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Developmental Physiology of Bean Leaf Plastids III. Tube Transformation and Protochlorophyll(ide) Photoconversion by a Flash Irradiation[1]

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Abstract. **A light flash of about 1 millisecond duration elicits tube transformation in paracrystalline prolamellar bodies as well as maximal protochlorophyll(ide) photoconversion in etiolated bean leaves (*Phaseolus vulgaris* L.). These findings support a more detailed hypothesis on the linkage between tube transformation and protochlorophyll(ide) photoconversion than has been offered previously.**

Prolamellar bodies in proplastids of dark-grown bean leaves ultimately form paracrystalline arrays of conjoined tubular membranes. The initial light-induced morphological change in such prolamellar bodies has been described as the dissection of the tubular membrane elements into disordered clusters of vesicles and termed tube transformation (4, 7, 10). More recently, tube transformation in a paracrystalline prolamellar body has been interpreted as a structural change whereby the order and regularity of the membrane system is largely lost, but membrane continuity is retained without any dissection (5).

Tube transformation has been correlated with protochlorophyll(ide) photoconversion to chlorophyll(ide) *a*, because the action spectra and energy requirements of the 2 phenomena are similar or identical (4, 7, 10, 11). While it has been demonstrated that most or all of the photoconvertible protochlorophyll(ide) in etiolated barley leaves can be converted to chlorophyll(ide) by a light flash of about 1.5 msec duration (9), the minimum irradiation time shown so far to elicit tube transformation in etiolated bean leaves is 10 sec (11).

This paper presents further indirect evidence linking protochlorophyll(ide) conversion and tube transformation. A single, brief light flash can elicit tube transformation as well as protochlorophyll(ide) conversion in etiolated bean leaves. A physicochemical link between protochlorophyll(ide) photoconversion and tube transformation is proposed and discussed.

Materials and Methods

Kentucky Wonder bean seedlings (*Phaseolus vulgaris* L.) were grown as described previously (6). After 13, 15, or 17 days, primary leaves were harvested. All operations requiring vision were performed under a dim green safelight until the leaf material was fixed or pigments were extracted with acetone.

By slitting both primary leaves along the midrib, the effects of 4 different treatments on half-leaves from a single plant can be compared. During 3 separate experiments, 2 half-leaves from each of a total of 5 plants were compared electron microscopically after receiving no experimental irradiation or a single flash of light, respectively. The other half-leaves from some of the plants were given 100 sec of tungsten filament illumination at intensities of 30 or 40 ft-c or 3 flashes of light spaced 1 min apart. Half-leaves with their abaxial sides in contact with water-saturated Whatman number 1 filter paper were maintained in petri dishes from the time they were cut until the beginning of the fixation procedure.

The light source for flash irradiations was a Honeywell Strobonar 65c xenon photographic strobe lamp. The energy input per flash was about 50 joules supplied from a 500 μf capacitor charged to 450 to 470 v before each discharge. Less than 0.1 msec elapsed between the initiation of a flash and the rise to full intensity. Exponential dieaway to one-third of the full intensity occurred in 0.7 msec. The duration of a flash will be approximated as 1 msec, though the actual situation is more complex. An aperture at the front of the strobe lamp, a collimating lens, and a focussing lens limited the flash to a circle about 28 mm in diameter at a distance

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of 38 cm from the lamp. Experimental half-leaves in petri dishes were placed on this target circle for flash irradiations.

A tungsten filament lamp (75 or 100 w) supplied longer irradiations. To reduce the temperature rise in the leaf material, the light was filtered through a 3 to 4 cm water layer, and a fan moved air across the system.

Samples of leaf tissue, 1 to 2 mm^2 and free of large veins, were fixed for 2 hr at 0° with 3 % (w/v) potassium permanganate made up in 0.1 M phosphate buffer (pH 7.5). Irradiated leaf tissue was fixed promptly after the light treatment. The fixed leaf pieces were then dehydrated with a stepped series of increasing acetone concentrations, embedded in an Epon plastic mixture and sectioned. Staining of sections with barium permanganate sometimes preceded electron microscopy with a Philips EM 200 at 60 kv.

In 1 of the experiments, the percent of protochlorophyll(ide) photoconverted to chlorophyll(ide) was compared for leaves given a 1 msec light flash *vs.* a 10 min irradiation. One hundred and sixty half-leaves without midribs, weighing 1.81 g, were each given a 1 msec light flash during a 30 min period. Protochlorophyll(ide) photoconversion was determined also on 6 g of leaves that received only green safelight and on 3 g of leaves that received 10 min of light at 400 ft-c. In the latter 2 treatments, the leaves, spread in a single layer in glass-covered moist chambers, remained intact and folded until their pigments were extracted.

Each of the 3 batches of leaf material was added to 100 ml of cold acetone and ground for 5 min in an Omnimixer with the cup in an ice bath. The homogenates were stored in darkness at 4° overnight. Then, in dim white or green light, they were filtered through Whatman No. 1 filter paper. After the pigments were transferred from acetone to ether, spectra were obtained with a Bausch and Lomb Spectronic 505. Blank readings were taken at 690 mμ and subtracted from the optical densities at 663 and 624 mμ. The percent of protochlorophyll(ide) converted to chlorophyll(ide) *a* was calculated using the formula of Koski *et al.* (8).

Results and Discussion

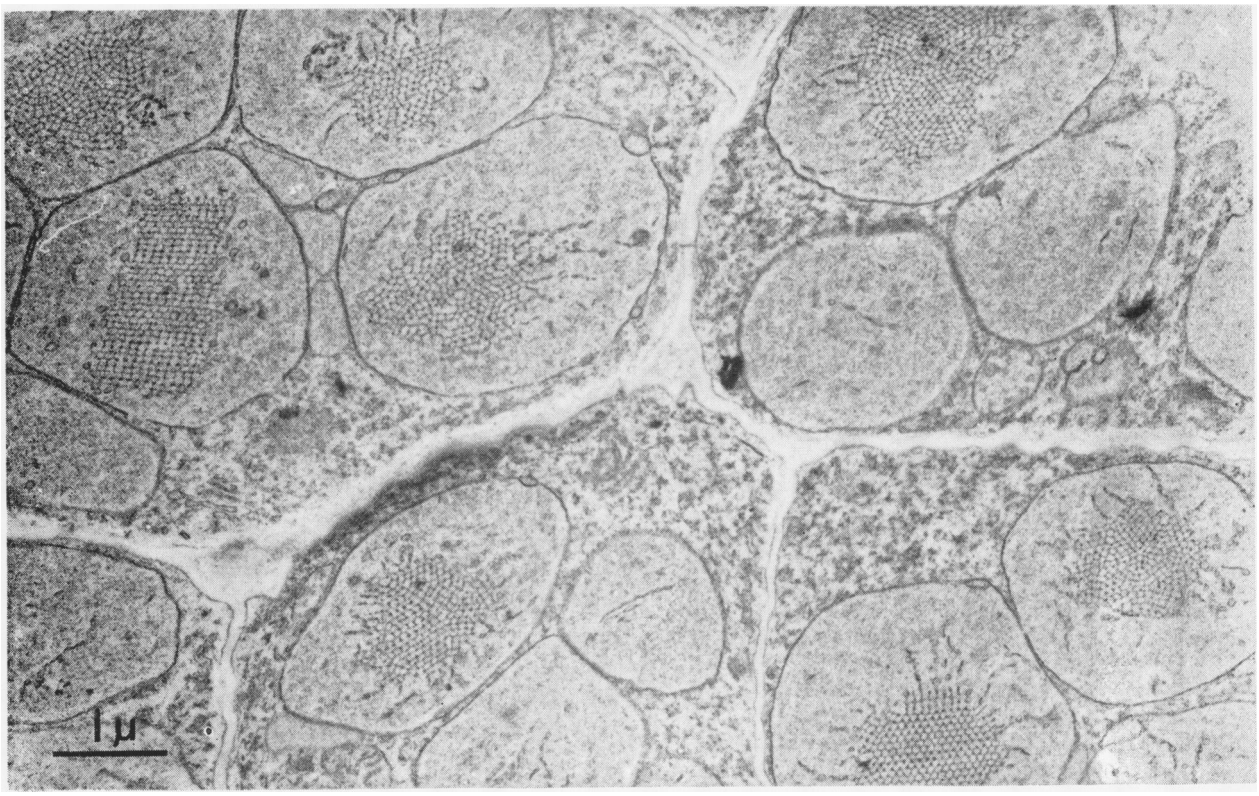
Tube Transformation. Half-leaves that received no experimental irradiation contained characteristic paracrystalline prolamellar bodies. The highly ordered, conjoined tubes of such prolamellar bodies are shown in figure 1. Another half-leaf from the same plant represented in figure 1 received a 1 msec light flash. The aspect of its prolamellar bodies appears in figure 2. Tube transformation, recognized by a disordering of the prolamellar body membranes, resulted from the light flash, as it did in 4 other tests. When 3 light flashes or tungsten filament irradiations were administered, tube transformation occurred, also.

Some comments concerning the detection of tube transformation and fixation methods are necessary. Disordering of prolamellar body membranes has been shown after a 5 min illumination at 750 to 1000 ft-c when followed by glutaraldehyde-osmium fixation (5). However, using the half-leaf comparison method and a number of irradiation times and intensities, I have in many cases failed to detect tube transformation after glutaraldehyde-osmium fixation, although tube disordering was evident after permanganate fixation (unpublished data). Two alternative explanations of these results are: 1) Tube disordering is only *triggered* by a very brief illumination and occurs physiologically after some delay or more rapidly through the action of permanganate, or 2) tube disordering actually occurs during a brief irradiation, and if a stabilizing process does not follow, the transformation is reversed by glutaraldehyde-osmium fixation but not by permanganate fixation. For the present discussion, it does not matter whether a 1 msec light flash causes immediate tube transformation or merely *potentiates* a loss of paracrystallinity subsequently and perhaps during permanganate fixation.

Protochlorophyll(ide) Photoconversion. Ether extracts of leaves from the same group of plants represented in figures 1 and 2 gave the absorption spectra in figure 3. Control leaves that received no experimental irradiation had 3 % of their protochlorophyll(ide) converted to chlorophyll(ide) by the green safelight. Leaves that were irradiated with a msec light flash or 10 min of light at 400 ft-c had 69 or 70 %, respectively, of their protochlorophyll(ide) converted to chlorophyll(ide). The latter light dose exceeded more than 25-fold the minimum dose required for tube transformation and saturating protochlorophyll(ide) photoconversion in similar leaves (unpublished data).

While this study proves that a light dose which elicits tube transformation and maximal protochlorophyll(ide) photoconversion in etiolated bean leaves can be delivered in about a msec, it provides no evidence for simultaneity of the 2 phenomena. The results do fulfill a prediction of the previously postulated (4, 7, 10, 11) close relationship between protochlorophyll(ide) photoconversion and tube transformation: a 1 msec light flash which elicits maximal protochlorophyll(ide) conversion also causes tube transformation.

To explain the apparent relationship between protochlorophyll(ide) photoconversion to chlorophyll(ide) *a* and tube transformation, a physicochemical linkage between the 2 events provides an attractive hypothesis. Several lines of information are significant in this regard. Protochlorophyll(ide) is the light absorbing molecule for its own conversion to chlorophyll(ide) *a* (8), and the action spectrum for tube transformation is consistent with photoreception by protochlorophyll(ide) (4, 7, 10). Tube transformation is detectable after doses of monochromatic red light that yield maximal or somewhat



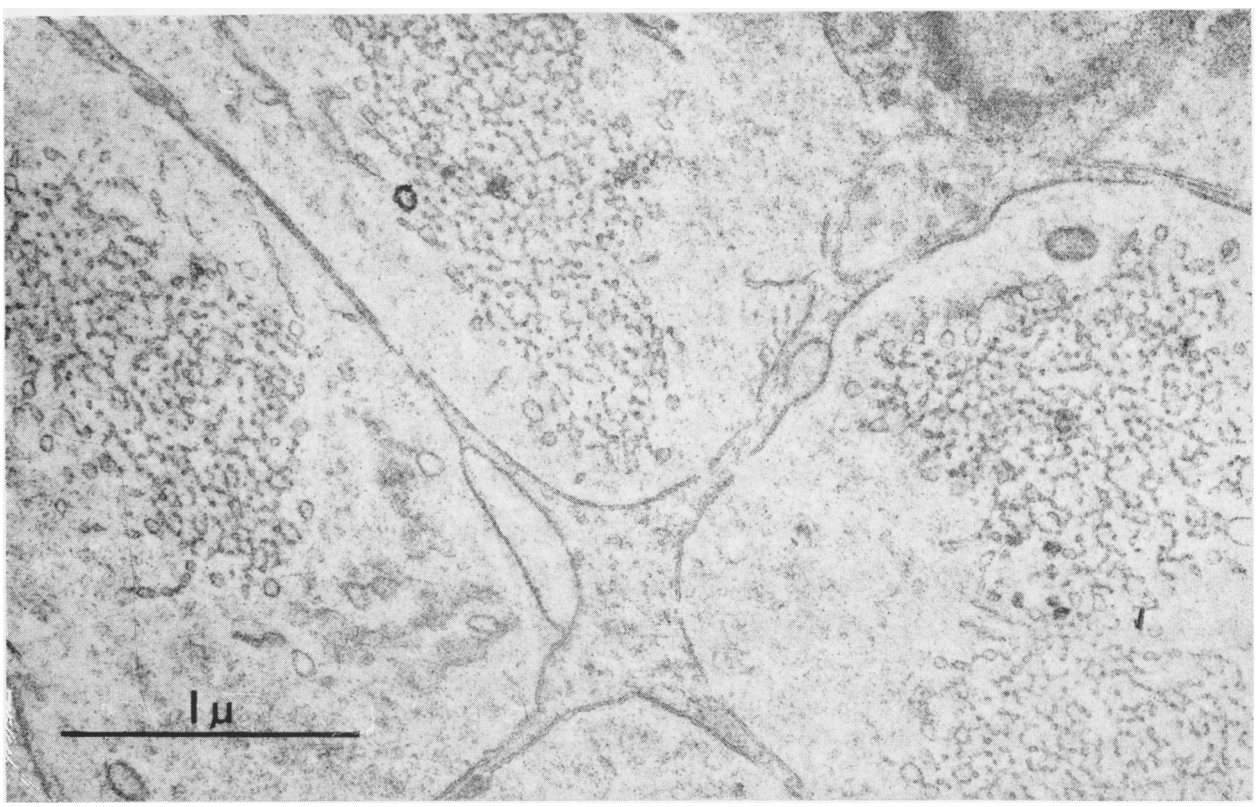


FIG. 1. (top). Paracrystalline prolamellar bodies in a dark-grown leaf piece which received no experimental irradiation. 15,000×.

FIG. 2. (bottom). Prolamellar bodies with disordered membrane elements (tube transformation) in a leaf piece from the same plant represented in figure 1, but subjected to a 1 msec flash of light before fixation. 39,000×.

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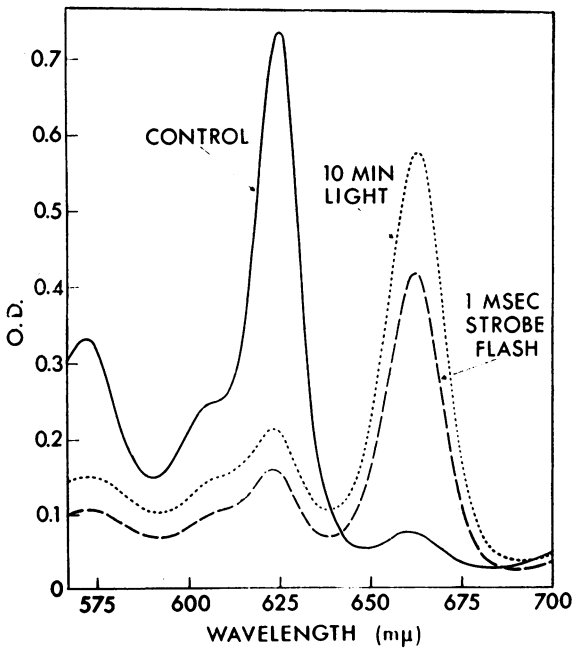

FIG. 3. Absorption spectra of leaf extracts in ether. Protochlorophyll(ide) and chlorophyll(ide) absorption maxima occur at 624 and 663 mμ, respectively.

less than maximal protochlorophyll(ide) conversion (10). The presence of protochlorophyll(ide) in prolamellar bodies has been indicated by several investigations (2, 3, 6, 7). There is evidence that protochlorophyll holochrome is a constituent of the tubular membrane elements of prolamellar bodies (6). Boardman has suggested that the macromolecular protein portion of protochlorophyll holochrome supplies the 2 hydrogen atoms for the photoconversion of the single protochlorophyll(ide) molecule in each pigment-protein complex (1).

The following hypothesis is consistent with the above information. Light energy absorption by protochlorophyll(ide) activates the photoconversion of protochlorophyll holochrome, an integral component of the tubular membrane of prolamellar bodies. Hydrogen atom donation to protochlorophyll(ide) from the membrane-contained protein coincides with the formation or breaking of 1 or more bonds within or among the protein macromolecules. After sufficient time, or through the mediation of permanganate fixation, this changed bonding among the protein macromolecules permits the membranes to assume a new, less ordered configuration. Thus, protochlorophyll(ide) photoconversion and tube transformation (or its potentiation) may represent a single, photodependent process.

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