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# Properties and localization of the photoreceptor active in displacements of chloroplasts in *Funaria hygrometrica*

## IV. Electron microscopy

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## INTRODUCTION

As it was shown in the recent studies the photoreceptor active in the photo-displacements of chloroplasts and absorbing in the blue region of the spectrum is regularly arranged in respect to the cell walls. This kind of arrangement was found in the cells of *Selaginella* (Meyer 1964), *Vallisneria* (Seitz 1966) as well as in *Funaria* (Zurzycki 1967 b). The question arises on which cell structures the molecules of the photoreceptor can be situated. By using light microscopy and fluorescence microscopy it was impossible to determine in which structure the photoreceptor is localized (Zurzycki 1967 c). These results made us investigate the organisation of *Funaria* cells on the electron microscopy level looking for the structures in the outer parts of the cell, which show enough great orientation and stability and which therefore could be the site of regularly arranged photoreceptor molecules.

## MATERIAL AND METHODS

Before fixation the plants were kept for 5 h in light of low intensity or 24 hours in darkness. The leaves were cut off from the stem with a razor blade and prefixed for 2 h in 6% glutar aldehyde buffered to pH 6,8 with 0,1 M phosphate buffer, then washed $3 \times 1$ h in phosphate buffer and fixed for 6 h. in 2% $OsO_4$ similarly buffered. After washing and gradual dehydration the leaves were embedded in epon 812 according to the method described by Luft (1961). Sections were cut on a Porter and Blum microtome by using a diamond knife. Staining of sections was performed with uranyl acetate and lead citrate according to Reynolds (1963). Sections were studied and electron micrographs were made through the JEM — 5Y electron microscope. Most pictures were taken at 17000 x primer magnification.

## RESULTS

Cell walls of several mosses are barely permeable for the substances of middle and high molecular weight. This phenomenon is known from slow and retarded plasmolysis (Beibl 1954, Steinlesberger 1959) as well as from the difficulties in embedding in polymers i.e. araldite (Falk and Sitte 1963). This was the reason why the duration of the fixation procedure was applied for a longer time than it is commonly used for other botanical objects. Dificult permeability for embedding media was also found in the course of embedding in epon. In some steps of embedding the outer cell walls colapse and the shape of the cell becomes abnormal. After a large prolongation of the embedding procedure sections of a natural cell shape but of a very destroyed inner structures were obtained. The present study is based on the preparations obtained by a usual procedure of embedding. In spite of a certain deformation of the outer cell shapes the ultrastructures of the cells show in this case no significant artefacts.

Cell walls. The lamina of the leaf of *Funaria* is composed of one layer of cells. The thickness of the outer (free) cell walls is comparable with that of the walls separating two neighbour cells, both ranging between 0,3—0,4 μ. Only at the edge curvatures the walls are thicker (fig 1). The exact determination of the thickness of the cell walls in living cells is not easy because of small dimensions but it seems to be bigger (0,5—0,8 μ — for comparison see Zurzycki 1967 c fig 1 *a*). Some schrinkage of the thickness of cell walls in the course of fixation and embedding can therefore not be excluded. On some sections two (for the outer cell walls) or three (for the walls separating two cells) layers in the cell walls may be distinguished. The outer layer of the outer cell wall shows in that case greater density for electrones than the inner one. Both layers are of constant thickness and the boundary between them is sharp. The darker layer becomes triangular in shape at the edge of the cell and runs on as a thin layer along the central part of the side cell wall (fig. 1).

Usually, however, the cell walls show great density on their whole thickness, without differentiation into an outer and inner layer. Very often they are quite dark on the electronograms (fig. 4). A havy darkening of the walls shows that they contain some materials which are able to reduce $OsO_4$. This property was just found previously (Zurzycki 1967 c) when cells fixed in osmium tetroxide proved to be unsuitable for densitometric measurements because of a strong darkening of their walls.

At middle magnifications the cell walls are either homogenous across their thickness, with some structural inhomogenity at the middle lamella, or show a slight fibrillar appearance (fig. 4). Similar fibrillar

structures were found in the cell walls of *Elodea* after staining with lead (Falk and Sitte 1963).

In the side walls the plasmodesmata of an irregular spindle shape were found. Their lumen is widest in the central parts of the cell wall, where their dimension reaches about 10—15 nm and become narrow approaching the surface of the walls (fig. 3, 4). This shape seems to be common among mosses ie. it was found for *Atrichium undulatum* (Falk and Sitte 1963). The opening of plasmodesmata looks as if it were a little errected above the plain of the cell wall, but this picture may be connected with the shrinkage effect of the cell walls.

Outer plasma membrane. On the suitable sections the outer plasma membrane is visible as a double membrane consisting of two dark lines separated by a clear layer*. The thickness of this double mebrane is about 90—100Å. Usually the plasmalemma does not adhere closely to the cell wall. A gap between these two structural components ranges about 100—300Å and contains dispersed granulations (fig. 2). The lack of close contact between the cell wall and the plasma membrane although found in all cases where the last one was well visible, could be caused by $OsO_4$ fixation as it was stated by Drawert and Mix (1963) for *Allium* cells, or could be connected with the shrinkage of cell walls in the course of embedding.

At the border of the lumen of plasmodesmata also a double membrane may be visible (fig. 3). Its thickness is comparable with that of the plasmalemma but its density is usually much weaker. The connections between membranes lining the inner surface of plasmodesmata and the outer plasma membranes were not studied in details.

Cytoplasm. The cytoplasm shows a granular appearance of different stage of density. On some sections or some parts of the cell the structures of cytoplasm are rather disperse, the granulations form loose clouds leaving empty spaces between them (fig. 5, 6, 7) but very often especially near the cell walls the granulations are very rich (fig. 4, 6). Bigger granules of diameter 60—120Å may be identified as free ribosomes. Their number and accumulation is rather great as for a developed vacuolised plant cell.

Endoplasmic reticulum is rather scarce. It is to be seen as smaller or bigger vesicles or longer membranes of smooth type (fig. 5). The membranes of an endoplasmic reticulum are of about 50Å thick, they are not very densely contrasted and make the impression of single membranes. ER penetrates through the plasmodesm in form of

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* In the description of electronograms we use the term single membrane for a single dark line, and double membrane for the structure composed of two close and parallel dark lines with clear space between them (Weier and Thomson 1962).

a double layered structure (fig. 4) as it was found by Falk and Sitte (1963) for *Atrichium*.

Mitochondria are found usualy in close neighbourhood to chloroplasts. Their shape is of irregular tubes of 120—260 nm diameter and till 5 μ long (fig. 5). At the ends the mitochondria are a little blown out (fig. 4, 5, 6). The walls of mitochondria consist of two single membranes. The distance between them varies from place to place (fig. 6). The inner structure of mitochondria is rather scarce. In some places tubes can be seen (fig. 6). On some cross sections ring structures as described by Falk and Sitte (1963) for mitochondria of *Elodea* were found (fig. 7). Inside the mitochondria deep stained granules of 240—300Å occur in more or less regular distances. The nature and significance of these structures is not clear.

Chloroplasts. The chloroplasts are surrounded by a double membrane ob about 100Å thickness (fig. 6). Their inner structure does not differ from the descriptions given for *Funaria* (Sun 1963) as well as for other mosses (Sitte 1963a, Paolillo 1964). The lamellar system is divided by spindle shaped granular stroma inclusions. Between outer membrane of chloroplast and inner lamellar system a granular layer of peristromium can be seen (fig. 5, 7). Grana can be distinguished but they are not very distinctly separated as structural units. In the stroma inclusions of osmophilic globuli can be found (fig. 5, 7).

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Plate I

Fig. 1. The cell wall between two neighbour cells showing layers of different density. ×13000.

Fig. 2. Fragment of the cell wall and outer plasma membrane. ×150000.

Fig. 3. The plasmodesm with double membranes lining its inner surfaces. ×107000.

Fig. 4. Fragment of the cell. Cell wall with plasmodesm, outer plasma membrane, granulations of cytoplasm, fragments of two chloroplasts and mitochondrium. ×76000.

Plate II

Fig. 5. Chloroplast and long mitochondrium. Above mitochondrium the endoplasmic reticulum. ×34000

Fig. 6. Fragment of a chloroplast surrounded by a double membrane and fragment of a mitochondrium. ×56000.

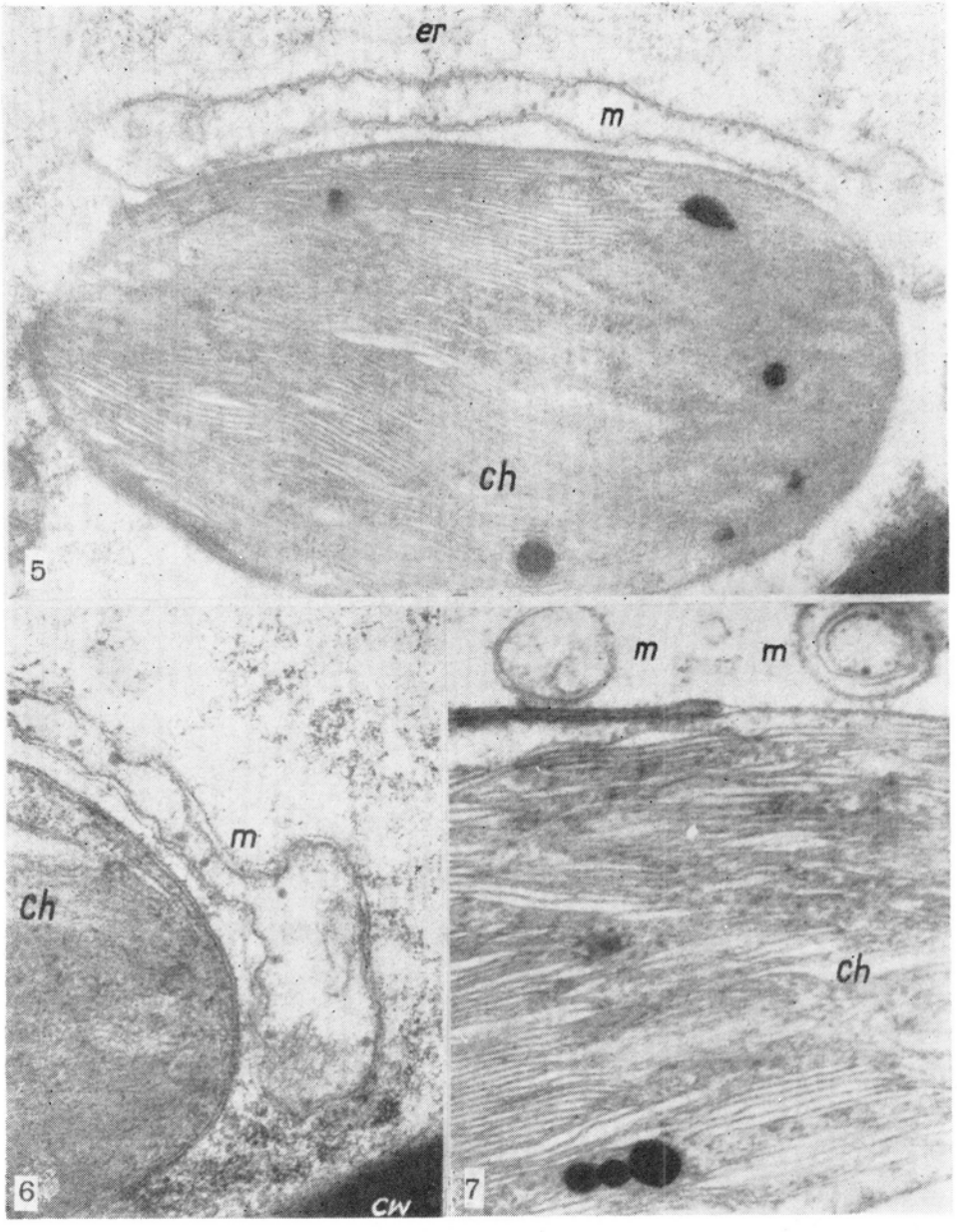
Fig. 7. Fragment of a chloroplast. In the upper part — cross section of two mitochondria. ×56000.

Abbreviations.

*cw* — cell wall; *pl* — outer plasma membrane; *m* — mitochondrium; *ch* — chloroplast; *er* — endoplasmic reticulum.

Plate I

Plate II



## DISCUSSION

Several facts show that the photoreceptor active in the displacements of chloroplasts is localized not in chloroplasts but outside them in the outer parts of the cell (Fischer-Arnold 1963, Zurzycki 1967 a). The orientation of the photoreceptor molecules in respect to the plane of the cell wall (Meyer 1964, Seitz 1966, Zurzycki 1967 b), suggests that there must be some stable structures in the cell on which the pigment molecules should be situated. Analysis of the ultrastructures of *Funaria* cell described in the present paper could point on two such structures: cell wall and outer plasma membrane. No other oriented structures close to the plasmalemma were found. One may speculate on the existence of such structures and its desapearance in the course of fixation and embedding. The method of the treatment of the cells prepared for EM study may have some influence on the picture of its ultrastructure. It was found for instance that fixation in $KMnO_4$ is suitable for preservation of the endoplasmic reticulum and the same material fixed in $OsO_4$ shows only poor ER in the cytoplasm (Falk and Sitte 1963). Probably also the reticulum in *Funaria* cells is reacher than it was shown on our electronograms. On the other hand the existance of ER was demonstrated even in the present study. As it was shown by Wohlfarth-Bottermann (1961) the basic structural aspect of the structure of protoplasm is not changed by different methods of fixation. The structures shown in the present study are in general agreement with the results of many other studies made on plant cells. It seems very improbable that they may exists some ultrastructures in the outer region of the cell which can not be detected with anyone method of treatment used. Taking into consideration the orientation of ER which is only seldom parallel to the cell wall and the great dynamics of this structure in the living cell (Girbardt 1965), the localization of the photoreceptor in endoplasmic reticulum seems not probable. Enough stabilisation and orientation is offered only by cell wall and outer plasma membrane. The localisation in the cell walls is not very probable for two reasons;

1. the "active" photoreceptor should be in close contact with the living cytoplasm where the mechanism of the chloroplasts movements is certainly localized. Localization of the molecules of the photoreceptor in the cell wall especially in its deeper parts will result in breaking of such contact.

2. Experiments on chloroplasts displacements in plasmolised cells (Zurzycki 1967 d) showed that even the parts of protoplast which are separated from the cell walls are the site of the oriented photoreceptor. Electron microscopy studies on plasmolysed cells (Sitte 1963 b) showed that the plasmalemma exists in those conditions and therefore we may

postulate that the molecules of the photoreceptor active in photo--displacements of chloroplasts are regularly arranged on outer plasma membranes.


## SUMMARY

The ultrastructure of the cells of *Funaria hygrometrica* after glutar-aldehyde, $OsO_4$ fixation was studied. The structure of cell walls, plasmalemma, cytoplasm, endoplasmic reticulum, chloroplasts and mitochondria is described. The analysis of the obtained results shows that only cell walls and the outer plasma membrane offer enough great orientation and stability to be the site of photoreceptor molecules. From the results of studies on plasmolysed cells a conclusion may be drawn that the photoreceptor molecules are regularly arranged on the outer plasma membranes.



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## *Własności i lokalizacja fotoreceptora czynnego w ruchach chloroplastów u Fanaria hygrometrica*

### *IV. Badania w mikroskopie elektronowym*

Streszczenie

Zbadano ultrastrukturę komórki *Funaria hygrometrica* stosując utrwalanie w aldehydzie glutarowym i czterotlenku osmu. Opisano strukturę ściany komórkowej plazmolemy, cytoplazmy, retikulum endoplazmatycznego, chloroplastów i mitochondriów. Analiza uzyskanych rezultatów wskazuje że jedynie ściany komórki i zewnętrzna błona plazmatyczna wykazują dostateczną orientację i stabilność aby mogły spełniać rolę struktur, w których są zlokalizowane i kierunkowo uszeregowane cząsteczki fotoreceptora. Na podstawie doświadczeń z komórkami poddanymi plazmolizie należy wnosić że fotoreceptor zlokalizowany jest w zewnętrznej błonie plazmatycznej.