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Ultrastructural observations on the pyrenoid of *Bryopsis duplex* De Notaris.

Introduction

The pyrenoid, early described by light microscopists as a dense and refractive body of the algal chloroplast (Schmitz, 1882; Carter, 1919; Geitler, 1926; Czurda, 1928; Chadefaud, 1941; Bose, 1941; Steinmann, 1951) is a well-defined organelle found in or associated with the plastid of most algal cells.

Recently investigations on the pyrenoid are concerned chiefly either with his ultrastructural (Leyon, 1954; Gibbs, 1962 a, b; Manton, 1966) or physiological and biochemical aspects (Griffiths, 1970; Holdsworth, 1968, 1971; Dodge, 1973).

Among the coenocytic Chlorophyceae, members of the Siphonales so far investigated present plastids with pyrenoids characterized by a granular matrix generally surrounded by a sheath of starch plates.

Studies on the representatives of this order were carried out by Hori et al. (1967) on *Halimeda discoidea* and other Siphonales, Santisi et al. (1969) on *Bryopsis duplex*, Burr et al. (1970) on *B. hypnoides*, and Wheeler et al. (1974) on *Derbesia tenuissima.*

The pyrenoid matrix, which is mainly proteic in nature (Holdsworth, 1968, 1971), is traversed by one or more plastidial thylakoids which may pass into the pyrenoid without or with peculiar structural modifications.

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LEYON (1954) in a number of green algae found that the chloroplast thylakoids traverse the pyrenoid without structural modifications, and such a situation has been detected in several algae belonging to different groups (UEDA, 1961, in *Porphyra tenera;* MURAKAMI et al., 1963, in *Chlorella pyrenoidosa;* KOWALLIK, 1969, in *Prorocentrum micans*).

Nevertheless, either in green algae or in other algal divisions, the thylakoid membranes may appear swollen or ondulated as they enter the pyrenoid matrix (STRUGGER et al., 1961; GIBBS, 1962 b; HORI, 1973; SILVERBERG et al., 1974; SILVERBERG, 1975).

The structural features of the intrapyrenoidal thylakoids are reported modified or atypical in *Closterium lunula* (LEYON, 1954). In this species groups of thylakoids may fold back on themselves, whereas in *Carteria* (LEMBI et al., 1965) and *Chlorococcum polymorphum* (BROWN et al., 1969) the thylakoid membranes penetrate the pyrenoid matrix in a rather contorted manner. As MANTON et al. (1961) reported, the behaviour of the pyrenoid thylakoids become more twisted and convoluted during the early stages of division in *Chrysochromulina minor.*

However there are no specific papers dealing with the structural modifications of the membranous inclusions lying in the pyrenoid of coenocytic algae.

In the present work, some ultrastructural aspects of the chloroplast and pyrenoid in the coenocytic marine green alga, *Bryopsis duplex* are presented, with emphasis on the fine structural modifications of thylakoid membranes found in the pyrenoid matrix.

Material and Methods

The intertidal benthic alga, *Bryopsis duplex* De Notaris (= *B. disticha* Kütz.) was collected on January 1976 at Portici, near Naples.

For electron microscope purposes whole siphons of the coenocytic organism were fixed in 2% glutaraldehyde in 0,1 M phosphate buffer (pH 7,2) for 2 h at 4°C.

Segments were then cut into 1-2 mm lenght and placed in fresh fixative solution for 1 h, washed in buffer, and post-fixed in 1% osmium tetroxide for 3 h (Millonig, 1961).

After dehydratation in a graded series of ethanol, embedding in Epon followed. The sectioning was carried out on a LKB ultramicrotome provided with glass knives; the material was stained with uranyl acetate and lead citrate (Reynolds, 1963), mounted on coated copper grids, and examined in a Siemens 1 A electron microscope.

Observations

Electron micrographs confirm the general features previously described in this species (Santisi et al., 1969).

The chloroplasts (up 8-12 μm long) are the most conspicuous bodies of this alga. In the plastidial stroma the thylakoids appear stacked in clusters, each of two to five discs (Pl. I, Fig. 2). Single or paired thylakoids interconnecting the adjacent stacks of membranes were also recognized. Starch plates and plastoglobuli lie between them.

In this region, sometimes electron-transparent areas enclose a network of fibrous denser material identified as D N A; therefore these areas are genophores.

As shown in Fig. 1, the pyrenoid body consists of an electron-opaque central matrix, whose structure appear essentially granular, about 2 μm in diameter, devoid of peripheral membranous envelope. A sheath of starch plates lies around it; thus, the whole size of the body may result of about 4 μm in diameter, ranging to 5 μm or more.

The sheath is not continuous, but interrupted several times so that the plastid thylakoids coming from different directions may penetrate the pyrenoid matrix with a right or more meandering course across the space free of starch plates.
Stacks of three to six or more thylakoids were observed in this space near the periphery of the pyrenoid (Pl. II, Fig. 3).

At this level the stacking appears reduced to three thylakoids (Fig. 5) because the upper and the inner peripheral discs seem to end by a margin before they enter the pyrenoid.

A feature of noticeable interest is that three thylakoids penetrate the matrix appearing structurally modified. As found in a transection of the thylakoid disc (Fig. 5), the one medial shows typical reel-like structures each about 30-40 nm high and 20-30 nm wide, lying in the lumen. Besides each of these structures presents a narrowing in the middle.
In Fig. 4 a short segment is showed with three paired thylakoids running inside the pyrenoid matrix; as may be seen, no ultrastructural modification has affected this thylakoidal segment.

In tangential sections, the thylakoid surface may be detected (Pl. III, Fig. 6). Then, the surface is seen supplied with small pores 20-30 nm in diameter and peripherically bounded by an electronopaque ring. The pores, arranged in parallel arrays, correspond likely to the profiles termed reel-like structures in transverse sections, found in the swollen lumen of the pyrenoid thylakoids.

Therefore in *B. duplex* the pyrenoid is characterized by the presence of thylakoid discs by threes, whose only the medial one seems modified by small pores; besides is to be outlined that some widening interests only this thylakoid disc.

Discussion

The thylakoid membranes found in the chloroplast of *Bryopsis duplex* confirm the typical ultrastructural aspects detected in other marine algae belonging to the Siphonales.

The chloroplast, bounded by an envelope composed of a double unit membrane, contains stacks each of two to six appressed thylakoids separed by a granular stroma.

This pattern is rather similar to the one observed in the mature chloroplast of *Halimeda* (Borowitzka et al., 1974), and *Bryopsis hypnoides* (Burr et al., 1970). However in the latter the thylakoid discs appear more swollen whereas in *Caulerpa prolifera* (Dawes et al., 1967) the stacking is more marked, the number of stacked thylakoids in this species reaching 3-12 units.

In two green algae, *Chlamydomonas* (Ris et al., 1962), and *Bryopsis* (Steffensen et al., 1965) the chloroplast D N A was found located in regions of low electron density adjacent to the pyrenoid.

The D N A-containing areas or genophores (Bisalputra et al., 1969), enclosed between the thylakoid stacks in the chloroplast of *B. duplex* often were recognized around the differentiated starch plates of the pyrenoid sheath. Therefore, according to the suggestions of Ris et al. (1962) and Mikulska et al. (1974) on the presence of enzymes responsible for starch formation in these regions, they may correspond to sites of starch synthesis around the pyrenoid in the chloroplast.

The fine structure of the pyrenoid of *B. duplex* is characterized by a dense and homogeneously granular matrix traversed by quite a few thylakoid discs paired by threes and surrounded by a sheath of starch plates.

A similar type of pyrenoid was termed « polypyramidal » by Chadefaud (1941) who distinguished it from the « bilenticular » one which is bounded by only two starch plates.

GRIFFITHS (1970) classified the pyrenoids into two general morphological types, « embedded» and « projecting » pyrenoid, the former consisting of a spherical, granular, fibrillar or paracrystalline body (the matrix) deeply set within the chloroplast stroma, while the latter is a body which protrudes from the plastid surface.

Recently DODGE (1973) characterized a type of pyrenoid as « globular pyrenoid entirely embedded in the plastid, surrounded by a starch sheath, and traversed by a number of thylakoid membranes ».

The fine structural evidence leads us to state that the embedded pyrenoid detected in the present material belongs to this type and therefore it may be classified according to the latter definition.

As is known, many groups of green algae generally show in proximity of the pyrenoid a drastic reduction in the number of the thylakoid discs. Likely, in the pyrenoid outer regions this evenience constitutes the sole observed modification.

Such a situation occurs in *B. duplex* at the periphery of the pyrenoid matrix where the number of stacked thylakoids rapidly decreases before they penetrate in the organelle.

According to LEMBI et al. (1965), the limiting membrane of the appressed thylakoids is derived by the external membrane of a cluster of thylakoids.

It is noteworthy that inside the pyrenoid matrix of the present material the thylakoid discs may appear rather swollen; this agrees with the findings of GIBBS (1962 a) in *Chlamydomonas*, MURAKAMI et al. (1963) in *Chlorella*, BROWN et al. (1970) in *Tetracystis*, and SILVERBERG (1975) in *Stichococcus*.

However, among the siphoneous green algae, the single thylakoids of *Derbesia* sp. (HORI et al., 1967), and the two paired thylakoid membranes found in the pyrenoid of *Derbesia tenuissima* (WHEELER et al., 1974) are reported as not modified by swelling but as made up of « tubules ».

In some red and green algae LEYON (1954) and GIBBS (1962 a) found an increase in the intrathylakoid space at the membrane entry inside the pyrenoid. The larger spacing found in the pyrenoid discs of these organisms corresponded to the medial band of the tripartite membrane, i.e. the loculus (*sensu* WEIER et al., 1965), as in the present material.

Thus in *B. duplex* too, the loculus might provide the spacing for the formation of the reel-like structures seen in transections of the discs.

The small pores arranged in parallel or subparallel arrays likely appear when sectioned in a plane parallel to the major surfaces of the pyrenoid lamellar inclusions.

In Fig. 7 a diagrammatic representation of these structures is given.

In the paper of BURR et al. (1970) on the vegetative and reproductive stages of *B. hypnoides*, a micrograph is presented showing a thylakoidal portion within the pyrenoid ground substance, and traversed by structures termed « pores ». Nevertheless the above researchers give no a detailed account on the ultrastructural modifications affecting the intrapyrenoidal thylakoids.

As the fine structural features of that pyrenoid are very similar to those described in transections of the pyrenoid thylakoids of *B. duplex*, they might correspond to those termed « reel-like structures » in the present material.

Therefore the term « small pores » might be ascribed to the structures as seen in longitudinal tangential sections of the thylakoid disc.

LEYON (1954) and GRIFFITHS (1970) pointed out that a reduction of chlorophyll pigment in the membranous pyrenoid inclusions may play a role in the structural diversity between the plastidial and pyrenoidal membranes.

Nevertheless GIBBS (1962 b) and BROWN et al. (1969) suggested that chlorophyll *a* and *b* was contained in the membranes traversing the pyrenoid matrix.

In *Eremosphaera viridis* (HOLDSWORTH, 1971) pyrenoid membranes were found qualitatively similar to thylakoids in the rest of the chloroplast, because extracts of chloroplast fragments showed an absorption spectrum similar to that of the pyrenoid extracts. Therefore is suggested that these thylakoids play a role in the photosynthetic processes.

Since the thylakoid membranes might affect the continuity of the pyrenoid matrix, then the reel-like structures or small pores might serve to establish important interconnections between different pyrenoidal compartments.

Thus, these structures are more likely concerned with translocation processes either to the pyrenoid, which may act as a protein store, or with transport of metabolites away from the pyrenoid towards the plastid stroma and cytoplasm.

As this paper describes the ultrastructural features of the pyrenoid inclusions in a single algal species, until further work is done on other green algae belonging to the Siphonales, the question whether it is of general occurrence in these organisms still remain open.

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SUMMARY

Vegetative filaments of the coenocytic marine green alga, *Bryopsis duplex* De Notaris have been examined by means of electron microscopy.

In the chloroplast one or more pyrenoids of embedded type were found. The pyrenoid matrix is traversed by thylakoid discs which become structurally modified in a way that the medial one shows « small pores » 20-30 nm in diameter on his upper and inner surfaces.

These structures correspond to « reel-like structures » running perpendicularly to the major axis of the thylakoid disc.

According to the assumptions of some researchers, the ultrastructural modifications found in the pyrenoid discs might be related with the chlorophyll content or more likely with the translocation processes from or towards the pyrenoid core.

RIASSUNTO

Osservazioni ultrastrutturali sul pirenoide di Bryopsis duplex De Notaris

È stata studiata l'ultrastruttura del pirenoide dell'alga verde cenocitica *Bryopsis duplex* De Not., con particolare riferimento agli inclusi membranosi.

La matrice del pirenoide è attraversata da tilacoidi i cui dischi subiscono una riduzione numerica al momento della loro entrata in essa: per tale motivo, all'interno della matrice pirenoidale i tilacoidi si presentano costantemente ridotti a tre elementi appaiati. Inoltre, quello mediano è interessato da caratteristiche modificazioni strutturali.

Tali modificazioni possono venire osservate sia in sezione trasversale, sotto forma di « pori » di 20-30 nm di diametro, allorquando il tilacoide intrapirenoidale viene interessato da una sezione tangenziale, oppure sotto forma di caratteristiche formazioni qui denominate « reel-like structures » (strutture a rocchetto), se la sezione è invece trasversale alla membrana tilacoidale (e in tal caso le strutture si presentano sezionate longitudinalmente).

Le strutture a rocchetto decorrono perciò nel lume dei dischi tilacoidali, perpendicolarmente alla loro superficie.

Le modificazioni ultrastrutturali osservate nel pirenoide di questa specie potrebbero venire correlate sia con il contenuto in clorofilla dei tilacoidi intrapirenoidali, sia con un processo di traslocazione di metaboliti dall'interno del pirenoide verso la matrice del cloroplasto, e viceversa.

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PLATE I

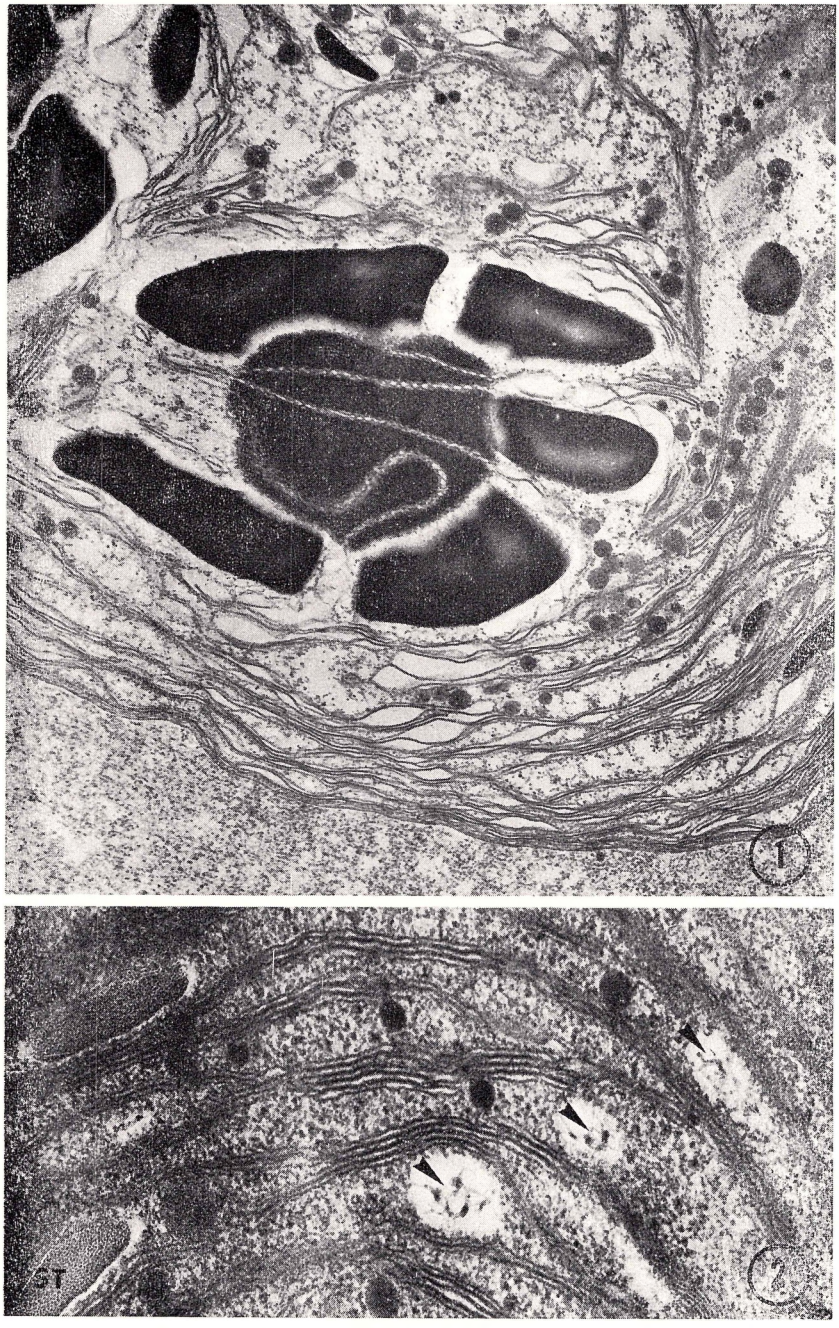

Fig. 1 Embedded pyrenoid equipped with a sheath of five starch plates. Osmiophilic globules are scattered around it between the plastidial thylakoids. Note the direct or meandering course of the membranous inclusions within the granular matrix. x 15.200.

Fig. 2 - Plastid thylakoids. Note the genophores (arrowheads), and the small starch plates (ST) in their proximity. x 65.000.

PLATE II

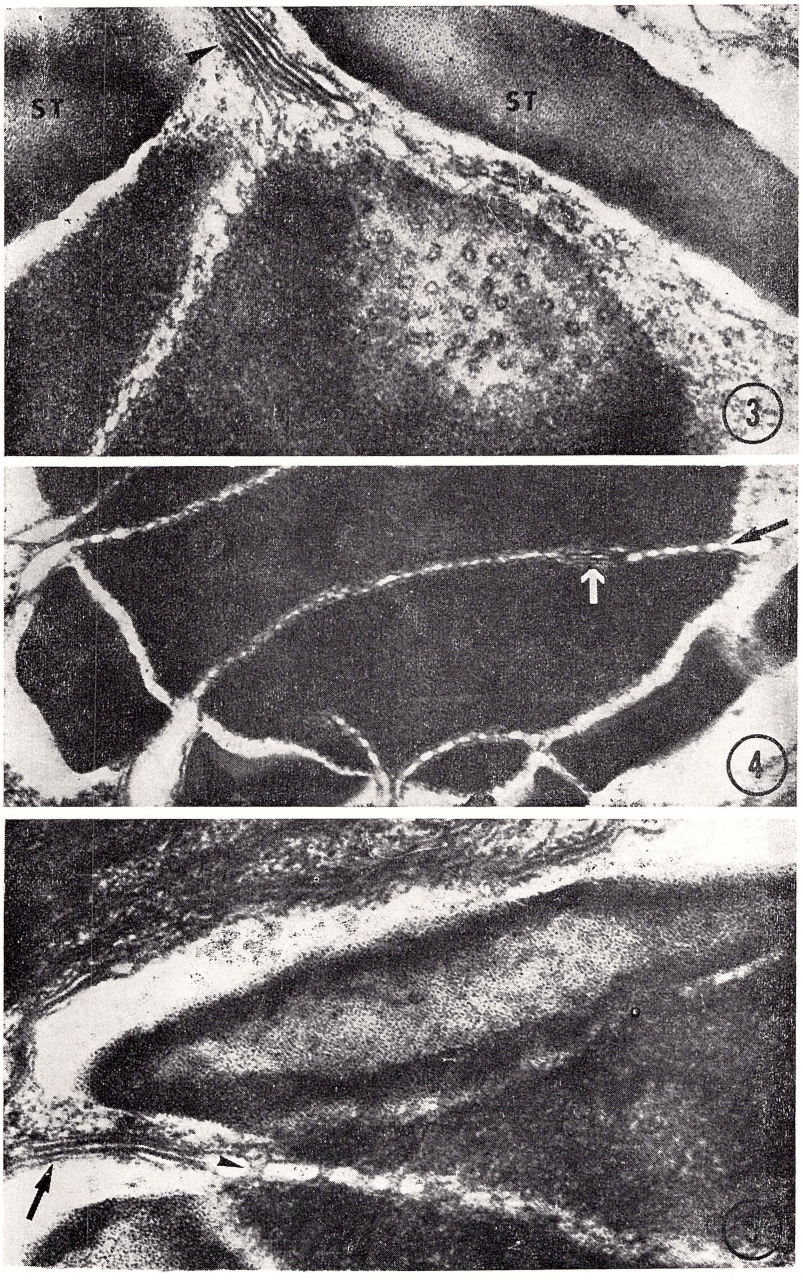

Fig. 3 - Stacked membranes (arrowhead) running towards the pyrenoid matrix between two large starch plates (ST); a field of small pores may be seen. x 68.000.
Fig. 4 - Pyrenoid matrix showing exceptionally three thylakoid discs (white arrow) not modified by the reel-like structures (black arrow). x 38.000.
Fig. 5 - A detail of three thylakoid discs (arrow) penetrating the pyrenoid matrix. The whole depth of the reel-like structures (arrowhead) is seen. x 70.000.

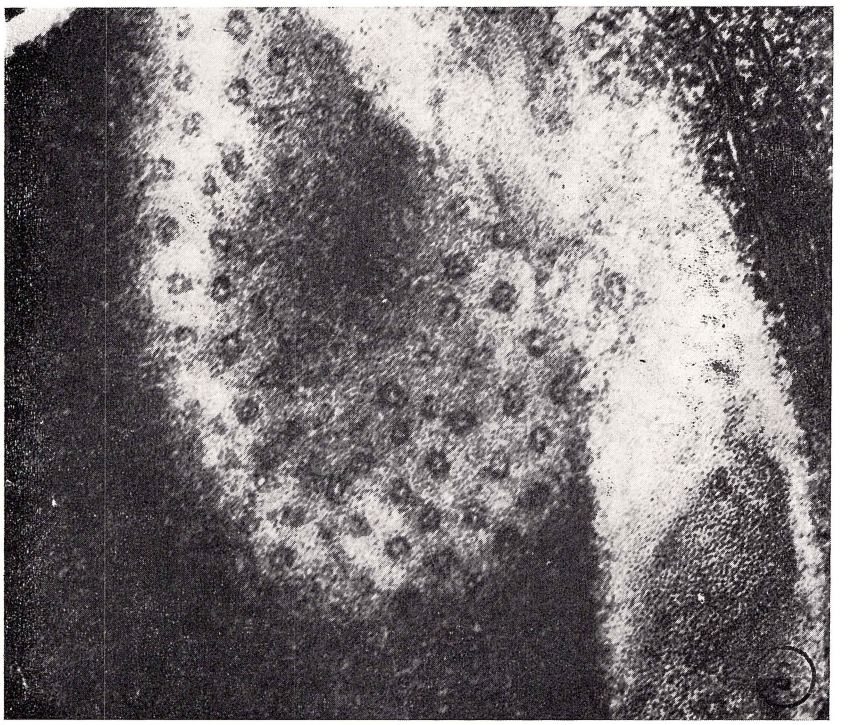

Fig. 6 - The same as in previous micrograph when seen in tangential section; thus the reel-like structures may be detected as small pores in transection. At the periphery, the pores display an electron-opaque ring, and in the whole field they appear arranged in arrays. x 77.000.

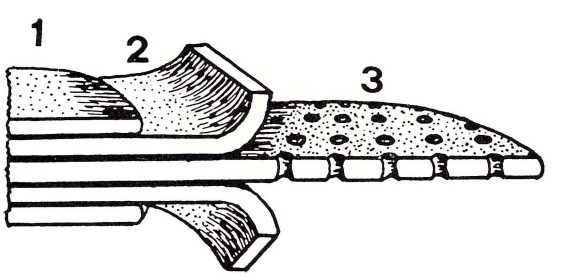

Fig. 7 - A diagrammatic representation of the pyrenoid membranous inclusions and their modifications.
In 1: five thylakoid discs are presented. In 2: the reduction of the discs as they penetrate the pyrenoid matrix. In 3: the medial thylakoid disc modified by small pores and reel-like structures is shown. In order to displaying the pores on the surface of the medial disc, upper and inner thylakoids are presented folded outward and sectioned. The widening of the lumen in the medial thylakoid disc is not represented.