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Ultrastructural Study on Differentiation of Plastid in *Panax ginseng* Root Tip

Byung-Kap Jeong and Woo-Kap Kim*
Department of Biology, Kosin University, Pusan 606-080, Korea
**Department of Biology, Korea University, Seoul 136-701, Korea*
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Abstract□The ultrastructural changes and differentiation mechanism of chromoplasts and leucoplasts from proplastids in root tip cells of *Panax ginseng* seedlings were studied with transmission electron microscope. Initial cells have so many proplastids with a few osmiophilic droplets and a lot of mitochondria at early stage of germination, therefore electron density of cytoplasm is generally higher than that of the other cells just like periblem, plerome and root cap. Proplastids are observed in the initial cells, but only leucoplasts appeared in the central root cap cells. Because root cap cells are derived ultimately from initial cells, the cell organells in the root cap cells are directly related by those of initial cells. This result postulates that leucoplast is diferentiated from proplastid, and this is the same with other's concepts. On the contrary, the precise observations of chromoplast with crystalline inclusions in the peripheral root cap cells can conclude the direct pathway of chromoplast development from proplastid. Because of the differences of these result from those of other experiments, new scheme of plastid development, direct differentiation of chromoplast from proplastid, can be postulated. And this is the originality of this research on the differentiation of plastids.

Key words□Ultrastructural changes, differentiation, *Panax ginseng*, plastids, root tip

Introduction

Chloroplasts are generally known to be developed from proplastids in plant leaf, but in some cases, the reversion of chloroplast into proplastid was reported by several authors.[1,4] Schimper[5] stated that chromoplasts, lecucoplasts and chloroplasts are developed from one another and gradually this concept gained general acceptance.[6] However Frey-Wyssling *et al.*[7] questioned this concept and they proposed the following scheme for plastid development.

Proplastid → Leucoplast → Chloroplast → Chromoplast

They suggested that development was in one direction, and that there was no reversion of chromoplast to chloroplast, or chloroplast to leucoplast. Thomson *et al.*[1] reported that the regreening of Valencia oranges(*Citrus sinensis* L.) is partly influenced by temperature, nutrition and potassium gibberellate. The regreening of oranges from maximum orange color was induced by two different mechanisms. One, the chromoplasts revert to chloroplast which would support Schimper's contention; or the other, new chloroplasts are formed in some manner[8] which would be support for the concept of Frey-Wyssling *et al.*[7]

The plastid is probably the best named of all cell organelles for the name indicates the plasticity of both its structure and its function. Furthermore, many forms of plastids, both green and nongreen, are developmentally interrelated.[9] Whatley[10,11] proposed a model for the interrelated pathway of plastid development. In this model she has indentified seven stages in plastid development; eoplast, amyloplast, amoeboid plastid, pregranal plastid, mature chloroplast, senescent chloroplast nd gerontoplast.

Thomson and Whatley[12] concluded that the development of plastid is influenced by genetic factors and metabolic conditions, but the development of crystalline chromoplast from proplastid, leucoplast,

chromoplast was early postulated by Spurr and Harris.[2]

Therefore, this study of the ultrastructural and developmental changes of plastid during germination of *Panax ginseng* was undertaken to clarify which of the above theories was valid in the differentiation of plastid, especially that of chromplast in the ginseng seedlings. And the possibility of the development of chromoplast directly from proplastid was examined by electron microscopy.

Materials and Methods

Fully ripened red fruits of 4-year old ginseng(*Panax ginseng* C.A. Meyer) were collected from ginseng paddy at Korea Institute of Ginseng and Tobacco in Daejeon. The sarcocarps of fruits were removed and seeds were mixed with sands at the ratio of 1 to 5 to get dehisced seed. The dehisced seeds were sown on the moist filter paper in petri dishes and kept in the dark at 4 ℃. After two monts, seeds with the primary root about 1 cm long were transfered to the fixative and then 3 mm length from tip was cut off under the dissecting microscope. The tips were fixed for 2 hours in paraformaldehyde-glutaraldehyde(phosphate buffer, pH 6.8, Karnovsky)[13] at 4 ℃ and then post fixed in 2% osmium tetroxide for 2 hours. After dehydration, root tips were embedded in Epon-Araldite mixture and the median longitudinal ultrathin sections were made using Sorvall Porter Blum MT-2 and LKB-V ultramicorotomes. Thin sections were stained with uranyl acetate and lead citrate[14] and observed under the JEM-100CX II(100 KV) transmission electron microscope.

Results

Initial cells in the quiescent center have a large nucleus with one prominent nucleolus and a little heterochromatin scattered in the nucleoplasm. The cytoplasm is full of mitochondria, ribosomes, small vacuoles or vesicles and a lot of plastids, but endomembranaceous cell organells, such as endoplasmic reticulum and Golgi complex, are not well differentiated in the initial cells compairing to the peripheral root cap cells(Fig. 1). Proplastids are globular or rod-shaped of 1.5-2.7 μm in length. The prolamellar bodies or fibrilar sturcture are scarcely developed at early stages of plastid differentiation and osmiophilic droplets(arrows) are well observed in the matrix of proplastids(Fig. 2).

Mitochondria show a little differentiation of cristae and, vaculoes appear at the periphery of cytoplasm(Fig. 1, 2). Osmiophilic droplets(Fig. 3 arrows) congregated in the central portion of proplastid are characteristically observed. The cell wall between initial cells has no plasmodesmata and has no winding shape.

Central root cap cells(Fig. 4) have a lot of leucoplasts with starch grains. Osmiophilic droplets are also observed in the leucoplast, but the congregation of them are not appeared compairing to the proplastids in the initial cells. Most of the cytoplasm show no difference from those of the initial cells. The leucoplasts in the central root cap cells show no distinctive features comapiring to the neighbouring periblem cells and the other general storage tissues(Fig. 4).

In peripheral root cap cells, large vaculoes with fibrilar materials appeared in the cytoplasm. The vaculoles thought to be increased in size as a results of fusion. Besides the increase in vacuolar size, other cytological changes, such as appearance of fibrilar materials and crystalline inclusions, also become evident. Especially mitochondria are not observed in the cytoplasm and small vacuoles, on the contray, are greatly increased in number. Irregular shaped crystalline inclusions are characteristically observed in the vacuole of peripheral root cap cells. Starch grains start to disappear within the plastids and, chromoplasts are begin to differentiated from the other plastids(Fig. 5).

Chromoplast in the peripheral root cap cells is surrounded by distinct double membrane and, one crystalline inclusion in each chromoplast is characteristically observed. Observations of one hundred or more sections revealed that no one chromoplast has more than two crystalline inclusions. The hexagonal shape of crystalline inclusion is a common occurance(Fig. 6). Osmiophilic droplets adjacent to the crystalline inclusion are particulary observed in the elongated chromoplast(Fig. 7).

The lattice pattern of the crystals varies in the

different views because of the different orientation of the lattice with reference to the plane of section. The lattice pattern may be that of paralled lines (Fig. 8) or of two sets of lines crossing each other at various angle(Fig. 6). Crossing of lines at right angle to one another also was recorded but is not represented in the selected illustrations. The planes of crystallization are not different from the other parts of the same chromoplast, which was reported by Thomson and Whatley.[12] The gap between lattice is about 5 nm and the thickness of lattice is about 3 nm. High magnification of electronmicrograph shows somewhat regular orientiations of lattice pattern of crystalline inclusions of chromoplast.

Discussion

Only three kinds of plastid(proplatid, leucoplast, chromoplast) were reported in the root tip and root cap at frequences of 20 to 40 per cell by Possingham.[15] Proplastids are commonly found in the tips of dark-grown roots, and these form chlorophyll may become chloroplasts when exposed to light. In addition to the proplastids and chloroplast, central root cap cells contain amyloplasts differetiated from proplastid or from chloroplast as well.[16]

On the contrary, chromoplasts are plastids which lack chlorophyll but which accumulate carotenoids. They provide several colors in many plant organs as well as roots. Chromoplasts can have many different shapes and often appear to be amoeboid.[6] Possingham[15] classified into four groups of chromoplast according to the form in which the carotenoids taken; 1. globular, those chromoplasts concentrated with the carotenoids in plastoglobuli; 2. tubular, those whose main structural characteristic is the presence of numerous tubules; 3. membranous, those which contain multiple arrays of membrane; 4. crystalline, those in which the carotrnoids are organized in the form of crystals.

During chromoplast development it is clear that degradation of photosynthetic membranes can take place at the same time as the synthesis of such new structure as plastoglobuli, crystals, tubules and membrances.[12]

The root cap cells of ginseng are differentiated from dermartocalyptrogen cells which were derived from the initial cells in the quiescent center.[16] Proplastids, leucoplasts and chromoplasts are all observed in root tip. But only proplastid is observed in the initial cell, and only leucoplast is observed in the central root cap cells, and only chromoplast is observed in the peripheral root cap cells. This result postulate that leucoplast is differentiated from proplastid, and this pathway of plastid development is the same aspect with Schimper[5] and Frey-Wyssling *et al.*[7]

On the contrary, only chromoplast observations in the peripheral root cap cells can raise new questions about developmental scheme of plastid. Therefore we think that the differentiation of chromoplast directly from proplastid can be taken place in the peripheral root cap cells of ginseng seedlings. And this conclusion can be supported by Spurr and Harris.[2] They suggested that crystalline chromoplast can be developed from proplastid, amyloplast, chloroplast.

On the component of crystalline inclustions, Walles and Hudak[17] reported that the crystals were composed primarily of β-caroten or lycopene. These materials were formed within or in association with the locules of thylakoid membrances. Esau[18] reported the diverse shape and formation process of crystalline inclusions in the mesophyll cells of spinach leaf.

In this experiment the components of crystalline inclusions can not be identified by this normal electron microscopy, but the lattice pattern is parallel or crossing over with one another. High magnification on the appearance of crystalline lattice reveals that such parallel lattice are repeated at intervals of 8 nm, and in case of crossing lattice at right angle and at acute can be observed in this expermient. Because of the composition of crystals are known as proteinaceous materials by some author, more detailed study must by required on the crystalline inclusions of chromoplast.

요 약

전 색소체로부터 분화되는 백색체와 잡색체의 미세구조적 변화와 발달과정을 규명하기 위하여 인삼 종자를 발아시킨 근단을 재료로 전자현미경을 이용하여 관찰하였다.

시원세포는 Osmiophilic droplet가 함유된 전색소체를 다수 가지고 있었으며 미토콘드리아가 많이 관찰되므로 주변의 다른 세포에 비해 전자밀도가 높게 나타났다. 백색체는 중앙부 근관세포에서만 관찰되었으며 이 세포들은 시원세포에서 분화되는 세포이므로 중앙부 근관세포의 백세포는 전색소체에서 분화되는 것으로 확인되었다.

단백질 결정체를 가지고 있는 잡색체는 측부 근관세포에서만 관찰되었으며 이 세포들도 역시 시원세포에서 분화되는 바 잡색체는 전색소체에서 직접 분화되는 것으로 생각된다. 따라서 인삼의 근관에서는 잡색체가 중간과정을 거치지 않고 전색소체서 곧바로 분화될 수 있다는 결론을 얻을 수 있었고 이것은 근단에서의 색소체 분화에 대한 새로운 모델이 제시될 수 있는 가능성을 나타낸다고 할 수 있다.

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Explanation of Figures

Fig. 1. Electron micrograph of initial cells in the quiescent center shows prominent nucleouls(NU) with nucleolar vacuole and a few heterochromatin in the central portion of nucleus(N). Proplastids(P) are rod shaped and have osmiophilic droplets(arrow). CW: Cell Wall, M: Mitochondria. ×13,000

Fig. 2. Cytoplasm of initial cell is full of mitochondria(M), proplastids(P) with osmiophilic droplets(arrows), fibrilar structures in the vacuole and a lot of free ribosomes. ×18,000

Fig. 3. Same electronmicrograph as Fig. 1 and 2, but the congregations of osmiophilic droplets(arrows) in the central matrix of proplastid(P) are characteristically observed at the periphery of nucleous(N). M: Mitochondria. ×13,600

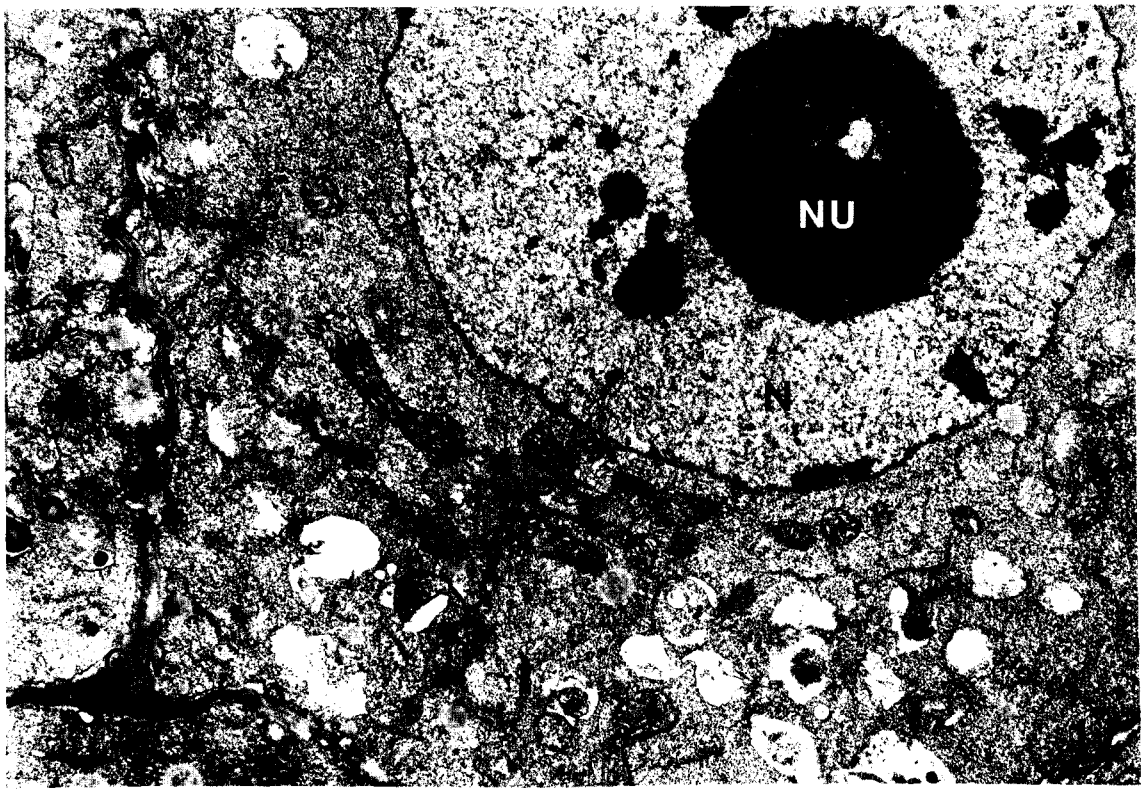
Fig. 4. No one proplastid appeared in the central root cap cells, but leucoplasts(L) containing both of the starch grains(S) and osmiophilic droplets appeared in them. These leucoplasts thoughts to be differentiated from proplastid. G: Golgi complex, M: Mitochondria, L: Lipid droplet. ×17,000

Fig. 5. Peripheral root cap cells have large vacuoles with irregular shaped crystalline inclusions(CR). Note the fibrilar structure in the vacuoles(V). CW: Cell Wall. ×34,000

Fig. 6. Chromoplast(C) in the peripheral root cap cell shows hexagonal shaped crystalline inclusion(CR) and prominent double membrane. The lattice pattern of crystalline inclusion is crossing one another with acute angle. ×54,000

Fig. 7. The chromoplast(C) of elongated shape is thought to be differentiated directly from proplastid which is well appeared in the initials cells. Note the osmiophilic droplet(arrow) and one crystal(CR) in each chromoplast. M: Mitochondria. ×36,000

Fig. 8. High magnification of single crystall in each chromoplast shows parallel pattern of lattice orientiations. ×220,000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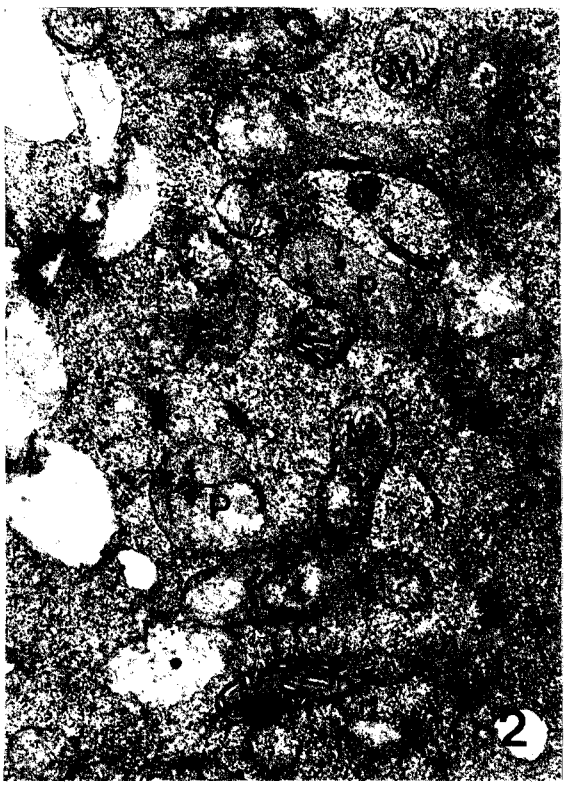
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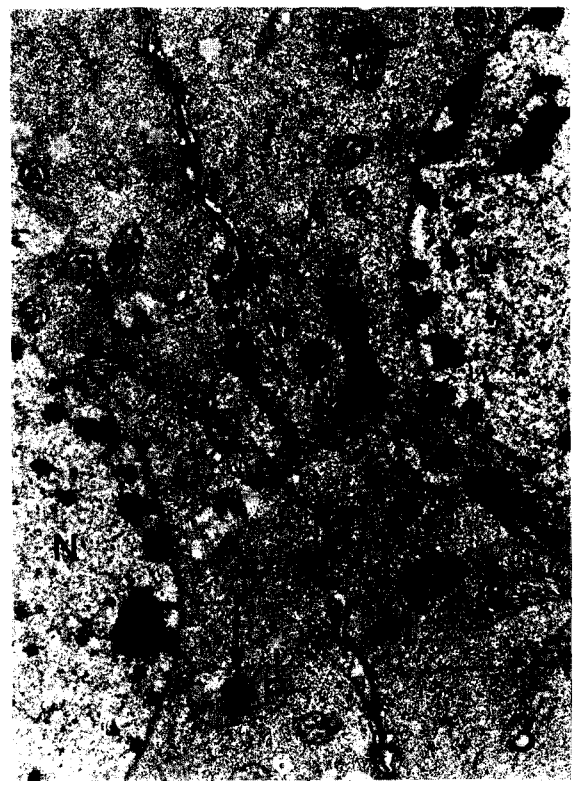
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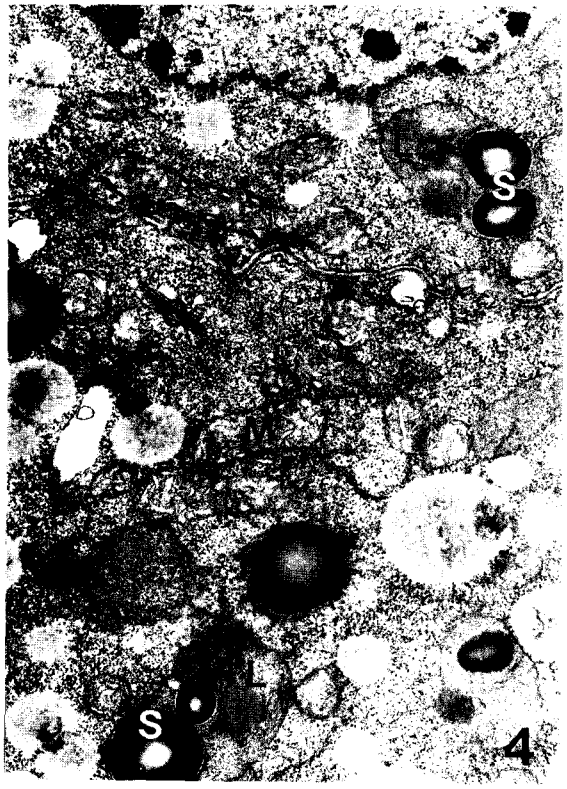
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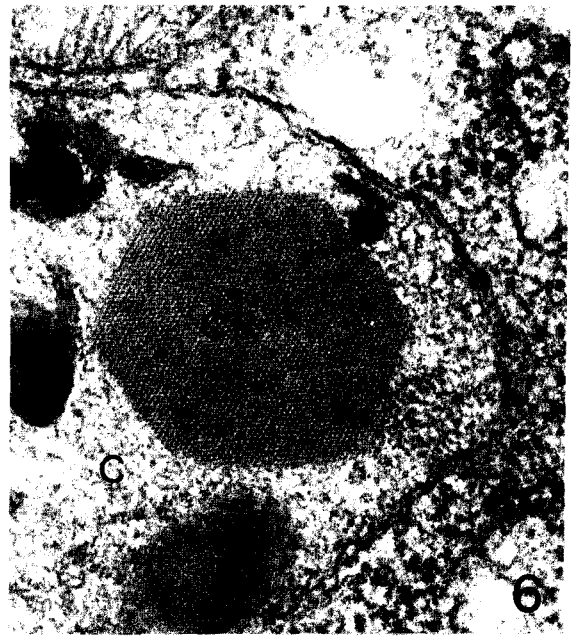
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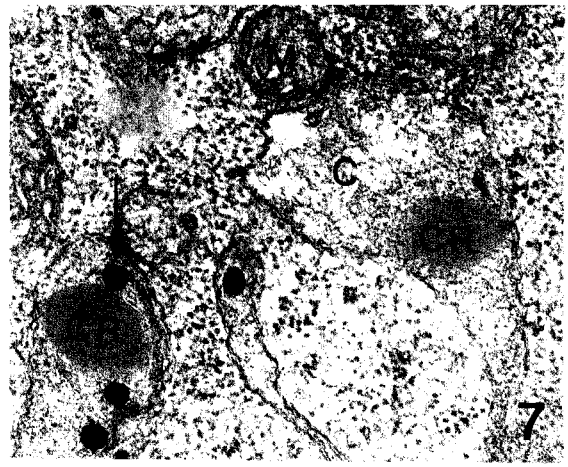
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