chloroplast DNA [8, 12], homologous sequences related to DNA replication have not been found, although about 30 open reading frames in the sequence have not yet been identified. Probably, nuclear-encoded proteins are important for the amplification.

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