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THE REGREENING OF TUBULOUS CHROMOPLASTS IN FRUITS OF *CUCURBITA MAXIMA* DUCH. CV. *TURBANIFORMIS**

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Introduction

According to the investigations of Schimper (1885), leucoplasts, chloroplasts and chromoplasts are in fact homologous cell organelles which can be transformed into one another. This theory of reversibility of plastid metamorphosis was generally adopted in the following years. On the other hand Frey-Wyssling et al. (1955) postulated that plastid metamorphosis proceeded linearly, wherefore chromoplasts were viewed as degenerated end products of plastid development; consequently, chromoplasts could not revert to chloroplasts. The authors mentioned believe that in regreening tissues the chloroplasts originate from plastid intermediates between chloroplasts and chromoplasts or from proplastids. In spite of this controversy the conversion of chromoplasts to chloroplasts has been demonstrated in a variety of tissues: Valencia oranges (Thomson et al. 1967); tobacco leaves (Ljubešić 1968); pumpkin fruits (Matienko et al. 1969; Devidé and Ljubešić 1972, 1974); carrot roots (Grönegress 1971; Wrischer 1972); spathe tissues and sepals (Grönegress 1974); *Chrysosplenium* leaves (Sitte 1974); soybean cotyledons (Huber and Newman 1976); leaves of *Euonymus* (Ikeda 1979) etc.

Chromoplasts are very heterogeneous in structure. According to their fine structure Sitte (1974) distinguishes five general types. The reversion of chromoplasts to chloroplasts was described in globulous, and very rarely in cristalline type of chromoplasts. In membranous, tubulous and

* Dedicated to Professor Zvonimir Devidé on the occasion of his 60th birthday.

reticulo-tubulous type of chromoplasts the regreening has not been described yet.

The red proximal part of the fruit of *Cucurbita maxima* cv. *turbaniformis* contains chromoplasts of the tubulous type (Ljubešić 1977). Very rarely this part can regreen in spring. In this study the conversion of tubulous chromoplasts to chloroplasts has been investigated in this regreening proximal part of the fruit.

Material and Methods

Ripe fruits of *Cucurbita maxima* Duch. cv. *turbaniformis*, grown under garden conditions, were used for experiments. The reversible transformation of plastids was observed only in the outer cell layer (subepidermis) of the proximal part of the fruit. Ripe fruits with red proximal ends were stored in the laboratory room near the window (1000—3000 lx, and 20° C). The material was studied by light and electron microscopy.

Hand-cut, unfixed tangential sections of subepidermal tissue were examined under light microscope.

For electron microscopic investigations the material was fixed in 1% glutaraldehyde at pH 7.2 (cacodylate buffer) for 30 minutes. After fixation the material was washed in cacodylate buffer and postfixed in 1% OsO_4 (2 hours). Fixed material was dehydrated in ethanol and embedded in Araldite. The sections were prepared on a Reichert Om U2 ultramicrotome, stained with uranyl acetate and lead citrate and observed with a Siemens Elmiskop I and Elmiskop 101.

Figs. 1—9. Plastids from subepidermal cell layers of fruit of *Cucurbita maxima* Duch. cv. *turbaniformis*.

Fig. 1. Chromoplast from the mature red fruit. Large peripheral reticulum, big plastoglobules, remnants of thylakoids, and numerous tubules are present. 20,000 : 1.

Fig. 2. The peripheral part of a chromoplast with invaginations of the inner portion of the plastid membrane. Tubules are connected with plastoglobules. 25,000 : 1.

Figs. 3. and 4. Plastids at the beginning of the regreening process. Small grana with swollen peripheral thylakoids (arrows). Ribosomes form an array along the thylakoids (double arrow). 49,000 : 1.

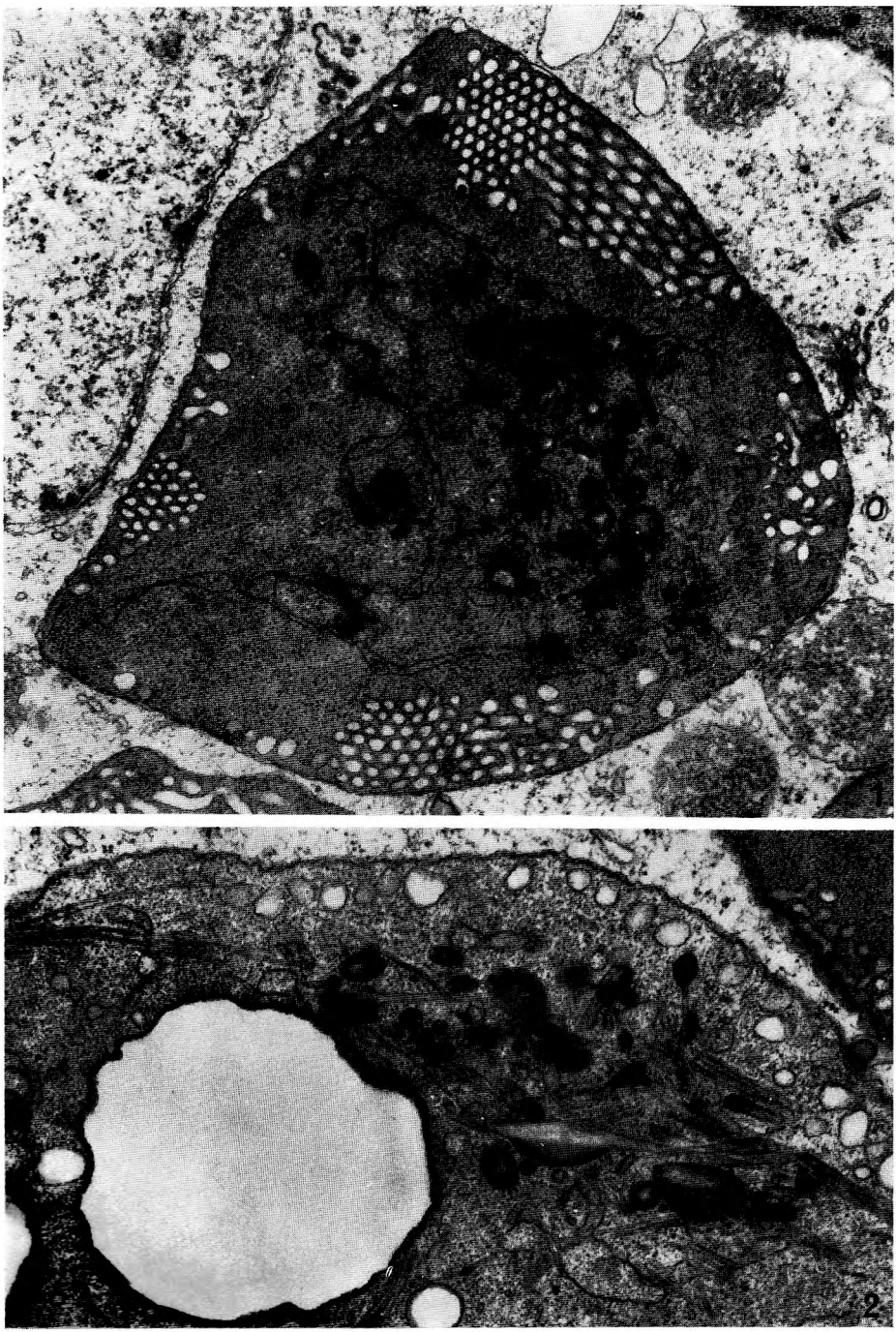
Fig. 5. Plastid with numerous small grana (arrows) in the peripheral region. 16,000 : 1.

Fig. 6. Chlorochromoplast with numerous newly formed grana. Plastoglobules are in the middle of the plastid. 21,000 : 1.

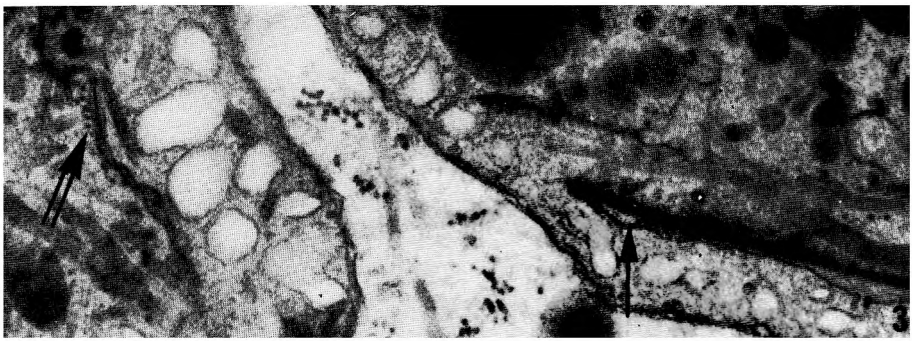
Fig. 7. Part of a chlorochromoplast with grana and plastoglobules. Thylakoids in a granum are different in length. 42,000 : 1.

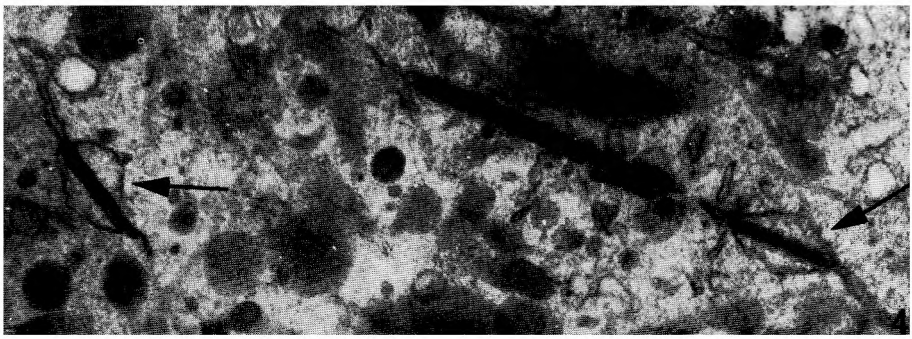
Fig. 8. Chloroplast from the regreened fruit. Grana are large and numerous. Among them big plastoglobules are still located. 20,000 : 1.

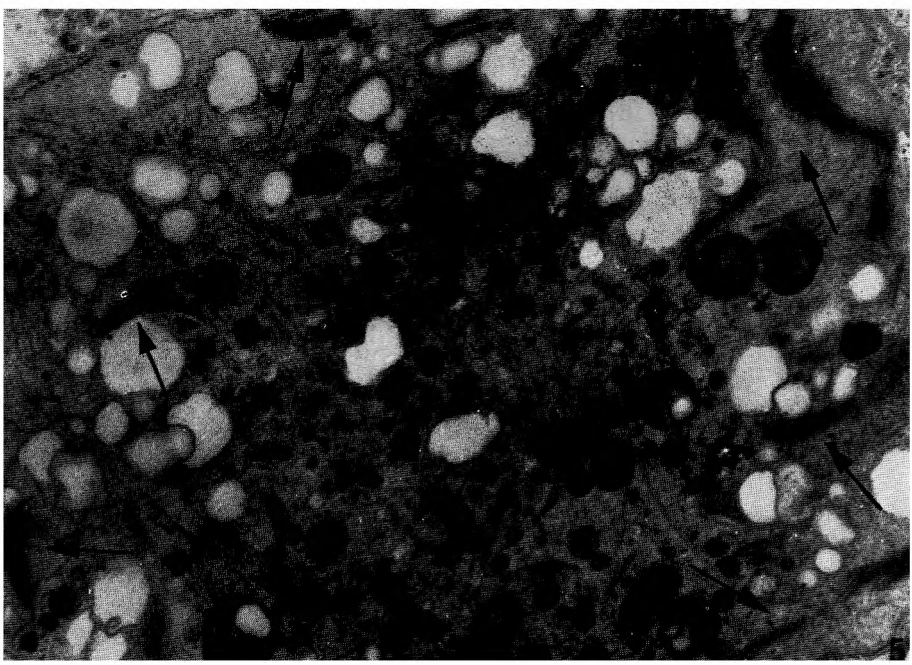
Fig. 9. High magnification of grana from the regreened chloroplast. 68,000 : 1.



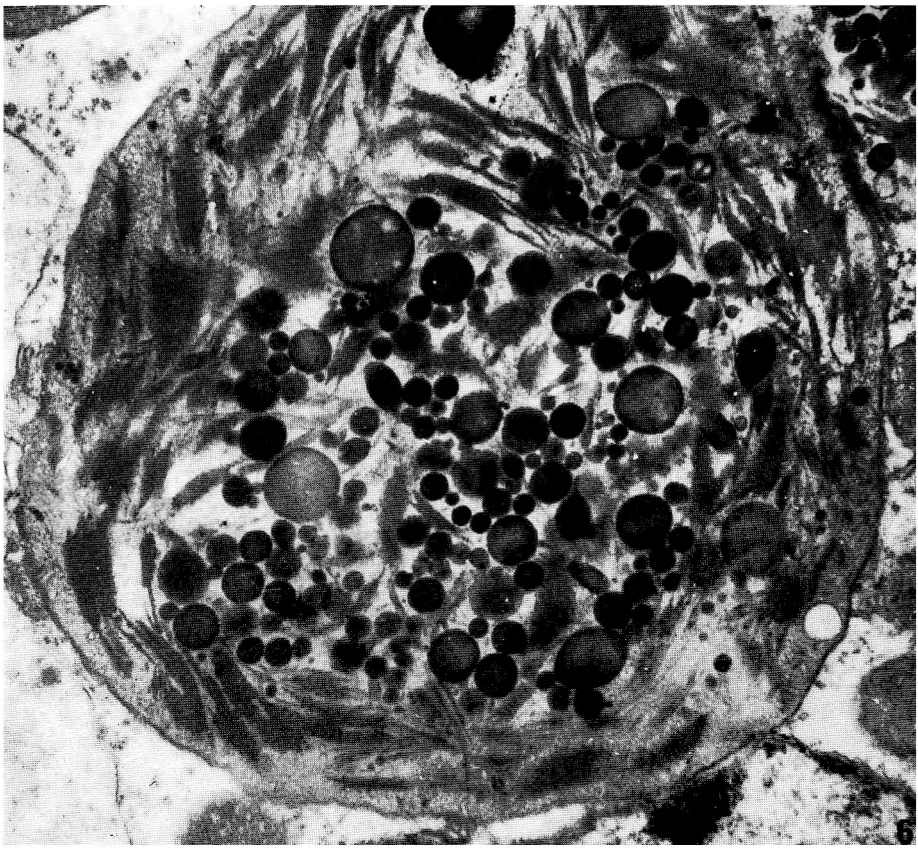
Figs. 1—2.

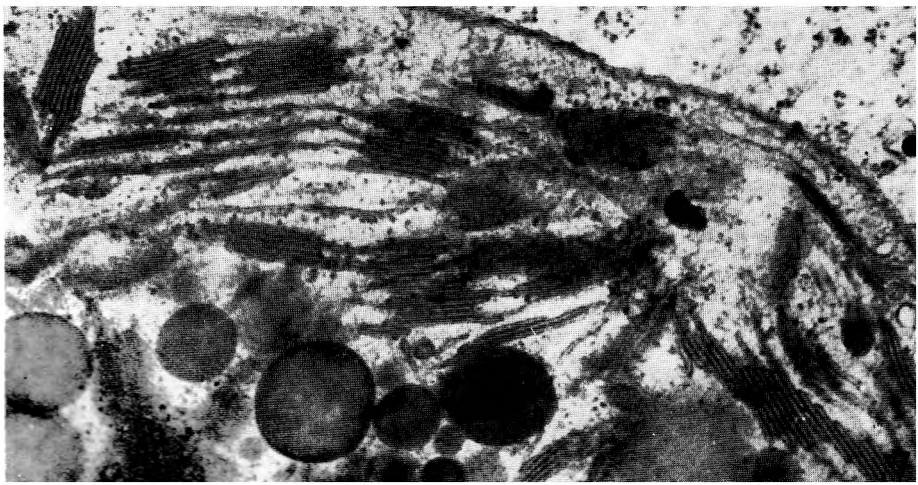




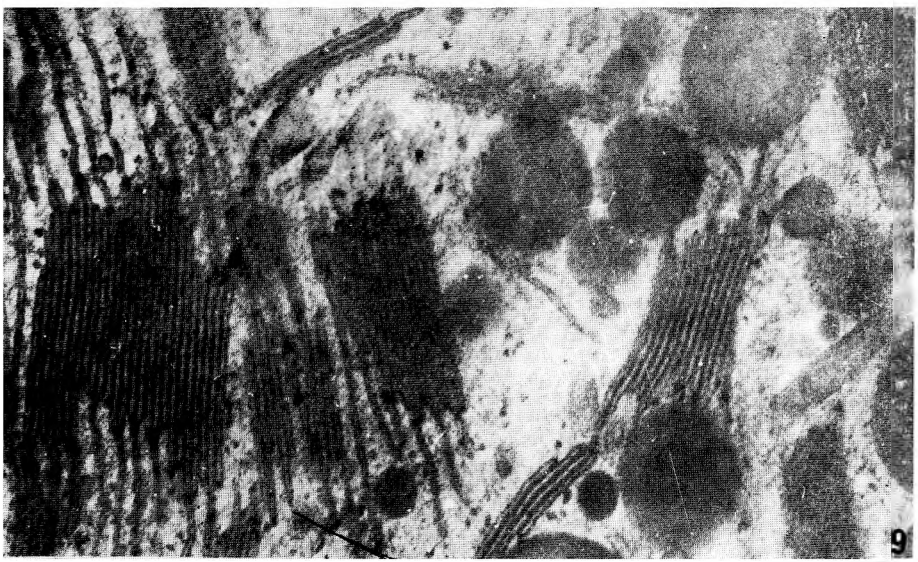


Figs. 3—5.





Figs. 6—7.



Figs. 8—9.

Results

Macroscopic observations. The proximal part of the fruit of *Cucurbita maxima* cv. *turbaniformis* is intensely coloured. Young unripe fruits are green, but during the process of fruit ripening they lose chlorophyll and become gradually orange and red. Ripe fruits are intensely red.

At the end of the winter period or at the beginning of spring in a very small number of fruits the red proximal part begins to regreen if exposed to light. The process of regreening is slow and lasts several weeks. At the end of the process the regreened area is intensely dark green.

Light microscopic investigations. Numerous, big and red chromoplasts are present in the subepidermis of the red proximal part of ripe fruits. Usually small irregular and intensely coloured structures are visible in chromoplasts.

The process of regreening occurs only in 1 to 3 upper subepidermal cell layers. The deeper layers contain orange and red chromoplasts. The regreened chloroplasts are intensely green and with clearly visible grana structures. Their diameter is more than 10 μm.

Electron microscopic investigations. The ripe fruit contains a typical tubulous type of chromoplasts in the red proximal part (Fig. 1) (Ljubešić 1977). In the first stages the stroma is dense and contains numerous ribosomes. Later stroma is dense too, but the ribosomes are not clearly visible any more. A large peripheral reticulum, sometimes regularly arranged, occurs along the border of the chromoplasts. The remnants of thylakoids are single and perforated. In the peripheral region of the chromoplast there are bundles containing 20 to 100 tubules each. The central part of chromoplasts is occupied by an accumulation of plastoglobules. Some of them are extended into tubular structures (Fig. 2). At the border of the chromoplasts numerous invaginations of the inner portion of the plastid membrane are present in the form of vesicles (Fig. 2).

When the fruit begins to regreen, significant changes appear in the ultrastructure of the chromoplasts. Small groups of thylakoids appear among the plastoglobules and tubules (Figs. 3,4), but at the periphery of the chromoplast. These groups contain 3 to 5 thylakoids, the peripheral ones usually swollen. Often arrays of ribosomes are visible along the thylakoids (Fig. 3). The thylakoids multiply very fast and form grana (Fig. 5). At first the grana contain thylakoids of various lengths. The length of the outer thylakoids decreases gradually from the center to the border of the granum (Figs. 6, 7). Later the grana get their typical shape (Figs. 8, 9). The number and the dimensions of the grana increase very rapidly. At the end of the regreening process more than a half of chloroplasts is filled with grana which are connected with numerous stroma thylakoids (Figs. 8, 9). The majority of grana is located at the border of chloroplasts. In the center, there is an accumulation of the rest of the plastoglobules and tubules. Bundles of tubules do not exist. The majority of tubules and part of plastoglobules disappear during the process of regreening. However, the reduction in volume of the plastoglobules and tubules is smaller than the volume of the newly formed grana and so the dimensions of the chloroplasts increase. The volume of the regreened chloroplasts is about 20% larger than the chromoplasts before regreening.

Discussion

Investigations on the reversion of chromoplasts into chloroplasts have been done by many authors, especially over the last 15 years. They have proved the correctness of Schimper's theory of possible reversibility of plastid metamorphosis. But in spite of numerous investigations, many questions have not been solved yet. The origin of the thylakoids and the grana in the regreened plastids is quite a complex problem. It seems that the thylakoids in the regreened chloroplasts of *Cucurbita maxima* cv. *turbaniformis* can be formed in several ways (Devidé and Ljubešić 1974). The majority of them are formed from thylakoid fragments and long perforated thylakoids which are located in each chromoplast. New thylakoids can be developed from vesicles originated from the invaginations of the inner portion of the plastid membrane (Thomson et al. 1967; Huber and Newman 1976). Lipoprotein tubules exist in chromoplasts of *Cucurbita maxima* cv. *turbaniformis* (Ljubešić 1977). The bundles of these tubules are formed directly from the material of the reorganized thylakoids during the development of chromoplasts from chloroplasts (Smith and Butler 1971; Falk 1976). It is possible that this material may thus be utilized directly for the formation of new thylakoids in the process of reversion of chromoplasts into chloroplasts. The fact that the bundles of tubules disappar after the regreening supports this opinion.

Plastoglobules contain a large amount of lipids, which are used in the formation of new thylakoids (Thomson et al. 1967; Devidé and Ljubešić 1974; Huber and Newman 1976 etc.). However, in *Cucurbita maxima* cv. *turbaniformis* the amount of plastoglobules diminishes partially after the reversion of chromoplasts into chloroplasts. These facts as well as the increase in the volume of chloroplasts after regreening indicate that the materials (lipids and proteins in the first place), which are present in chromoplasts of *Cucurbita maxima* cv. *turbaniformis* are only partially utilized in the formation of new thylakoids. Partially they must be synthesized during the process of regreening.

It is already known that tubulous and cristalline type of chromoplasts have the ability of regreening (for literature review see Introduction). The reversion of tubulous chromoplasts in the proximal part of fruit of *Cucurbita maxima* cv. *turbaniformis* proves that this type of chromoplasts can also be transformed to chloroplasts. However, the chromoplasts of membranous and reticulo-tubulous types have not been adequately investigated yet and therefore it is still unknown whether they can be reversed into chloroplasts or not.

Summary

In subepidermal cell layers of the red proximal part of fruits of *Cucurbita maxima* cv. *turbaniformis* the reversion of chromoplasts into chloroplasts has been investigated. Before the process of regreening the subepidermal cells contain only chromoplasts of the tubulous type. The chromoplasts are filled with a large peripheral reticulum, plastoglobules and numerous tubules.

During the process of regreening, the tubules and part of plastoglobules disappear, while new thylakoids are formed simultaneously. The thylakoids increase in number and form grana structures. The results of

the process of regreening are chloroplasts with normal grana structures and numerous, big plastoglobules. The investigations suggest that the material contained in the new thylakoids originates partially from tubules and plastoglobules, but is partially synthesized during the process of regreening. The observations are discussed with special reference to the origin of the newly formed thylakoids and grana.

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SAŽETAK

OZELENJAVANJE TUBULARNIH KROMOPLASTA U PLODOVIMA VARIJETETA *CUCURBITA MAXIMA* DUCH. CV. *TURBANIFORMIS*

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Istražena je pretvorba kromoplasta u kloroplaste u stanicama subepiderme crvenog proksimalnog dijela ploda varijeteta *Cucurbita maxima* Duch. cv. *turbaniformis*. Prije ozelenjavanja subepidermske stanice sadržavaju samo kromoplaste tubularnog tipa. Kromoplasti su ispunjeni s velikim perifernim retikulumom, plastoglobulima i brojnim tubulima.

Za vrijeme ozelenjavanja tubuli, a djelomično i plastoglobuli, nestaju uz istodobni nastanak novih tilakoida. Broj tilakoida raste i nastaju grana. Rezultat ozelenjavanja su kloroplasti s normalnim granama, ali s mnogobrojnim plastoglobulima. Ta istraživanja upućuju na to da materijal za izgradnju novih tilakoida potječe djelomično iz tubula i plastoglobula, a djelomično je bio sintetiziran tijekom procesa ozelenjavanja. Posebno je razmotren način postanka novih tilakoida i grana.

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