A DIRECT LIGHT EFFECT ON MAINTAINING PHOTOSYNTHETIC ACTIVITY OF *NITELLA* CHLOROPLASTS

I. W. CRAIG and A. GIBOR

From the Biology Department, University of California, Santa Barbara, California 93106

ABSTRACT

The chloroplasts of internodal cells of *Nitella* are fixed to a stationary layer of cytoplasm whereas the nuclei and most of the cytoplasm stream along the longitudinal axis. Isolated internodal cells were maintained for several days with half the cell kept in the dark, the other half kept under continuous light. Photosynthetic activity of the cells was checked by placing the cell evenly illuminated in a $^{14}CO_2$ atmosphere. Chloroplasts of the previously dark half of the cell were found to fix only half as much CO_2 as the chloroplasts which were continuously illuminated. These results are discussed in relation to the possible direct effect of light on biosynthetic reactions of mature chloroplasts.

INTRODUCTION

In higher plants and in some algae several light reactions are required for the development of photosynthetic chloroplasts. One of these reactions is the conversion of protochlorophyllide to chlorophyll. Other light reactions, probably mediated via the phytochrome system, initiate the synthesis of other components of the photosynthetic apparatus. It was found, for example, that different enzymes of the carbon fixation pathway are synthesized in response to light. However, the fact that they appear at different rates suggests that light triggers a complex system of controls over the development of chloroplasts (2).

Since protochlorophyllide is found only within plastids it is obvious that the first of these light reactions, i.e., the absorption of light by protochlorophyllide, occurs within the plastids. It is not known whether the other light effects are also initiated within the plastids.

The metabolic activity of fully developed chloroplasts also appears to be under control. For example, rhythmic changes of photosynthesis have been reported in several organisms (7) The role of the nucleus in maintaining these photosynthetic rhythms was indicated by nuclear transplantation in *Acetabularia* (5).

Turnover of chlorophyll in higher plants has been demonstrated (6); perhaps other components of mature chloroplasts may also turn over. The anabolic phase of such a turnover might require light.

The experiments reported in this paper were undertaken to investigate whether light is required for the maintenance of photosynthetic activity in fully developed chloroplasts and whether other cell organelles possibly are involved in this phenomenon.

To investigate these questions we experimented with the alga *Nitella*. This alga has unique properties which make it most suitable for this study. *Nitella* belongs to the order Charales of the green algae. The chloroplasts of these coenocytic cells are adjacent to the cell wall and attached to a stationary layer of cytoplasm. They can, therefore, be exposed to different light regimes and

then be observed for any resultant changes in their properties. The nuclei and most of the cytoplasm stream along the longitudinal axis of the cell below the chloroplast layer. When a portion of a cell is kept in the dark, the nuclei and cytoplasm are exposed intermittently to light or dark because of the active streaming. However, the chloroplasts of the portion of the cell which is kept in the dark do not receive direct illumination because they are stationary.

It is possible, therefore, with *Nitella*, to study directly whether chloroplasts lose their photosynthetic capabilities when they are kept in the dark while the nuclei and cytoplasm are exposed to light. This can readily be accomplished by keeping one half of a cell in the dark for several days, then taking the cell out, and exposing the entire cell to light in the presence of $^{14}CO_2$. The fixation of CO_2 provides a measure for the photosynthetic efficiency of the chloroplasts which were kept previously in light or in dark.

MATERIALS AND METHODS

Cultures of *Nitella* sp. were obtained from the Carolina Biological Supply Co., Burlington, N. C., and were maintained at 20°C in a medium of the following composition: KNO_3, 100 mg; K_2HPO_4, 20 mg; $CaCl_2$, 0.5 mg/liter of distilled water. Soil extract was added to a faint yellow color and the pH was adjusted to 7.0.

Internodal cells about 5 cm long were isolated from the middle sections of growing filaments and, after the removal of lateral cells which arise at the nodes, adhering epiphytes were removed by brushing with a camel's hair brush. The cells were then washed with sterile water and examined; only those which exhibited active cytoplasmic streaming were used for experimentation.

For the experimental period the cells were maintained in a culture medium and either the cells were evenly illuminated under constant light, or one half of each cell was kept in the dark and the other half was kept under continuous light. The uneven illumination was achieved by threading the cells through small perforations in a paraffin wax partition which separated the culture chamber into two compartments. The wax partition was made black by incorporating charcoal powder into the molten wax. One of the compartments was covered with black polyethylene sheeting whereas the other was left open.

Light intensity was adjusted to either 40 or 100 ft-c, as indicated for the specific experiments, and was provided by a tungsten lamp mounted above the culture chamber. At the end of the experimental period, the cells were removed from the chambers and their photosynthetic activity was measured by exposure to $^{14}CO_2$.

Measurement of $^{14}CO_2$ Incorporation

The procedure for $^{14}CO_2$ incorporation followed that described by Gibor (3). The cells were immersed in a solution of 0.005 M phosphate buffer, pH 7.0, which contained soil extract and was supplemented with $NaH^{14}CO_3$, 10 μCi/ml (specific activity 20 μCi/μM). The cells were evenly illuminated by a tungsten lamp providing 5×10^5 erg/sec cm^2 for a period of 30 min and the temperature was maintained at 20°C.

At the end of 30 min the cells were washed with water, fixed for 3 hr in a solution of 4% glutaraldehyde, and subsequently washed with 1% (v/v) acetic acid. The nodal regions were removed and sections of equal length (4 mm) were cut from both ends of the cell. These sections were again washed with 1% acetic acid and then placed into scintillation vials. In the vials the pigments of the section were oxidized by covering the section with two drops of 15% hypochlorite solution (clorox). After drying, toluene liquifluor was added and the vials were counted in a Packard liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

Fixation of $^{14}CO_2$ into the cells was also checked by radioautography. For this purpose the remaining middle sections of the cell were placed onto microscope glass slides and were coated with stripping film (Kodak AR 10). After several days the slides were developed and the relative distribution of silver grains over the length of the cells was examined.

It was possible to remove chloroplasts as continuous sheets from the fixed sections of the cell (20–40 mm). In some experiments such layers of chloroplasts, with adhering protoplasm, from measured lengths of cell sections were taken for direct counting in the scintillation counter and for radioautography.

Chlorophyll Estimation

Measured cell sections were suspended in a known volume of acetone: 0.01 M $NaHCO_3$ 4:1 (v/v) and disrupted with a glass on glass homogenizer. Pigments were extracted in the dark at 0°C for 30 min, and the cell debris was then removed by centrifugation. Chlorophylls a + b in the supernatant were estimated by employing the procedure of Arnon (5).

Photosynthetic Oxygen Evolution

Cells were maintained in a closed chamber with a known volume of normal *Nitella* medium supplemented with 0.05% $NaHCO_3$ and allowed to photosynthesize under an illumination of 1000 ft-c supplied by daylight fluorescent lamps. Temperature was maintained at 20°. Changes in oxygen concentration of the medium were measured with a Clark oxygen

TABLE I

Photosynthetic $^{14}CO_2$ Fixation by Nitella Cell Parts

Half of each cell was kept in darkness while the other half was under continuous illumination. The light intensity incident on the illuminated section was 40 ft-c. After the indicated time, the cells were removed from the experimental chamber and exposed to $^{14}CO_2$ for 30 min while the entire cell was evenly illuminated.

Time	Light half	Dark half	Ratio (light/dark)
	cpm/4 mm cell fragment		
5 Days	19,400	11,600	1.7
	20,100	7,700	2.6
11 Days	6,600	1,600	4.1
	4,100	2,500	1.6

electrode assembly. Total oxygen changes were corrected for respiration measured in the dark, to give "photosynthetic oxygen exchange rates."

RESULTS

Nitella cells were kept for 5–11 days, half of the cell in the dark and the other half of the cell illuminated. The cells were then taken out of the experimental chamber and exposed to even illumination in the presence of $^{14}CO_2$ for 30 min (see Methods).

Table I summarizes the results obtained from direct counting of segments of equal length cut from such cells after fixation. A radioautograph of the middle segment of such a cell is presented in Fig. 1.

It is apparent from Table I and Fig. 1 that the portions of the cells which were maintained in the dark lost 40–75% of their ability to fix carbon photosynthetically.

As the cultures of *Nitella* which we used were contaminated with various photosynthetic epiphytes, it was considered possible that the observed differences (Table I) were due to the accumulation of these organisms on the illuminated half of the cell. To eliminate this possibility, an experiment was performed as follows: cells were kept half in the dark and half in the light for 7 days. To determine the photosynthetic capacities of the two halves, the entire cell was exposed to $^{14}CO_2$ and illuminated for 30 min. Then the cells were fixed, the measured segments of the two ends of the cells were cut off, and the chloroplast sheet from these segments was dissected out and counted directly. The cell walls with their contaminating epiphytes were thus eliminated. The results of this experiment are presented in Table II. It is apparent that the differences between the illuminated and dark parts of the cells are due to the chloroplasts themselves and not to contaminating organisms.

Control experiments were conducted to investigate the distribution of photosynthetic ac-

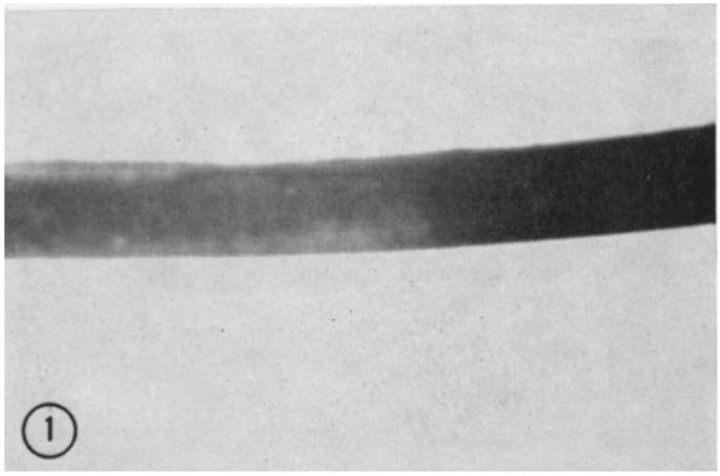

FIGURE 1 Radioautograph from the middle section of a *Nitella* cell, the left half of which was maintained in darkness for 5 days. Subsequently the whole cell was exposed to $^{14}CO_2$ for 30 min under even illumination. The relative increase in development of silver grains on the right side indicates a greater activity in photosynthetic CO_2 fixation. × 30.

TABLE II

Photosynthetic $^{14}CO_2$ Fixation by Chloroplast Sheets from the Two Parts of the Cell

Half of each cell was kept for 7 days in the dark while the other half was kept under continuous light (100 ft-c). $^{14}CO_2$ fixation was checked as before (Table I). Chloroplasts were removed from 4 mm segments of each cell and counted.

Light half	Dark half	Ratio (light/dark)
cpm/chloroplast sheet from a 4 mm cell section		
6,900	2,800	2.5
5,800	4,900	1.2
7,100	2,900	2.4
5,100	3,400	1.5

TABLE III

Photosynthetic $^{14}CO_2$ Fixation by Chloroplasts from Evenly Illuminated Cell Parts

Cells were kept for 7 days evenly illuminated at an incident light intensity of 100 ft-c. The abilities of apical and basal cell sections to fix $^{14}CO_2$ were then compared. Conditions were as described in text.

Apical half	Basal half	Ratio (apical/basal)
cpm/chloroplast sheet from a 4 mm cell section		
4,800	4,700	1.0
4,900	4,300	1.1
5,900	5,800	1.0
7,000	6,900	1.0
5,900	5,600	1.0
9,400	12,300	0.8

tivity along the length of the cell grown under even illumination. Isolated cells were maintained under continuous illumination for 7 days. Their photosynthetic capacity was then checked as before; chloroplast sheets from segments of the apical and basal ends of a cell were excised for counting. The results (Table III) indicated that, under conditions of even illumination, no gradient of photosynthetic potential existed along the longitudinal axis of the cells.

Microscopic examination of cells so maintained that half of the cell was kept in the light and half in the dark for up to 11 days did not reveal any obvious morphological differences between the darkened and the illuminated halves of the chloroplasts. The chlorophyll contents of the illuminated halves were observed to be only slightly and probably not significantly greater than those of their nonilluminated counterparts (see Table IV).

To investigate whether the loss of photosynthetic activity of the "dark" chloroplasts was reversible, the following experiment was performed. The rates of photosynthetic oxygen evolution from samples of cells were measured. After

TABLE IV

Chlorophyll Content of Cell Parts

Chlorophyll content of cell halves after maintenance of half cell in light and half in dark for 7 days (Part A). Cells maintained under even illumination for 7 days (Part B).

Cells	Chlorophyll content	
	μg chlorophyll a + b/fragment	
	Light half	Dark half
Part A		
1	1.01	0.85
2	0.79	0.75
3	1.18	0.94
4	0.95	0.91
	Apical half	Basal half
Part B		
1	0.68	0.64
2	0.78	0.72
3	0.43	0.42
4	0.83	0.76

TABLE V

Recovery of Ability to Form O_2 Photosynthetically after a 7 Day Interval in the Dark

The rate of O_2 evolution from groups of cells was measured with a Clark O_2 electrode. The cells were then kept in the dark for 7 days and reilluminated for 3 more days. Results are expressed as a percentage of the initial rate of O_2 evolution. Measurements were corrected for respiratory oxygen uptake and were performed at the same time of day in each case to obviate problems associated with possible rhythmicity in photosynthetic activity.

	Initial value	After 7 days in dark	After 3 days' reillumination
Sample 1	100	33	73
Sample 2	100	49	82

an initial rate had been established, the cells were maintained in the dark for a period of 7 days and then returned to continuous illumination (100 ft-c) for an additional period of 3 days. The rates of photosynthetic oxygen production were measured after the dark treatment and again after the 3 days of reillumination. The results from this experiment (Table V) indicated that the drop in photosynthetic activity (measured by rates of oxygen evolution) observed after placing cells in darkness was reversible.

The reduction of photosynthetic activity in the darkened half of the cell is not due to total inactivation of only some of the darkened chloroplasts and retention of activity in others. Radioautographs of chloroplast sheets from experimental cells revealed that the darkened chloroplasts were uniformly less effective in photosynthesis than the illuminated chloroplasts.

DISCUSSION

The results presented here indicate that light is required for the maintenance of the photosynthetic capacity of developed chloroplasts. In the same cell, chloroplasts kept in the dark for 1 wk fixed $^{14}CO_2$ at about half the rate that those chloroplasts kept under continuous light did.

It is apparent that light stimulation of the nuclei and cytoplasm alone is not sufficient for the maintenance of full photosynthetic activity in chloroplasts because in the same cell the cytoplasm and nuclei were streaming past both the chloroplasts in the dark and those in the light.

The depletion of low molecular weight metabolic intermediates from the chloroplasts which were kept in the dark is unlikely to be the cause of the observed drop in activity. Such metabolites could probably diffuse freely from the illuminated chloroplasts into the dark half of the cell.

One possible explanation of the decreased CO_2 fixation of the unilluminated chloroplasts is that the permeability of these chloroplasts was modified to prevent the penetration of some molecules into them, thereby causing an alteration in their enzymatic composition. As in mitochondria, it is probable that many proteins of the plastids are coded by nuclear DNA and that much protein must be transferred from the cytoplasm to the plastids. Another possibility is that light may be required to maintain the levels of specific enzymes of the chloroplasts by direct stimulation of the biosynthesis within the organelle.

We do not yet know if DNA-mediated protein synthesis is associated with the observed direct light effect on the chloroplasts. Evidence from experiments with maize (2) and radish cotyledons (4) indicates that chloroplast RNA is synthesized in dark-grown cotyledons at only a fraction of the rate observed in the light. It has been found also that light stimulates the synthesis and accumulation of chloroplast RNA about four times more than it does the synthesis and accumulation of cytoplasmic RNA.

These experiments and observations confirm a previous report (3) on the existence within the same *Nitella* cell of phenotypically differing chloroplasts. Furthermore, such heterogeneity can be induced in a cell by direct action of light on the organelles themselves.

These observations suggest the possibility that environmental factors interact directly with cellular organelles in the processes which cause differentiation at the cellular level.

This work was supported by a National Science Foundation Grant #GB 5461.

Dr. Craig is holder of a N.A.T.O. post-doctoral fellowship.

Received for publication 2 July 1969, and in revised form 15 September 1969.

REFERENCES

1. ARNON, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol escidase in *Beta vulgaris*. *Plant Physiol.* **24**:1.
2. BOGORAD, L. 1967. Control mechanisms in plastid development. *Soc. Develop. Biol. Symp. 26*. M. Locke, editor. Academic Press Inc., New York. 1.
3. GIBOR, A. 1967. Phenotypic variations among chloroplasts of a single cell. *Science (Washington)*. **155**:327.
4. INGLE, J. 1968. The effect of lights and inhibitors on chloroplasts and cytoplasmic ribonucleic acid synthesis. *Plant Physiol.* **43**:1850.
5. SCHWEIGER, E., H. H. WALLROFF, and H. G. SCHWEIGER. 1964. Endogenous circadian rhythm in cytoplasm of *Acetabularia*. *Science (Washington)*. **146**:658.
6. SIRONVAL, C. 1963. Chlorophyll metabolism and the leaf content in some other tetrapyrrole pigments. *Photochem. Photobiol.* **2**:207.
7. SWEENEY, B. M. 1969. *Rhythmic Phenomena in Plants*. Academic Press Inc., New York. 18.