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FINE STRUCTURAL STUDIES OF PLASTIDS DURING THEIR DIFFERENTIATION AND DEDIFFERENTIATION*

(A Review)

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A survey of fine structural characteristics of various plastid types, their variability and interconvertibility, based on the authors' experience is given. In this article the fine structure and development of the main plastid types — chloroplasts, etioplasts, chromoplasts and leucoplasts — are described in several plant systems.

The differentiation of chloroplasts proceeds either directly from proplastids or through the etioplast stage. Various environmental factors are shown to influence this process. The adaptability of chloroplasts to environmental changes is shown in *aurea* mutants. These plants promptly adapt their structure and function to the surrounding light conditions. The development of the photosynthetic activity in the thylakoids during chloroplast differentiation can be demonstrated by a cytochemical method. The fine structure of various chromoplast types is described. The evolution of their pigment containing structures can be successfully studied by applying specific inhibitors which block or alter their normal differentiation.

The plastid dedifferentiation is demonstrated by several examples. These include changes from leucoplasts to chloroplasts and several types of chromoplasts to chloroplasts transformations. The significance of these transformations is discussed.

* Dedicated to Prof. Zvonimir Devidé on the occasion of his 65th birthday. Prof. Zvonimir Devidé was Head of the Laboratory of Electron Microscopy, Ruđer Bošković Institute, Zagreb, for many years (from 1954 till 1973), and the initiator of the investigations on plastids.

Introduction

Plastids are typical organelles in most eucaryotic plant cells. They exist in many different forms and have different functions. Moreover, plastids are able to adapt their structure and function to various changes of environment. The mode of these adaptions has not yet been fully explained, because it depends on the interaction of genome and plastome of the cell (Kirk and Tilney-Bassett 1978, Devidé 1983).

All plastids develop from undifferentiated proplastids. Dependent on the plant organ and environmental conditions, proplastids are transformed into various plastid types, such as chloroplasts, etioplasts or chromoplasts. The developmental scheme of plastids is very complicated, because their differentiation can be reversed under certain conditions, or can take another direction. It is considered that all plastid types are interconvertible to some extent, i.e. »any kind of plastids can be shown to change into or be formed from one or more other kinds of plastids« (Kirk and Tilney-Bassett 1978).

This survey gives the results of the authors' 25-year experience of the problems of fine structural changes during plastid differentiation.

Proplastids

Proplastids are small colourless undifferentiated plastids, which occur in meristematic cells and are precursors of all other plastid types. These plastids contain only few internal structures — vesicles and short single prothylakoids — which develop through invagination of the inner membrane of the envelope (Fig. 1) (Wrischer and Vrhovec 1969). The internal membranes lack photosynthetic activity (Wrischer 1981). In some cases proplastids can accumulate phytoferritin (Knoth, Wrischer and Vetter 1980). This iron containing protein is thought to serve as storage for the synthesis of new thylakoids.

Differentiation of chloroplasts from proplastids can be best studied in monocotyledonous light grown leaves, which grow at their base. For the study of functional differentiation of plastids *in situ* photooxidation of diaminobenzidine (DAB) turned out to be a useful method*. Differentiation starts with the development of new prothylakoids. Some of them become photosynthetically active (they react positivelly with DAB) and then start to divide and stack to small grana. Further increase in the number of grana gives rise to the thylakoid system of chloroplasts, consisting of grana and stroma thylakoids (Fig. 6) (Wrischer 1981).

In roots and some fruits, proplastids may remain structurally undeveloped as *leucoplasts* (Fig. 2) or they may accumulate starch to form *amyloplasts*. Leucoplasts are not always the last step in plastid differentiation. In some underground organs, e.g. in onion bulbs when illuminated, leucoplasts of the mesophyll cell layers are promptly transformed into chloroplasts by developing new grana (Devidé, unpublished).

* Photooxidation of DAB is mediated by some component(s) of the photosynthetic apparatus lying in the thylakoid membrane in the vicinity of the photosystem I (Wrischer 1978a).

Etioplasts

Among all environmental factors light has the greatest influence on the differentiation of plastids. In leaves grown in darkness proplastids cannot change to chloroplasts, but etioplasts develop instead (Fig. 4). Etioplasts contain only few membranes (prothylakoids) and many tubules arranged in a semicrystalline structure, the s.c. prolamellar body (PLB) (Kirk and Tilney-Bassett 1978, Wellburn 1982). Neither the tubular network, nor the prothylakoids can photosynthesize (they are both DAB negative). When etiolated leaves are illuminated, the semicrystalline array of tubules is immediatelly transformed into an irregular coil. After 1—3 hours of illumination the prothylakoids become DAB positive, then start to divide and through their stacking the first grana are formed. At the same time the DAB negative tubular coil disappears (Wrischer 1978a).

The process of »greening«, that is the formation of grana, is one of the most critical steps in the chloroplast differentiation. It can be completely or partially inhibited by different external noxious factors, like ionizing radiation (Devidé 1967, 1969, Wrischer 1966a, Wrischer and Devidé 1964, 1967a, 1967b), anoxia (Devidé and Wrischer 1964, 1967), inhibitors of protein synthesis (Wrischer 1967a, Wrischer and Vrhovec 1972), different herbicides (Vrhovec and Wrischer 1970, Wrischer and Botka 1978), or pollutants, like heavy metals (Fig. 7) (Wrischer and Meglaj 1980, Wrischer and Kunst 1981).

Prolamellar bodies are not restricted to etioplasts. They also develop in young chloroplasts during the night (Wrischer 1966b, 1978a), or in weak light (Wrischer 1966b, 1981). It is generally accepted, that tubular aggregates (PLBs) develop in young chloroplasts whenever normal thylakoid formation is inhibited (Wrischer 1981, Lütz et al. 1984). The appearance of PLBs in ethionine (an inhibitor of protein synthesis) treated chloroplasts might be explained in the same way (Wrischer 1973d).

The degree of plastid autonomy is evident in studies with isolated etioplasts and young chloroplasts. When etioplasts are isolated in darkness and afterwards illuminated for several hours, their PLBs are partially transformed and dispersed, but the formation of new thylakoids in them does not occur (Wrischer 1973a). New thylakoids do not appear in isolated young chloroplasts (etiochloroplasts) held in light either. In this case small PLBs are formed *de novo*, instead of thylakoids (Wrischer 1973b). These results serve as a proof that an intact cell and a normal cooperation of genome and plastome is necessary for normal plastid differentiation (Kirk and Tilney-Bassett 1978, Devidé 1983).

Chloroplasts

Chloroplasts are the most common plastids and, owing to their important physiological function, the most widely studied. They are specially adapted to perform photosynthesis, i.e. to convert light energy into chemical energy. It is well known that the s.c. light reactions of the photosynthesis take place in the thylakoid membrane, and the dark reactions in the chloroplast stroma (Fig. 5). According to recent biochemical in-

vestigations the two photosystems of light reactions are localized separately in the thylakoid membrane: photosystem II predominantly in the grana thylakoids, and photosystem I mostly in the stroma thylakoids and peripheral grana thylakoids (Staehelin and Arntzen 1983). Cytochemical investigations — using photooxidation of DAB (for the detection of photosystem I) and photoreduction of thiocarbamyl nitro blue tetrazolium (TCNBT; for the detection of photosystem II) — could confirm these findings to some extent as well (Vaughn et al. 1983, Wrischer, unpublished).

The most abundant stroma protein — ribulose-bisphosphate carboxylase (Rubisco) — can be crystallized under certain conditions in chloroplasts *in situ*. In bean chloroplasts this crystallization can be induced by feeding the leaves with sucrose or by removing some water from the cells (Fig. 3) (Wrischer 1967b, 1970a, 1973c). In spinach chloroplasts, under stress conditions (detachment of the leaves), crystallization of Rubisco takes place intrathylakoidally (Wrischer 1970b, 1978b).

Peripheral reticulum is an ingrowth of the inner plastid membrane. This membrane has an important role in the formation of other membranes (thylakoids in chloroplasts, internal membranes and tubules in chromoplasts) and in the transport processes through the plastid envelope. The envelope and the reticulum can be distinguished from thylakoids on the basis of their DAB negativity (Keresztes and Wrischer 1977).

The organization of the thylakoid system reflects its function. This is most obvious in s.c. C_4 plants (e.g. in maize), containing dimorphic chloroplasts that have different functions in photosynthesis (Kirk and Tilney-Bassett 1978, Wrischer 1980, Wrischer and Ljubešić 1983).

Visible variations in the quantity and structure of grana exist between chloroplasts of leaves grown in the shade and those grown in the sun. Large grana in chloroplasts of shade leaves reflect the high concentration of light-harvesting complexes of photosynthetic reaction centers in grana thylakoids, that are necessary for an efficient capture of light energy in week light (Lichtenthaler et al. 1982, Wrischer and Ljubešić 1983).

Growth in strong light can result in degradation of some thylakoids and in accumulation of lipids and pigments in plastoglobules. This was observed in alga *Acetabularia mediterranea* when growing on sun-exposed grounds (Kleinig and Wrischer 1968).

However, overwintering blackberry leaves contain very large grana with much chlorophyll. This seems to be an adaptation to reduced illumination during autumn and winter months, although a certain influence of the senescence of leaves cannot be excluded (Wrischer and Modrušan, in preparation).

Unusual, and not yet explained, is the formation of pseudograna in the alga *Netrium digitus* growing in darkness. In light grown cultures of this desmidiacean alga grana have never been observed (Marčenko 1970b).

Senescence of *chloroplasts*. Senescence of leaves is always connected with their yellowing. On the ultrastructural level a gradual loss of the thylakoid system is observed; first of stroma, and then of grana thylakoids (Ljubešić 1968, Wrischer 1978b). Thylakoids of senescent chloroplasts retain some photosynthetic activity for a long time, as shown by their positive reaction with DAB (Wrischer 1977).

Simultaneously with the reduction of the number of thylakoids plastoglobules start to accumulate. They contain lipids and pigments (carotenoids), which are derived from disintegrated thylakoids. Ribosomes also disappear from the stroma (Fig. 9) (Ljubešić 1967, 1968, 1969). Large crystalline inclusions of phytoferritin in old blackberry leaves originate from degraded thylakoids (Ljubešić 1976). In alga *Euglena* accumulation of wax crystals is also considered as a characteristics of senescence (Marčenko 1978).

Senescence of chloroplasts can be accelerated when the leaves are detached from the plant. It also proceeds much quicker in darkness than in light. Addition of sucrose, probably as a nutritional source, can considerably prolong the survival of chloroplasts in detached leaves (Wrischer 1978b).

Old (senescent) chloroplasts are not always the last step in plastid differentiation. Detached senescent leaves, which have become yellow in darkness, can regreen when they are transferred to light (Wrischer 1978b). In old senescent tobacco leaves the process of regreening is induced by removing all young leaves from the plant (Ljubešić 1967, 1968), and in old detached blackberry leaves the same effect can be obtained through kinetin treatment (Ljubešić 1976). The regreening process starts with reappearance of ribosomes in the stroma. Plastoglobules and phytoferritin inclusions (in blackberry) slowly disappear and the synthesis of new thylakoids begins at the same time (Ljubešić 1967, 1976).

Chloroplast mutants. Mutations in plants are often expressed as a disturbance in function, structure and chemical composition of plastids. Structural components of plastids are coded for partly by the nuclear DNA, and partly by the plastid DNA, and therefore various interferences can happen in the course of differentiation (Devidé 1983).

For the study of interaction between mutations and environmental factors very useful objects are the s.c. conditional mutants (Hopkins, Hayden and Neufer 1980). *Aurea* mutants also belong to this group. The leaves of *aurea* mutants can green, i.e. develop chloroplasts, only when they are growing in the shade, while in the sunlight the leaves become yellow. In these yellow leaves the plastids contain only a poorly developed thylakoid system, which consists mostly of single thylakoids and thylakoids in degradation (Fig. 8). After a prolonged growth in the sun the leaves bleach, and then only the remnants of thylakoids remain in the plastids. Concentration of pigments, especially of chlorophyll, is very low in yellow leaves. Nevertheless their photosynthetic efficiency is several times higher than in shade-grown green leaves of the same plant, or in wild type plants (Wrischer, Ljubešić and Devidé 1975a, b, 1976, Antica and Wrischer 1982, Kunst 1983, Wrischer et al. 1986).

Plastids of *aurea* mutants posess the ability to adapt promptly to changes in light conditions. Alternate yellowing and greening is observed under natural conditions in succession of sunny and cloudy days, but it can also be experimentally induced. It has been shown that alternate yellowing and greening can occur several times in the course of one season (Kunst and Wrischer 1984).

Spontaneous mutations in *Euglena* can be induced when the cells are exposed to high light or high temperatures (Marčenko 1970a). One of these spontaneous mutants, the y-1 strain, has lost most of the chlorophyll and the plastids contain a very reduced thylakoid system

(Fig. 10) (Marčenko 1973). This mutant is more sensitive to high temperatures, strong light and some inhibitors of protein synthesis, than the wild type *Euglena* (Marčenko 1974a, b, 1980). Spontaneous mutation in *Euglena* can result also in a completely pigmentless mutant, which contains only residual thylakoids and some plastoglobules (Marčenko 1981).

Chromoplasts

Chromoplasts in yellow and coloured fruits, flowers and roots are usually the last stage in the process of plastid differentiation. Their physiological role is yet unknown, although their ecological function has been well stated. The structure of chromoplasts varies exceedingly, as it was observed already by Schimper (1885). Chromoplasts usually develop from chloroplasts, with an exception when they directly evolve from proplastids, as in the proximal part of the fruit of *Cucurbita pepo* cv. *pyriformis* (Ljubešić 1970a, b) and in young roots and callus cells of carrot (Wrischer 1972, 1974). Different types of chromoplasts can be best classified according to the carotenoid containing structures (Sitte 1974).

The most widely spread are *globulous chromoplasts* with pigments dissolved in lipid droplets, lying in the stroma, as in the fruits of *Cucurbita pepo* cv. *ovifera* (Fig. 12) (Devidé 1970a, b), in distal parts of the fruits of *Cucurbita pepo* cv. *pyriformis* (Ljubešić 1972), and in lemon fruits (Ljubešić 1984). The plastids of senescent yellow leaves, which contain large numbers of plastoglobules, should also be classified in this chromoplast type (Ljubešić 1968, 1969, 1976).

Fig. 1. *Sinapis alba*, proplastids from the root meristem. 24,000:1.

Fig. 2. *Allium cepa*, leucoplast from the bulb tissue. 38,000:1.

Fig. 3. *Phaseolus vulgaris*, protein crystalloids in the stroma of a leaf chloroplast. 100,000:1.

Fig. 4. *Phaseolus vulgaris*, etioplast from the dark grown leaf. 40,000:1.

Fig. 5. *Zea mays*, part of a leaf chloroplast. 42,000:1.

Fig. 6. *Zea mays*, leaf chloroplasts with positive DAB reaction in the thylakoids. 22,000:1.

Fig. 7. *Triticum vulgare*, chloroplast from the cadmium treated leaf ($CdCl_2$ 1 mmol). 17,000:1.

Fig. 8. *Fraxinus excelsior* var. *aurea*, part of a plastid from the yellow, sungrown leaf with degraded thylakoids. 66,000:1.

Fig. 9. *Nicotiana rustica*, part of a senescent leaf chloroplast. 42,000:1.

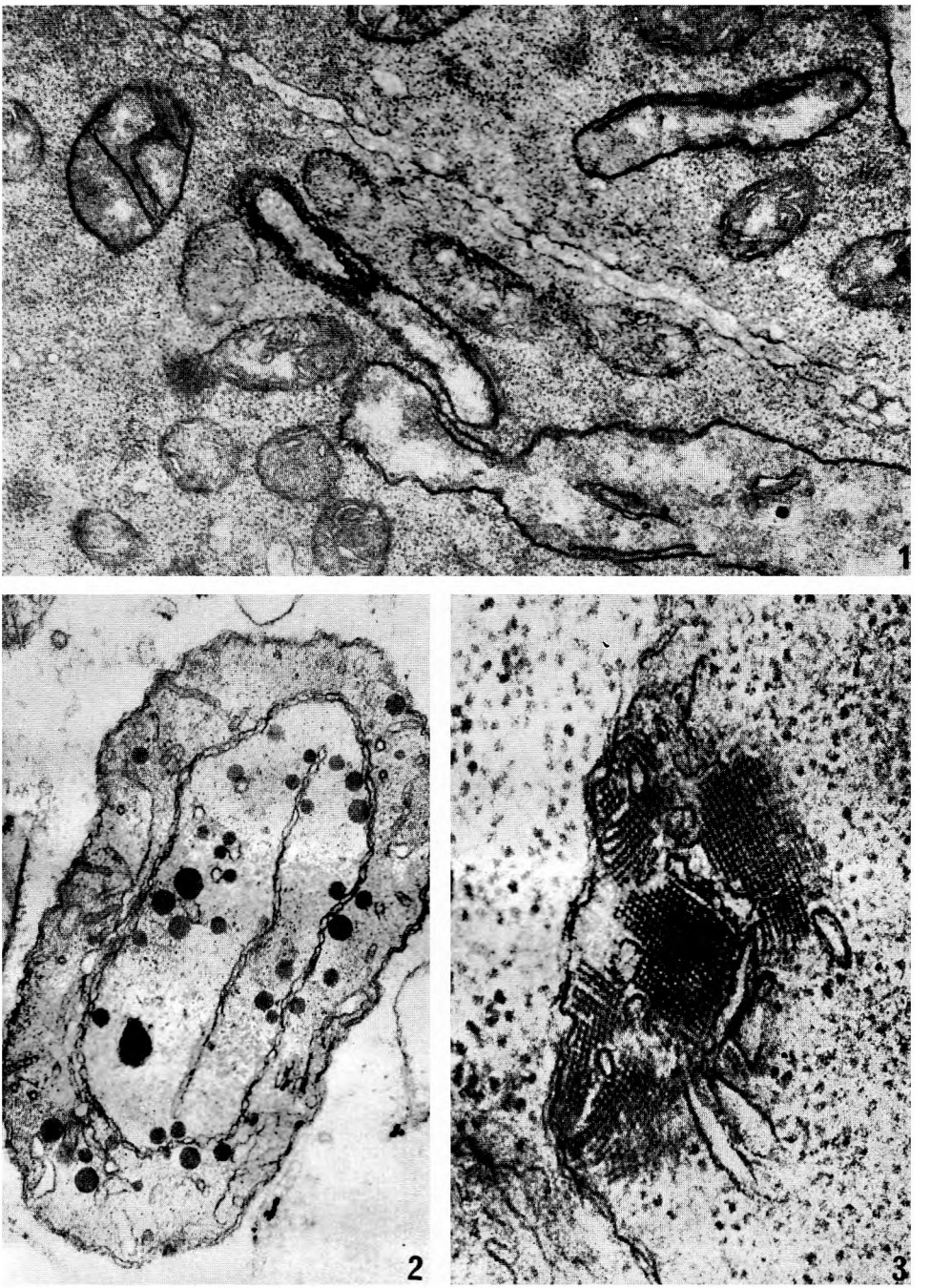
Fig. 10. Yellow (y-1) mutant of *Euglena gracilis* with undeveloped plastids. 8,000:1.

Fig. 11. *Cucurbita pepo* cv. *pyriformis*, chromoplasts from the proximal part of the fruit. 28,000:1.

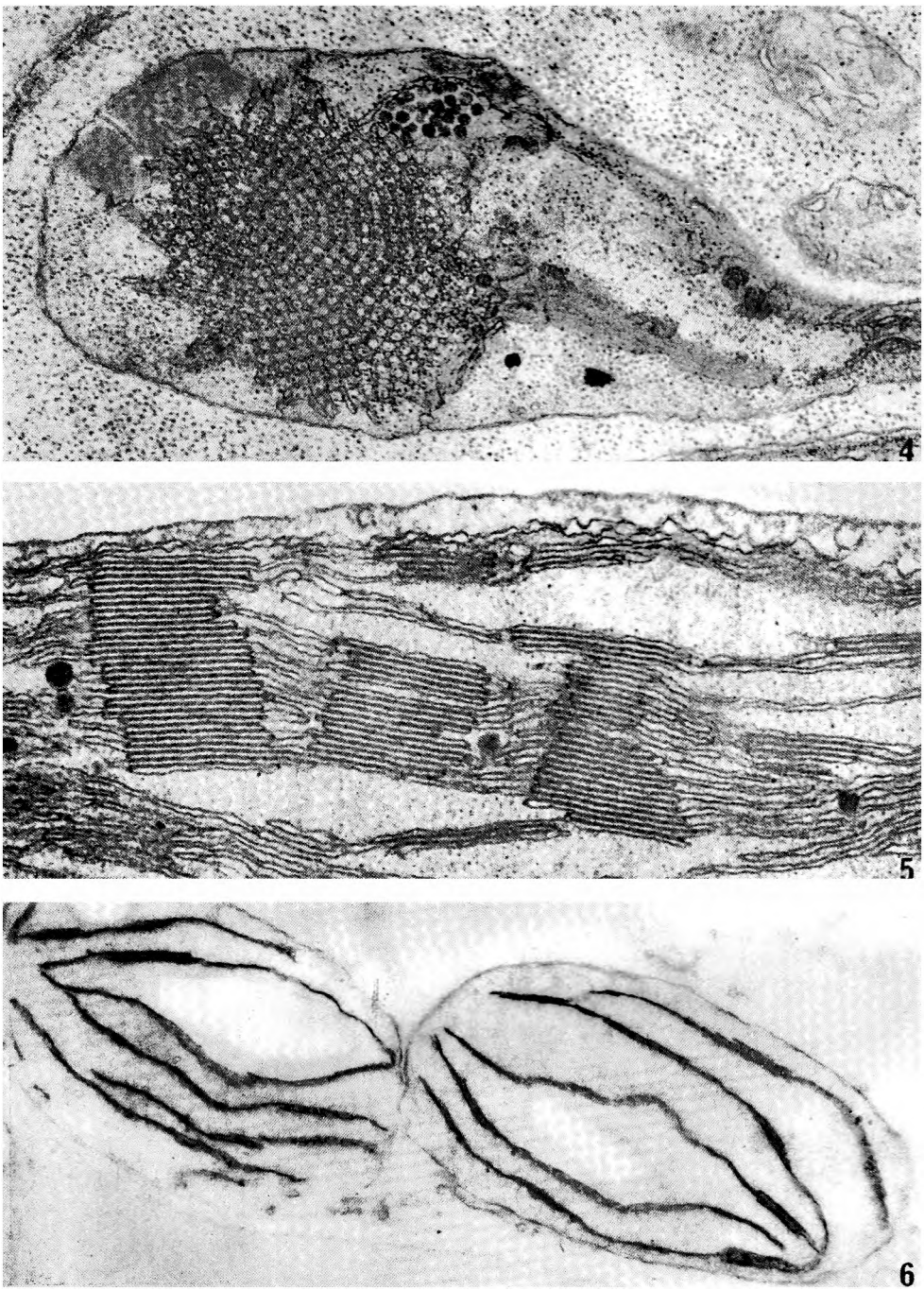
Fig. 12. *Cucurbita pepo* cv. *ovifera*, chromoplast from the yellow fruit. 16,000:1.

Fig. 13. *Cucurbita pepo* cv. *ovifera*, chloroplast from the regreened fruit. 17,000:1.

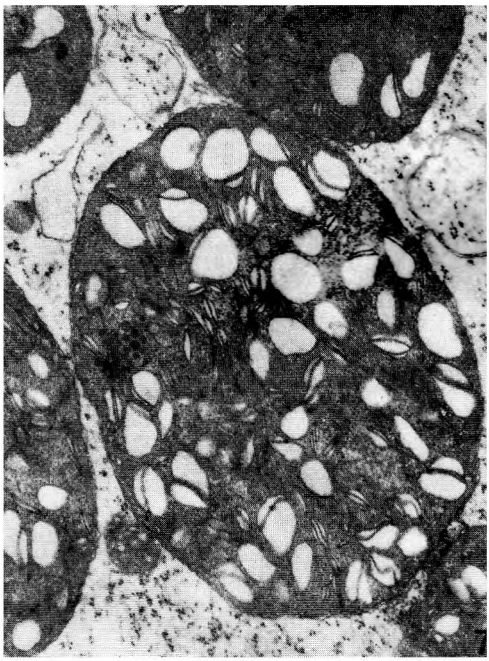
Fig. 14. *Liriodendron tulipifera*, chromoplast from the flower treated with SAN 9789 (0.02 mmol). 30,000:1.

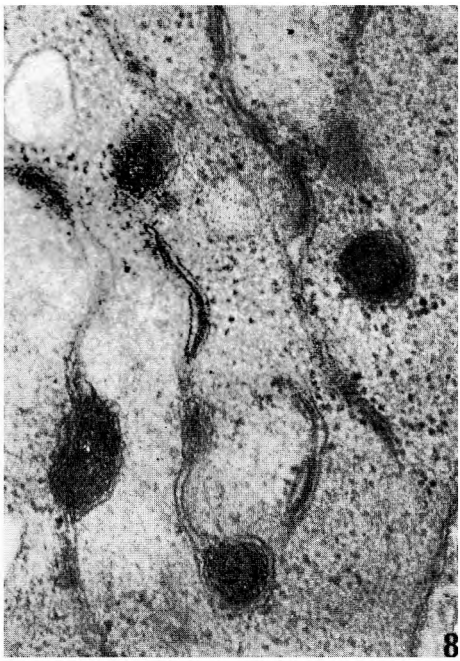


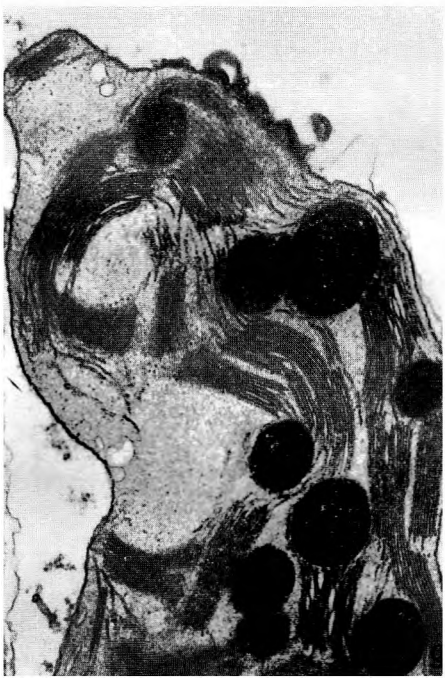
Figs. 1—3.

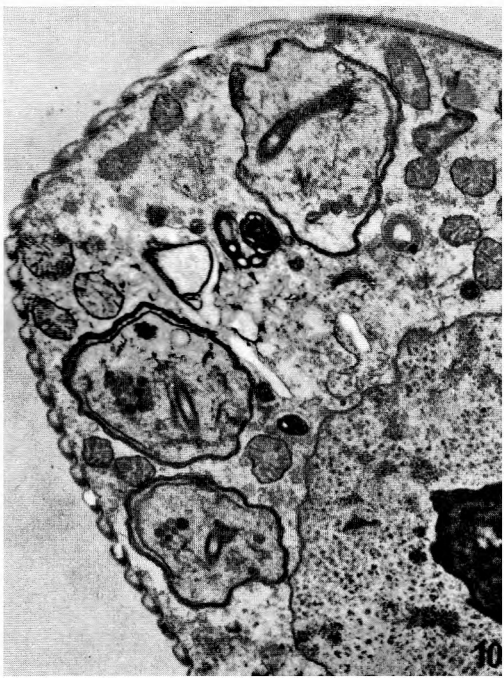


Figs. 4—6.

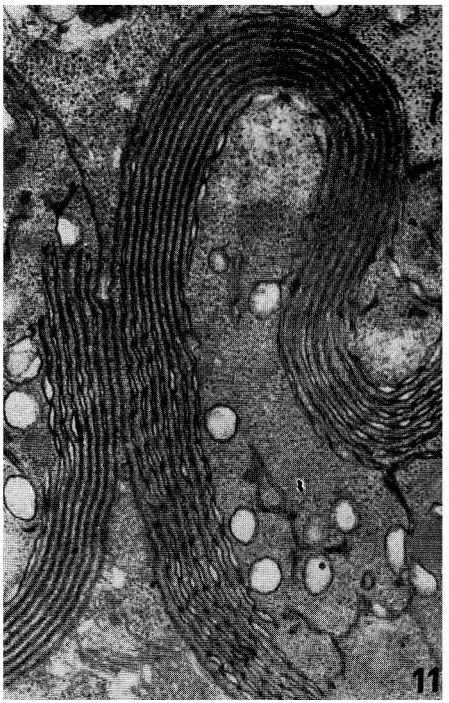


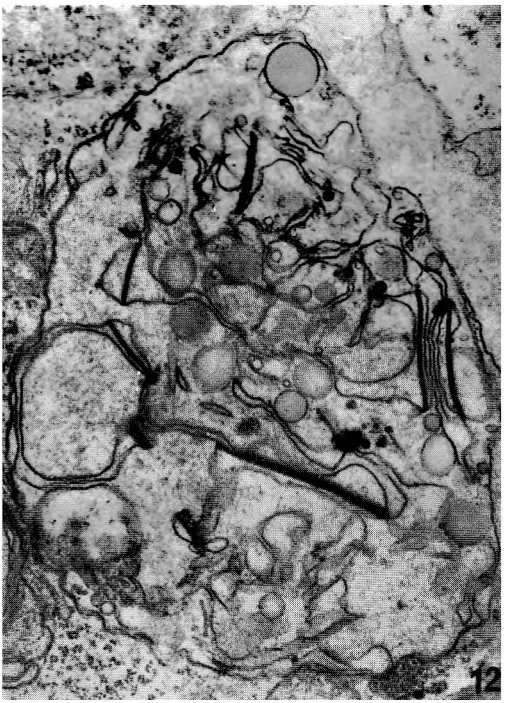


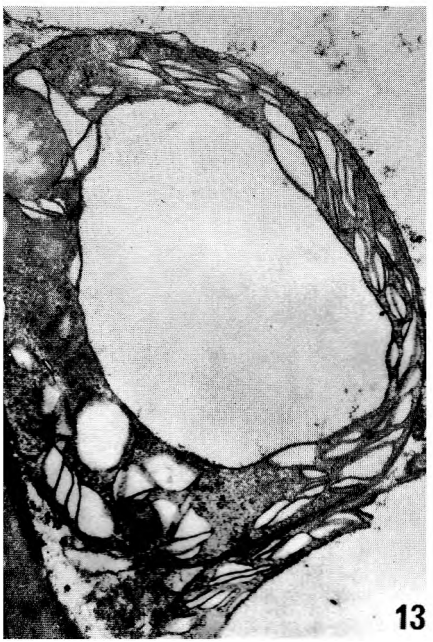


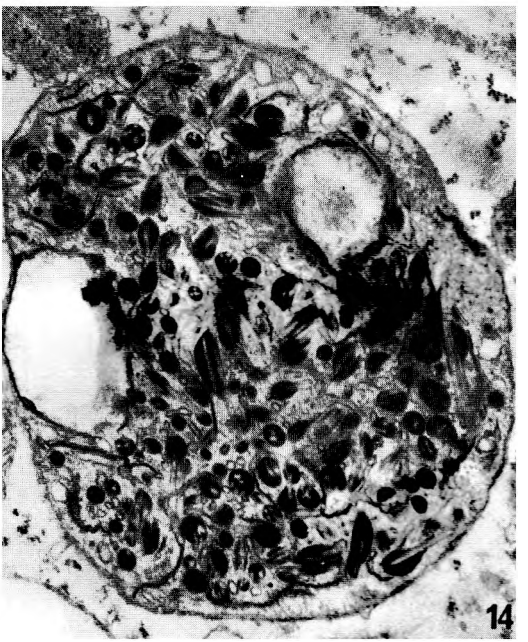


Figs. 7—10.









Figs. 11—14.

Presence of plastoglobules does not necessarily mean that they contain pigments. Leucoplasts in the fruits of *Cucurbita pepo* cv. *patisson* are filled with plastoglobules, but are pigmentless (Ljubešić 1973).

In *membraneous chromoplasts* carotenoids are located in special »internal« membranes, which are sometimes perforated (Fig. 11) (Ljubešić 1970a, b). In the course of chromoplast differentiation in *Calceolaria* petals it is possible to follow the development of these membranes by invagination of the inner membrane of the envelope (Wrischer and Ljubešić 1984).

In *tubulous (fibrilar) chromoplasts* pigments are present in numerous long tubules. The origin of these tubules is different. They appear either through reorganization from degraded thylakoids, as in the chromoplasts of *Forsythia* flowers (Ljubešić 1979b), or develop inside plastoglobules, as in the chromoplasts of *Sorbus aucuparia* fruits (Ljubešić 1982). Both types of tubular formations seem to be present in the chromoplasts of the fruits of *Cucurbita maxima* cv. *turbaniformis* (Ljubešić 1977).

Reticulo-tubulous chromoplasts are very rare. As yet they have been described in only two cases. One of them is the spadix of *Typhonium divaricatum* (Schnepf and Czygan 1966), and the other the orange zone of the *Liriodendron tulipifera* petals. These chromoplasts contain numerous branched and curved tubules, which form a large network. Plastoglobules are located among the tubules. It is impossible to say yet whether the pigments are located within the tubules, or the plastoglobules, or in both of them (Ljubešić 1979a).

In *crystalloid chromoplasts* numerous crystals of β-carotene or lycopene lie in the stroma enveloped by a membrane. The formation of these crystals starts already intrathylakoidally in chloro-chromoplasts of the *Narcissus poëticus* corolla (Hlušek-Radojčić and Ljubešić, unpublished). It should be mentioned that β-carotene crystals develop also in some other old chromoplasts, e.g. in reticulo-tubulous chromoplasts of the old *Liriodendron tulipifera* flowers (Ljubešić 1979a).

Although it is generally thought that chromoplasts form the last stage in plastid differentiation, it is well known that tissues which contain chromoplasts can regreen under certain conditions. Repeated regreening was observed in the ripe fruits of *Cucurbita pepo* cv. *ovifera* in the light. New thylakoids are formed either by division of small vesicles, present in the stroma, or by invagination of the envelope (Fig. 13) (Devidé and Ljubešić 1972, 1974). The regreening of the proximal part in completely red *Cucurbita maxima* cv. *turbaniformis* fruits and in ripe lemon fruits proceeds in a similar fashion (Ljubešić 1981, 1984). Carrot roots regreen promptly when illuminated. In chromoplasts of old roots new thylakoids are developed by division of vesicles, present in the stroma, and in young ones by invagination of the inner membrane of the envelope. Vesicles, which pinch off the membrane, are divided and then stacked to small grana (Wrischer 1972, 1974).

Treatment of plants with *inhibitors* offers an opportunity to study the genesis of different components of the chromoplasts and to learn more about their composition without destroying the organelles. By changing the direction of plastid differentiation this process can be made more comprehensive. When *Forsythia* flowers are treated with the herbicide isopropyl N-phenylcarbamate (IPC; a common inhibitor of protein synthesis) no tubules, but only numerous plastoglobules, are developed in the chromoplasts (Ljubešić and Radić 1979).

The herbicide SAN 9789 is a specific inhibitor of the synthesis of β-carotene. When the flower buds of *Narcissus poëticus* or *Liriodendron*

tulipifera are treated with this inhibitor, no crystals of β-carotene, but numerous plastoglobules, are developed in chromoplasts (Fig. 14). According to the analysis by thin layer chromatography these plastoglobules contain only xanthophylls and never β-carotene (Hloušek and Ljubešić 1985). Another herbicide of the same series, SAN 9785, inhibits only the synthesis of lipids. Therefore it does not inhibit the formation of β-carotene crystals in the chromoplasts of *Narcissus poëticus*, but it has some effects on other chromoplast components (Hloušek-Radojčić and Ljubešić, unpublished).

In the course of the years the authors have studied plastid differentiation in numerous plants, under various environmental conditions and treatments (light, temperature, specific inhibitors, ionizing radiation and pollutants). These investigations have provided a lot of data, but at the same time many new questions have arisen.

Conclusion

Plastids represent unique cell organelles in the living world. Only they contain photosynthetic membranes in which light energy is converted into chemical energy. Other important biosynthetic processes occur in plastids as well. Therefore a better knowledge of the processes taking place in these organelles is needed and is of manifold academic and practical interest, since all our food originates directly or indirectly from plastids.

Plastids are semiautonomous cell organelles and their structure and function is closely bound to the interaction of both plastome and genome of the cell. Their great variability and interconvertibility make them extremely suitable for various investigations. Valuable informations on plastid autonomy and its dependence on the genetic apparatus of the cell can be obtained by studying plastids isolated in their various developmental stages. A useful tool in studying plastid differentiation is without doubt the application of inhibitors with well known action, as specific inhibitors of protein synthesis or compounds which influence the synthesis of distinct components of the photosynthetic apparatus.

Conditional mutants, e.g. *aurea* mutants, are very convenient in the study of environmental influence on plastid differentiation and dedifferentiation. The response of plastids to environmental changes (e.g. to changes in light conditions) is much more strongly pronounced in these plants than in normal ones and therefore suitable to study.

In recent years another practical aspect connected with plastid investigations has arisen. It is evident that plastids, being very sensitive to different noxious substances, are excellent indicators of all ways of pollution.

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SAŽETAK

ELEKTRONSKO-MIKROSKOPSKA ISTRAŽIVANJA PLASTIDA TIJEKOM NJIHOVE DIFERENCIJACIJE I DEDIFERENCIJACIJE

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Prikazani su razni tipovi plastida, njihova varijabilnost i interkonvertibilnost, na temelju višegodišnjih istraživanja autora. Na većem broju objekata opisani su ultrastruktura i razvoj glavnih tipova plastida (kloroplasta, etioplasta, kromoplasta i leukoplasta).

Studirana je diferencijacija kloroplasta i istražen utjecaj različitih vanjskih čimbenika na taj proces. Razvoj fotosintetske aktivnosti u tilakoidima tijekom diferencijacije kloroplasta praćen je primjenom odgovarajuće citokemijske metode. Na primjeru mutanata tipa *aurea* prikazana je sposobnost prilagodbe kloroplasta na promjene okoliša (uvjete osvjetljenja). Opisana je ultrastruktura različitih tipova kromoplasta, a primjenom specifičnih inhibitora istražena je njihova diferencijacija.

Dediferencijacija plastida prikazana je na nekoliko primjera. Opisana je prijetvorba leukoplasta u kloroplaste i prijetvorbe više tipova kromoplasta u kloroplaste. Raspravljeno je značenje tih prijetvorbi.

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