

TRANSFORMATION OF PLASTIDS IN YOUNG CARROT CALLUS

Mit deutscher und kroatischer Zusammenfassung

MERCEDES WRISCHER

(Laboratory of Electron Microscopy, Institute »Ruder Bošković«, Zagreb)

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Introduction

It is well known that cells of some tissues of carrot roots, if wounded, are able to divide, and that the chromoplasts in such newly formed callus cells divide as well (K ü s t e r 1956). In the present work the fine structure of these chromoplasts and the way of their further transformation has been studied.

Material and Methods

Fully developed carrot roots (*Daucus carota*, cv. "Nantes") were cut with a razor-blade into 2 to 3 mm thick discs and put into Petri dishes on wet filter paper. The paper was changed daily and wetted with one of the following solutions: a) tap water, b) tap water + 0.1 M sucrose, c) nutrient medium (F a d e e l 1962), d) nutrient medium + 0.1 M sucrose, e) nutrient medium + 0.1 M sucrose + 10^{-6} indolylacetic acid.

The Petri dishes were kept either in darkness at 25 — 26 °C, or under a discontinuous white light (mercury bulb VTF — 250 W TEŽ, illumination intensity 5000 lx; periods of 15 hours of light and 9 hours of darkness daily).

Small pieces of callus tissue were fixed in 1% glutaraldehyde, post-fixed in 1% OsO₄ and embedded in araldite. Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I (at the Institute of Biology, University of Zagreb).

Thin, hand made sections of the callus tissue were currently examined also with a light microscope.

Results

At the time of wounding only typical chromoplasts with prominent carotene crystals can be observed in root cells with a light microscope.

One to two days after the wounding many small knots develop on the surface of the injured tissue, and they can be observed in the light microscope even at the lowest magnification. These structures multiply quickly and soon turn into grape like structures. At high magnification of the light microscope these structures are recognized as newly formed callus cells. New cells develop over the peripheral part of the central root cylinder first, then over the whole central part of the root, and soon also over the root cortex. Cell divisions proceed rapidly, so that a few days later almost the whole cut surface of the root is covered with new callus cells. Such cells contain — as observed in the light microscope — either chromoplasts with prominent carotene crystals, or more often small colourless plastids, probably proplastids.

Five- to six-day-old samples exposed to light turn green slowly. The greening starts over the central part of the root first, and later spreads over the root cortex. Observations under light microscope show that the colourless proplastids turn greenish, and the plastids with carotene crystals become scarce.

No differences in the division rate of callus cells, nor in the way of plastid transformation can be detected in samples cultivated on various media.

The changes in the fine structure of plastids proceed as follows:

In intact carrot roots only typical chromoplasts are present. They contain some plastoglobules, rare single thylakoids, the so-called "tubular complex" (Newcomb 1967), and empty sharp-edged vacuoles (Fig. 1). In live plastids these vacuoles contained carotene crystals which were dissolved during the dehydration of the tissue (Ben-Shaul, Treffry and Klein 1968).

In young, two- to four-day-old callus tissue most of the plastids are proplastids, of an amoeboidal shape, containing only few thylakoids and some cisternae as invagination of the inner part of the outer membrane (Fig. 2). Sometimes small plastoglobules also appear. True chromoplasts with carotene crystals (i.e. vacuoles) are rare. Chromoplasts are more often found in callus cells lying over the root cortex (Fig. 8), where the division of the cells proceeds at a somewhat slower rate than over the central part of the root.

In samples exposed to light for four or five days the plastids become elongated, and the internal membrane structures differentiate into a system of thylakoids, sometimes already arranged in stacks. The lumina of the thylakoids are more or less dilated (Figs. 3, 4, 5) and often dark, or filled with a finely granular substance (Fig. 5). In seven- to eight-day-old callus tissue young chloroplasts can already be observed (Fig. 6). Grana are still poorly developed, containing not more than three thylakoids in a stack. The plastid stroma is rich in ribosomes. Small plastoglobules also appear. Cisternae extending from the inner part of the outer membrane towards the thylakoids can be seen in several places (Figs. 3, 5, 6, 6 inset). In the following days the number of grana becomes somewhat higher (Fig. 7). In about-a-month-old callus tissue the chloroplasts contain large plastoglobules.

The callus cells develop in darkness also, but the plastids in them remain at the proplastid stage or rarely, at the chromoplast stage. If illuminated, they soon turn into chloroplasts in a way similar to that described for samples developing only when exposed to light.

The fine structure of other organelles in callus cells is typical of very active cells. The cytoplasm is rich in ribosomes and in elements of the endoplasmic reticulum. The Golgi apparatus is always surrounded by a ring of vesicles and mitochondria contain a considerable number of tubules (Fig. 6).

Discussion

No data are known about chromoplast divisions in intact root cells. After wounding the tissue, the chromoplasts in the newly formed callus cells obviously regain the ability of multiplying by division. Dividing rapidly they turn gradually into proplastids. During these divisions different inclusions, mostly carotene crystals, which do not multiply, obviously become scarcer at the same time. As seen under the light microscope, carotene crystals, which are otherwise the most characteristic features of carrot chromoplasts, are still found in plastids after several cell divisions, sometimes even in young chloroplasts. Grönegress (1971), who has studied the fine structure of regreened intact carrot roots, states that carotene crystals are desintegrated during the regreening process. The same has been found for carrot cells in tissue cultures by some authors working with the light microscope (Buvat 1942, 1944/45, Küster 1956). The present investigation could not confirm these statements, at least not for young callus cells.

Ribosomes become numerous and distinct at the moment when the greening of the tissue begins, i.e. at the moment of the grana development. In some pictures (Fig. 3) the way of grana formation described by Wehrmeyer (1966) can be observed. It is not certain, at present, to what extent the inner layer of the outer plastid membrane supplies the material for the new thylakoids, as it is generally considered (Newcomb 1967, Thomson, Lewis and Coggins 1967). Dilatations seem to be a characteristic feature of newly formed thylakoids, for they have been observed e.g. also in other plant materials during the regreening process (Devidé and Ljubešić 1972). In our case the lumina of the dilated thylakoids are often dark. Most probably the thylakoids are filled with a substance which under certain conditions reduces osmium-tetroxide during the fixation process. This phenomenon is not rare, and appears e.g. in plastids of carrot tissue cultures (Israel and Steward 1967) or in bean and spinach plastids (Newcomb 1967, Wrischer 1970). According to the data by these authors such inclusions should be proteinaceous deposits.

In old callus tissue the chloroplasts accumulate large plastoglobules. Accumulation of plastoglobules is a general feature of plastids in senescing tissues (Ljubešić 1968, Dodge 1970).

As expected, light does not have a direct influence on the division rate of callus cells, because divisions start both under illumination and in darkness. The greening of the plastids in such cells, i.e. the transformation of proplastids into chloroplasts, is on the contrary — as has been expected — strongly dependent on light.

In extensive studies on the development of cells and plastids in carrot tissue culture Israel and Steward (1966, 1967) have found that plastids could develop into chloroplasts only if the tissue is cultured on a complete and very rich medium. It is therefore somewhat surprising that in this study the composition of the medium, on which the root slices were kept, did not play a significant role in the callus development and in the transformation process of the plastids. It is possible that the differences between the results obtained by Israel and Steward and these obtained here are the consequence of the fact that the pieces of tissue used by those authors were probably smaller than the ones used in the present work. The mobilisation of starch reserves in root cells after wounding (Buvat 1965), as well as the mobilisation of auxines, usually induced in injured tissue, are most probably the main reasons for this behaviour of plastids in callus tissue.

Fig. 1. Chromoplast from a fully grown, intact root. Plastoglobules and carotene crystals (arrow) in the stroma. 40,000 : 1.

Sl. 1. Kromoplast iz izraslog intaktnog korijena s plastoglobulima i karotenskim kristalima (strelica) u stromi. 40 000 : 1.

Fig. 2. Proplastids from a 4-day-old callus with cisternae (c) and plastoglobules. 27,600 : 1.

Sl. 2. Proplastidi iz 4 dana starog kalusa s cisternama (c) i plastoglobulima. 27 600 : 1.

Fig. 3. Plastid from a 4-day-old callus with rows of thylakoids and some cisternae. 45,000 : 1.

Sl. 3. Plastid iz 4 dana starog kalusa s nizovima tilakoida i cisternama. 45 000 : 1.

Fig. 4. Plastid from a 4-day-old callus. Swollen thylakoids arranged in small stacks. 50,000 : 1.

Sl. 4. Plastid iz 4 dana starog kalusa. Nabubreni tilakoidi složeni u malene svežnjeve. 50 000 : 1.

Fig. 5. Plastid from an 8-day-old callus. Swollen thylakoids filled with dark granular substance and empty cisternae (c). 50,000 : 1.

Sl. 5. Plastid iz 8 dana starog kalusa. Nabubreni tilakoidi ispunjeni tamnim, granuliranim sadržajem, cisterne (c) prazne. 50 000 : 1.

Fig. 6. Young chloroplasts from an 8-day-old callus with small grana, plastoglobules and ribosomes in the stroma. 40,000 : 1. Inset: Cisternae extending from the plastid membrane towards the thylakoids. 80,000 : 1.

Sl. 6. Mladi kloroplasti iz 8 dana starog kalusa s malenim granama, plastoglobulima i ribosomima u stromi. 40 000 : 1. Umetak: Cisterne se protežu od membrane plastida prema tilakoidima. 80 000 : 1.

Fig. 7. Young chloroplast from an 11-day-old callus with well developed grana. 36,000 : 1.

Sl. 7. Mladi kloroplast iz 11 dana starog kalusa s dobro razvijenim granama. 36 000 : 1.

Fig. 8. Chromoplast from an 11-day-old callus lying over the root cortex. Large carotene crystals (arrow) and a few plastoglobules. 27,600 : 1.

Sl. 8. Kromoplast iz 11 dana starog kalusa (iznad kore) s velikim karotenskim kristalima (strelica) i nekoliko plastoglobula. 27 600 : 1.

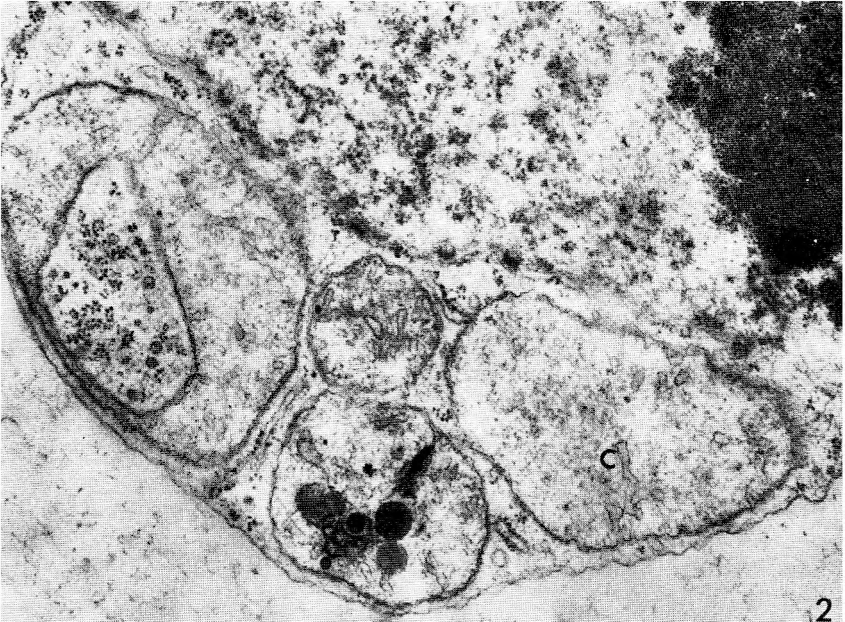
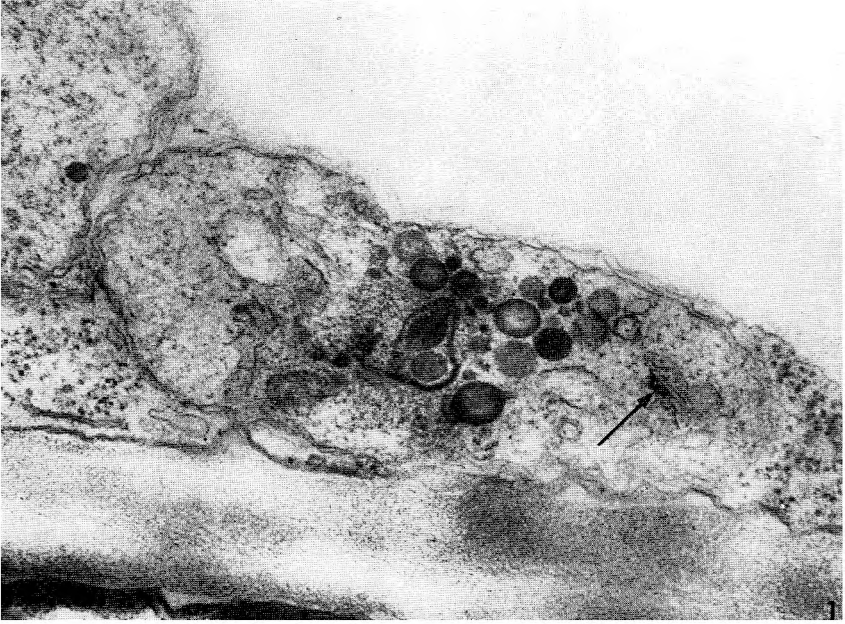


Fig. 1—2.

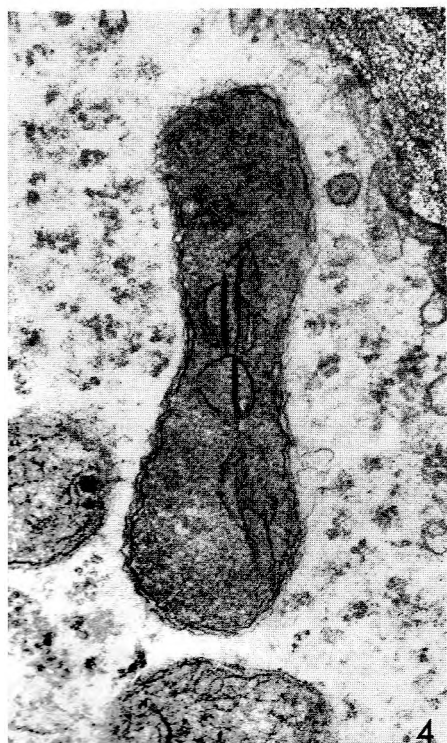
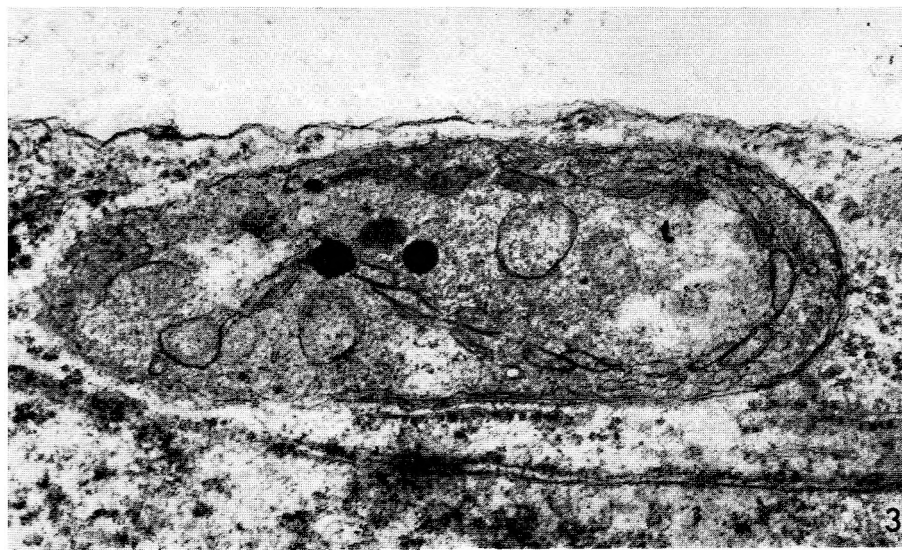
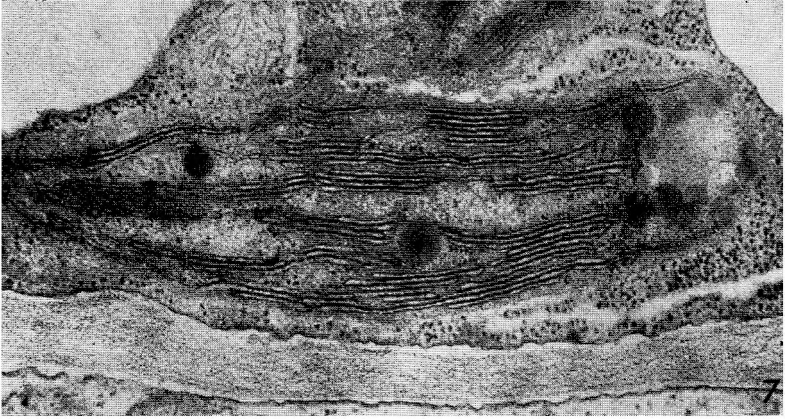


Fig. 3—5.



Fig. 6.



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Fig. 7—8.

The transformation of the plastids in callus cells starts with the dedifferentiation of chromoplasts, which divide and change into proplastids. These probably undergo several divisions before differentiating finally into chloroplasts. This way of plastid transformation is somewhat different from that during the greening processes described recently in intact carrot roots (Grönegress 1971) and in orange and pumpkin fruits (Thomson, Lewis and Coggins 1967, Devidé and Ljubešić 1972), in which chromoplasts develop to chloroplasts directly, i.e. without any preceding division.

Summary

Ultrastructural changes, appearing during the transformation of the plastids in young callus cells of carrot roots, are described. Chromoplasts divide, and during these divisions transform successively into proplastids, which after a few days of exposure to light differentiate into chloroplasts. These plastid transformations do not seem to be dependent on the composition of the nutrient medium on which the root slices are kept.

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ZUSAMMENFASSUNG

PLASTIDEN-UMWANDLUNGEN IM JUNGEN KALLUS DER KAROTTE

Mercedes Wrischer

(Institut »Ruder Bošković«, Zagreb)

Veränderungen in der Feinstruktur der Plastiden in Kalluszellen der Karotte wurden untersucht. Die Chromoplasten dieser Zellen teilen und wandeln sich sukzessiv zu Proplastiden um, die sich, nach entsprechender Belichtung, weiter zu Chloroplasten entwickeln. Diese Plastiden-Umwandlungen scheinen von der Zusammensetzung des Mediums weitgehend unabhängig zu sein.

SADRŽAJ

PRETVORBE PLASTIDA U MLADOM KALUSU MRKVE

Mercedes Wrischer

(Institut »Ruder Bošković«, Zagreb)

Istražene su promjene u finoj građi plastida u kalusnim stanicama mrkve. Kromoplasti kalusnih stanica dijele se i sukcesivno pretvaraju u proplastide, koji se nakon nekoliko dana osvjetljavanja razviju dalje u kloroplaste. Procesi plastidnih pretvorbi u kalusu, čini se, ne ovise o sastavu medija na kojem se komadi korijena uzgajaju.

Dr Mercedes Wrischer
Institut »Ruder Bošković«
Bijenička 54, p. p. 1016
41001 Zagreb (Jugoslavija)