Chromoplasts – the last stages in plastid development

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ABSTRACT The results of investigations on the development of chromoplast fine structures in various plants are reviewed. Emphasis is placed on the specific pigment-containing structures and their development during chromoplast formation. There is a large variety of these structures, although four fundamental types can be discerned. These are plastoglobules, membranes, crystals, and tubules. During chromoplast development, various types of structure follow one after the other, or they may even be present simultaneously in the same chromoplast. Depending on the structures present in chromoplasts their pigment content also varies. It is still not clear whether the type of structure defines the pigment content of the chromoplast or *vice-versa*. Various possible ways of chromoplast development and dedifferentiation are discussed.

KEY WORDS: *chromoplasts, development, pigment-containing structures*

Introduction

Plastids are specific organelles of plant cells. In higher plants they exist in various forms and have manifold functions. Plastids develop from undifferentiated proplastids present in meristematic tissues. Depending on the plant organ and its functions these proplastids develop into various other plastid types. The most widespread and best known are the green, chlorophyll-containing chloroplasts, which are the only ones able to photosynthesize, *i.e.* to convert light into chemical energy. Their elaborate membrane system of flattened vesicles, s.c. thylakoids, contains chlorophyll and all other components necessary for carrying out the light-dependent part of photosynthesis. In higher plants for an efficient photosynthesis these thylakoids are arranged into stacks, s.c. grana. Several important biosynthetic processes, especially the «dark» part of photosynthesis, are located in chloroplast stoma. The components of the genetic apparatus of chloroplasts (DNA, RNAs, etc.) are present in the stroma as well. Chloroplasts are semiautonomous organelles, *i.e.* they are only able to encode and synthesize a part of their constituents.

Much less is known about other plastid types. Chromoplasts are defined as plastids which are photosynthetically inactive but contain various carotenoids and thus give the tissue a yellow, orange or red hue. They are typical plastids of flowers and fruits, as well as of some carotenoid-bearing roots, *e.g.* carrot roots. The physiological functions of chromoplasts are still not completely known. They are usually the last stage in the process of plastid development. Some authors therefore place chromoplasts together with the senescent forms of chloroplasts - the gerontoplasts - into one group. Both leaf gerontoplasts and chromoplasts of flowers, roots and fruits are devoid of chlorophyll and are unable to photosynthesize. However, there are important differences between them (Sitte *et al.*, 1980). Gerontoplasts appear only in senescent cells. They always develop from fully grown, *i.e.* old, chloroplasts and are unable to multiply. In contrast, chromoplasts present in flower, fruit and root cells have an active metabolism. They develop mostly from young chloroplasts and seldom from proplastids, *e.g.* in some flower petals (Modrusan and Wrischer, 1988), in some fruits (Ljubesic, 1970) and in carrot roots (Wrischer, 1972, 1974).

Chromoplasts have an active metabolism and possess the ability to synthesize some of their structural constituents. They *e.g.* contain a complete mechanism for fatty acid synthesis (Kleinig and Liedvogel, 1980) and are especially active in the synthesis and accumulation of various carotenoids, which are the most obvious characteristics of these organelles. Chromoplast stroma also contains several copies of circular DNA. The number of DNA copies is however lower than in chloroplasts. Chromoplasts are able to multiply, at least in the early stages of maturation (Sitte *et al.*, 1980). During chromoplast maturation ribosomes gradually disappear from the stroma. Supposedly, at this time, enzymes which synthesize carotenoids are encoded in the cell nucleus, translated on the cytoplasmic ribosomes and integrated in the chromoplast (Hansmann *et al.*, 1987; Carde *et al.*, 1988).

There is great diversity in the morphology of chromoplasts, especially in the structures that contain carotenoids. The development of chromoplasts proceeds therefore in various ways. According to the kind of carotenoid-bearing structures some investigators have developed a systematics of chromoplasts (Sitte *et al.*, 1980; Whatley and Whatley, 1987; Tilney-Bassett, 1989). Usually, however, there is more than one type of pigment-containing

Abbreviations used in this paper: CIMs, chromoplast internal membranes.

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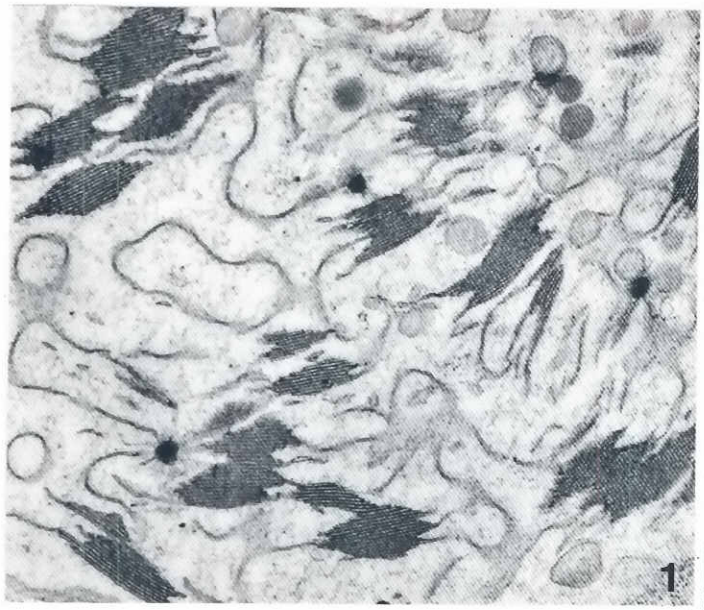
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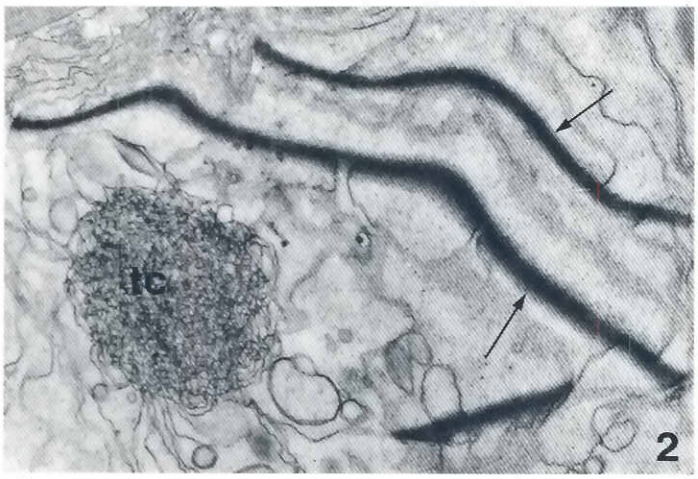
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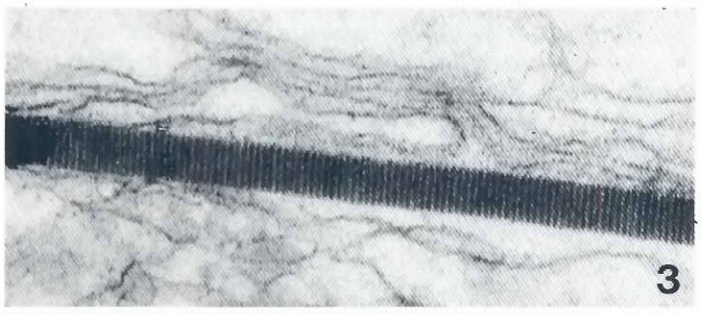
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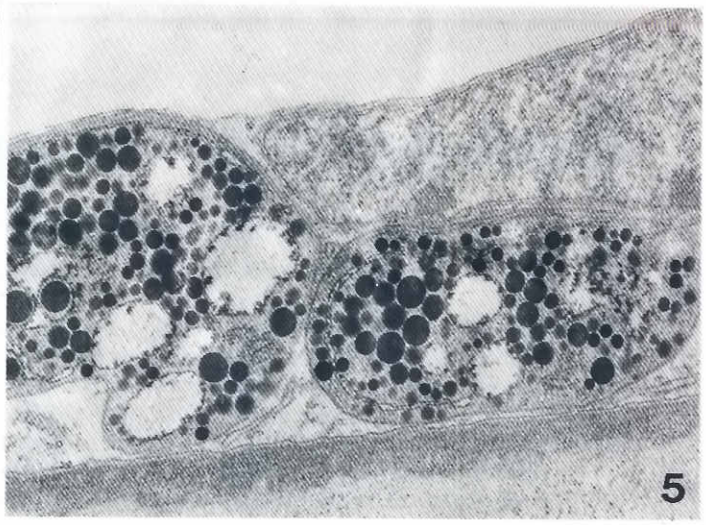
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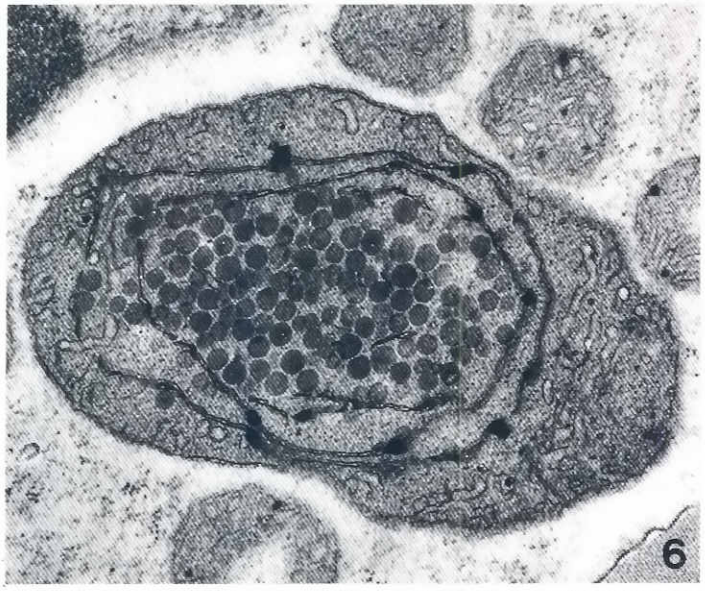
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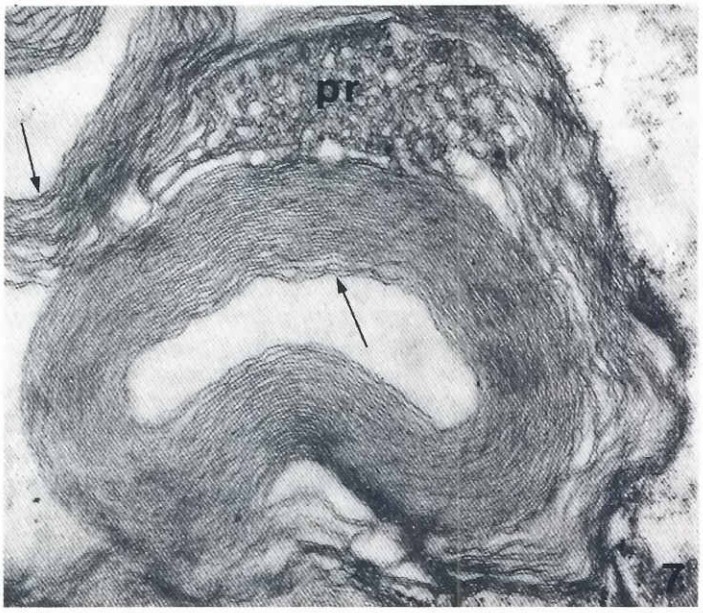
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structure present in a chromoplast. Therefore an exact classification of chromoplasts on this basis is not reliable.

In this review true gerontoplasts will be omitted and only our own investigations concerning the development of chromoplasts *s.s.* will be reported.

Degradation of thylakoids

The most conspicuous process during the early part of chloroplast-chromoplast transformation is the degradation of the photosynthetic apparatus. There are various types of thylakoid degradation observed in these organelles. The most frequent is the gradual decomposition of the thylakoids, consisting of their unstacking and disappearance. A transient appearance of «oblique» grana is often observed at this stage (Fig. 1). It is supposed that the thylakoids are decomposed from the periphery of the grana stacks, so that the peripheral thylakoids or their margins diminish first (Devide, 1970; Ljubesic, 1972).

A special type of thylakoid degradation was found in some ripening pumpkin fruits (Devide, 1970; Ljubesic, 1972). In the beginning grana thylakoids of young chloroplasts lose their lumina and then stick together (Fig. 2). Later on a fine striation (period 12 - 13 nm) is found on cross sections of these tightly stuck thylakoids (Fig. 3). In plane sections isometric particles appear (about 8 nm in diameter), which are very regularly arranged in a hexagonal pattern. The distance between particles is 12 - 13 nm (Fig. 4). The next step in degradation is the complete disappearance of these structures.

Similar pictures of degraded thylakoids have also been observed in leaf chloroplasts of plants treated with the herbicide aminotriazole (Wrischer and Ljubesic, 1989), which stops the cyclization of carotenes and therefore inhibits the synthesis of ß-carotene. Unprotected chlorophylls are then photooxidized. Due to this inhibition the reaction centers of the photosynthetic apparatus become inactive and degrade, while the protein components of the antennae stick together and, for geometrical reasons, form a hexagonal pattern. We suppose that as a consequence of deviation of carotene synthesis similar processes appear in pumpkin chloro-chromoplasts as well, which is in agreement with the data reported for the s.c. lycopenic leaf mutants in which the synthesis of carotenes is stopped at the level of lycopene. In plastids of these mutants similar tight sticking of thylakoids has been demonstrated (Walles and Hudak, 1975).

Formation of structures containing carotenoids

Simultaneously with the degradation of the thylakoid system in chloro-chromoplasts, an active synthesis of carotenoids begins and special carotenoid-bearing structures start to develop. Since these structures are structurally very different and several types are usually present in one and the same organelle, the development of chromoplasts can proceed in various ways. In addition, some carotenoid-containing structures are transient and disappear again in senescent chromoplasts.

Plastoglobules

The most common carotenoid-containing structures encountered in the chromoplasts are globules, generally called plastoglobules (Lichtenthaler and Sprey, 1966). These are spherical lipid droplets lying singly or in groups in the chromoplast stroma (Figs. 5,9). Their diameter ranges from 0.1 µm to one or more µm (Devide, 1970; Ljubesic, 1972, 1984). The dimensions and number of plastoglobules usually increase during the maturation of chromoplasts. Plastoglobules can be isolated and their chemical constituents investigated (Steinmüller and Tevini, 1985). Their content varies greatly depending on the object and developmental stage of the chromoplasts. In addition to lipids (mostly triglyceroles), various carotenoids (mostly xanthophylls) have been found. Carotenoids originate only partly from degraded thylakoids and are mostly synthesized anew during chromoplast maturation.

In the late stages of chromoplast development inside some plastoglobules, or connected with them, carotenes can accumulate either as crystals or as long tubules (fibrils) (Devide, 1970; Ljubesic, 1977). These types of inclusion will be described later.

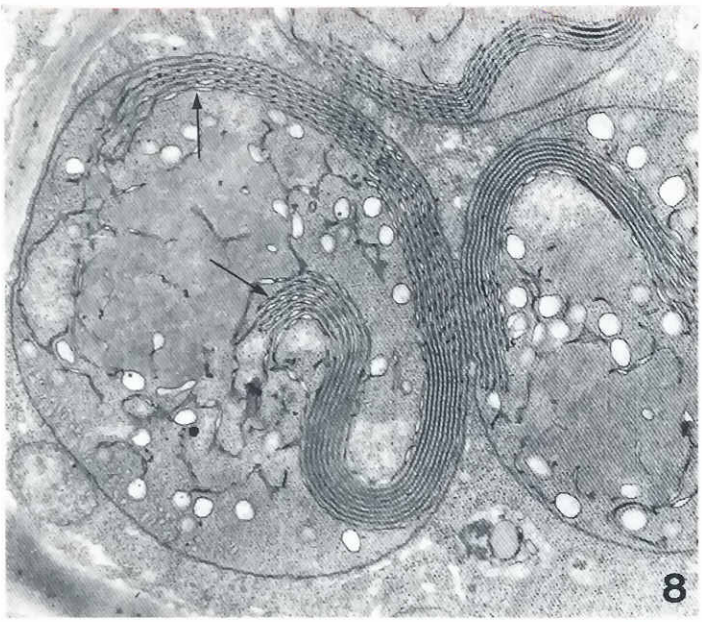
Plastoglobules do not necessarily contain carotenoids. There are plastids which are practically without pigment, although their stroma is filled with numerous plastoglobules. This is the case *e.g.* in the white pumpkin fruit (Fig. 6) (Ljubesic, 1973).

Membranes

In many chromoplasts special membranes, called *chromoplast internal membranes* (CIMs) (Sitte *et al.*, 1980) develop. The best known are CIMs in chromoplasts of some flower petals (Wrischer and Ljubesic, 1984; Hlousek-Radojcic and Ljubesic, 1988). They form many concentrically arranged layers in plastid stroma lying usually at the periphery (Figs. 7,12). These membranes develop *de novo* by invagination of the inner membrane of the plastid envelope and not directly from the degraded photosynthetic membranes. This can be demonstrated by differential staining with diamino-benzidine. This method could also state that CIMs are devoid of photosynthetic activity (the activity of photosystem I) (Wrischer, 1989), and are thus completely different from the chloroplast thylakoids.

The chemical content of CIMs was studied in certain flowers. In addition to lipids (mostly galactolipids) and special proteins (Hansmann and Sitte, 1984), they bear carotenoids but never chlorophyll. The membranes of fully developed chromoplasts of *Calceolaria* petals contain *e.g.* 7 times more carotenoids than young chloro-chromoplasts; 90% of these carotenoids are lutein, 5% ß-carotene and 5% an unidentified carotene, which is not present in leaf chloroplasts (Wrischer and Ljubesic, 1984). In CIMs carotenogenic enzymes have been found (Kreuz *et al.*, 1982) and

Figs. 1-7. Electron micrographs of developmental stages of chromoplasts. (1) Cucurbita pepo *cv.* pyriformis, *fruit. Degradation of thylakoids with «oblique» grana in a chloro-chromoplast. x30,000.* **(2)** Cucurbita pepo cv. ovifera, fruit. *Degradation of thylakoids in a chloro-chromoplast with stuck thylakoids (arrows) and a tubular coil (tc). x20,000.* **(3)** Cucurbita pepo *cv.* ovifera, *fruit. Degradation of thylakoids in a chloro-chromoplast. Cross section of a tightly stuck granum showing fine striation. x75,000.* **(4)** Cucurbita pepo *cv.* ovifera, *fruit. Plane section of a tightly stuck granum with hexagonally arranged particles. x75,000.* **(5)** Crataegus oxyacantha, *fruit. Chromoplast with numerous plastoglobules. x18,000.* **(6)** Cucurbita pepo *cv.* patisson, *white fruit. Plastid with numerous plastoglobules. x30,000.* **(7)** Calceolaria rugosa, *flower petal. Chromoplast with internal membranes (arrows) and peripheral reticulum (pr). x26,000.*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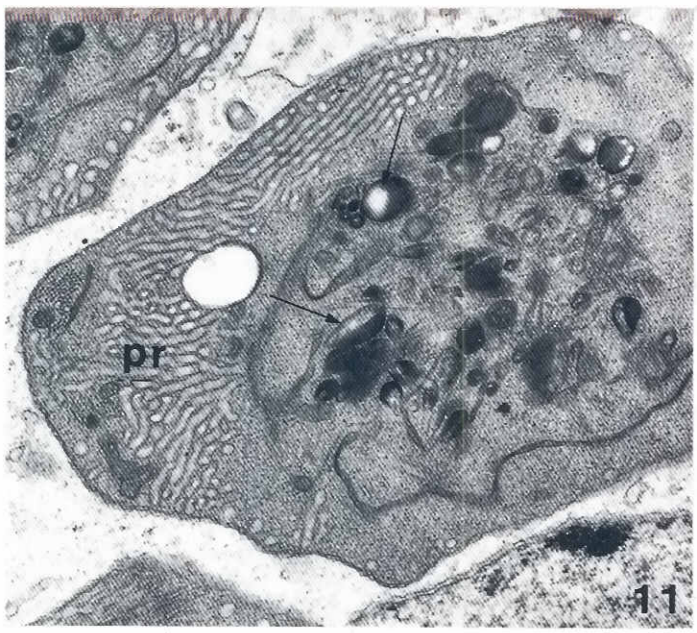
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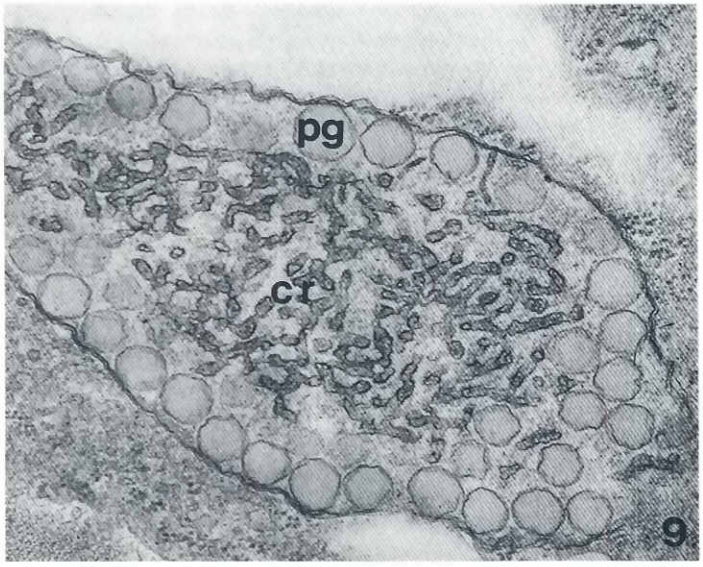
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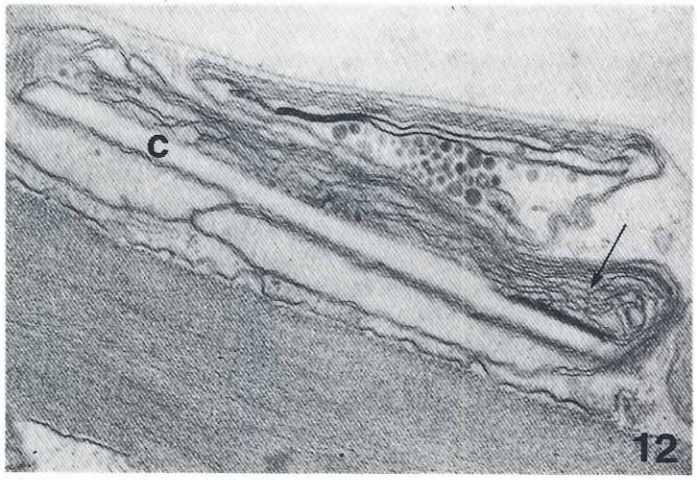
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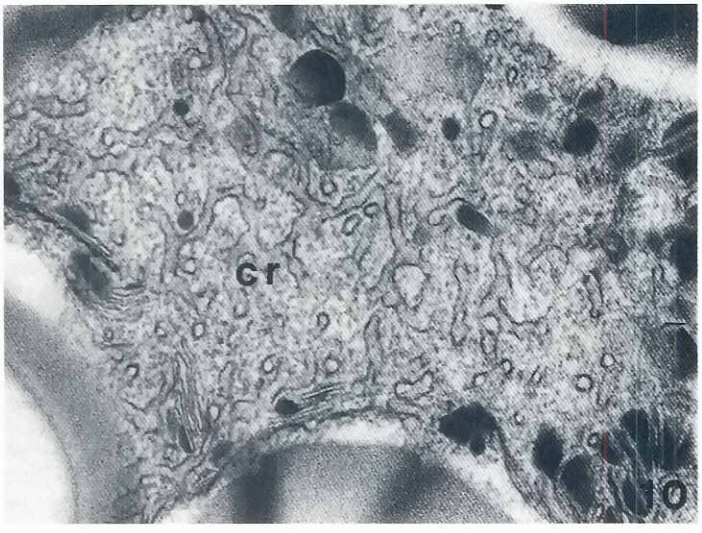
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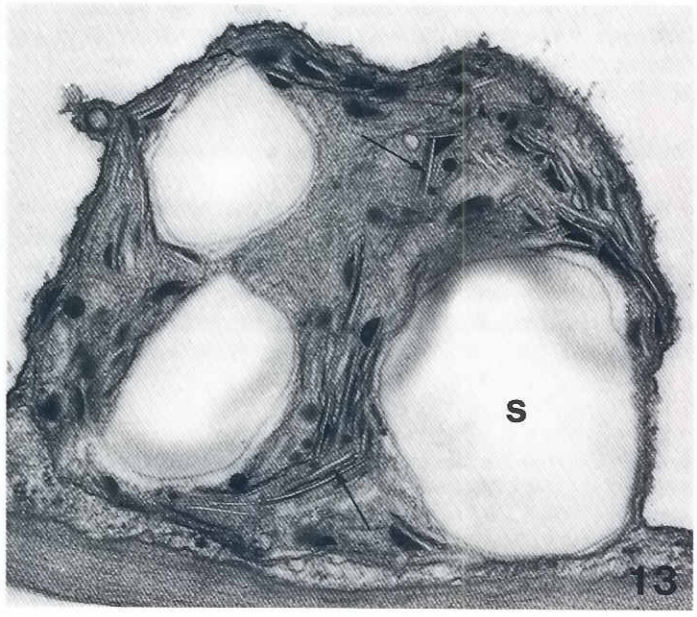
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these membranes are the site of carotenoid synthesis.

In some chromoplasts, *e.g.* in pumpkin fruits, there are other types of membrane which seem to develop by a «remodeling» of degrading photosynthetic membranes. These are either large membranous (tubulous) coils (Fig. 2) or long single, sometimes perforated thylakoids (Fig. 8), Ljubesic, 1970, 1977). Although these membranous structures look similar to chloroplast thylakoids, they never contain chlorophyll. This type of membrane is a peculiarity of intermediate stages in chromoplast development. In mature chromoplasts such membranes usually disappear.

A special, seldom found type of CIMs is the *chromoplast reticulum*. It consists of a net of branched tubules of about 30 nm in diameter (Figs. 9,10). The reticulum appears in some varieties of *Capsicum* (Carde *et al.*, 1988) and is also abundantly present in the stroma of some flower chromoplasts (Ljubesic, 1979a). The reticulum contains various carotenoids. During chromoplast formation the reticulum is produced anew from the inner membrane of the envelope. A contact with the envelope has sometimes been observed. The reticulum does not contain chlorophyll and has no photosynthetic activity, which can be illustrated by its negative reaction with diaminobenzidine (Ljubesic, unpublished).

The increase in the quantity of the *peripheral reticulum* (Figs. 7,11), which is the invagination of the inner membrane of the envelope (Ljubesic, 1977), is a transitional phenomenon that characterizes certain stages of developing chromoplasts. It is supposed that the abundance of this reticulum reflexes the amount of the transport activity of the envelope. A similar increase in the quantity of peripheral reticulum appears in some young stages of developing chloroplasts as well (Wrischer *et al.*, 1986). As in other plastid types, the inner membrane of the envelope of chromoplasts has a two-fold function: first in the transport of different metabolites into and out of the organelles, and secondly as the site of the synthesis of new membranous structures.

The differentiation of membranous chromoplasts can be strongly modified when plants are treated with the «bleaching» herbicide SAN 9789, which very specifically inhibits the synthesis of ß-carotene. In chromoplasts of daffodil and *Calceolaria* petals SAN 9789 stops the formation of CIMs and at the same time greatly lowers the content of ß-carotene (Hlousek-Radojcic and Ljubesic, 1988; Modrusan *et al.*, 1988). Similarly, in chromoplasts of tulip tree flowers after a treatment with SAN 9789 and with aminotriazole (another «bleaching» herbicide) the chromoplast reticulum is only purely developed and much less ß-carotene is synthesized (Hlousek-Radojcic and Ljubesic, 1985). These results indirectly confirm the finding that the majority of carotenoids are located in the membranous structures of the chromoplasts.

Crystals

During the maturation of some chromoplasts the concentration of carotenes can increase so much that they crystallize. The best known are the large crystals of ß-carotene in carrot roots (Wrischer, 1972, 1974) and in daffodil flowers (Hlousek-Radojcic and Ljubesic, 1988), as well as lycopene crystals in tomato fruits (Bathgate *et al.*, 1985). The peculiarity of these crystals is that they develop intrathylakoidally, *i.e.* inside the lumina of some thylakoids (Fig. 12). The crystallization starts already in chloro-chromoplasts. The crystals remain enveloped by a membrane even in mature chromoplasts, which are completely devoid of photosynthetic membranes.

In chromoplasts of daffodil petals, in addition to CIMs, ß-carotene crystals are also present (Fig. 12). These crystals disappear completely if flowers are treated with the herbicide SAN 9789. On the other hand, after a treatment with SAN 9785, which blocks the synthesis of lipids and not of ß-carotene, the crystals are present but the quantity of CIMs is much reduced (Hlousek-Radojcic and Ljubesic, 1988). This is understandable because the lipid basis of the membrane is missing.

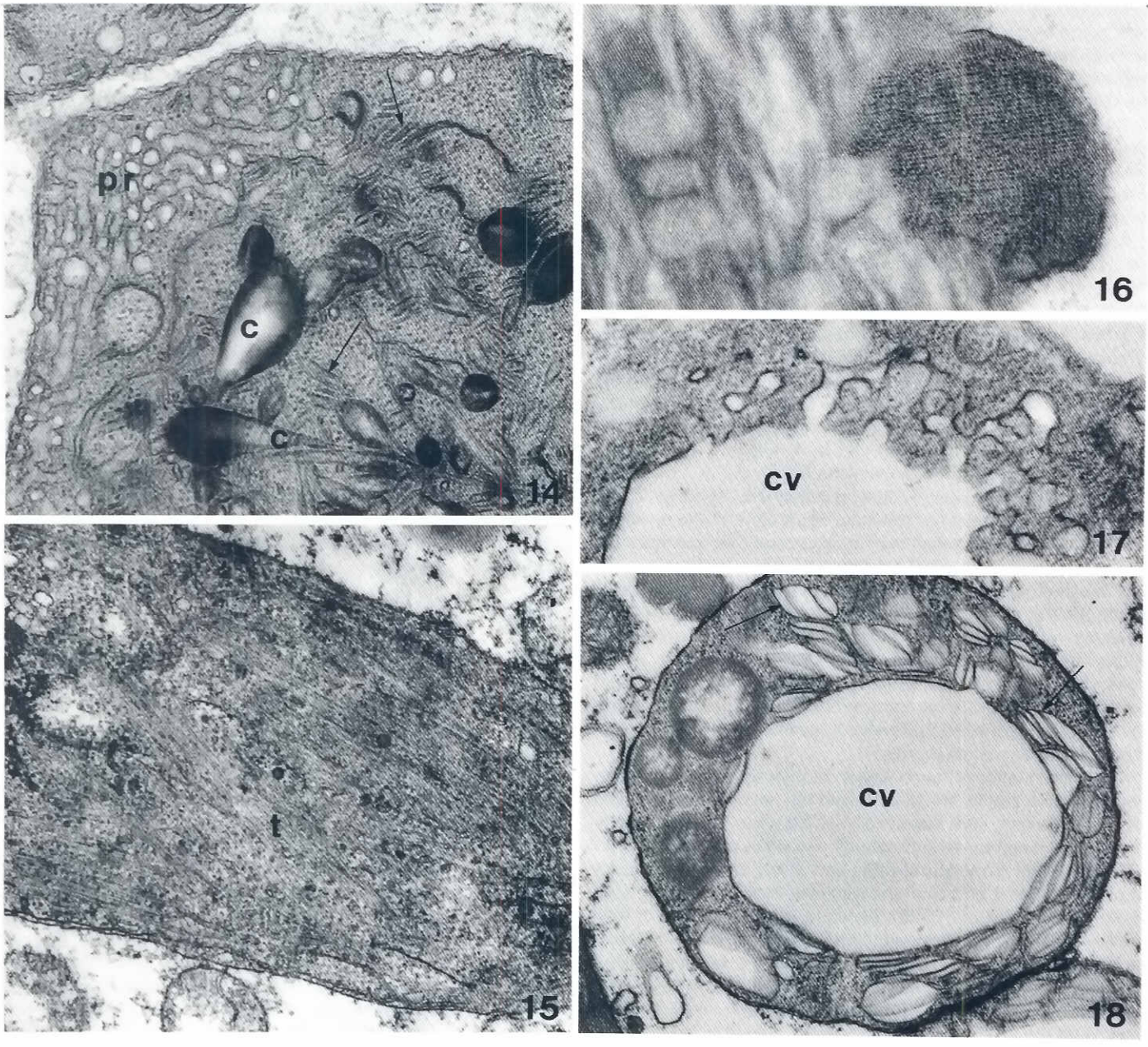
A rarer mode of formation of carotene crystals has been found in mature chromoplasts of tulip tree flowers (Ljubesic, 1979a). Here small crystals develop inside the plastoglobules (Fig. 13). According to the pigment analysis, these crystals are ß-carotene. Structurally somewhat different types of crystals develop inside plastoglobules in the chromoplasts of the fruit of *Cucurbita maxima* cv. *turbaniformis* (Ljubesic, 1977). In these chromoplasts long crystals grow out of plastoglobules, so that they assume the shape of a tadpole (Figs. 11, 14). The crystals probably do not contain ß-carotene, but still unidentified carotene.

Tubules

Tubules (fibrils) are another group of chromoplast-specific structures which contain carotenoids. These are thin (diameter 20-40 nm) and very long cylinders (fibrils) with an electron dense core (Figs. 14, 15). Their length can reach 10 µm (Ljubesic, 1977, 1982). According to the fine structure and chemical composition there should be several types of tubules (Sitte *et al.*, 1980). It seems that transitional forms between tubules and crystals could also exist (Ljubesic, 1977). Tubules are usually in contact with plastoglobules; in most cases plastoglobules are pierced by long straight tubules (Ljubesic, 1977; Modrusan *et al.*, 1988). On the other hand, in the chromoplasts of *Forsythia* petals, no contacts between tubules and plastoglobules have been found (Ljubesic, 1979b). These tubules are not straight but undulated and have an irregular shape in the cross section. The chemistry of tubules has been studied on isolates (Sitte *et al.*, 1980). It is known that tubules are not pure pigments, like crystals. In addition to various carotenoids they contain galactolipids as well as some chromoplast-specific proteins.

The dependence of chromoplast structures upon the type of pigments can be well studied when the synthesis of carotenoids is experimentally stopped or deviated. A treatment with «bleaching» herbicides stops the development of one type of structure in chromoplasts, but at the same time other types of inclusion appear. In tulip tree flowers treated with SAN 9789, instead of ß-carotene crystals, long thin tubules growing out of plastoglobules develop. In

Figs. 8-13. Electron micrographs of different types of chrmoplasts. (8) Cucurbita pepo *cv.* pyriformis, *fruit. Chromoplasts with perforated membranes (arrows). x16,000.* **(9)** Taraxacum officinale, *flower petal. Chromoplast with plastoglobules (pg) and chromoplast reticulum (cr). x44,000.* **(10)** Liriodendron tulipifera, *flower petal. Part of a chromoplast with chromoplast reticulum (cr). x69,000.* **(11)** Cucurbita maxima *cv.* turbaniformis, *fruit. Chromoplast crystals inside plastoglobules (arrows) and peripheral reticulum (pr). x29,000.* **(12)** Narcissus poëticus, *flower corona. Chromoplast with internal membranes (arrow) and a long carotene crystal (c). x28,000.* **(13)** *Liriodendron tulipifera, flower petal. Chromoplast with crystals (arrows), which are in connection with plastoglobules, and starch grains (s). x33,000.*



Figs. 14-18. Electron micrographs of different chromoplasts. (14) Cucurbita maxima *cv.* turbaniformis, *fruit. Part of a chromoplast with crystals (c) protruding from the plastoglobules, tubules (arrows), and a well-developed peripheral reticulum (pr). x44,000.* **(15)** Capsicum annuum, *fruit. The chromoplast is filled with numerous tubules (t). x29,000.* **(16)** Sorbus aucuparia, *fruit. A large phytoferritin crystalloid in the chromoplast stroma. x60,000.* **(17)** Cucurbita pepo, *cv.* ovifera, *a regreening fruit. Formation of thylakoids by invagination of the central vacuole (cv) in a chromo-chloroplast. x26,000.* **(18)** Cucurbita pepo *cv.* ovifera, *a regreening fruit. Chromo-chloroplast with small grana (arrows) and a central vacuole (cv). x24,000.*

these chromoplasts the content of ß-carotene is very reduced and at the same time the content of other carotenoids increases (Hlousek-Radojcic and Ljubesic, 1985).

Other structures present in chromoplasts

Starch grains (Fig. 13) are only seldom found in chromoplast stroma (Keresztes and Schroth, 1979; Ljubesic, 1979a), although the enzyme system for synthesis seems to be present, at least in some types of chromoplasts. It is known that in tissue grown *in vitro* chromoplasts are able to synthesize starch when glucose is added to the growth medium.

In some immature chromoplasts large protein inclusions wrapped by a membrane have been found. They are probably spare material and disappear during the maturation of the chromoplasts (Ljubesic, 1979b; Modrusan and Wrischer, 1988).

Fig. 19. Fruits of *Cucurbita pepo*, cv. *ovifera*. *Top: ripe (yellow) fruit; bottom: regreened fruit.*

Phytoferritin, the non-toxic complex of protein and iron, has been found in the stroma of some chromoplasts (Ljubesic, 1982), where it builds crystalloid aggregates (Fig. 16). It is supposed that phytoferritin derives from cytochromes and ferredoxin of the degraded thylakoids. Very large phytoferritin aggregates are characteristic of mature and senescent chromoplasts. It should be mentioned that crystalloids of phytoferritin are present in some senescent chloroplasts (gerontoplasts) as well (Ljubesic, 1976).

Dedifferentiation of chromoplasts

Although chromoplasts are usually considered to be the last stage in the process of chloroplast-chromoplast transformation, a reversible development of chromoplasts into chloroplasts has been observed in some plants. It is known that some yellow tissue in fruits and roots, but never in flowers, can regreen under certain conditions. Very useful objects for these studies are lemons (Ljubesic, 1984). When these fruits are left for two seasons on the tree, their yellowing and regreening can be repeated several times. The process is slow: yellowing of the fruits occurs in the winter months, their regreening in spring and summer and the second yellowing in autumn. The chromoplasts of yellow fruits contain many large plastoglobules and only remains of thylakoids. No photosynthetic activity could be detected in these fruits. During regreening the number of thylakoids greatly increases, grana are rebuilt and the photosynthetic activity is again detectable. In the stroma large plastoglobules, already present in chromoplasts of yellow fruit, remain also in chromo-chloroplasts. In addition to these, many small plastoglobules appear anew.

Regreening has been observed also in ripe pumpkin fruits (Devide and Ljubesic, 1972, 1974; Ljubesic, 1981) when they are exposed for several weeks to the light. In ripe yellow pumpkins chromoplasts are filled with large plastoglobules and have neither chlorophyll nor photosynthetic activity (Dvojkovic-Penava, 1973). During regreening there is an increase in chlorophyll content and the photosynthetic activity starts again. In the stroma of these chromo-chloroplasts large plastoglobules remain.

The regreening of chromoplasts is also a normal process in carrot roots when they grow in light. During the regreening the carotene crystals partly disappear from the chromo-chloroplasts. According to light microscopic investigations new chloroplasts, which develop by division of chromo-chloroplasts, are already without carotene crystals (Wrischer, 1972).

The process of regreening seems to proceed similarly in all investigated objects. Parallel with the start of chlorophyll synthesis new thylakoids develop by division (multiplication) of small vesicles (remains of thylakoids), which are already present in the chromoplast stroma. This type of regreening has been observed *e.g.* in lemon fruits (Ljubesic, 1984). New vesicles could also be punched off from the inner membrane of the chromoplast envelope as in carrot roots (Wrischer, 1974), or from the membrane of a «central vacuole» (Fig. 17) as in some pumpkins (Devide and Ljubesic, 1972, 1974). The multiplication of vesicles continues and they are arranged into stacks, small grana (Fig. 18). Ribosomes again become visible in the stroma and are often attached to the stroma thylakoids and peripheral thylakoids of the grana, indicating thus an active protein synthesis in these chromo-chloroplasts.

It is not yet clear which agents induce the regreening of a yellow tissue and the dedifferentiation of their chromoplasts. Light is surely the most important factor that, probably via the phytochrome system, induces the regreening. It is possible that growth substances play a dominant role in these processes (Ljubesic, 1976). The regreening of the tissue is very slow, usually lasting for many weeks. This is probably the reason why in flowers, which have a short life span, the regreening of their chromoplasts does not occur.

Concluding remarks

The various physiological functions of the chromoplasts are just beginning to be understood. Recent investigations have further revealed that chromoplasts are organelles with an active metabolism leading to the accumulation of various pigments (carotenoids) and to the formation of special structures that contain the pigments. The correlation between these structures and their chemical constituents (pigments) is not always clear. In most cases a stop in the synthesis of carotenoids (*e.g.* by mutation or by special herbicides) leads to the loss of characteristic chromoplast structures. Otherwise, there are also uncolored plastids which contain typical chromoplast inclusions (membranes or plastoglobules) but are completely devoid of carotenoids.

A large variety of pigment-containing structures exists even in one and the same chromoplast. Some of these structures are transient, being present only in unripe chromoplasts. In most ripe and senescent chromoplasts plastoglobules are the prevailing inclusions, which bear, in addition to lipids, various carotenoids.

Chromoplasts are usually the last step in plastid development, which finishes with their senescence and death. There are however many objects for which a reversible transformation leading back to chloroplasts is confirmed. Factors which correlate these processes

are still not well understood, since intensive biochemical and genetic investigations on chromoplasts have been started only in the last few years.

The ecological significance of chromoplasts is on the other hand well stated and has been known for a long time. Due to its intensive color, the tissue that contains chromoplasts has an important function in pollination of flowering plants and in the dispersal of their fruits and seeds by various animals.

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