

A LARGE SCALE QUASI-CRYSTALLINE LAMELLAR LATTICE IN CHLOROPLASTS OF THE GREEN ALGA *ZYGNEMA*

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ABSTRACT

A quasi-crystalline lamellar lattice was observed in chloroplasts of the filamentous green alga *Zygnema*. The lattice does not appear in the cells until cultures are at the end of the log phase of growth. Pseudograna are also present and become more numerous towards the middle of the log phase. The three-dimensional lattice superficially resembles the configuration of cubic prolamellar bodies but is about 10 times larger and is entirely different in internal structure. The lattice is composed of one or two appressed thylakoids in a stroma matrix which is bounded on each side by a single thylakoid membrane. This multilayered sandwich of membranes and matrix occupies a position equivalent to the single membrane of a cubic prolamellar body.

INTRODUCTION

The chloroplasts of green algae, although more complex in their internal structure than most other algae, do not contain true grana. Sager and Palade (9), for instance, have described the chloroplasts of normal *Chlamydomonas reinhardi* cells in which the thylakoids frequently occur in stacks of two to twenty, resembling the grana of higher plants though being more irregular in their size and orientation. Sager and Palade noted that intrathylakoid space may vary from 40 to 700 Å, and that in these intrathylakoid spaces there is a "homogeneous material of rather low density." This description could apply to the chloroplasts of many green algae. However, unusual occurrences have been observed in the chloroplasts of various other genera. Lembi and Lang (6) observed, in the chloroplasts of *Carteria*, thylakoids which opened wide and then folded in on themselves many times

to give the appearance of stacks. They termed these thylakoid formations pseudograna. Pseudograna were also observed quite regularly by Berkaloff (1) in chloroplasts of *Protosiphon botryoides*. One of his micrographs also shows rings of two concentric thylakoids, in addition to the pseudograna. *Mougeotia*, a filamentous green alga in the Zygnematales, studied by Puisieux-Dao and Levain (8), also possesses thylakoids with wide intrathylakoid spaces and pseudograna (7).

This investigation reports an unusual lattice occurring in the chloroplasts of *Zygnema* when in the stationary phase of growth in axenic culture. *Zygnema*, also in the Zygnematales, is characterized and best known by the twin stellate plastids in each of its cells. These plastids are suspended in the central portion of the cylindrical cell, and lobes of the plastid radiate to the periphery. The pyre-

noid, located in the center of each plastid, is traversed by numerous lamellae and is surrounded by starch grains. The nucleus lies between the two plastids. No definitive work has been done on the fine structure of this alga, and there are no reports of structures comparable to the quasi-crystalline lattice of its chloroplasts. Lang and Rae (5), however, have reported a lattice resembling a prolamellar body in cells of the blue-green alga *Anabaena* in the stationary phase of growth. Chloroplasts of *Chlorella pyrenoidosa* grown under heterotrophic conditions revert to a proplastid condition with a decrease in the number of thylakoids and the eventual appearance of a prolamellar body (2).

MATERIALS AND METHODS

Zygnema species No. 3 (an isolate so-numbered in our culture collection) was originally isolated from a pond in the Austin, Texas area and maintained in axenic culture. It was grown in soil water medium (6) at 22°C under a 12–12 hr light-dark regime at 300–400 ft-c. The filaments were embedded in 1.5% agar and then fixed for 1 hr in 3% glutaraldehyde–3% acrolein (1:1) with 0.2 M cacodylate buffer, pH 7.2. After washing several times during 1 hr with the same buffer and distilled water (1:1), the cells were treated with 4% osmium tetroxide and buffer (1:1) for 1 hr. (In some cases the cells were fixed in 2% KMnO₄ for 2 hr in the cold.) The material was then washed four times with distilled water, placed in 0.5% uranyl acetate, and left overnight in the refrigerator. After being dehydrated in an ethanol series and acetone, the cells were gradually infiltrated with Epon-Araldite. Sections were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome (Ivan Sorvall Inc., Norwalk, Conn.) and poststained in uranyl acetate and lead citrate. Micrographs were taken on RCA-EMU-3F and Siemens Elmiskop I electron microscopes.

RESULTS

Fig. 1 shows a light micrograph of a portion of a *Zygnema* filament. The two stellate plastids are apparent in each cell. Fig. 2 is an electron micrograph of a median longitudinal section through a *Zygnema* cell. This alga has large vacuoles. The cytoplasm and its organelles are concentrated in the center of the cylindrical cell and are connected to the periphery by strands; a layer of cytoplasm coats the cell periphery. Radiating lobes of the stellate chloroplast, which are contained within the cytoplasmic strands, are mostly out of the plane of section shown in Fig. 2. A lattice-like configura-

tion is adjacent to the pyrenoid in the central part of each chloroplast.

The lattice appears to consist of aligned rings and rippled lines in a regular repeating pattern (Figs. 3 and 4). Sections examined under a polarizing microscope did not show the lattice area to be birefringent. Serial sections were made through several planes of the lattice, and a series of four sections is presented in Figs. 5–8. Clay models were constructed of each section, and the sections were positioned sequentially atop one another. The resulting three-dimensional configuration is presented in the illustration in Fig. 9. This represents only eight units of the lattice, each unit being the confluent junction of tubules lying in the three planes of a cubic lattice. Sections have been observed to have 10 or more units running the length and width of the lattice revealing at least 100 units in that plane of section. If this number persists in depth also, then a lattice could consist of as many as 1000 units. The resulting configuration of the lattice has a cubical appearance. The diameter across the lumen of a tubule or arm (95 m μ) is the same as the distance between two adjacent arms in the same place.

The walls of each tube or arm are multilayered, as seen in Fig. 10, which represents a close-up of a section through part of a lattice revealing the internal composition. The internal structure of the lattice is shown in Fig. 11. One can see from the various figures that this internal organization is consistent throughout the lattice. The only variation is the presence either of one or of two intralattice thylakoids (see Fig. 14). When two thylakoids or sacs are present, little or no space is observed between them; they lie close to each other

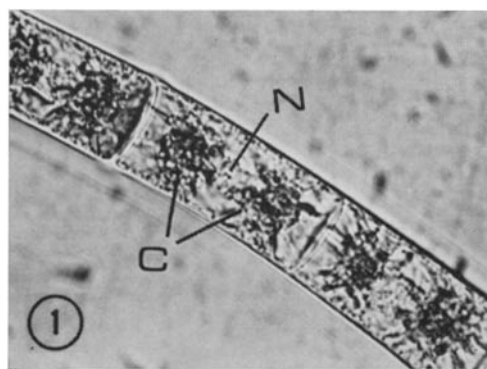


FIGURE 1 A light micrograph of part of a *Zygnema* filament. N, nucleus; C, chloroplast. $\times 830$.

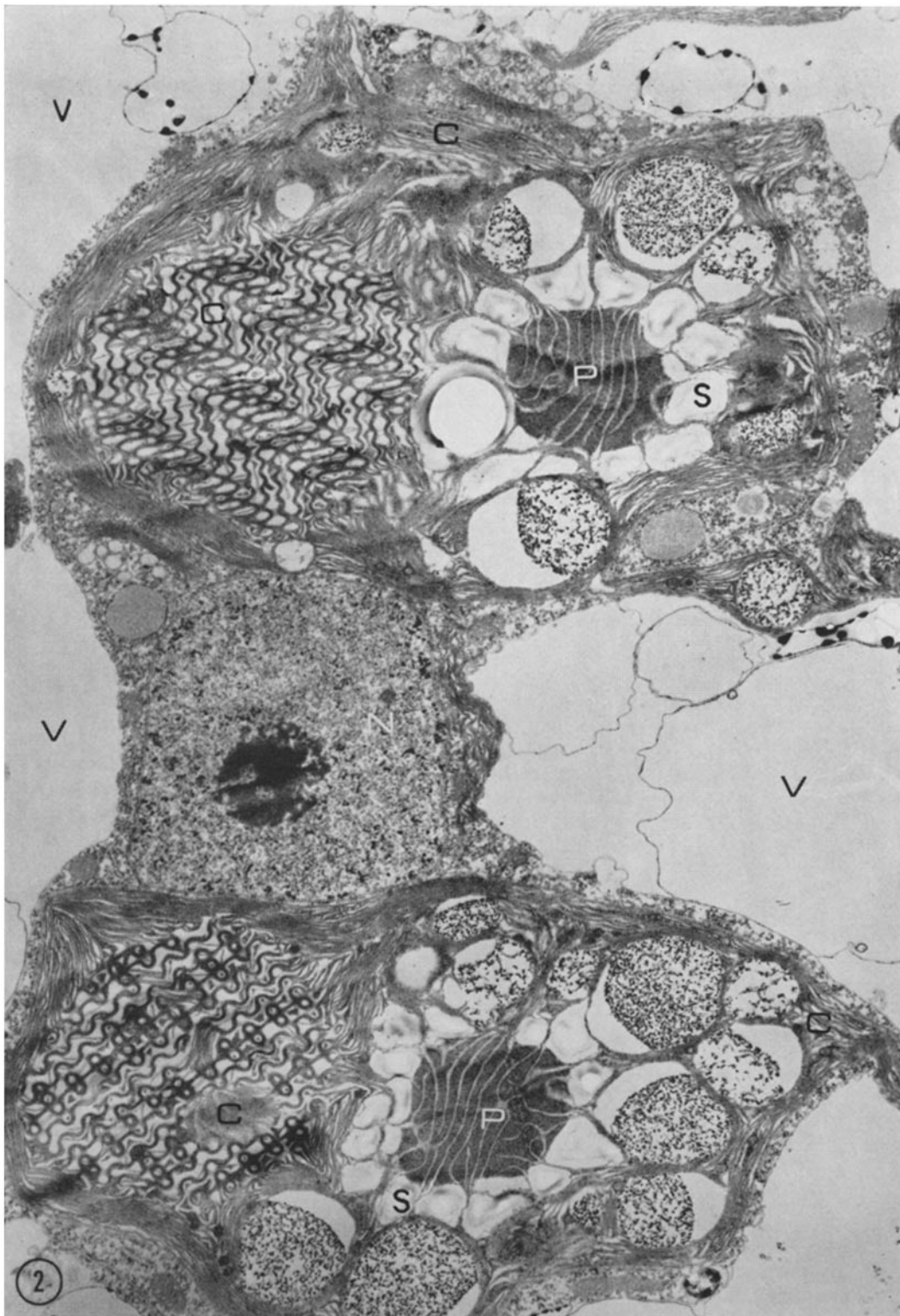


FIGURE 2 An electron micrograph of a median longitudinal section through a *Zygema* cell with the lattice apparent in the chloroplast. *C*, chloroplast; *N*, nucleus; *P*, pyrenoid; *S*, starch grain; *V*, vacuole. $\times 10,400$.

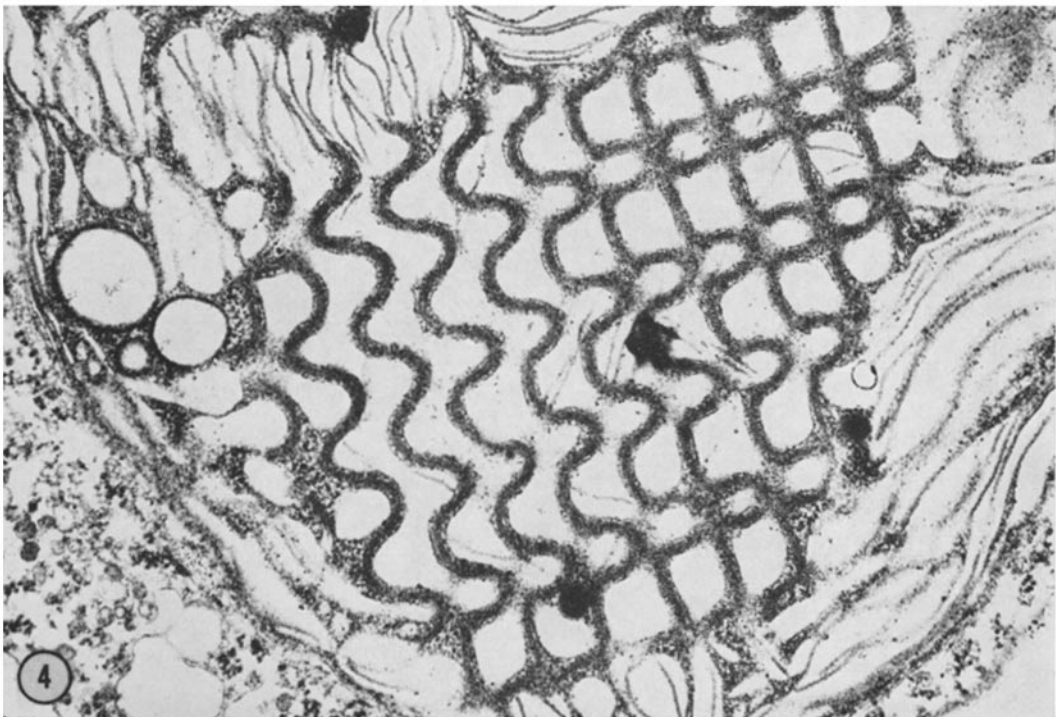
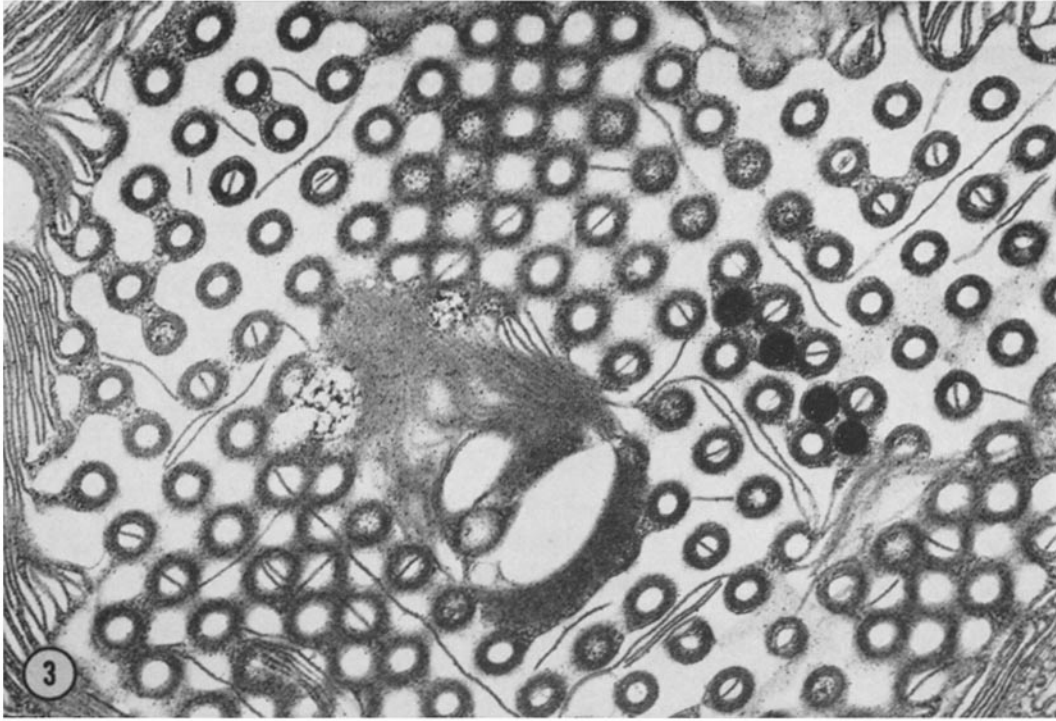
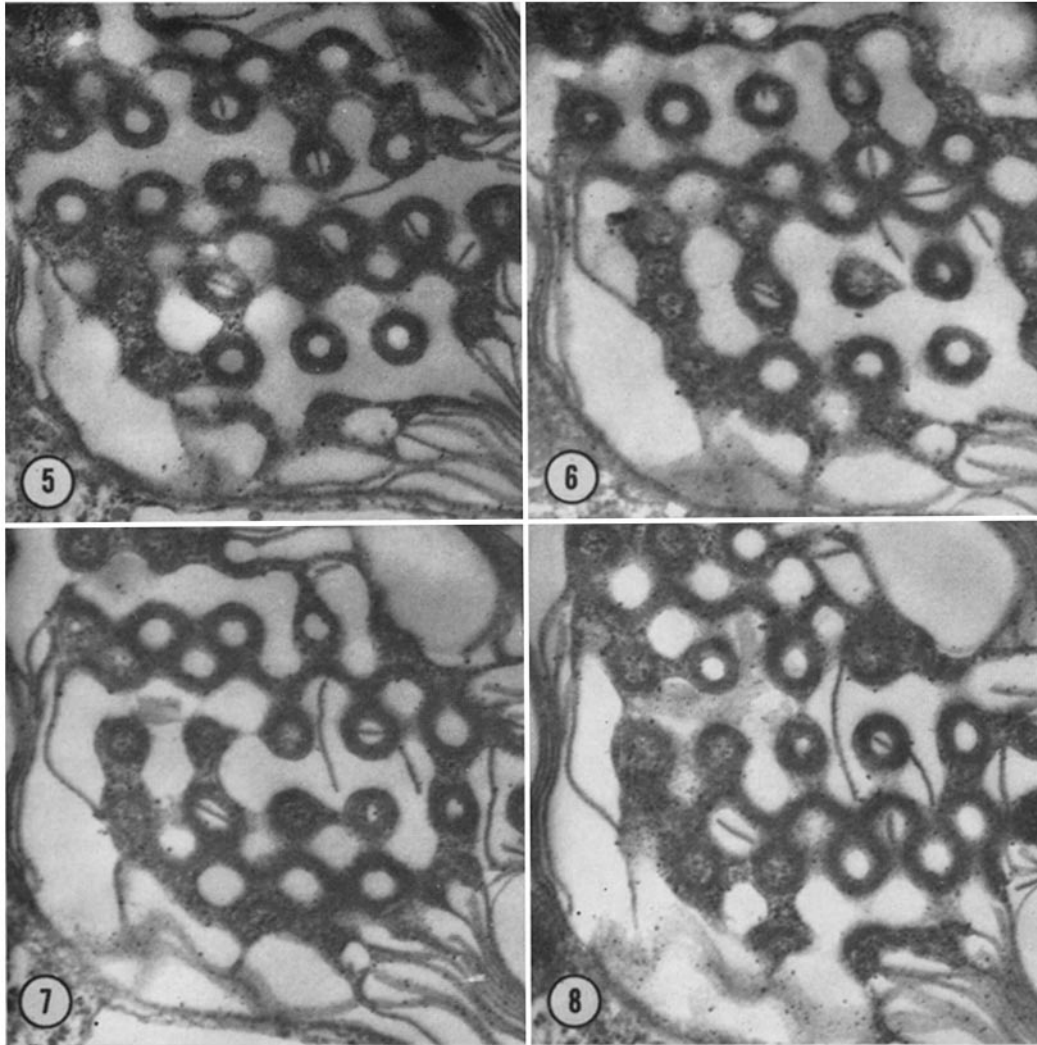


FIGURE 3 An approximately transverse section through the lattice (see Fig. 9). $\times 23,200$.

FIGURE 4 A section through the lattice (see Fig. 9). $\times 46,000$.



FIGURES 5-8 Serial sections through the lattice of *Zygnuma* taken from Butvar-coated grids. $\times 30,000$.

throughout the lattice (*c* in Fig. 11). These intralattice thylakoids are surrounded by stromal material (*b*). Enclosing the multiple layers of stromal material and thylakoids are two single lamellae (*a*). The area which is not very electron opaque and which appears to form a matrix for the lattice is probably filled with water. The space that the water occupies is, in fact, intrathylakoid space, the thylakoid membranes of which are the single lamellae that enclose the stromal material and intralattice thylakoids. This electron-transparent area is continuous with the distended intrathylakoid spaces outside the lattice structure where pseudograna are present.

In order to determine how the lattice is formed, soil water bottles were inoculated with *Zygnuma* and were incubated for 6 wk. Twice a week, cells were fixed in 2% KMnO_4 . The lattice was not observed to form until cultures were at least 24 days old, that is, near the end of the log phase of growth. In cultures 10 days old, cells contained chloroplasts which had thylakoids with wide intrathylakoid spaces (Fig. 12). In addition, thylakoids, in terms of lattice formation, could be observed. As the cultures age, the chloroplasts appear to form more pseudograna (Fig. 13). At 24 days, the first lattices are observed (Fig. 14). Pseudograna are still present. The lattices seem to

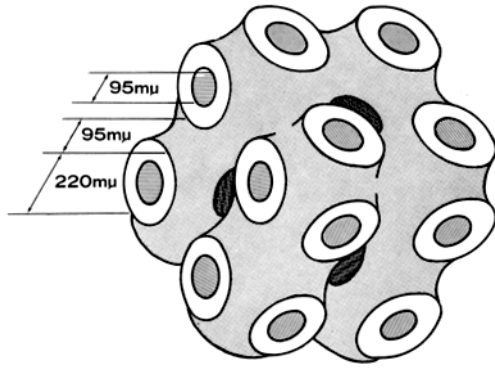


FIGURE 9 A three-dimensional reconstruction of part of the lattice in *Zygnema* chloroplasts.

begin to form by the process of a thylakoid evaginating and sliding between two other thylakoids (Fig. 14). Presumably, in lattices with two intralattice thylakoids (Fig. 10), either two thylakoids intrude or else one thylakoid evaginates twice. Between the intruding thylakoids and the enclosing membrane is stromal material. The enclosing membranes are themselves part of thylakoids with wide intrathylakoid spaces. Fig. 15 is a diagram indicating the initiation of the lattice structure.

Although the lattice seems to persist in cells from cultures up to 6 wk old, the actual fate of the lattice is not known. Because of heavy lipid accumulation and thickening of the cell walls, good fixation of the resting cells or akinetes is difficult to achieve. Resting cells will germinate to produce new filaments when they have been transferred to media containing fresh nutrients.

DISCUSSION

The lamellar lattice of *Zygnema* is only superficially similar in organization to the gross structure of the prolamellar body reconstructed by Gunning and Jagoe (4) and found usually in etiolated higher plants. Both types of structures are composed of interconnected six-armed units. One of the most obvious differences is size; there is nearly a 10-fold difference in size between the prolamellar body of higher plants and the lamellar lattice of *Zygnema*. For instance, the diameter of the tubules of nodal units of the prolamellar body is 210 Å, whereas the tubules or arms of the units in the *Zygnema* lattice have a diameter of 220 μ m. This is due to the fact that nodal units of the prolamellar body are composed of a single membrane, while units in the *Zygnema* lattice have one or two thyla-

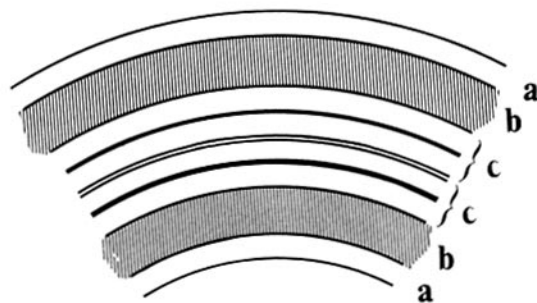
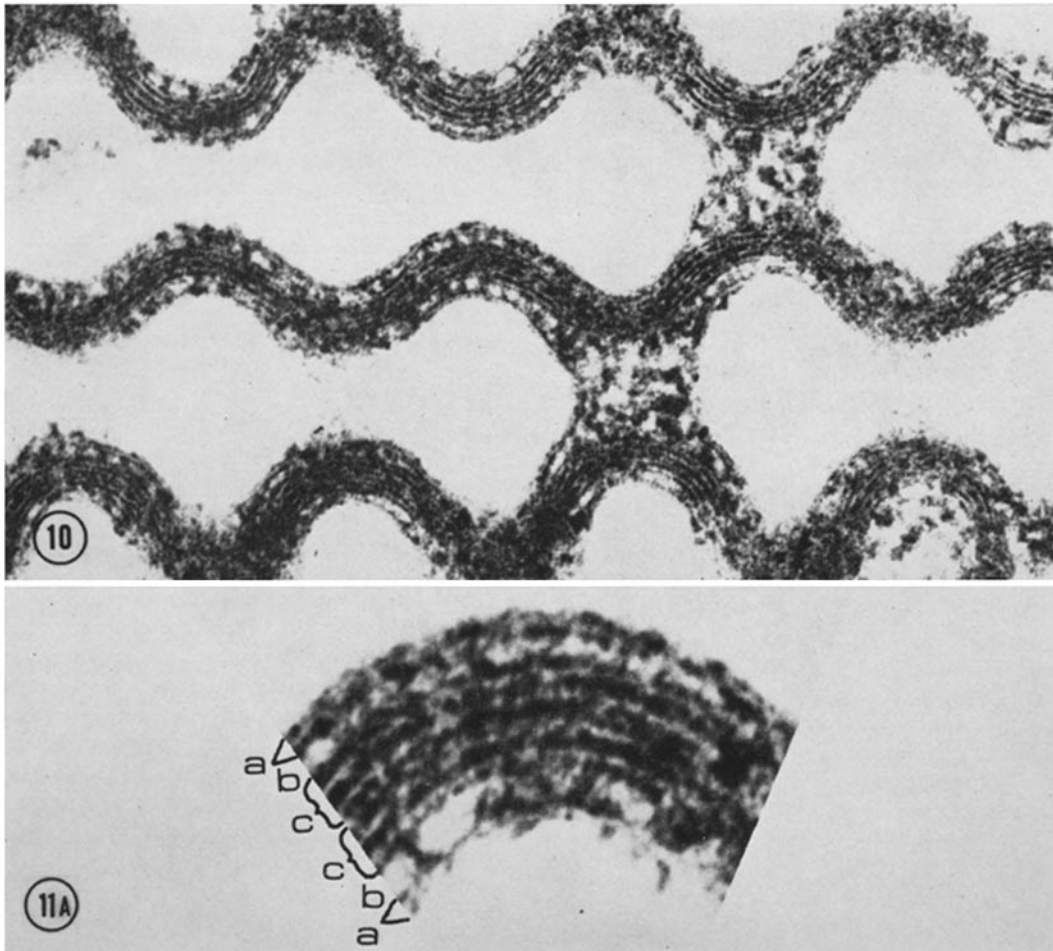
koids and two bounding membranes making up their basic structure. The lattice of *Zygnema* is obviously not a prolamellar body since thylakoids are already found within it. Also, the lattice was observed in cultures incubated under what are considered normal conditions of 12 hr light and 12 hr dark rather than grown in complete darkness as seems necessary for prolamellar body formation.

It appears that one or two thylakoids slide between two neighboring thylakoids to initiate the lattice (Figs. 14 and 15). The thylakoid membranes must then assume the three-dimensional configuration of the lattice. Gunning and Jagoe (4) have presented theoretical evidence concerning the formation of the prolamellar body and the maintenance of its three-dimensional configuration. Because of the differences in size and composition, it does not seem likely that their explanation of the configuration of the prolamellar body would apply to that of the *Zygnema* lattice.

There seems to be less stromal material in *Zygnema* chloroplasts than in those of many other plants. By contrast, whatever substance (water?) is contained in the intrathylakoid spaces and lattice matrix occupies a large portion of the chloroplast volume. A question that comes to mind is whether the large amount of water is due to an osmotic effect. This may be discounted because of the repeated appearance of the lattice in cells from old cultures. Also, the condition of other organelles in the cells appeared normal even when the lattice was present.

Pseudograna, so clearly described by Lembi and Lang (6) in *Carteria* chloroplasts, are also common in chloroplasts of *Zygnema*. Pseudograna form by an invagination of thylakoid membranes. Lattice development is initiated by an evagination and subsequent intrusion of a thylakoid into a stroma matrix which is between two other thylakoid membranes. The evagination procedure seems to be the opposite of the invagination process in pseudogranum formation. An interesting fact is that the pseudograna become more common at about the middle of the log phase of growth, whereas lattices are not seen until the end of the log phase of growth. The relationship of the two configurations is not known.

Since pseudograna have been observed by Puiseux-Dao (7) in *Mougeotia*, by Lembi and Lang (6) in *Carteria*, and by Berkaloff (1) in *Protosiphon*, one wonders if these organisms, like *Zygnema*, have the potential for producing a lamellar lattice. The



(11 B)

FIGURE 10 High magnification of thylakoid layering in *Zygnema* lattice. $\times 130,000$.

FIGURE 11 A Portion of Fig. 10 enlarged to show sandwich layers in *Zygnema* lattice. *a*, unit membrane; *b*, stroma; *c*, thylakoid. $\times 350,000$.

FIGURE 11 B Diagrammatic representation of thylakoid layering (not to scale). *a*, unit membrane; *b*, stroma; *c*, thylakoid.

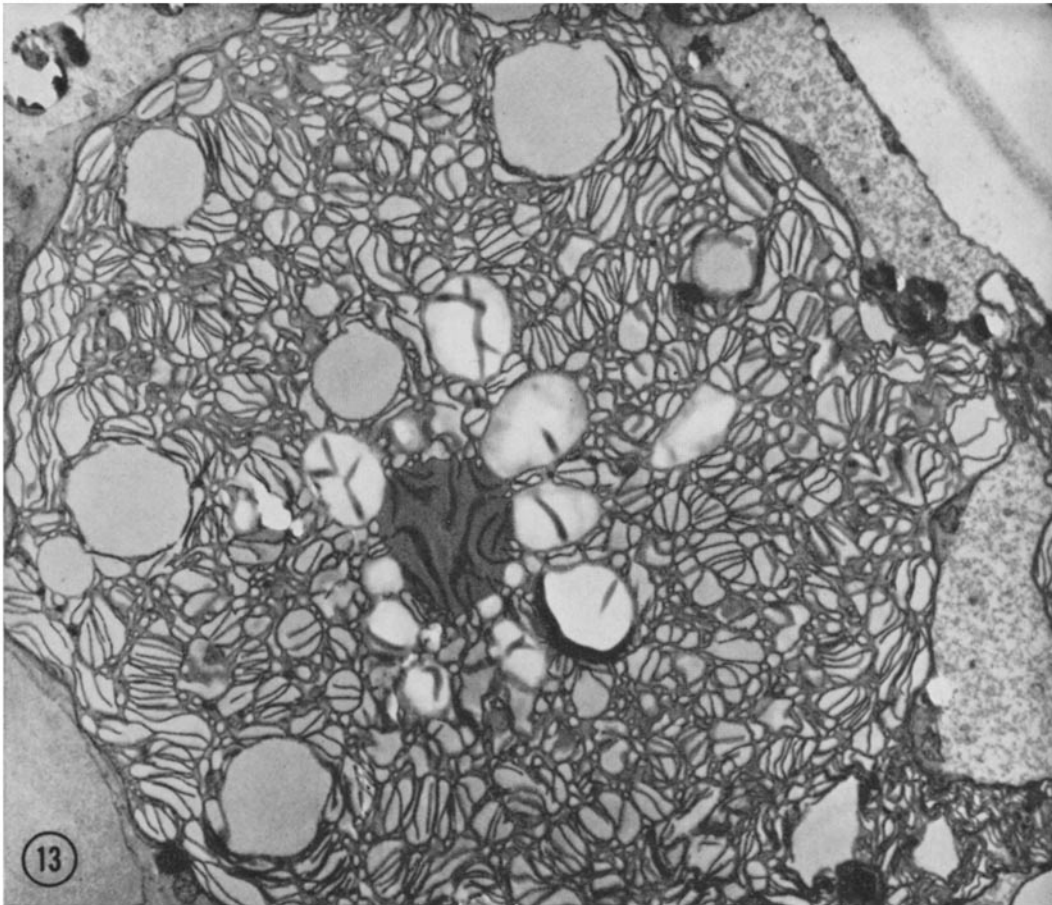
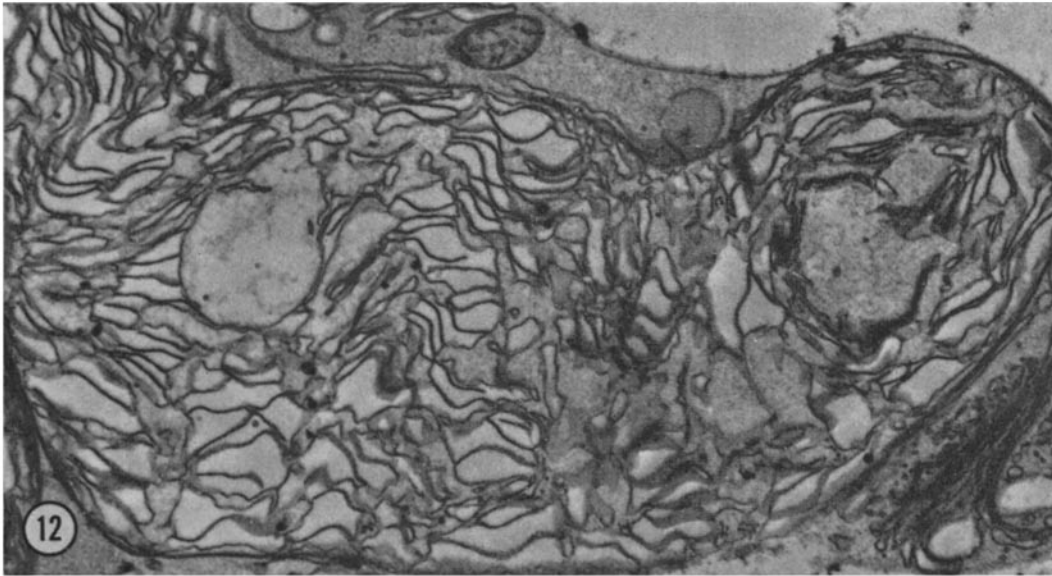


FIGURE 12 Portion of a chloroplast of a cell from a 10 day old culture showing lack of organization and large intrathylakoid spaces. $\times 16,800$.

FIGURE 13 Numerous pseudograna appear in this cell from an 18 day old culture. $\times 12,500$.

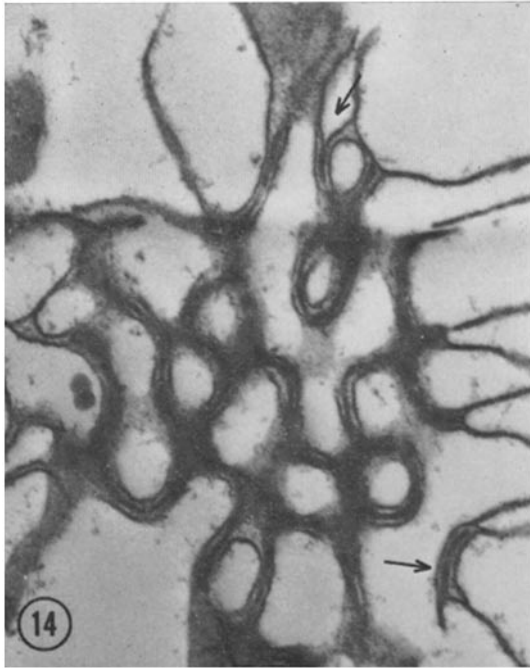


FIGURE 14 After 24 days in culture, cell chloroplasts demonstrate initiation of the lattice (arrows). $\times 43,000$.

appearance of two concentric thylakoids in *Protosiphon* (Berkaloff, 1967; Fig. IV a) is identical to that seen during the initiation and early stages of lattice development in *Zygnema*. This presents the possibility that a lattice was indeed beginning to form in *Protosiphon*.

A lattice similar to that of *Zygnema* was recently observed by us in a report of Granhall and Hofsten (3) on the blue-green alga *Anabaena variabilis* which was under attack by viruses. They thought that in their disorganized nature the thylakoids were similar to a proamellar-like body observed by Wildon and Mercer (11) and Lang and Rae (6) in heterocysts of *Anabaena*. However, closer examination of Granhall and Hofsten's micrographs shows the configurations not to be prolamellar-like, but similar to the lattice of *Zygnema*. Since the lattice in *Anabaena* was apparently due to the virus attack, one wonders if the same might be true in *Zygnema*. We observed no viruses on the cell walls of *Zygnema*. However, a membrane structure

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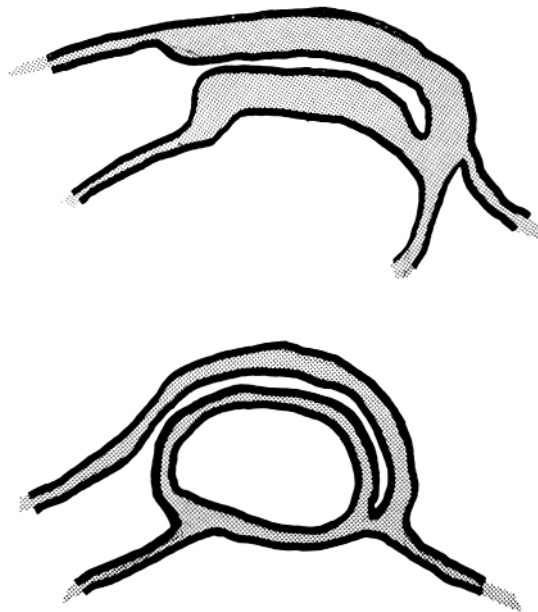


FIGURE 15 A diagrammatic interpretation of initiation of the lattice. A thylakoid intrudes into stroma which is bordered by membranes of two adjacent thylakoids.

similar in some ways to the *Zygnema* lattice was observed in cells of virus-induced tumors of a subhuman primate (10). Perhaps a virus attack on *Zygnema* cannot be ruled out since the sheath covering the wall of *Zygnema* would make it difficult to recognize viruses.

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