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Development of Photosystem I and Photosystem II Activities in Leaves of Light-grown Maize (*Zea mays*)[1]

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ABSTRACT

To compare chloroplast development in a normally grown plant with etiochloroplast development, green maize plants (*Zea mays*), grown under a diurnal light regime (16-hour day) were harvested 7 days after sowing and chloroplast biogenesis within the leaf tissue was examined. Determinations of total chlorophyll content, ratio of chlorophyll *a* to chlorophyll *b*, and O_2-evolving capacity were made for intact leaf tissue. Plastids at different stages of development were isolated and the electron-transporting capacities of photosystem I and photosystem II measured. Light saturation curves were produced for O_2-evolving capacity of intact leaf tissue and for photosystem I and photosystem II activities of isolated plastids. Structural studies were also made on the developing plastids. The results indicate that the light-harvesting apparatus becomes increasingly efficient during plastid development due to an increase in the photosynthetic unit size. Photosystem I development is completed before that of photosystem II. Increases in O_2-evolving capacity during plastid development can be correlated with increased thylakoid fusion. The pattern of photosynthetic membrane development in the light-grown maize plastids is similar to that found in greening etiochloroplasts.

Etiochloroplasts (15) in greening etiolated tissue are the most frequently chosen plastids in which to characterize plastid development. The development of the light-harvesting apparatus and the electron transport system has been studied in many etiochloroplasts (6, 14, 16), but in the plastids of only one plant (cocoa) in which the leaves have been grown in a natural diurnal light regime (4, 5). The studies of cocoa suggested that the biogenesis of functional photosynthetic membranes in naturally grown leaves may be very different from the patterns of development in an artificially created greening etiolated system. It has also been known for some years that the ultrastructural changes associated with the transformation and dispersion of the prolamellar body of an illuminated etioplast are grossly different from the changes during normal development of a proplastid into a chloroplast in a developing leaf primordium (17, 28). It has recently been re-emphasized that even before illumination etioplasts develop in size and morphological complexity as a plant grows in the dark. On illumination the kinetics of the appearance of photosynthetic activity varies considerably for etioplasts at different developmental stages (15).

The relevance of functional studies on greening (etiolated) tissues and plastids to an understanding of natural plastid development is frequently questioned, but experimentalists are deterred from examining natural proplastid development because of the logistical problems of handling and examining very young plastids in the tiny leaf primordia of the temperate dicotyledonous tissues most frequently used in photosynthetic research (*e.g.* spinach, pea, and bean) (4). The leaves of those monocotyledons, which develop from a basal intercalary meristem, would seem to be a very good tissue in which to examine natural plastid development. In the leaves of *Zea mays*, for example, the cells are in linear array, with the youngest cells nearest the base of the leaf and the oldest nearest the tip (14). Electron microscopic studies of the structure of the plastids in a young leaf of maize have shown that there is also a progressive sequence of plastid differentiation from the base to the tip of the leaf (17). Methods are now available for the isolation from successive leaf segments of suspensions of functional plastids which provide a sequential developmental series which can be used to study the developing plastid in maize (18, 19).

The work described in this paper is an analysis of the development of the O_2 evolution capacity and the development of photosystems I and II electron transport capacities of plastids in *Z. mays* leaves grown in a normal night/day light regime.

MATERIALS AND METHODS

Plant Material. Caryopses of *Z. mays* (var. Kelvedon Glory, Thompson and Morgan, Ipswich, England) were washed in running water for 15 hr, surface-sterilized and grown in John Innes No. 1 potting compost at 28 C under an irradiance of 0.8×10^3 erg cm^{-2} sec^{-1} and a 16-hr photoperiod. Sixteen-cm plants were harvested after 7 days from planting by cutting just above the mesocotyl insertion. In all experiments the coleoptile and the first outermost leaf were removed, and the two (rolled) inner leaves used for experimentation.

Measurement of Photosynthetic Oxygen Evolution from Intact Leaf Tissue. For measurements of O_2 evolution from intact leaf tissue, 3-mm-wide segments were cut transversely across the leaf at various positions from the base. The inner leaf was then removed from the segment leaving only the outer of the two inner leaves. This procedure was used to take samples up to 6 cm from the base of the plant. To assay leaf tissue above this region, leaf discs of 0.5 cm diameter were cut from the lamina of the outer leaf. It was essential to harvest the plant and sample the leaf tissue as quickly as possible, since the O_2-evolving capacity of the leaf samples decreased rapidly with time after harvesting. All measurements were completed within 5 min of harvesting the leaf. The relative rates of O_2 evolution (from the leaf samples) at 25 C were determined by placing them directly onto a Clark-type O_2 electrode (25). The leaf tissue was irradiated with red light of energy 1×10^5 erg cm^{-2} sec^{-1} produced from a helium-neon laser, the beam being spread to cover the entire leaf sample. Photosynthetic rates were determined by comparing the O_2 evolution during irradia-

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tion with O_2 uptake in the dark. Light saturation curves from the tissues were determined from the photosynthetic rates under different irradiances, produced by the use of glass neutral density filters.

Plastid Isolation. Plastids were isolated from leaf segments 2 cm wide following the rapid procedure previously described (18). The homogenization procedure used for the plastid isolation from the first 4 cm of tissue above the leaf base was different from that used for leaf tissue higher up the plant, in order that the yield of plastids from the younger segments was increased. Tissue from the first 4 cm of leaf was homogenized in a mortar with ice-cold 0.5 M sorbitol in 67 mM K-Na phosphate buffer at pH 8 containing 1 mM $MgCl_2$. The leaf homogenate was filtered through five layers of cotton organdy or 10 layers of butter muslin and five layers of 25-μm nylon bolting cloth before centrifugation for 90 sec at 3,000g to precipitate the plastids. Plastids from leaf tissue above 4 cm from the base were isolated in the same medium, but the leaves were homogenized in a M.S.E. Atomix at full speed for 5 sec followed by a second 5 sec after remixing. Filtration and centrifugation procedures were the same as for the younger tissue. Plastid pellets were resuspended in the isolation medium.

Measurement of Photosystem I and Photosystem II Activities. The electron-transporting capacity of PSI was estimated by following electron flow from reduced TMPD[3] to methyl viologen. A Clark-type O_2 electrode was used to monitor O_2 uptake from a 3-ml reaction chamber containing the following reaction mixture: 30 mM K-Na phosphate buffer (pH 8), 3 mM sodium isoascorbate, 0.2 mM TMPD, 0.1 mM methyl viologen, 3 mM $MgCl_2$, 15 μM DCMU. Plastids containing between 15 and 30 μg of Chl were added and the initial rate of O_2 uptake measured in white light of irradiance 1×10^6 erg cm^{-2} sec^{-1} produced from a quartz-iodine source. O_2 uptake in the light was corrected for any dark rate of O_2 consumption. Dark rates of O_2 consumption were generally found to be negligible in these plastid preparations.

PSII activity of the isolated plastids was determined by following the photoreduction of DCPIP or TCPIP. The reaction mixture for DCPIP reduction was 3 ml in volume and contained 30 mM K-Na phosphate buffer (pH 6.8), 0.1 mM DCPIP, and plastids containing 15 to 30 μg of Chl. Photoreduction of the DCPIP was determined by measuring the absorbance decrease at 600 nm after irradiation with 1×10^6 erg cm^{-2} sec^{-1} of white light for 1 min. Correction was made for any dark reduction of DCPIP. TCPIP photoreduction was estimated in a 3-ml reaction mixture of 30 mM K-Na phosphate buffer (pH 7.8), 23 mM NaCl, 23 μM TCPIP, and plastids containing 15 to 30 μg of Chl by following the absorbance decrease at 645 nm after irradiation as for the DCPIP photoreduction. Light saturation curves for both PSI and PSII activities were produced by reducing the exciting light intensity with neutral density filters.

Chl contents and Chl a/b ratios were determined using the methods of Arnon (1). Plastid numbers/unit Chl were estimated using phase contrast microscopy and a hemocytometer slide (16).

Electron Microscopy. For electron microscopy, 1-mm-thick slices of tissue were cut from the leaves of three plants. Each segment was fixed for 2 hr at 0 C in 2.5% (v/v) glutaraldehyde in K-Na phosphate buffer (33 mM, pH 7.3) containing 0.5 M sucrose, then washed successively in K-Na phosphate buffer containing 0.5 M, 0.35 M, and 0.2 M sucrose before postfixation for 2 hr at room temperature in 1% (w/v) OsO_4 in K-Na phosphate buffer containing 0.15 M sucrose. The tissue was washed for 20 min in 30% (v/v) acetone containing 0.1 M sucrose, and dehydrated rapidly in acetone (17). Two changes in 100% acetone were made at 1-hr intervals before embedding the tissue in Spurr's resin (24).

The sections were stained for 30 min in 1% (w/v) aqueous uranyl acetate and 10 min in Reynolds' lead citrate (22) and examined with a Perkin-Elmer Hitachi HU 12A electron microscope.

RESULTS

Throughout this paper the major concern was to establish the pattern of change of measured photosynthetic parameters during plastid development in maize leaves which were on a normal day-night cycle. The data are presented from single experiments. In all cases experiments were repeated a minimum of 10 times and it should be stressed that the pattern of change in the observed parameters was always the same, although the absolute values for a given parameter often varied from one batch of plants to the next.

Experiments with Leaf Segments. Changes in the Chl/leaf segment, Chl/plastid, and Chl a/Chl b ratio as a function of position within the leaf are given in Figure 1. Chl content of the leaf on a unit tissue or plastid basis increases from the leaf base to the tip and confirms that the leaf of *Z. mays* offers a useful natural system with which to examine chloroplast development. No significant change is seen in the Chl a/Chl b ratio throughout the leaf; this finding is consistent with observations on the development of light-grown cocoa and cottonwood leaves (4, 10). During the development of etiochloroplasts the Chl a/Chl b ratio decreases (27).

The capacity for light-stimulated O_2 evolution was determined for several ages of tissue from the bottom 6 cm of the greening leaf. The stomata in all of the sections were open during experimentation. The results are given in Figure 2 expressed as O_2 evolution/leaf segment and /unit Chl. The capacity for light-stimulated O_2 evolution is already present in the first cm of tissue above the leaf base. Previous studies on the CO_2-fixing capacity of these leaves indicated that no CO_2 fixation is detected in tissue below the 4th cm from the leaf base (9). Thus, photosynthetic O_2 evolution in developing maize plastids can be detected before photosynthetic CO_2 fixation. The 4th cm from the leaf base shows a large increase in O_2-evolving capacity and this corresponds to the region in which CO_2

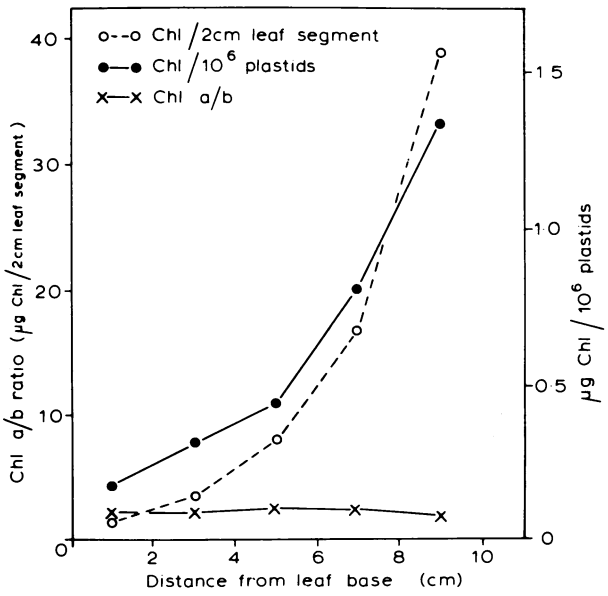

FIG. 1. Chl content/2-cm leaf segment, Chl content/10^6 plastids, and Chl a/b ratio as a function of distance from the leaf base in young green maize leaves.

[3] Abbreviations: TMPD: N,N,N',N'-tetramethyl-p-phenylenediamine; DCPIP: 2,6,-dichloroindophenol; TCPIP: 2,3',6-trichloroindophenol.

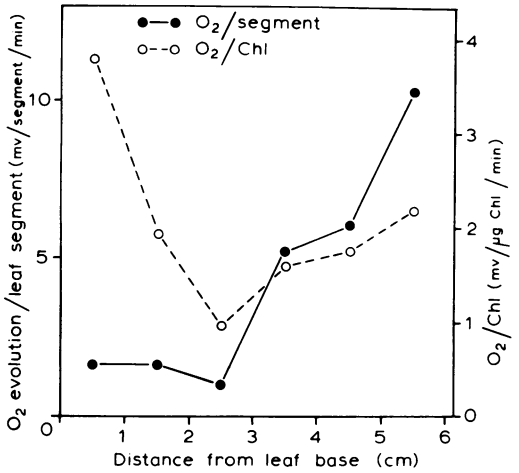

FIG. 2. O_2-evolving capacity per leaf segment and per unit Chl as a function of distance from the leaf base in young green maize leaves.

fixation is first detected (9). The bottom 3 cm of leaf tissue exhibit a capacity for photosynthetic electron transport but not for CO_2 fixation.

The initial decrease in the O_2-evolving capacity of the leaf/unit Chl suggests that the number of light-harvesting Chl molecules is increasing with respect to the number of reaction centers during greening. This situation has been observed repeatedly in greening etiolated leaves (3, 13). However, in tissue above 3 cm from the leaf base, the capacity to evolve O_2/unit Chl increases with increasing Chl content, suggesting an unexpected decrease in photosynthetic unit size. It is possible that physiological factors other than the photosynthetic electron transport system and the light-harvesting apparatus are affecting the capacity of O_2 evolution from the intact leaf. A lowering of the diffusive resistance to gas flow could account for such increases in O_2 evolution/unit Chl, and also would be compatible with the fact that CO_2 fixation is not detected within tissue 3 cm from the leaf base; CO_2 exchange would be more severely restricted than O_2 exchange by a high diffusive resistance of a tissue to gas flow. Measurements on intact leaf material do provide useful information on the physiological capacity of the tissue for photosynthesis however, and the data in Figure 2 clearly show that it is only in tissue above 3 cm from the leaf base that the maize leaf exhibits high photosynthetic rates.

Intact leaf material can be used to determine the pattern of development of the light-harvesting apparatus provided that variations in physiological factors involved in the determination of the maximum photosynthetic rate can be avoided. This can be achieved by comparing the light saturation curves for O_2 evolution at different stages of plastid development. The light saturation point for O_2 evolution in a tissue describes the efficiency of the light-trapping systems with respect to radiant flux density, and a comparison of several saturation points gives an indirect measure of the relative number of Chl molecules associated with each reaction center at different times during development, *i.e.* relative photosynthetic unit size (5). The light saturation curves for tissue from different regions of the maize leaf are given in Figure 3. The curves have been normalized for ease of comparison of their light saturation points. The light saturation point decreases with increasing age of the tissue, indicating an increase in the efficiency of light trapping and thus an increase in the average number of light-harvesting Chl molecules with respect to the number of reaction centers. This developmental pattern is analogous with the pattern observed in greening etiolated tissues, but unlike that found in light-grown cocoa leaves where the light saturation point increases with increasing Chl content (4).

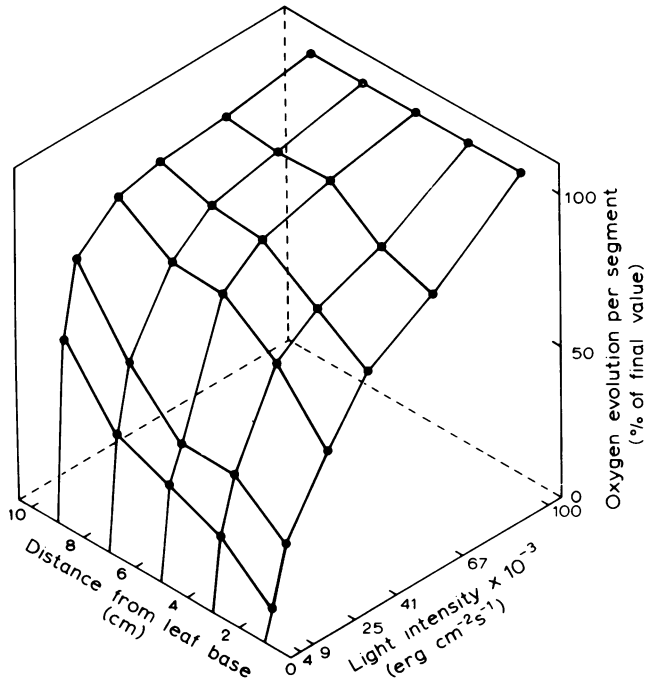

FIG. 3. O_2-evolving capacity/leaf segment as a function of light intensity and distance from the leaf base in young green maize leaves. Curves were normalized for ease of comparison of the light saturation points.

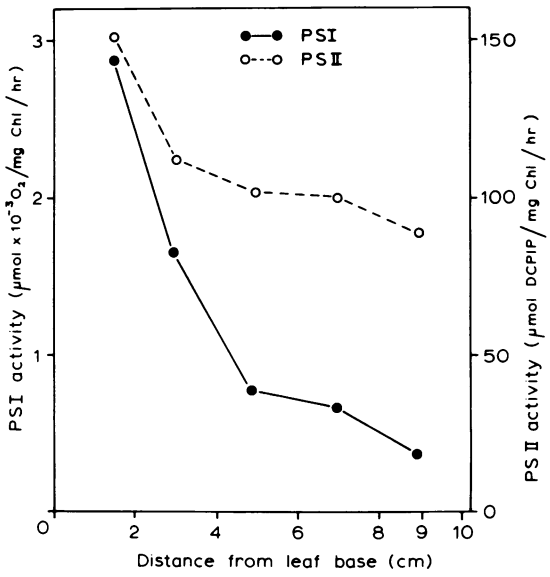

FIG. 4. PSI and PSII activities/unit Chl of isolated plastids as a function of distance from the leaf base in young green maize leaves.

Experiments with Isolated Developing Plastids. A more critical analysis of the development of the photosynthetic apparatus can be made by examining the electron transport capacity of isolated plastids as a function of Chl content and light intensity. Figure 4 shows that the electron transport capacity/unit Chl of PSI and PSII decreases with increasing age of the tissue, hence the average functional photosynthetic unit size of PSI and PSII is increasing during greening. If these figures are expressed in terms of electron transport capacity/plastid, then PSI activity exhibits no dramatic changes during greening, whereas the actvity of PSII on a plastid basis increases markedly (Table I). This indicates that the development of PSI activity is completed

Table I. PS I and PS II activities of isolated plastids from young green maize leaves (per 10^6 plastids)

Leaf segments used for plastid isolations (cm from leaf base)	0-2	2-4	4-6	6-8	8-10
PS I activity (pmol $O_2/10^6$ plastids/hr)	14.4	19.4	9.5	17.2	14.8
PS II activity[6] (pmol DCPIP/10^6 plastids/hr	0.77	1.32	1.23	2.56	3.56

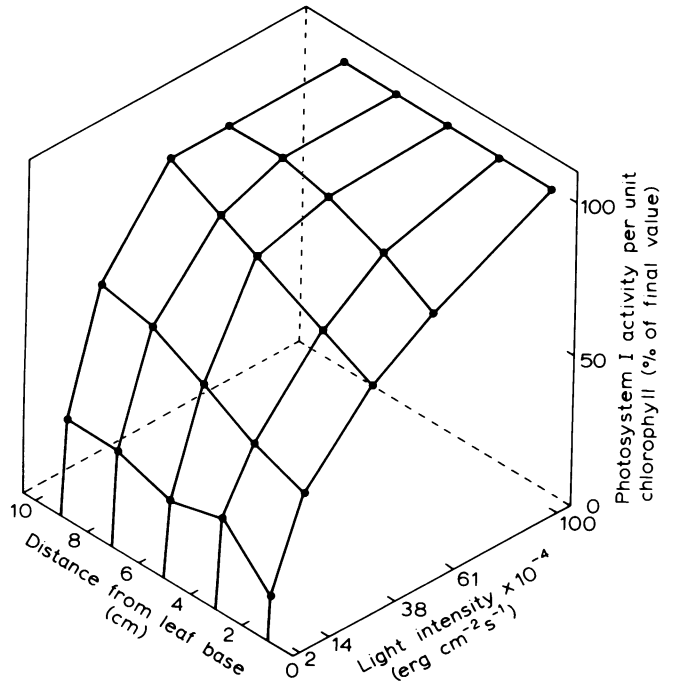

FIG. 5. PSI activity/unit Chl of isolated plastids as a function of light intensity and distance from the leaf base in young green maize leaves. Curves were normalized for ease of comparison of the light saturation points.

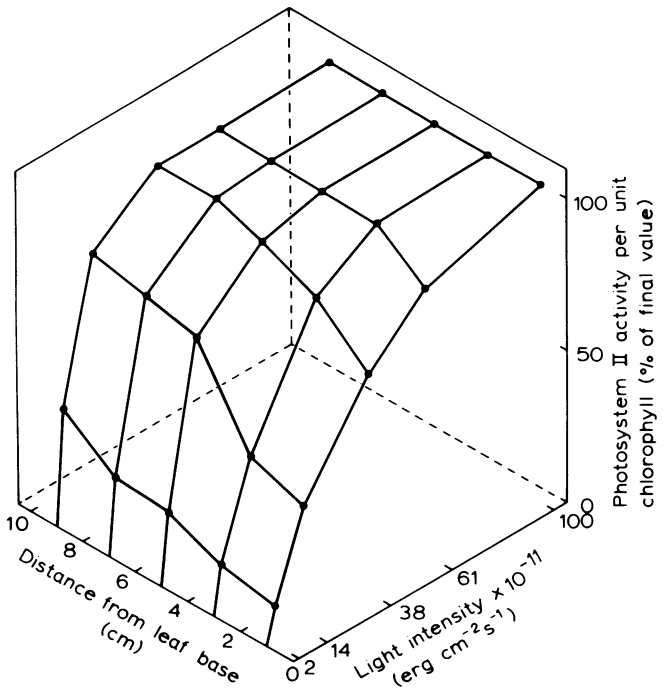

FIG. 6. PSII activity/unit Chl of isolated plastids as a function of light intensity and distance from the leaf base in young green maize leaves. Curves were normalized for ease of comparison of the light saturation points.

before that of PSII in developing maize plastids, again a situation similar to greening etioplasts where PSI activity appears and reaches a maximum before PSII activity (1, 8, 13, 20, 21).

The light saturation curves for PSI and PSII electron transport capacities of developing green plastids are given in Figure 5 and Figure 6, respectively. The curves were normalized for ease of comparison of the light saturation points. For both PSI and PSII activities the light saturation point decreases with increasing Chl content, confirming that the average photosynthetic unit size of both photosystems increases during development. The findings from the isolated plastids are compatible with those obtained from the intact leaf tissue. Similar results for the development of PSII activity were obtained when TCPIP was used as the electron acceptor of PSII instead of DCPIP.

Recently Strasser and Butler (26) have suggested that the fusion of thylakoids is a prerequisite for efficient O_2 evolution in bean leaves grown under a flash regime. A similar hypothesis can be applied to the developing maize leaf. Figure 7 shows a series of electron micrographs of plastids at different stages of development. All of the plastids contain fused thylakoids and even the youngest plastids, in the basal 0.5 cm of leaf tissue, contain fused thylakoids. It is apparent, however, that the degree of membrane fusion in the plastids within the first 3 cm of leaf tissue is considerably less than in plastids from the rest of the leaf. The number of grana/plastid profile and the number of thylakoids/granum can give a good indication of the degree of fusion in developing plastids (Table II). In leaf tissue 3.5 cm from the leaf base the characteristic dimorphic chloroplasts of maize are found, and thus complications can arise in the interpretation of data on the development of photosynthetic membranes. Table II shows that there is a significant increase in membrane fusion in both mesophyll and bundle sheath chloroplasts with increasing distance from the leaf base. The degree of thylakoid fusion increases in a way similar to the increase in the O_2-evolving capacity of the tissue (Fig. 2). Previous studies on maize have shown that plastids found in the 4th cm of tissue from the leaf base show synchronous division (17) and hence it is possible that division is also associated with the observed increase in the O_2-evolving capacity of the tissue.

DISCUSSION

The changes in photosynthetic capacity/unit Chl and the progressive decrease in the light saturation point during normal development of maize chloroplasts indicate that the development of functional Chl-protein complexes in light-grown maize is comparable with the situation in the greening etiochloroplast, *i.e.* an initial rapid synthesis of all reaction centers is followed by a slower well synchronized synthesis of the light-harvesting Chl associated with each of the reaction centers.

The characteristics of the development of photochemical capacity in light-grown maize differ considerably from those of the cocoa leaf, the only other light-grown system previously examined. During the development of the cocoa leaf, the increase of the light saturation point and the constancy of the photosynthetic capacity/unit Chl and of the Chl *a*/Chl *b* ratio suggest that the average size of the photosynthetic units remains constant and that each light-harvesting Chl-reaction center complex is synthesized prior to the next (5). It is possible that these differences in plastid membrane biogenesis may be associated with differences in the site of production and growth of leaf

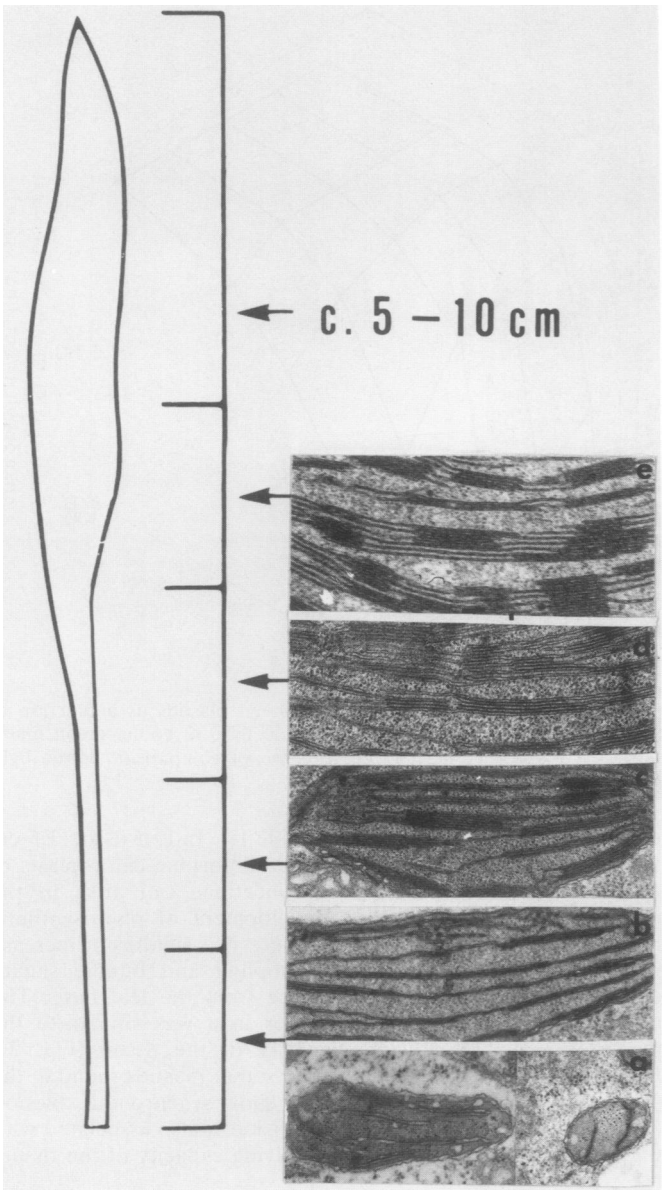

FIG. 7. Electron micrographs of plastid thylakoid membranes in progressively older cells of a green maize leaf. The plastids are in cells at measured distances from the leaf base. a: 0 cm; b: 0.5 cm; c: 1.5 cm; d: 3 cm; e: 5 cm. Preparation for microscopy was as described in the text (× 30,000).

Table II. Number of grana and average number of thylakoids per granum measured in plastid profiles in electronmicrograph sections from young green maize leaves

Leaf slice[1] distance from leaf base (cm)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	5.0 m[2]	5.0 bs[3]	7.0 m[2]	7.0 bs[3]
Average number of grana per micrograph profile[4]	0.2	1.7	3.0	3.0	4.5	8.4	13	16	24	18	32	33
Average number of thylakoids per granum	2.1	3.1	3.5	3.6	2.9	3.8	4.0	3.4	3.7	2.6	6.2	2.9

1. A thin (1mm) slice was cut transversely across the leaf and fixed for electron microscopy
2. m - plastids from mesophyll cells
3. bs - plastids from bundle sheath cells
4. Each average number represents at least 100 measurements

primordia in young maize plants and young cocoa plants. In maize, the young leaf primordia inside the base of older leaves are heavily shaded during early development but in cocoa the plastids are not subjected to shading during early stages of development. The absence of a dark period at the onset of plastid development in cocoa may be associated with the subsequent lack of synchrony of membrane development in contrast to dark-grown illuminated etioplasts of many plants and to plastids in normally grown monocotyledon leaves.

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