A PROLAMELLAR BODY-LIKE STRUCTURE IN *CHLAMYDOMONAS REINHARDI*

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The prolamellar body (PLB) appears in the etioplasts of dark-grown higher plants (3, 16, 18, 21). It has a well-defined structure consisting of a central core built of an array of interconnected tubules, from which peripheral tubular elements extend into the plastid matrix. The tubular elements of the central core can be arranged in a crystalline (3, 7) or random pattern (19). The peripheral elements consist of tubules, which may have a smooth appearance or be lightly corrugated or helically coiled (22). During the greening process of etiolated plants the PLB undergoes a series of structural transformations, the tubular elements giving rise to flattened vesicles and finally to lamellae or thylakoids which eventually fuse to form the grana system of the chloroplast

(7, 16, 17, 19). Thus, the PLB is apparently a pool of readily convertible precursors of chloroplast lamellae (18).

As opposed to its regular appearance in etioplasts of higher plants, the PLB was not observed in unicellular algae for many years (2, 6, 18). Only recently the presence of PLB-like structures was reported in *Euglena* (26), *Anabaena* (20), and *Chlorella* (4, 5). The presence of PLB or PLB-like structures in *Chlamydomonas* has not been reported to date, although the ultrastructure of this alga was investigated in detail by many workers (10–14, 23–25). However, occasionally an array of tubules in an arrangement slightly reminiscent of a tangential section through a "PLB" was observed in dark-grown *Chlamydomonas reinhardi* y-1 cells (9).[1] During a recent study on biogenesis of the photosynthetic lamellae of *Chlamydomonas reinhardi* y-1, it was observed that in several unrelated cultures of dark-grown cells the plastid contained a structure which seemed to be identical with a PLB. In the present communication the morphological features and the occurrence of a PLB-like structure in dark-grown cells and in cells at different stages during the greening process are described.

MATERIALS AND METHODS

Chlamydomonas reinhardi y-1 cells were grown in the dark for 5–6 days on a mineral medium (23). Cells in the logarithmic phase of growth were harvested in dim fluorescent light or daylight (23), resuspended in fresh growth medium containing 2.5 mM KH_2PO_4 (9) to a final concentration of 8.3×10^6 cells per ml, and further incubated either in the light or in the dark. Samples of cells were taken at different times, the chlorophyll content was measured as described by Arnon (1), and cells were fixed with OsO_4 and processed for observation by electron microscopy as described (24). Thin sections were examined with a Philips EM-300 operated at 60 or 80 kv, at magnifications of × 20,000–50,000.

RESULTS AND DISCUSSION

The PLB-like structure (PLB) in dark-grown *Chlamydomonas* cells consists of a central dense core formed of a network of short tubular-like elements with no definite tridimensional orientation (Fig. 1). The tubules seem to have a round cross section of about 25 nm, their walls being about 5–6 nm thick. These dimensions are similar to those reported for the tubules of PLB in higher plants (15). Osmiophilic granules of variable size, density, and shape, which were considered to contain carotenoids in higher plants (27), are also found in the core region (Figs. 2, 3). Occasionally, they are located at the periphery of the core and seem to give rise to, or to be at the base of, tubular and lamellar elements protruding outward from the PLB core (Fig. 4). The degree of packing of the central core elements varies from a very dense mesh, in which the boundaries of the tubules are distinguished only with difficulty (Figs. 2–4), to a loose or spread packing which allows the recognition of the individual tubules (Figs. 1, 5). The size of the central core varies in different sections from about 0.5 to 1.5 μ. Usually, only one but occasionally two or more (Figs. 4, 7, 8, Table I) and up to seven PLB-like structures have been observed in a single cell section. From the periphery of the central core, tubular-like or lamellar elements extend into the plastid matrix and form curved arches of different lengths (Figs. 1, 2, 3, 8). The abundancy of the peripheral elements is variable, their number and length varying with the time of exposure to the light (Figs. 1, 2, 5). Also, it seems that in the dark-grown cells corrugated tubular-like elements prevail (Figs. 1–3) while after exposure to light the peripheral elements are clearly of a lamellar structure (Figs. 4–8). The peripheral elements seem occasionally to be in close apposition with the tubular elements of the pyrenoid (Fig. 7), or to form bridges between the adjacent PLBs when more than one PLB is present in a plastid (Figs. 4, 7), as reported also in higher plants (15). In no case, however, has a connection been observed between the PLB tubules or lamellae and the inner membrane of the plastid envelope. The central core of the PLB in higher plants was reported to have a crystalline pattern centered around organizing particles which have been considered by Gunning to be ribosomes (15, 16). Particles similar in size to the plastid ribosomes have been observed within the central core of the PLB in *Chlamydomonas*, but it seems that they are located between the tubular elements at random (Fig. 1). In higher plants the crystalline pattern of the central core loses its organisation on exposure to light, even at very low light intensities and short exposure times (7, 8, 17, 19). We have so far not observed such a crystalline arrangement in the central core of the PLB in *Chlamydomonas*. It is possible that this is due to the fact

[1] Ohad, I. Unpublished results.

that the harvesting of the cells and their preparation before the greening experiment in our work was routinely carried out in dim artificial light or daylight (see Materials and Methods). Since the PLB was not usually observed in dark-grown *Chlamydomonas* cells (10, 12, 13, 23, 25), the question arises whether this structure is found in every cell from cultures exhibiting the presence of a PLB and, if so, what its fate is during the greening process. The PLB can be recognized only in sections passing through its central core. The diameter of the central core of the PLB in dark-grown *Chlamydomonas* varies from about 0.1 to 0.13 of the cell diameter. Thus, one would expect to find it in about 10%–13% of the cell sections. Indeed, 7%–11% of a total of 1096 sections of dark-incubated cells contained a PLB (Table I). A reduction in the frequency of occurrence of a PLB might be due to its complete disappearance or to a gradual reduction in the diameter of the central core. In order to evaluate the frequency of occurrence of the PLB in a cell population, one can compare it with that of another subcellular organelle of constant size and occurrence. For such a comparison we have chosen the pyrenoid, which in *Chlamydomonas* is also located in the plastid and has a diameter about 0.16 of that of the cell (24). If there is only one pyrenoid per cell, one would expect to find it in about 16% of the sections. Indeed, 16%–19% of the sections of dark-grown cells contained a pyrenoid (Table I). The ratio of occurrence of PLB to pyrenoids in section from cells incubated for 4.5 hr and 8 hr in the dark was 0.7 and 0.3, respectively, while the ratio expected from their relative diameter was 0.5 to 0.8, which might be taken as an indication that there is one PLB in each cell. However, the possibility that some cells have more than one such structure while others have none cannot be excluded since sections have been found containing five PLBs (Table I) or more. The occurrence of the PLB seems to decrease in the light-incubated cells. Thus, after 4.5 hr of illumination their frequency is reduced to about 38%, an additional decrease being observed after 6.5 hr of exposure to the light, and the amount of peripheral elements increases concomitant with the increase in chlorophyll content (Figs. 4, 5, 8). However, a decrease in frequency by about 50% is also observed in the cells incubated in the dark for 8 hr.

The decrease in occurrence and relative frequency of the PLB as compared with the frequency of the pyrenoid might be due to a decrease in the relative diameter of the PLB and an increase in the diameter of the pyrenoid. Indeed, the diameter of the PLB in the greening cells is about 0.5–0.6 μ (0.04–0.05 of the cell diameter). The structural transformations which the PLB undergoes during greening of etiolated higher plants are very rapid (7, 17, 19) as compared with the sluggish changes observed in the PLB of greening *Chlamydomonas* cells. It appears that the prolamellar body-like structures of *Chlamydomonas* as well as those found in other algal cells (4, 5, 20, 26) are not obligatory intermediates in the greening process. The presence of the PLB has been observed so far in our laboratory in four separate cultures originating from different stock culture plates including an

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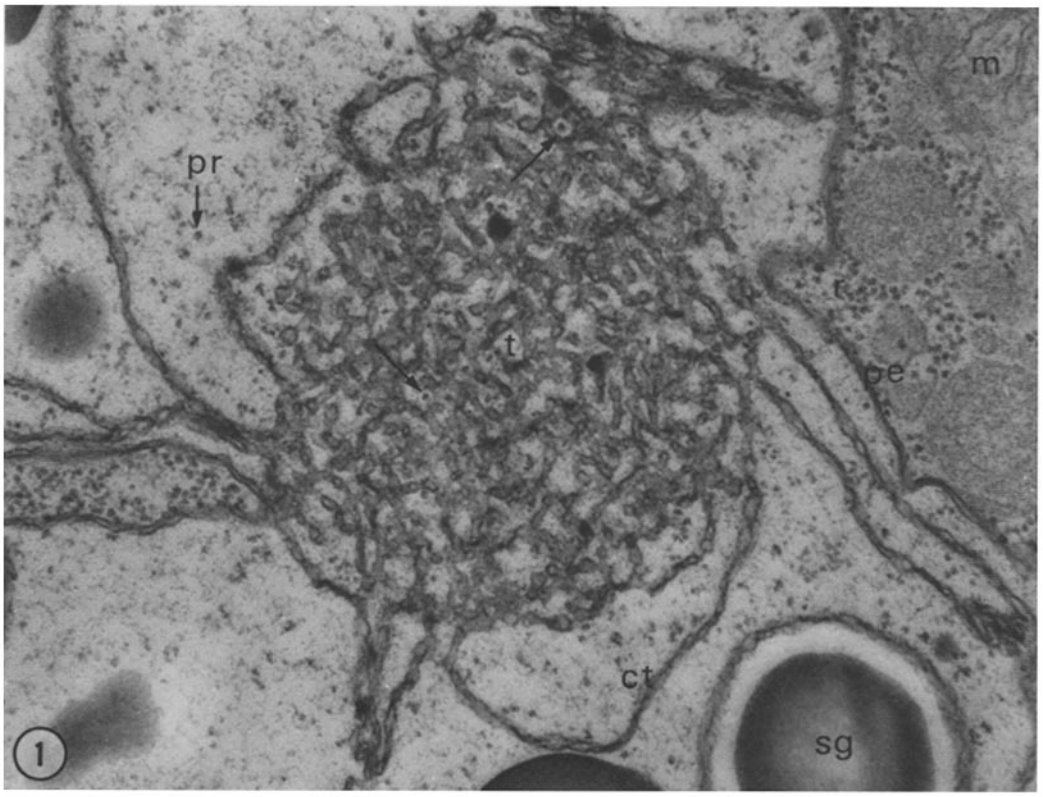
The following abbreviations are used in all figures:

ct, corrugated or twisted peripheral tubular elements;
cw, cell wall;
es, eye spot;
m, mitochondria;
og, osmiophilic granules;
pe, plastid outer envelope;
pm, plasma membrane;
pr, plastid ribosome-like particles;
py, pyrenoid;
r, cytoplasmic ribosomes;
sg, starch granules;
t, central core tubules.

FIGURE 1 A prolamellar body in a dark-grown cell. Short, loosely packed interconnected tubules (*t*) form the central core. Corrugated tubular elements (*ct*) extend into the plastid matrix. Notice the presence of plastid ribosome-like particles within the irregular mesh formed by the tubules in the central core (arrows). × 41,000.

FIGURE 2 The central core of a PLB in a dark-grown cell showing tightly packed tubular elements and randomly dispersed osmiophilic granules (*og*). × 44,000.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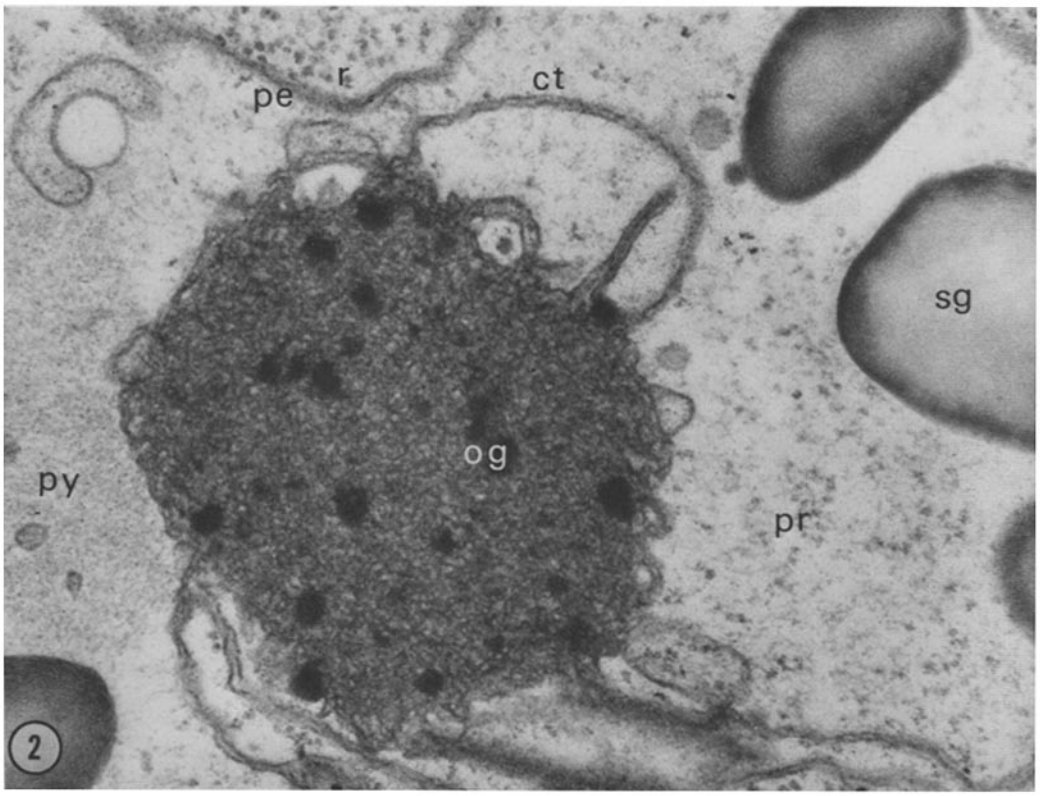
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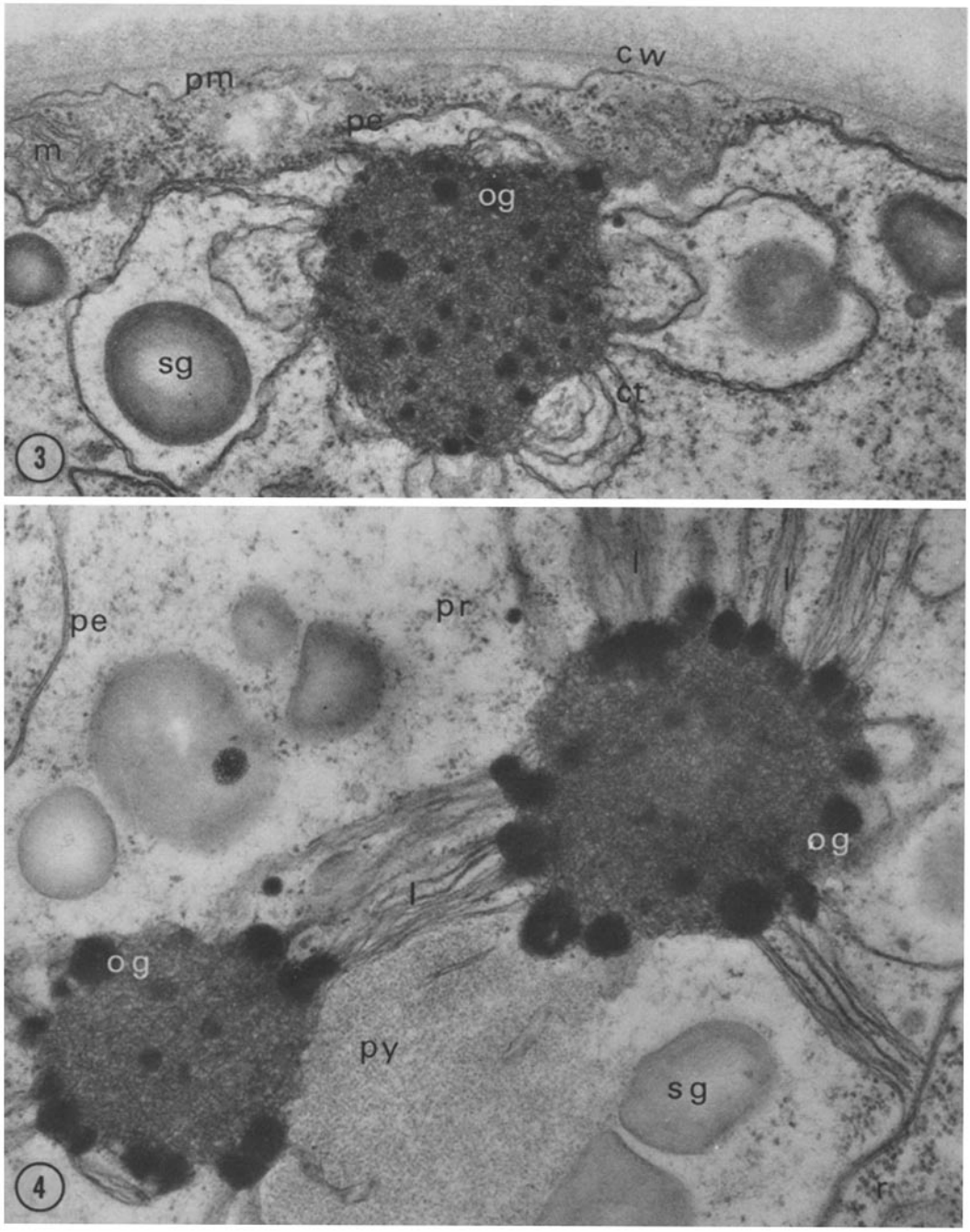

FIGURE 3 A prolamellar body in a dark-grown cell showing randomly distributed osmiophilic granules (*og*) and peripheral closed arches formed of corrugated or twisted tubular elements (*ct*). × 30,000.

FIGURE 4 A section showing two prolamellar bodies interconnected by lamellar extensions (*l*) originating from the central core. Notice the peripheral disposition of osmiophilic granules (*og*) coinciding with the origin of the lamellae and the close apposition of the PLB complex with the pyrenoid (*py*). The lamellar extensions do not fuse with the plastid outer envelope. The section is from a dark-grown cell exposed to the light for 5 hr. × 44,000.

TABLE I

The Frequence of PLBs and Pyrenoids in Sections of Dark-Grown Chlamydomonas Reinhardi y-1 during the Greening Process

Hr of incubation		μg chlorophyll per 10^7 cells	No. of cell sections counted	No. of cell sections containing a pyrenoid	No. of PLBs counted	Per cent of cell sections containing PLBs (No of PLBs per cell section)					Pyr* cell section	PLB‡ cell section	PLB§ Pyr
Dark	Light					1	2	3	4	5			
4.5	—	1.33	516	85	102	6.79	2.91	0.97	0.58	0.39	0.165	0.116	0.706
8.0	—	1.05	580	113	63	5.52	0.35	0.52	0.35	0.35	0.195	0.071	0.362
—	4.5	6.55	588	168	32	3.23	0.85	0.17	—	—	0.286	0.043	0.149
—	5.0	6.95	911	202	49	2.74	0.55	0.44	—	—	0.222	0.037	0.168
—	6.5	10.10	529	136	27	3.21	0.95	0.38	—	—	0.257	0.045	0.176
—	8.0	17.00	860	257	18	1.86	0.12	—	—	—	0.299	0.020	0.066

* No. of sections containing pyrenoid per total number of sections counted.

‡ No. of sections containing PLBs per total number of sections counted.

§ No. of sections containing PLBs per section containing pyrenoid.

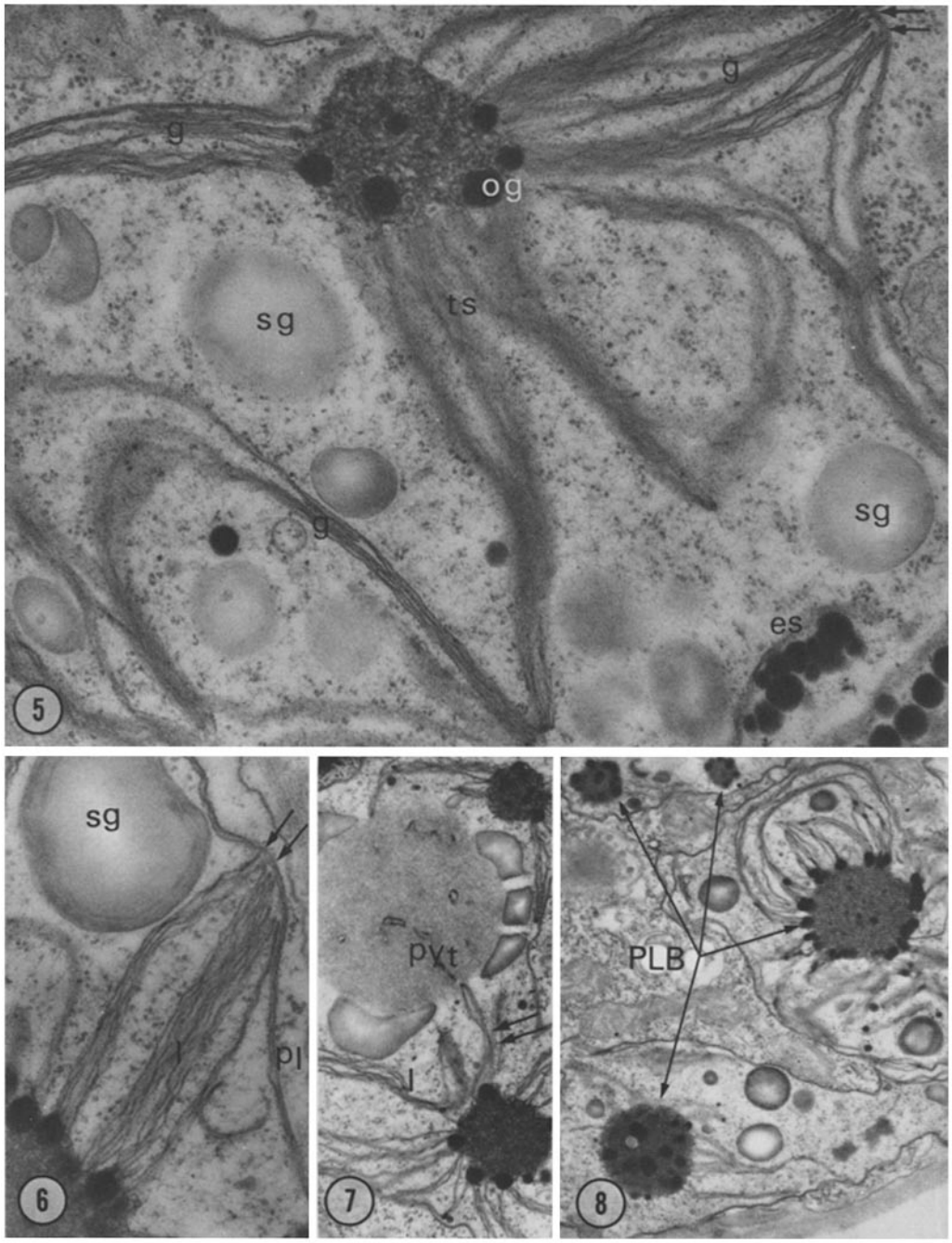

FIGURE 5 A section through the PLB in a dark-grown cell exposed to the light for 5 hr. Notice the abundance of the lamellae protruding from the small central core and the presence of osmiophilic granules (*og*) at the origin of the lamellae, some of which are sectioned tangentially (*ts*). The lamellae are fused in places forming grana (*g*) but do not fuse with the plastid outer envelope (arrows). × 41,000.

FIGURE 6 Same as in Fig. 4, showing the PLB peripheral lamellae distinct from the outer envelope of the plastid (*pl*) (arrows). × 41,000.

FIGURE 7 A section through a dark-grown cell exposed to the light for 5 hr, showing connections through lamellar processes (*l*) between the PLB and the tubular system (*pyt*) of the pyrenoid (arrows). × 21,000.

FIGURE 8 Same as in Fig. 7, showing four prolamellar bodies (arrows). × 16,000.

arginine-requiring mutant. The reasons for its sudden and erratic appearance are not yet understood and remain to be studied. It is usually accepted that the PLB is found in plastids of higher plants under conditions of inhibited development (21). In the case of *Chlamydomonas* cells, growth in the dark, by itself, seems to be insufficient to induce the formation of this structure. Further work will be necessary in order to define the conditions required for the formation of a prolamellar body in *Chlamydomonas* cells and to establish to what extent this structure can be considered as an equivalent to the prolamellar body of higher plants.

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