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Chromoplasts of *Tropaeolum majus* L.: Structure and Development *

Heinz Falk

Biologisches Institut II der Universität, Lehrstuhl für Zellbiologie, Schänzlestr. 9, D-7800 Freiburg i.Br., Federal Republic of Germany

Summary. The fine structure of chromoplasts in epidermal cells of flower petals of *Tropaeolum* has been investigated by light, polarizing, and electron microscopy at different stages of development. The pale greenish-yellow petals still enclosed in the bud contain barely differentiated chloroplasts with few, irregular grana. The chromoplasts of unfolding petals show differently oriented bundles of tubules with variable diameters (mean: 17 nm). Thylakoid membranes become reduced more and more. The tubular bundles are intermingled with numerous isodiametric bodies of ca. 50 nm diameter; these bodies are better discernible at later stages when the chromoplasts possess a less dense matrix. The chromoplasts of open flowers are in a state of disorganization at a time when the cytoplasm still appears normal. A comparison is made between chromoplast tubules and tubular structures described from other kinds of plastids. The observations are discussed in view of chromoplast typology and with regard to possible processes underlying chromoplast differentiation in flowers.

Introduction

Following Schimper's extensive investigations of the homologies between chloroplasts and other kinds of plastids in plant cells (1885), the carotenoid-containing chromoplasts have been shown to be the most heterogeneous group among plastids. The application of polarizing microscopy and especially of electron microscopy has made it possible to classify them on the basis of morphological features. At present, we can distinguish at least five different types of chromoplasts with respect to their pigment-bearing structures (for a brief review, see Sitte, 1974). Among these categories, the less abundant "tubulous" chromoplasts are defined as containing bundles of (at least partly) parallel, unbranched tubules in the diameter range of 15 to 20 nm. Chromoplasts described earlier by others as possessing bundles of fibrils or filaments have been included in this group (the reasons for doing so will be discussed later). Chromoplasts of fruits seem to be predominantly of this type, *e.g.* those of *Solanum capsicastrum* (Steffen and Walter, 1955, 1958), *Capsicum annuum* (Frey-Wyssling and Kreutzer, 1958; Kirk and Juniper, 1967; Spurr and Harris, 1968; Suzuki, 1974), *Rosa canina* (Steffen and Walter, 1955), *Celastrus scandens* (Bornman, 1968), as has been shown by electron microscopy. Most probably, further species the fruits of which have so far been investigated only by light or polarizing microscopy (Schimper, 1885; Zurzycki, 1954) will have to be added to this type. Among chromoplasts of flowers, only those of two species have been found to belong to the tubulous category, viz. *Cucumis sativus* (Smith and Butler, 1971) and *Strelitzia reginae* (Grönegress, 1974; Simpson *et al.*, 1975).

This paper deals with the structure of the deep-yellow to orange plastids of the petals of *Tropaeolum majus* which represent an additional example of the tubulous type of chromoplasts in flowers. Moreover, the fine structural changes of the plastids during the development of flowers are described.

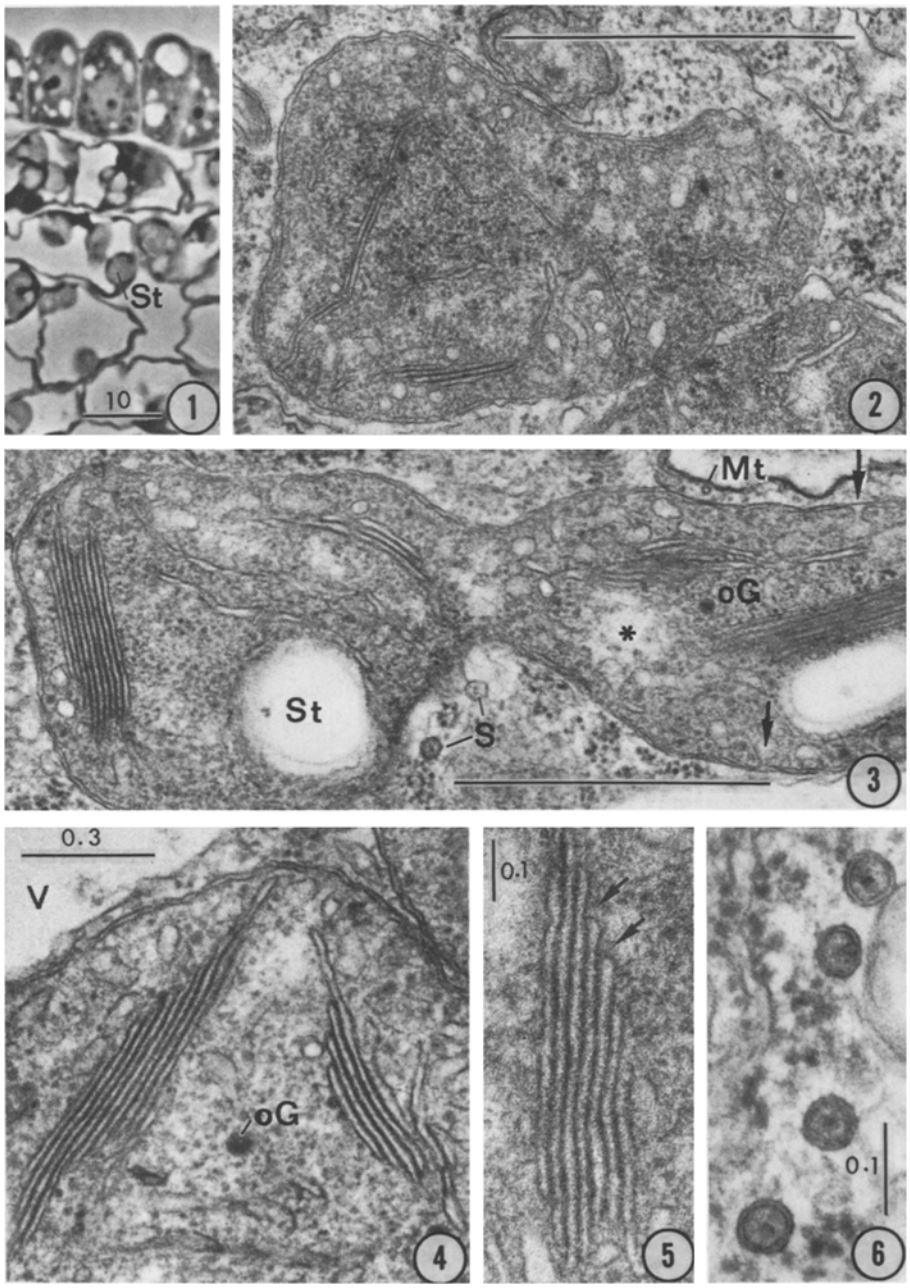
Material and Methods

Tropaeolum majus L., cv. 'Goldglanz' (lacking anthocyanins in the flowers) was cultivated from seeds (O. Hambrecht KG., Freiburg i.Br., Germany) under natural conditions. Flower buds were selected according to progressive length and grouped into three classes (length measurements without spur):

Stage I: Bud length ≤ 10 mm, petals still enclosed in the bud. In this class, buds somewhat differing in size but without marked difference in fine structure have been combined.

Stage II: Bud length ca. 20 mm, unfolding petals just visible at the tip of the bud.

* *Abbrevations in Figures:* Chr = chromoplast, CT = chromoplast tubules, Cy = cytoplasm, D = dictyosome, IB = isodiametric body, M = mitochondrion, MT = microtubule, oG = osmiophilic globule, S = "S-body", St = starch grain, V = vacuole. All micrographs from glutaraldehyde-OsO_4-fixed material, unless otherwise specified. The bar designates 1 μm (multiples or fractions of it indicated).



Figs. 1–5. Early stages of chromoplast development in young petals within the flower bud

Fig. 1. Cross section of a petal with large starch grains in the mesophyll cells. The epidermal layer contains only small vacuoles. × 1,100

Fig. 2. Very young chloroplast from an epidermal cell with few thylakoids and peripheral vesicles in the ribosome-containing stroma. × 47,000

Fig. 3. Further developed chloroplast, probably being engaged in division. Grana, small starch grains, and some transparent (DNA-)areas (*) are visible. Note peripheral vesicles in continuity with the inner envelope membrane (↑). A microtubule and some "S-bodies" are discernible in the cytoplasm. × 42,000

Figs. 4 and 5. Oblique grana from young plastids formed by staggered arrangement of thylakoids (↑). Note the very low number of osmiophilic globules within the plastids. × 61,500, × 81,000, respectively

Fig. 6. S-bodies at higher magnification in the cytoplasm of an epidermal cell. × 118,000

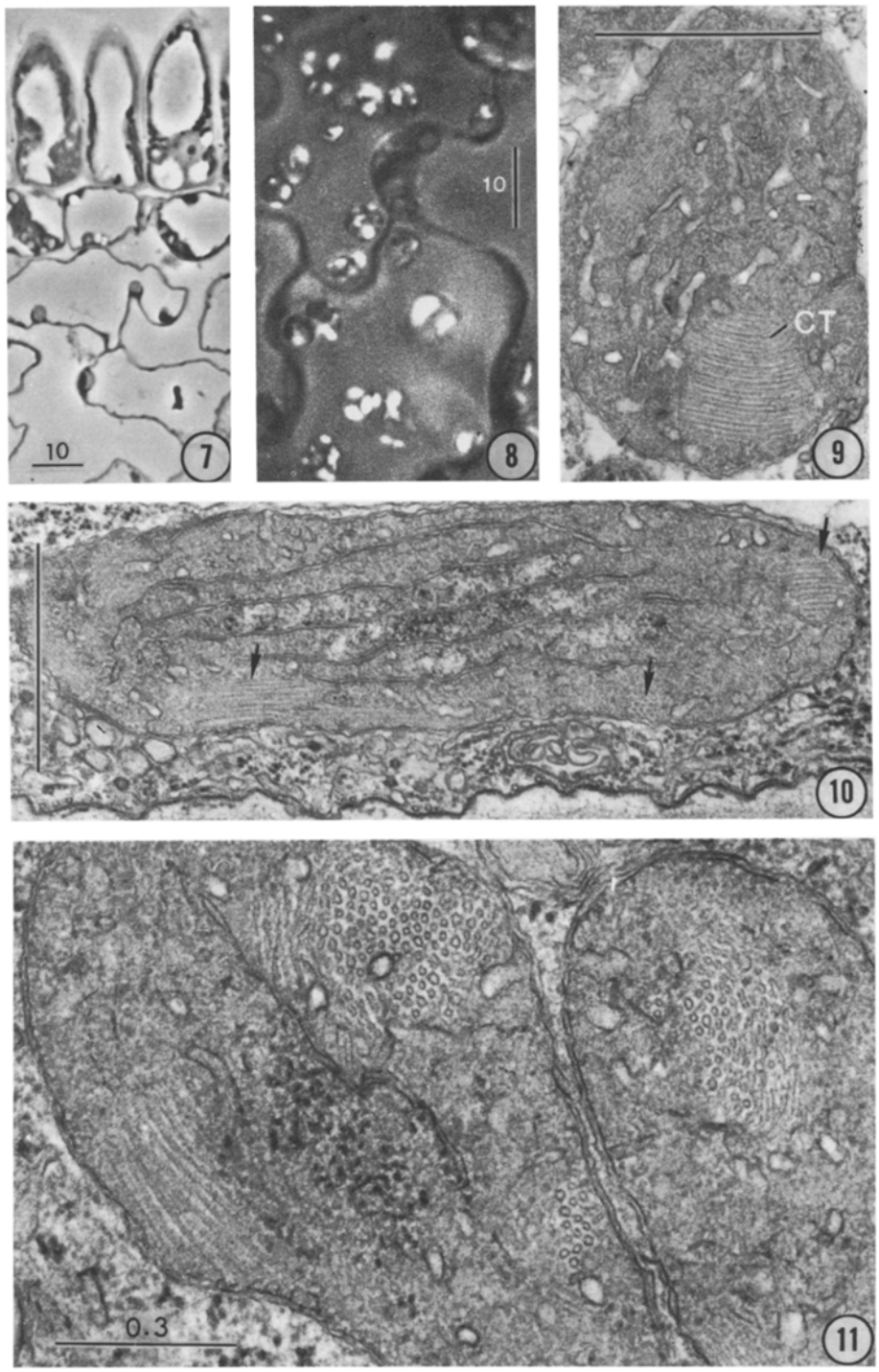
Stage III: Open flowers.

Discs of 1.0 mm diameter were punched out of the petals according to the method of Berlin and Bowen (1964) and immediately fixed for 3 h in ice-cold 3% glutaraldehyde in 50 mM cacodylate buffer, pH 7.2. After several washings, postfixation with 2% OsO_4 in the same buffer was carried out for 1 h at 0° C, and 2% unbuffered uranyl acetate, recommended for better preservation of membrane lipids (*e.g.* Silva *et al.*, 1971), was added after careful washing with distilled water. Dehydration in an ethanol series and embedding in Epon 812 were carried out according to conventional methods. Ultrathin sections (stained with uranyl acetate and lead citrate) were observed in a Siemens electron microscope (Elmiskop 101). Occasionally, unbuffered 2% $KMnO_4$ solution was used as a fixative at 0° C for 0.5–1 h. In these cases no further staining was applied, except with lead citrate on ultrathin sections.

Results

1. Young Petals (Stage I)

At stage I of bud development the young petals have a very pale greenish-yellow color. There exists a considerable difference with respect to the state of differentiation between mesophyll and epidermal cells. The former contain prominent vacuoles and numerous large starch grains in a narrow cytoplasmic layer; the latter show in contrast a more meristematic aspect (Fig. 1), possessing the usual complement of organelles and cytoplasmic structures. Occasionally, even single



Figs. 7–11. Chromoplasts from the epidermal cells of yellow, unfolding petals

Fig. 7. Part of a cross section of an unfolding petal. × 700

Fig. 8. Face view of the lower epidermis in the polarizing microscope. Note birefringent areas within the chromoplasts. × 1,200

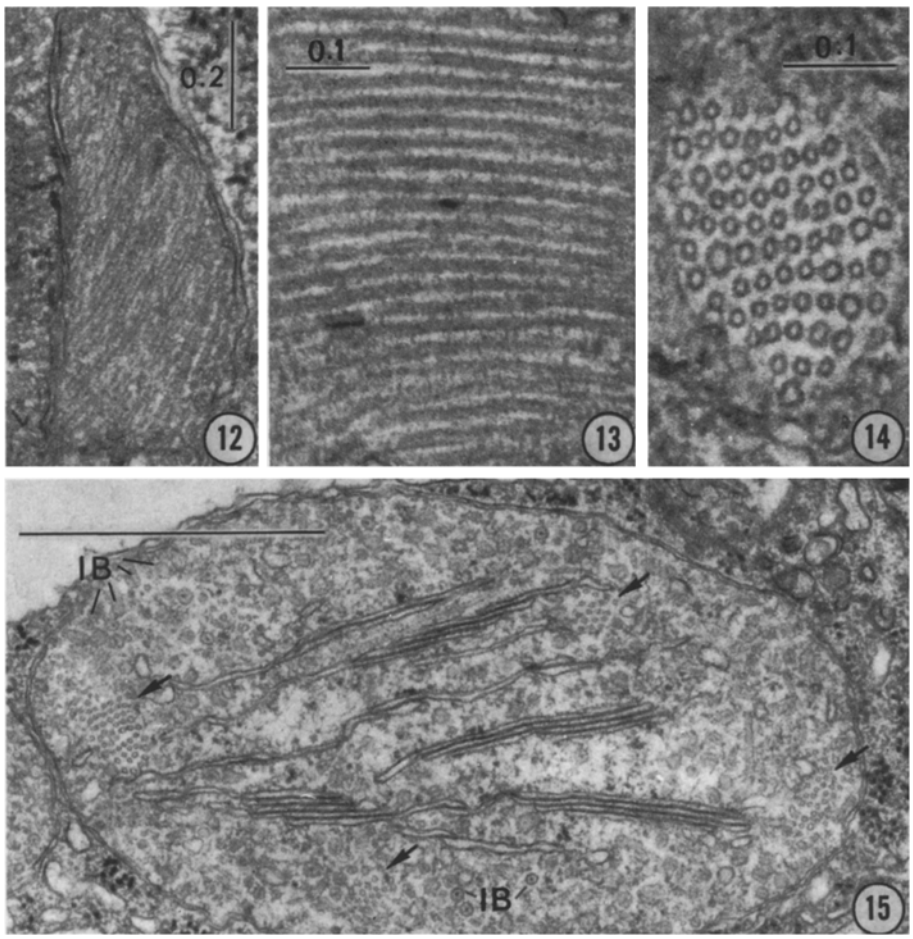
Fig. 9. Transverse section of a chromoplast showing an area consisting of parallel tubules presumably giving rise to birefringence. Note the network formed by elongated and fused vesicles. × 31,000

Fig. 10. Longitudinal section of a chromoplast showing restriction of thylakoids, DNA-containing regions, and ribosomes to the central part. Compare the contrast of cytoplasmic microtubules (bottom) with that of chromoplast tubules (↑). × 32,000

Fig. 11. Part of two chromoplasts containing tubular bundles in different directions. × 83,000

mitoses can still be seen. Moreover, at all stages of development particles which had been described as "S-bodies" in *Tropaeolum* (Ie, 1972) could be detected as being scattered throughout the cytoplasm of all specimens investigated (Figs. 3, 6, 21).

The small plastids (diameter 2–3 μm) of growing epidermal cells show one or several clear areas which contain fine filaments (Fig. 3). By comparison with similar structures depicted in the literature these can be assumed to consist of DNA. The stroma is rich in ribosomes. A few thylakoids (up to 15, but in most cases less) are stacked to form a small number of grana in such a way that each thylakoid is either laterally dislocated, or shortened to a certain extent in relation to the preceding one. In this manner, what we call "oblique" grana are formed the borders of which in



Figs. 12–18. Chromoplasts from the epidermal cells of yellow, unfolding petals

Fig. 12. Chromoplast tubules extending between thylakoid and inner envelope membrane. ×75,000

Figs. 13 and 14. High power micrographs of chromoplast tubules in longitudinal and transverse view, respectively. Note the variable diameter and the irregular outline of tubules which are connected by fine strands. The tubules are located in a transparent matrix. ×110,000, ×150,000, respectively

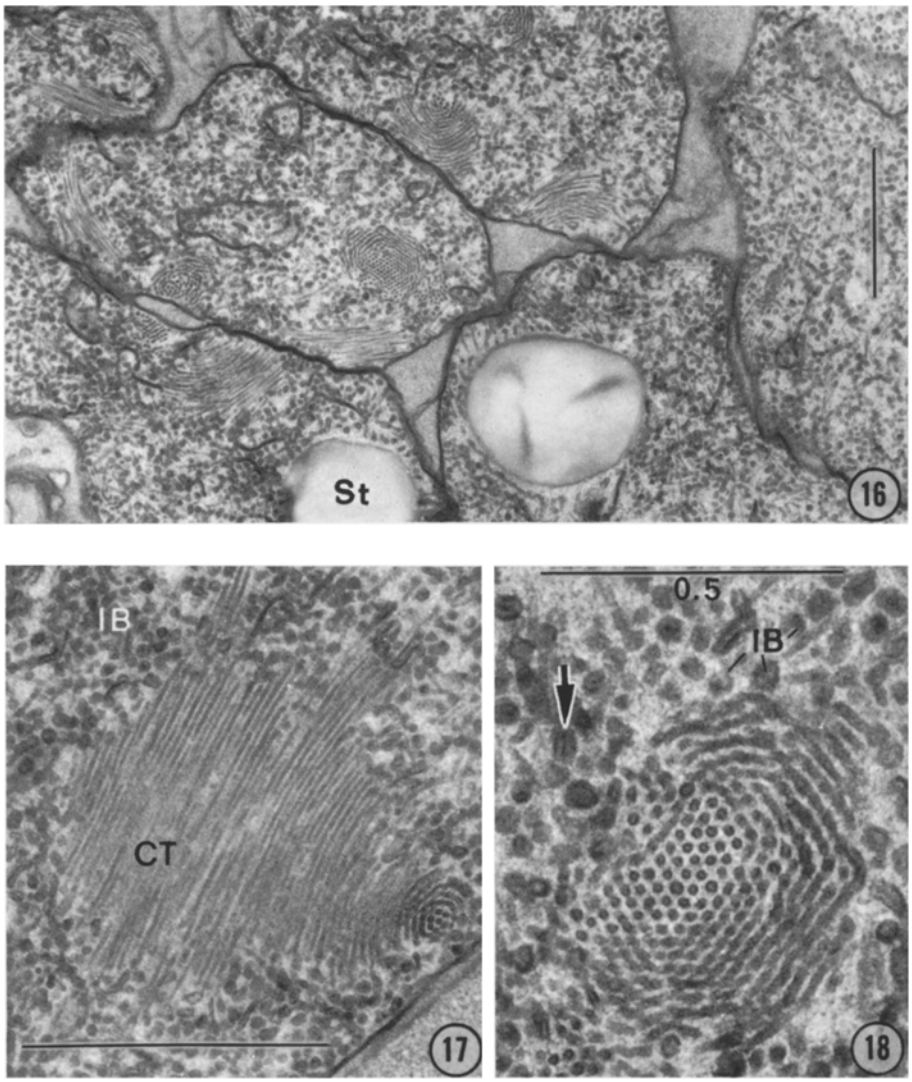
Fig. 15. More advanced stage of development. Apart from chromoplast tubules (↑) which are more dispersed than at earlier stages, numerous isodiametric or somewhat elongated bodies are visible, some of which contain a central dot. ×40,500

transverse sections have a staggered appearance (Figs. 4, 5). The peripheral stroma of immature plastids is occupied by irregularly shaped, electron-transparent vesicles which may be derived from the plastid envelope (Fig. 3). Sometimes they look elongated and form a network by local fusions. A remarkably low number of small osmiophilic globules is present in the matrix. Occasionally small starch grains can be found. As regards the ultrastructure of the plastids at this stage of development, there is nothing yet discernible which would indicate a prospective chromoplast (with the possible exception of the "oblique" grana).

2. Unfolding Petals (Stage II)

The color of the petals, still enclosed in the green sepals with only the foremost tips breaking through, is now bright yellow. Transverse sections show that the number of starch grains in the mesophyll is reduced to some extent (Fig. 7). The upper and lower epidermal cells have approximated their final, papillate form. They contain vacuoles considerably increased in volume, and nuclei which are predominantly lying in the basal region of the cytoplasm. The cytoplasm is filled with clustered ribosomes. Yellow plastids have accumulated near the nuclei. They usually contain several birefringent areas of different size (Fig. 8; compare Schimper, 1885) which show dichroism *in vivo*. The birefringent areas appear to consist of bundles of a variable number of tubules (Fig. 9) which are straight or slightly bent, and unbranched. Their diameter varies within a relatively wide range (14–20 nm). Their outline, as seen in cross sections, is rather irregular (Figs. 11, 14). Occasionally cross-bridges can be observed in the form of electron-dense strands (Fig. 14). The bundles as a whole are slightly twisted, as evident from stereomicrographs of thick sections. The arrangement of tubules within a bundle appears more or less hexagonal (Figs. 11, 14, 16, 18) although they do not seem to be closely packed. Remnant thylakoid membranes can often be seen to be connected to each other or to the chromoplast envelope by groups of parallel tubules (Fig. 12).

During further development, the normal internal structures of plastids are more and more restricted to the central region (Figs. 10, 11, 15). Areas of fine (DNA-)filaments, irregularly arranged and sometimes swollen thylakoids, and ribosomes are surrounded by



Figs. 16–18. Stage of development similar to Fig. 15 but fixed with $KMnO_4$. Note the excellent preservation of chromoplast structures. Fig. 16: ×20,000. Tubules and isodiametric bodies are shown at higher magnification at Fig. 17 (×41,000) and 18 (×80,000). The central dot takes the form of a line in elongated IBs (↑)

a peripheral zone crowded with bundles of tubules which are embedded in a densely packed mass of roundish particles of ca. 50 nm diameter, with considerable variation (Fig. 15). These bodies are of medium contrast and can be recognized for the first time at this developmental stage; they often contain a central dot which is visible after both fixation techniques (glutaraldehyde-OsO_4 and $KMnO_4$). Occasionally these particles are more elongated in shape, and in these instances the central dot assumes the form of a line (Fig. 18). Nothing can be said as regards the significance of this central structure. After glutaraldehyde-OsO_4 fixation the particles are often somewhat obscured by an amorphous matrix material. On the other hand, in sections of $KMnO_4$-fixed petals these bodies are clearly discernible but their appearance is slightly altered (Figs. 16–18): their diameters appear to be more uniform, they exhibit an increased contrast, and they are less densely packed (probably as a result of poor preservation of the matrix material, as well as swelling of the chromoplast). These bodies represent, together with the tubules already described, the most prominent fine-structural elements in the *Tropaeolum* chromoplasts at this stage. In $KMnO_4$-fixed preparations, single tubules of approximately the same size as the ones assembled into bundles, and also thicker ones can be observed extending throughout the chromoplast matrix in different directions (Figs. 16, 18). It should be noted that, in sharp contrast to the well-known cytoplasmic microtubules, the chromoplast tubules are very well preserved by $KMnO_4$.

The thylakoid membranes are further reduced at this developmental stage. The irregularly-shaped, electron-transparent vesicles described above for the peripheral part of young plastids after glutaraldehyde-OsO_4-fixation now traverse the entire matrix as a more or less extended network (Fig. 11). They are not seen in $KMnO_4$ preparations, probably as a result of an

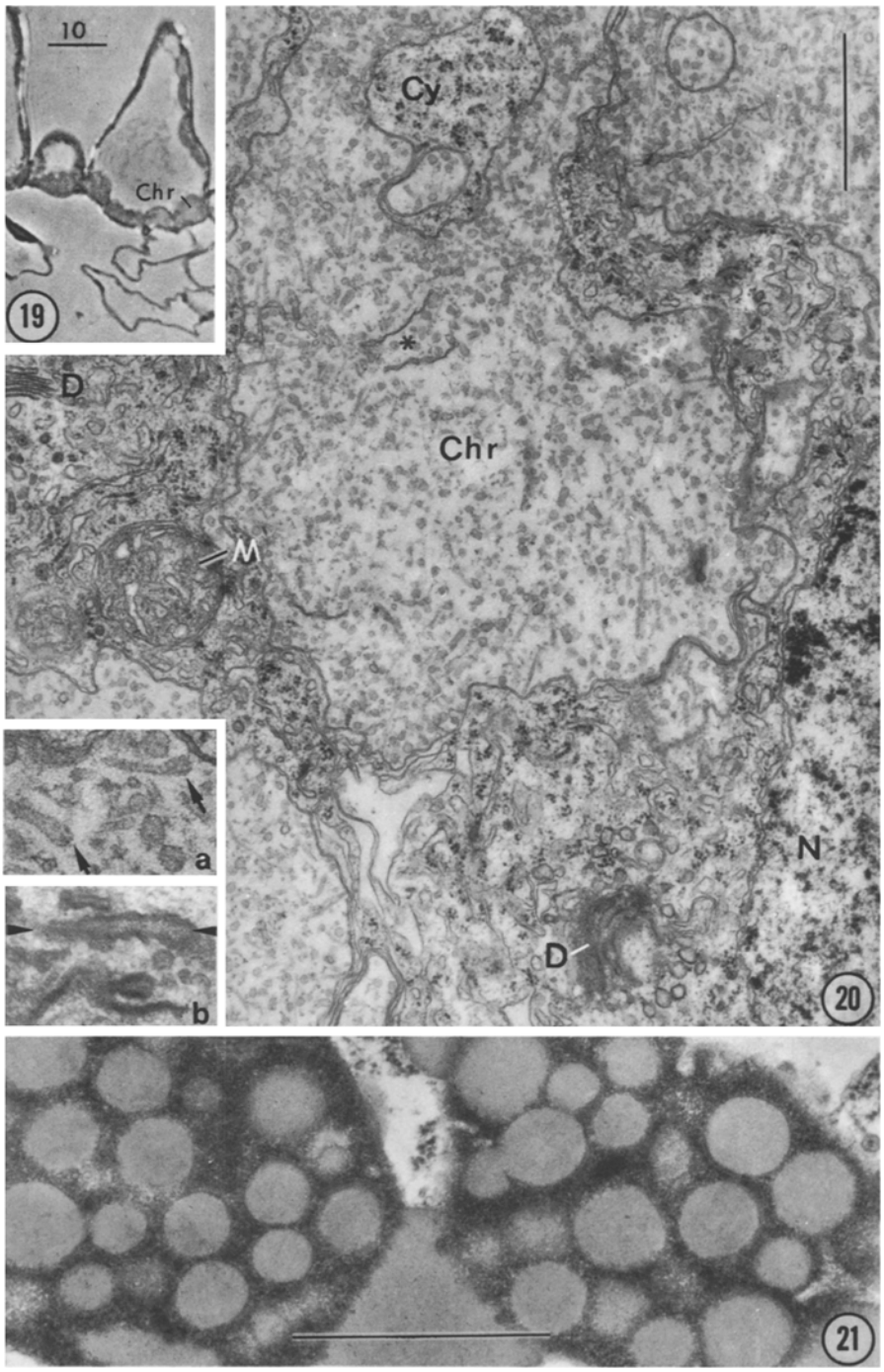


Fig. 19. Part of a cross section of a petal from an open flower. Note the chromoplasts in the epidermal cells and the absence of starch in the mesophyll. ×800

Fig. 20. Greatly enlarged chromoplasts (same stage as Fig. 19) with irregular outline, containing predominantly IBs, some of which show characteristic elongations or outgrowths (insets a and b, arrows). At the point marked by an asterisk remnants of internal membranes are seen. Chromoplast tubules have dispersed at this stage. Note the normal appearance of cytoplasm and organelles. ×21,500. (a) ×70,000, (b) ($KMnO_4$) ×80,000

Fig. 21. Senescent plastids from a yellow-colored autumn leaf containing numerous globules. ×35,000

advanced development rather than of poor preservation. Osmiophilic globules have completely disappeared. Occasionally, a few small starch grains are still present in some of the chromoplasts.

The starch-containing plastids of the mesophyll cells, the volume of which is nearly completely occupied by starch, contain in their small stroma portion the same structures as are found in the epidermal chromoplasts.

3. Petals of the Open Flower (Stage III)

Transverse sections of the deep-yellow petals of fully opened flowers show that the starch in the mesophyll cells has completely disappeared (Fig. 19). The now highly vacuolated epidermal cells exhibit their definitive papillate shape. Chromoplasts are the most prominent organelles in the cytoplasm. They have further increased in volume but possess now a more or less

irregular outline. Usually several chromoplasts lie close together at the base of the cells, interlocked by indentations of their envelopes. The chromoplast interior is obviously swollen and contains, except for a few slender membrane profiles, tubules and spherical bodies as the only structures; the tubules are more randomly oriented than in earlier stages (Fig. 20). Sometimes, the (normally more or less isodiametric) spherical bodies possess elongations or outgrowths (Fig. 20a, b). The bundles of tubules, which were so prominent in the preceding stage, are now apparently disorganized and the number of tubules seems to be reduced. The chromoplast matrix which was rather electron-dense in the earlier stages, has become transparent. The general impression of the chromoplasts at this stage is one of disorganization. This is consistent with Schimper's observation that, in *Tropaeolum* petals, disorganization of chromoplasts takes place long before wilting. In contrast, nuclei and cytoplasm still exhibit an entirely normal state; there are still clusters of ribosomes, and dictyosomes are active in detaching vesicles (Fig. 20), and neither mitochondria nor microbodies show any unusual characteristics.

Discussion

The most conspicuous structural elements of mature chromoplasts in petals of *Tropaeolum majus* which can be seen in the electron microscope are unbranched tubules which are often arranged in bundles. These bundles of tubules show birefringence in polarized light. They are at least part of the pigment-containing structures of the chromoplast as can be demonstrated by the existence of dichroism (compare also Schimper, 1885, who upon observing dichroism assumed the presence of "pigment crystals"). Because of these characteristics, the chromoplasts of *Tropaeolum* have to be classified as belonging to the "tubulous" type of the categories recently proposed by Sitte (1974). This type comprises also the "fibrillar" (Steffen and Walter, 1958) and the "filamentous" one (Frey-Wyssling and Kreutzer, 1958).

The reasons for the new, unifying terminology are twofold. 1. As stated already by Sitte (1974) there is evidence from results obtained in our laboratory that structures described earlier as being filaments – e.g. in chromoplasts of the rose hip (Steffen and Walter, 1955) – appear as tubules having a dark wall and a light core after improved fixation techniques (Wuttke, unpublished). 2. The term "filament" generally denotes a homogeneous structure consisting of only one component whereas a "tubule" is composed of at least two, namely wall and core material. In a subsequent paper, biochemical evidence will be presented showing that the chromoplast tubules in *Tropaeolum* are made up of two classes of components (Winkenbach *et al.*, 1976).

Tubular structures occurring in plastids have been repeatedly described by electron microscopists. In addition to those of typical chromoplasts there have been observations of aggregated tubules in chloroplasts and also in proplastids which clearly cannot be remnants of prolamellar bodies (e.g. Schnepf, 1961; Sitte, 1962; Jensen, 1965; Pickett-Heaps, 1968; Sprey, 1968; Gifford and Stewart, 1968; Mittelheuser and Van Steveninck, 1971; Mikulska *et al.*, 1973; Ponzi and Pizzolongo, 1973; Lunney *et al.*, 1975). Normally these tubules are rather short and (in the majority of cases) they are found in a restricted peripheral zone of the plastid, in some instances apparently attached to inner membrane of the plastid envelope, and arranged in a (quasi-)hexagonal pattern. There can be little doubt, because of the striking structural resemblance, that these tubules have some features in common with the chromoplast tubules, but there is no well-established information concerning the conditions of formation nor the possible similarities in chemical composition of the two structures. In this regard it is noteworthy that a certain kind of intraplastidal tubules is not preserved by fixation with $KMnO_4$ (Zimmermann, 1973; Brandão and Salema, 1974). This is a strong indication of a quite different chemical composition[1]. Unfortunately, only few investigators have tested the sensitivity of the plastid tubules to $KMnO_4$ treatment. In all cases where this has been examined, *chromoplast* tubules have been found to be stable, possibly as a consequence of their high lipid content.

The *differentiation* of chromoplasts in the petals of *Tropaeolum* starts from immature chloroplasts containing only a few "oblique" grana. A lateral dislocation of thylakoids in the grana (compare Steffen and Walter, 1958) may be the first indication of a degradation process of the thylakoids, the final result of which is the almost complete absence of internal membranes in chromoplasts of *Tropaeolum* at the stage of the open flower. This process is very similar to that taking place in the buds of *Cucumis sativus* flowers (Smith and Butler, 1971). In both species the appearance of tubules is not correlated with a previous accumulation of osmiophilic globules which, in contrast, is found as an intermediate stage during the development of fruit chromoplasts of the tubulous type or the tubulous chromoplasts of sepals of *Strelitzia reginae*. For this reason Smith and Butler assume a "direct" utilization of material from thylakoid degradation in the production of tubules. The same could be true in *Tropaeolum*. In this connexion reference may be made to the hypothetical considerations put forward by Franke (1971)

[1] Nevertheless it does not seem advisable to use the term "microtubules" in this connexion (e.g. Brandão and Salema, 1974). The essential criterion for microtubules is that they consist of tubulin. They have never been shown to exist in prokaryotes and it seems unlikely that they are components of cell organelles such as mitochondria or plastids.

concerning animal cells. Franke supposes that compounds derived from the breakdown of intracellular membranes may become rearranged into tubular or filamentous structures by a process of self-assembly. Alternatively, the formation of rod- or tubule-like crystallites of lipids under proper conditions could likewise offer an explanation for the appearance of tubules in chromoplasts on the basis of self-assembly.

However, the observation that senescent chloroplasts of bright yellow autumn leaves of *Tropaeolum* (Fig. 21) contain voluminous lipid globules without any trace of tubules or of isodiametric bodies appears to be somewhat contradictory to the assumption of a purely self-regulated assembly of degradation products. In fact, these leaf chromoplasts exhibit an organization which is completely different from that of the yellow chromoplasts in the flowers. They have the appearance of chromoplasts of autumn leaves of other plants so far investigated (e.g. Toyama and Ueda, 1965; Stearns and Wagenaar, 1971), all of which belong to the globulous type. In view of the obvious organ-specificity one can agree with Smith and Butler (1971) that the events in chromoplast differentiation most likely "follow a controlled, repeatable pattern, perhaps mediated through the genetic system".

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