

The Electron Microscopic Investigation of Changes in Cytoplasmic Structures in Leaves of *Cucumis sativa* L. Infected with Cucumber Virus 4

IVAN HRŠEL

Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha*

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Změny cytoplasmatických struktur v listech okurky infikované Cucumber virus 4

Buňky listu okurky (*Cucumis sativa* L.) zdravé a infikované Cucumber virus 4 byly studovány za živa a fixované, pod světelným a elektronovým mikroskopem. Pod elektronovým mikroskopem byly zjištěny u infikovaných listů změny v struktuře ergastoplasmy, Golgiho aparátu, mitochondrií, útvarů s hustou osmiofilní matrix a chloroplastů. Chloroplasty jeví známky tukové degenerace, která postihuje především lamelární systémy gran a intergran. Slabě osmiofilní globule, hojně v poškozených chloroplastech, vznikají patrně degenerací gran. Chloroplasty infikovaných rostlin se podobají některým přechodovým stádiím plastidů zjištěným při tvorbě chromoplastů. Byla diskutována otázka lokalizace virů v buňce a degenerace.

Summary

Leaf cells of healthy cucumber and cucumber infected with Cucumber virus 4 were studied living and fixed, using optical and electron microscopy. Under the electron microscope the infected leaves displayed changes in the structure of the ergastoplasm, Golgi apparatus, mitochondria, bodies with an osmiophil matrix and chloroplasts. The chloroplasts exhibit symptoms of fatty degeneration which affects above all the lamellar systems of grana and intergrana. Weakly osmiophil globules, abundant in damaged chloroplasts, are probably formed through degeneration of grana. Chloroplasts of infected plants resemble some transitional stages of plastids observed during chromoplast formation. The problems of localization of viruses in the cell and degeneration are discussed.

* Address: Na ovičišti 2, Praha-Dejvice

Introduction

Changes in cytoplasmic structures of leaf cells of cucumber infected with Cucumber virus 4 had not hitherto been described in the literature. In a previous paper (BRČÁK and HRŠEL, 1961) the localization of virus inclusions was examined and an orientation observation of degenerated chloroplasts made. In the present paper the changes of cytoplasmic structures will be taken up in some detail.

Methods

Observation with optical microscope:

Free-hand sections of leaves, healthy and infected with the virus (BRČÁK and HRŠEL, 1961) were observed with optical and fluorescence microscope in Knop's solution. Chloroplasts of infected plants contain light-refractive granules and show a weakened red fluorescence when observed with a fluorescence microscope. Some cells exhibited no red fluorescence whatever. Leaves were also fixed with Zirkle's fixative and passed through tertiary butanol to paraffin. The paraffin sections were stained with iron haematoxylin.

The objects were fixed with buffered osmium tetroxide (pH 7.2) and embedded in methacrylate to be used for electron microscopy. Some thicker sections were placed on a glass slide, stained with iron haematoxylin and observed under a light microscope. A comparison of the methods of optical and electron microscopy used may be seen in Fig. 2, 3.

Observation with electron microscope:

Small pieces of cucumber leaf about 1—2 mm. in size, using 8 cm. leaves of healthy and infected plants (BRČÁK, HRŠEL 1961) were fixed in buffered osmium tetroxide (pH 7.2) and passed through alcohol to a mixture of butyl and methyl methacrylate (93 : 7). Polymerization was carried out at 60° C. Sections were prepared by the usual technique. The preparations were photographed on electron microscopes in the Laboratory for Electron Microscopy, Institute of Biology, Czechoslovak Academy of Sciences, Prague, in the Institute for Apparatus Engineering, Czechoslovak Academy of Sciences, Brno, and on the RCA microscope of the Laboratory for Electron Microscopy and Experimental Morphology, Czechoslovak Academy of Sciences, Prague.

Results

E r g a s t o p l a s m

Control plant: Cytoplasm contains freely dispersed Palade granules (Fig. 1a). Sometimes the Palade granules are arranged in spiral systems (Fig. 4a, b) as described by STRUGGER (1957). Cytoplasm also contains isolated vesicular elements (Fig. 4b).

Infected plant: Palade granules are either arranged in the spiral systems (Fig. 5b, 7) or in irregular clusters. Occasionally numerous small and larger vesicular elements can be observed (Fig. 1b, 4c). Cytoplasm also contains irregular masses of lamellar structures (Fig. 13) apparently formed on decomposition of chloroplasts.

G o l g i a p p a r a t u s

Control plant: The Golgi apparatus has the same appearance as in other objects studied, i.e. it is composed of a bundle of 5 or 6 membrane-cisternae terminated with light vesicles and with vesicles in the proximity (Fig. 1a).

Infected plant: Infected plants were found to contain the Golgi apparatus with clear vesicles and osmiophil globules either in connection with membranes or in the immediate proximity of the latter (Fig. 1b). Fig. 12 shows a Golgi apparatus with strongly osmiophil granules and globules, with hypertrophic clear vesicles and a large globule of weakly osmiophil material. The Golgi apparatus is very close to a virus inclusion.

Mitochondria

Control plant: Mitochondria of the control plant have the same appearance as in objects studied by other authors (Fig. 4a).

Infected plant: Mitochondria either have the same appearance as in healthy plants (Fig. 5a, 7) or are vacuolized (Fig. 5a). In one case a structure of the size of a mitochondrion was observed to enclose an irregular osmiophil mass (fatty degeneration) (Fig. 10a). In addition to mitochondria the infected plants were often observed to contain bodies, the common characteristic of which is a dense osmiophil matrix. In some of them a fine internal structure can be observed. Similar bodies were described by a number of authors examining healthy plants. Views concerning their function are not unified. They are considered to be sphaerosomes, young mitochondria, young proplastids or vacuole products. We may be dealing here with bodies of different origin. Bodies shown in Fig. 4c resemble the sphaerosomes described by PERNER (1958), the body (A) in Fig. 5b is similar to that described by GENEVES et al. (1958) (lysosome-like bodies). These bodies sometimes displayed vacuolization in the infected plants (Fig. 4c).

Chloroplasts

Control plant: The mesophyll cells of control plant leaves (Fig. 6) contain chloroplasts with finely granular stroma, in which lamellar systems of grana and intergrana with several strong osmiophil globules (O) about $0.15\ \mu$ in size are incorporated, such as were described in chloroplasts of different plants. The stroma also contained starch granules (S) of different size. The lamellar systems show fine strongly osmiophil granules (G) about $0.025\ \mu$ in size. These are especially concentrated at the edges of the grana.

Infected plant: The infected plants were found to contain chloroplasts in different degrees of disintegration. We find young chloroplasts (Fig. 7) containing lamellae of the grana and intergrana. The grana lamellae (D) are frequently indistinct, probably due to damage. Young chloroplasts contain a finely granular stroma with incorporated starch grains (S) and strong osmiophil globules (O) ($0.25\ \mu$). Older chloroplasts (Fig. 8, 9, 10, 11) contain, with the exception shown in Fig. 12, mostly reduced stroma. The remaining granular substance of the stroma occurs in clusters. The peristromium is frequently markedly extended (Fig. 8b, 9, 10b). Lamellar systems of the grana and intergrana are reduced in the less degenerated chloroplasts (Fig. 8, 9, 10b) and disintegrate in the heavily degenerated ones (Fig. 10a, 11). The number of large osmiophil globules $0.3\text{--}0.7\ \mu$ in size is striking (Fig. 8, 9, 10, 11, 12) the globules not being so intensely osmiophil as those occurring in chloroplasts of healthy plant leaves (Fig. 6). In addition to these weakly osmiophil globules a great number of enlarged strongly osmiophil granules (G) -about 0.02 to

0.05 μ in size can be observed, located on the lamellae of the intergrana and especially at the edges of grana. The enlarged osmiophil granules shown on the lamellar systems may represent a concomitant phenomenon during decomposition of lamellar systems. Fig. 11 and 12 show different phases of grana degeneration. In Fig. 12 the lamellar systems are altogether lacking, but groups of osmiophil granules (G) concentrated about the mass of former grana can be observed there (cf. with Fig. 8a and 9 where the osmiophil granules (G) are concentrated about still preserved grana). The osmiophil granules and the mass of former grana give rise to weakly osmiophil globules (O). The terminal phase of chloroplast decomposition is not the same in all objects. Fig. 9 shows the remains of a chloroplast with a large irregular osmiophil mass in the centre. The chloroplast contains a preserved limiting membrane and residues of lamellar systems. Fig. 10a shows a chloroplast with large globules as well as small osmiophil granules and residues of lamellar systems of the intergrana. Fig. 12 shows a chloroplast with a preserved limiting membrane, with dense stroma and with the previously described globules without lamellar systems. Fig. 13 shows irregular clusters of lamellar systems and large weakly osmiophil globules. In the proximity of these irregular clusters a virus inclusion (VI) can be observed. The lamellar clusters contain rodshaped structures (R) which can also be observed in chloroplasts in Fig. 10b. They were probably formed by splitting (S) of grana lamellae (Fig. 9).

Discussion

Cytoplasmic structures. The principal cytoplasmic structures, i.e. ergastoplasm with endoplasmic reticulum, Golgi apparatus, mitochondria, structures with an osmiophil matrix and chloroplasts in leaves infected with Cucumber virus 4 exhibit changes as compared with control plants. The ergastoplasm shows increased vesiculation (Fig. 1b, 4c, 5a) and fusion of Palade granules, Golgi apparatus reveals osmiophil globules and hypertrophic vesicles (Fig. 1b, 12) as atypical symptoms. The aberration of the Golgi apparatus of plant cells was taken up by WHALEY et al. (1959) who described hypertrophy of the cisternae of the Golgi apparatus in peripheral cells of maize root tips. WRISCHER (1960) describes deformed Golgi apparatus in the root tip of *Alium cepa* following irradiation with X-rays. ROSSNER (1960) applied streptomycin to the same object and observed a splitting of the bundles of lamellae. In animal cells, too, the pathology of the Golgi apparatus has received little attention. Changes in the Golgi apparatus of mammary gland carcinoma cells have been described when the virus particles are in direct contact with the Golgi apparatus. Hypertrophy of the Golgi apparatus is known from tumour cells (references in OBERLING 1959).

Mitochondria are vacuolized only in some cases of infected leaves, like the bodies with osmiophil matrix. Animal tissues also contain bodies with a dense osmiophil matrix (described as dense bodies, promitochondria, lysosomes etc.; references in OBERLING 1959, and ROUILLER 1960). They occur frequently in young or regenerating tissues. In the plant tissue their role in regenerative

processes might also be considered as in the infected cells of cucumber these bodies were very often observed. Also an isolated finding of a very young chloroplast in the infected cell should support the possibility of regenerative processes taking place in the infected cell.

Chloroplasts almost regularly display signs of degeneration which belong to the most pronounced symptoms of plant virosis. Changes of chloroplasts have also been described in leaves infected with tobacco mosaic virus, the changes being first characterized by vesiculation and then by general vacuolization of the chloroplasts (SHALLA 1959, GEROLA et al. 1960, personal observations made on leaves infected with TMV). Leaves of maize infected with the "raya blanca" virus displayed a loss of osmiophil globules from the chloroplasts and a general disintegration of the grana and intergrana (HEROLD et al. 1960). Chloroplasts of leaves infected with the Cucumber virus 4 exhibited changes resembling fatty degeneration. The large number of osmiophil granules in the lamellar systems of the grana and intergrana and large weakly osmiophil globules apparently formed by degradation of grana lamellae point to a gradual degradation of the lamellar systems when lipid components are released. A similar phenomenon was described by FREY-WYSSLING and KREUTZER (1958) in the electron microscopical study of the development of some chromoplasts. The authors distinguish between three types of chromoplasts: 1. Chromoplasts with carotenoid crystals, 2. chromoplasts with filamentous structure, 3. chromoplasts with globular structure. FREY-WYSSLING and KREUTZER (1958) studied the formation of chromoplasts with globular structure of perianth epidermis of *Ranunculus repens* L., LANCE-NOUGAREDE observed the same in flowers of *Chrysanthemum segetum* L. (1960). These authors found that chromoplasts are formed on degeneration of green chloroplasts. The development of chromoplasts is explained by FREY-WYSSLING and KREUTZER (1958) as a kind of lipophanerosis and the chromoplasts formed as degenerated plastids without their own metabolism, with only a passive function. The transitional stages in the development of globular chromoplasts, i.e. the stages between chloroplast and chromoplast containing reduced lamellar systems and multiplied osmiophil globules resemble degenerated chloroplasts of leaves infected with the Cucumber virus 4. In degenerated chloroplasts of leaves infected with this virus the lipophanerotic process ends with a variegated disintegration of chloroplasts (Fig. 9, 13) whereas the formation of chromoplasts is organized and ends with a fully developed chromoplast. The disintegration of chloroplasts of cells infected with the Cucumber virus 4 might be called pathological degeneration while the formation of chromoplasts would be a physiological degeneration.

Localization of virus particles

Virus inclusions were found only in the ergastoplasm and not in the chloroplasts. No individual virus particles were observed in the cells. Their minute size and low stainability represent an obstacle in their detection. Similar findings were made by other authors. HEROLD et al. (1960) studied virus particles of considerable size which might be possibly detected even if isolated in the ergastoplasm and chloroplasts. The finding of virus inclusions in the ergastoplasm only indicates that the degeneration of chloroplasts is probably

not a primary phenomenon but rather a reflection of the total damage of the cell due to the virus which in a differentiated cell is displayed in changes in the most active parts of the cell, i.e., in chloroplasts. We are apparently dealing here with a close relationship between ergastoplasm and chloroplasts. Virosis of this type does not appear to be a disease with chloroplasts as the centre as might be inferred from macroscopic phenomena (yellowing of leaves) and from light microscopy (changes in chloroplast shape). It appears that the centre lies in the ergastoplasm.

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И. Гршел (Институт экспериментальной ботаники Чехословацкой академии наук, Прага)

Изменения цитоплазматических структур в листьях огурцов, инфицированных *Cucumber virus 4*

Изучались клетки листьев огурцов, здоровых и зараженных *Cucumber virus 4* в живом состоянии, фиксированные, под оптическим и электронным микроскопом. Общее состояние фиксированных клеток не показывает при сравнении всех методов никаких важных морфологических изменений. Под электронным микроскопом установлены у инфицированных листьев изменения в структуре эргастоплазмы, аппарата Гольджи, митохондрий и хлоропластов. Хлоропласты проявляют признаки дегенерации липидов, которая захватывает прежде всего ламеллярные системы гран и интергран. Слабо осмофильные глобулы, частые в поврежденных хлоропластах, возникают, повидимому, вследствие дегенерации гран. Хлоропласты инфицированных листьев похожи на некоторые переходные стадии установленные при образовании хромопластов. Дискутируется вопрос о сущности изменений цитоплазматических структур, процессов регенерации, локализации вирусов в клетке и дегенерациях физиологической и патологической.

Fig. 2, 3. Mesophyll cells of cucumber leaf treated by methods of light and electron microscopy. Letters without prime (below) describe cells of healthy leaf, those with a prime (above) leaves infected with *Cucumber virus 4*. a, a' — free hand-cut sections observed in vivo (magn. 750×). b, b' — paraffin sections of objects fixed with Zirkle's fixative. Stained with iron haematoxylin (magn. 1800×). c, c' — methacrylate sections of objects fixed with buffered osmium tetroxide. Stained with iron haematoxylin (magn. 1800×). d, d' — ultrathin sections of the same object in an electron microscope treated as in c, c'. No staining. V = virus inclusion (direct magn. 3500×).