Cytological changes in phloem parenchyma cells of *Solanum rostratum* (Dunal.) related to the replication of potato virus M (PVM)

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Abstract

The first cytological symptom of infection of phloem parenchyma cells by potato virus M is the formation of clusters of endoplasmic reticulum cisterns in a cytoplasm containing numerous ribosomes. Randomly distributed PVM particles are found in the vicinity of the cisterns. As the infection progresses, inclusions made up of regularly arranged particles of PVM are formed. The cytoplasm of the cells becomes electron transparent because the ER cisterns disappear. Masses of homogenous substances containing single PVM particles appear. There are two types of deposits in the inclusions containing PVM virus particles — additionally coated particles and tubules.

Key words: potato virus M, cytological picture of replication, viral inclusions

INTRODUCTION

Solanum rostratum reacts systemically to infection by potato virus M (PVM). Necrosis occurs on the leaves (Rudzińska-Langwald 1986). The viral particles are not limited to the necroses and surrounding tissues. They are also observed in the cells of vascular tissues — phloem and xylem, not only in leaves but also in stems (Rudzińska-Langwald 1988).

The infection of a plant by a virus is a complex process. On one hand, the pathogene infecting the plant takes over its metabolism for its own needs. Processes involved in the multiplication of a virus cause specific changes in the structure of the cell in which they take place. On the other hand, all of these changes evoke several reactions in the cell, indirectly related to the propagation

of the virus in them, eg. in the case of *S. rostratum*, necroses. It seems that the phloem parenchyma cells do not undergo degeneration to any significant extent and rarely necrotise. Determination of the time-lapse between inoculation and the appearance of virus particles in these cells (Rudzińska-Langwald in press) has made it possible to trace the temporal relationship between the particular cytological changes. Phloem parenchyma cells are convenient to observe the cytological changes caused directly by the replication of PVM as well as by the large amount of particles produced in them.

This study describes only those cytological changes which seem to be involved in the propagation of PVM in cells and in the storage of its particles.

MATERIAL AND METHODS

Three-leafed *Solanum rostratum* seedlings were inoculated mechanically with an inoculum from *Lycopersicon esculentum* cv. Najwcześniejszy plants that were infected with potato virus M isolate 55a (PVM). The lowest, developed leaf was inoculated. Fragments of petioles were sampled 10, 13, 15 days and 3 weeks after inoculation.

Petiole samples were fixed in 3% glutaraldehyde and 4% paraformaldehyde in cacodylate buffer, pH 7.2 for 3 hours at room temperature. This was followed by post-fixation in 1% osmium tetroxide for 2 hours at 4°C. The material was dehydrated in rinsing concentration of ethyl alcohol (10-70%) acetone (70-100%) and propylene oxide. The samples were embedded in Epon 812 and sectioned on an LKB ultramicrotome, stained with uranyl acetate and lead citrate. The material was observed in a JEM 100 C electron microscope.

In order to label the capsid proteins of the potato mosaic virus, tissue samples were fixed by the method given above. Ultrathin sections were collected on nickel grids, digested in 10% hydrogen peroxide for 5 min and sodium periodate 5 min or 10% hydrogen peroxide for 10 min. After rinsing, the grids were transferred to normal goat serum in 0.05 M Tris buffer, pH 7.6 (1:3) containing 0.2% BSA for 15 min., then to a specific serum, anti-PVM serum produced by the Potato Institute's Gdańsk Laboratory — in Tris buffer as above, diluted 1:1, 1:10 for 1 hour at 20°C. After this time, the sections were washed in 0.02 M Tris buffer containing 0.1% BSA at pH 8.2 and placed in anti-rabbit globulin goat serum conjugated with gold colloid (Jensen) in Tris buffer, pH 8.2 (1:30) containing BSA, for 30 min. After washing with Tris buffer (pH 8.2) and redistilled water, the grids were contrasted with uranyl acetate and lead citrate.

RESULTS

The first changes related to infection of petiole phloem parenchyma became visible 10 days after inoculation. Characteristic clusters of endoplasmic reticulum cisterns began to appear in the parenchyma cells of the phloem

(Figs. 1-3). These cisterns were scattered throughout the cytoplasm. They were short and had a dark, homogenous content. The majority belonged to the rough endoplasmic reticulum. Polysomes, occasionally dictyosomes and rare vesicles were observed in the basic cytoplasm in the vicinity of the cisterns. Most of these vesicles were electron empty. Only sporadically did vesicles with a fibrillar content appear (Fig. 4).

The cytoplasm described above contained PVM particles scattered randomly in it. Their size corresponded to that given in literature (Brandes et al. 1959).

The area of the described inclusions was, for all practical purposes, very weakly labelled with colloidal gold, and the arrangement of the gold globules did not suggest the presence of viral capsid proteins.

Three days later, that is, 13 days after inoculation, various-sized clusters of viral particles appeared in the cytoplasm and formed bunches of loosely arranged virions (Figs. 5-7). The cytoplasm around and between the particles was devoid of organelles, whereas in the neighbourhood of the clusters of particles, concretions of electron dense material in the form of long strips (Figs. 5 and 6) appeared. Single PVM particles were well-visible inside of these concretions because each of them was surrounded by a lighter area.

PVM particles which occurred in greater clusters became well-labelled with colloidal gold (Figs. 8, 10). No other areas of the cell became labelled.

Three weeks after inoculation, phloem parenchyma contained large clusters of viral particles which formed paracrystalline structures, in which each particle was surrounded by six equally distant virions (Fig. 8).

Cross sections of the potato virus M clusters showed the structure of its particles (Fig. 7). The PVM particle was composed of an internal, dark ring and a somewhat lighter external part. The diameter of the internal ring was 4.0-4.5 nm. In all, the thickness of the particles measured on the electron micrographs was 12-13 nm, which is in agreement with the value given by Brandes et al. (1959).

Longitudinal sections through the virus clusters revealed a parallel arrangement of particles having an indistinct internal structure. These clusters were the same length as PVM particles (Fig. 10) and occasionally formed "whirlpool" structures (Fig. 11).

Deposits were found in the clusters of PVM particles. On cross sections these deposits resembled virus particles surrounded by an additional coat (Fig. 9). The diameter of these structures was 28-30 nm, and the diameter of their midpart, as well as its structure correspond to a PVM particle. These structures were found in small numbers in the cells and only after a longer period following inoculation (3 weeks). The other type of deposit in the viral particle clusters were tubules. They were well visible on the cross sections. They had a slightly larger diameter than a PVM particle and were characterised by an electron transparent interior (Figs. 12-14). These tubules were not labelled by colloidal gold.

The earliest stages of multiplication of PVM in phloem parenchyma cells. Fig. 1. Long PVM particles (arrows) and rough endoplasmic reticulum cisterns (ER) with a dark content are visible in the cytoplasm. 27 000 $\times$. Fig. 2. A fragment of a cell showing a cluster of rough endoplasmic reticulum and the scattered virus particles. 26 000 $\times$. Fig. 3. A grid labelled for the capsid viral proteins. Few globules of colloidal gold are visible in the cytoplasm among endoplasmic reticulum cisterns, polysomes (arrows) and PVM particles. 54 000 $\times$. Fig. 4. A part of a cell containing vesicles (asterisks) 66 000 $\times$

The second stage of cytological changes related to the multiplication of PVM. Fig. 5. Inclusion of PVM particles (v) cross sectioned (upper part of photograph). The arrangement of the particles is irregular. In the upper part of the photograph there is a dark, homogenous structure in which single viral particles are seen (arrow). The cytoplasm of these areas is devoid of ribosomes and endoplasmic reticulum cisterns. $70\,000\times$. Fig. 6. The deposits accompanying the virus inclusions are elongated, branched masses of a homogenous substance. Virus particles are seen in the deposit. $72\,000\times$. Fig. 7. A cross section through an inclusion of PVM particles. Each particle has a darker external ring and a lighter internal part. Two particles are encapsulated (arrows) $250\,000\times$.

Clusters of viral particles. Fig. 8. A fragment of an inclusion of PVM particles in paracrystalline arrangement. Each particle is surrounded by six, equally distant virions. Black globules of colloidal gold are visible over the inclusion — the grid was labelled for capsid proteins. 100 000 $\times$. Fig. 9. A fragment of a cell with a diagonally sectioned PVM inclusion (right side of photograph). On the left, the PVM particles are surrounded by an additional coat. 110 000 $\times$. Fig. 10. Longitudinal section through a PVM particle inclusion. The sample was labelled for capsid proteins — globules of colloidal gold are visible over the inclusion. 52 000 $\times$. Fig. 11. Virus inclusions in a whirlpool arrangement. 40 000 $\times$

PVM particle clusters along with tubules. Fig. 12. A cross section through a virus inclusion in which, in addition to viral particles, tubules are present. The gold colloid globules are visible only over the virus particles. $68\,000\times$. Fig. 13. A diagonal section through an inclusion containing PVM particles and tubules. Numerous gold globules over the virus particles, there is no labelling over the tubules. $44\,000\times$. Fig. 14. A longitudinal section through a PVM inclusion. The middle part of the inclusion containing tubules is not labelled with colloidal gold globules (arrow). $66\,000\times$

Thirteen, 15 and 21 days following inoculation, the majority of plant cells contained PVM clusters and, in addition, structures characteristic for the earliest stage of infection were also found. They were, however, increasingly rare and covered smaller areas in the cell as the time from inoculation increased.

DISCUSSION

The first sign of infection of cells with potato virus M is the appearance of clusters of endoplasmic reticulum cisterns. In later stages, the areas of the cytoplasm involved in the infection are lacking ribosomes and endoplasmic reticulum. Similar cytological pictures and order of events were observed by Atkinson and Cooper (1976) and Brunt et al. (1976) in cells of *Nicotiana megalosiphon* infected with poplar mosaic virus, which belongs to the same virus group as PVM.

At the same time, no vesicles, so characteristic for many groups of plant viruses and considered to be the place of replication of viral RNA (Martelli and Russo 1984), were observed. No vesicles were found either for any other virus belonging to the carlavirus group (Francky et al. 1986).

The early stages, then, of virus multiplication occur in cytoplasm which is rich in ribosomes and endoplasmic reticulum. The replication process does not significantly alter the appearance of any organelles. The accumulation of capsid proteins outside of the PVM viral particles was not observed in the cells.

The later stages of infection were characterised by a cytoplasm that was poor in organelles. These stages seem to represent the successive stages of packing the viruses in the form of inclusions, the purpose of which is to store the ready viruses. Lack of labelling in the cytoplasm indicates that the entire pool of capsid proteins was used in the production of PVM particles.

The tubules observed to be part of the viral inclusions did not become labelled, and therefore did not contain capsid protein. Single tubules were observed in the PVM inclusions in *Lycopersicon chilense* cells (Rudzińska-Langwald 1981) and in the related potato S virus (PVS) (de Box and Waterreus 1971). It is very probable that these tubules naturally occur in the cells and have become trapped in the PVM inclusions.

Unfortunately, the other type of deposit (the coated viral particles) is more puzzling. It occurs in small amounts in the inclusions. Its coat is thin and therefore it is difficult to identify it with the methods used in this study. Similar coated particles were observed in potato virus S (PVS) (Brzezicka-Szymczyk 1982).

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*Zmiany cytologiczne w komórkach miękiszowych
łyka Solanum rostratum (Dunal.) związane z namnażaniem
wirusa M ziemniaka (PVM)*

Streszczenie

Pierwszym cytologicznym objawem porażenia komórek łyka wirusem M ziemniaka jest powstawanie skupisk cystern retikulum endoplazmatycznego w cytoplazmie bogatej w rybosomy. W sąsiedztwie cystern obserwuje się bezładnie ułożone cząstki PVM. W miarę rozwoju infekcji wytwarzają się inkluzje zbudowane z regularnie upakowanych cząstek PVM. Cytoplazma komórek staje się elektronoprzezierna gdyż zanikają cysterny retikulum endoplazmatycznego. Powstają masy homogennych substancji, w których tkwią pojedyncze cząstki PVM. W inkluzjach zbudowanych z cząstek PVM istnieją dwojakiego rodzaju wtrąty — dodatkowo opłaszczone cząstki oraz tubule.