Electron Microscopy of Maize Rough Dwarf Virus Assembly Sites in Maize. Cytochemical and Autoradiographic Observations

By MARIA BASSI AND M. AUGUSTA FAVALI
Centro di Microscopia Elettronica, Politecnico di Milano, and Istituto di Scienze Botaniche, Università di Milano, Milano, Italy

(Accepted 5 April 1972)

SUMMARY

The following experiments have been carried out: (1) enzymatic digestion with pronase and RNase, to study the nature of the cytoplasmic inclusions (viroplasm and cytoplasmic tubules) induced by the virus, and of the smaller virus particles as seen in the viroplasm; (2) autoradiographic experiments after [H³]-uridine incorporation, to detect the possible sites of virus synthesis and/or assembly.

Enzymatic digestion of maize leaf tumours caused by maize rough dwarf virus (MRDV) showed that the viroplasm and cytoplasmic tubules induced by the virus are composed mainly of protein, and that the small particles embedded in the viroplasm are 'naked' RNA particles, since they were promptly digested by RNase, while the complete particles were not.

Autoradiographic experiments demonstrated that [H³]-uridine was incorporated almost exclusively into the viroplasm containing virus RNA particles, while no incorporation was detected in the viroplasmic regions free of virus particles and in the mature virus particles as seen in the cytoplasm.

It is concluded that the viroplasm is most probably the site of assembly of MRDV.

INTRODUCTION

The fine structural alterations caused by maize rough dwarf virus (MRDV) in host cells have been described in both plant and animal hosts (Gerola et al. 1966; Gerola & Bassi, 1966; Lovisolo & Conti, 1966; Vidano, 1966). Although some phases in the developmental cycle of the virus have been described in the planthopper host (Vidano, 1970), little is known about the morphogenesis of the virus in the plant host, and the function and chemical composition of the cytoplasmic inclusions induced by the virus in the plant host-cells, i.e. viroplasm and cytoplasmic tubules.

In an attempt to elucidate some of these points, we have carried out experiments, including enzymatic digestion and electron microscopic autoradiography, on leaf vein tumours of maize plants experimentally infected with MRDV. The results are reported in the present paper.

METHODS

Inoculated plants. The maize plants (Zea mays L. cv Wisconsin 641 AA’), experimentally infected with MRDV, were kindly supplied by ‘Laboratorio di Fitovirologia Applicata del C.N.R.’, Torino. The virus was transmitted by the vector Laodelphax striatellus Fallén to plants in the coleoptile stage (Conti, 1966). At the time of sampling (20 days after inoculation), vein tumours were clearly visible on the maize leaves.
**Enzymatic digestions.** For enzymatic digestion, leaf vein tumours were fixed for 1 hr in 3% (v/v) glutaraldehyde in 0.1 M-phosphate buffer, pH 6.9. After thorough washing in buffer, the samples were dehydrated and embedded in glycol methacrylate, following the method of Leduc & Bernhard (1967). The whole procedure was carried out at 4°C. The ultrathin sections were incubated for the appropriate length of time (from a minimum of 1 hr to a maximum of 48 hr), in the following enzyme solutions:

1. Pronase (*Streptomyces griseus*, activity 45,000 Kunitz units/g.), 0.1% in 0.1 M-phosphate buffer, pH 7.
2. Ribonuclease (bovine pancreas, Sigma Chemical Co., 5 x crystallized), 0.1% in distilled water adjusted to pH 6.8 with 0.01 N-NaOH.

Additional sections were incubated simultaneously in the appropriate enzyme-free media.

After incubation, the sections were rinsed in distilled water and stained for 30 min. in half-saturated uranyl acetate and for 2 min. in lead citrate (Reynolds, 1963).

**Electron microscopy autoradiography.** Strips of 2 x 4 mm. were cut from tumour-bearing leaves. Each strip contained one or more tumours. Two sets of strips were floated for 3 and 6 hr respectively at 27°C on sterilized water containing 500 µc/ml.[H³]-uridine (uridine-5-T, specific activity 28.4 c/m-mole).

After the removal of the isotope solution, the samples were washed for 45 min. in water containing unlabelled uridine (250 mg./ml.), fixed in phosphate-buffered 3% glutaraldehyde and washed in buffer overnight. Both the fixative and the buffer contained unlabelled uridine (250 mg./ml.). The samples were then post-fixed in osmium tetroxide, dehydrated in ethanol and embedded in Epon–Araldite. When in 75% ethanol, they were impregnated with uranyl acetate in half-saturated solution.

Ultrathin sections were mounted on gold grids, stained with lead citrate (Reynolds, 1963), coated with a thin carbon layer, and covered with Ilford L4 emulsion, according to the method of Caro & Van Tubergen (1962). The grids were exposed in the dark, at 4°C, for 3 weeks. The autoradiographs were developed with Microdol X, and examined in a Siemens Elmiskop IA.

**RESULTS**

All the features associated with MRDV infection in maize tumour cells, i.e. viroplasm, cytoplasmic tubules and virus crystals (Gerola & Bassi, 1966), were well preserved and easily recognizable in glycol methacrylate preparations (Fig. 1). Some virus particles were free or aligned in tubular arrangement in the cytoplasm (Fig. 1, 4), some were aggregated in crystals (Fig. 1, 6, inset a). All these particles had an inner darker core and an outer lighter coat (complete or mature particles); others, embedded in the viroplasm, seemed to lack an outer coat (naked or immature particles) (Fig. 1, 2). The viroplasm appeared as a mass of highly electron-dense material, sometimes containing bundles of fibrils (Fig. 2).

The structure of the cytoplasmic tubules was somewhat less clearly defined in glycol- than in Epon–Araldite preparations. In the latter, the tubules appeared as straight formations, about 80 nm. in diameter, which often contained a single row of virus particles (Fig. 1, inset a), and were surrounded by other particles in a spiral arrangement (Fig. 1, inset b). In cross-section, the tubules appeared as circular structures (Fig. 1, inset c).

**Enzymatic digestions**

After pronase digestion, the viroplasm became much less electron-dense, and the fibrils appeared disrupted and reduced to thin, short strands (Fig. 3). The virus particles outside
Fig. 1. Leaf vein tumour cells of maize experimentally infected with MRDV. Glycol methacrylate preparation. The three different kinds of inclusions caused by MRDV are easily recognizable: viroplasm (arrows), cytoplasmic tubules along and inside which the virus particles are aligned (double arrows), and part of a virus crystal (top left). Insets: Epon–Araldite preparations, showing: (a) a cytoplasmic tubule containing virus particles in single row; (b) a cytoplasmic tubule wrapped in a spiral of virus particles (arrows); (c) two cytoplasmic tubules in cross-section, showing a circular profile and contoured by seven virus particles; one particle is visible inside each tubule.
Fig. 2 to 5. Leaf vein tumour cells of maize experimentally infected with MRDV.

Fig. 2, 3. Comparable sections of a glycol methacrylate preparation. Fig. 2: control section, showing viroplasm with bundles of fibrils and immature MRDV particles. Fig. 3: pronase digestion: the viroplasm is much less electron dense, and the fibrils appear thinner and disrupted.

Fig. 4, 5. Comparable sections of a glycol methacrylate preparation. Fig. 4: control section, showing cytoplasmic tubules surrounded by or containing MRDV particles. Fig. 5: pronase digestion: the cytoplasmic tubules appear almost completely digested. The outer coat of the virus particles has disappeared.
Fig. 6, 7. Leaf vein tumour cells of maize experimentally infected with MRDV. Comparable sections of a glycol methacrylate preparation. Fig. 6: control section. The viroplasm (V) contains many immature virus particles and a few mature ones (arrows). Mature virus particles are seen in the cytoplasm. Ribosomes are visible at the edge of the viroplasm and among the free MRDV particles. Fig. 7: 1 hr RNase digestion. The immature particles in the viroplasm have disappeared, while the core of the mature particles in the cytoplasm is still recognizable (arrows). The ribosomes have disappeared. Inset a. Portion of a crystal made of mature MRDV particles. Inset b. Portion of a virus crystal, after combined pronase-RNase digestion. Only small rings, about 40 nm. in diameter, are left in place of the complete particles. Arrows point to the more clearly discernible rings.
Fig. 8, 9. Leaf vein tumour cells of maize experimentally infected with MRDV.

Fig. 8. Autoradiograph of an Epon–Araldite preparation. Portions of two cells are visible, in both of which the labelling is localized only on the viroplasm containing immature virus particles. The viroplasm regions free of virus particles (Vf) and the mature particles in the cytoplasm (arrows) are not labelled.

Fig. 9. Autoradiograph of an Epon–Araldite preparation. The labelling is present on the viroplasm, but not on the virus crystal next to it.
the viroplasm lost their outer coat, while the cytoplasmic tubules disappeared, leaving only thin, unoriented strands (Fig. 4, 5).

After RNase digestion, the particles embedded in the viroplasm disappeared completely in a short time (1 to 2 hr) (Fig. 6, 7), while all the other ones partly retained their dark core even after a prolonged treatment (24 to 48 hr). The viroplasm remained unchanged.

After a combined pronase–RNase digestion (2 hr pronase followed by 4 hr RNase), the virus particles were no longer recognizable as such, but only as small, empty rings having a diameter of about 40 nm. (Fig. 6, 7, insets). The viroplasm had the same aspect as after treatment with pronase alone.

**Autoradiography**

No difference was found in the localization of autoradiographic grains in the two series of experiments, i.e. in the samples kept in contact with the labelling solution for 3 and 6 hr respectively; in both cases, in all the observed cells the autoradiographic grains were localized over the masses of viroplasm containing virus particles. No grains were visible over the viroplasmic zones devoid of virus particles, or over the virus particles outside the viroplasm (Fig. 8). Also the virus crystals were always free of autoradiographic grains (Fig. 9).

**DISCUSSION**

The results of pronase digestion demonstrate that the bulk of MRDV-induced viroplasm is made of protein, and it can be postulated that the virus capsids are made from this protein. In fact, the virus particles embedded in the viroplasm and previously described as ‘immature’ on morphological grounds (Gerola & Bassi, 1966), seem really to be made of naked or incompletely coated RNA, since they disappeared after a short treatment with RNase, while the ‘mature’ or complete particles as seen outside the viroplasm required a longer treatment to lose their core. It is therefore quite probable that the viroplasm is the site where MRDV RNA becomes coated with protein, to produce mature MRDV particles. This is what happens with a similar plant virus, wound tumour virus (Shikata & Maramorosch, 1967).

Apparently, also the cytoplasmic tubules along which MRDV particles align in the cytoplasm are made mainly of protein, since they disappeared after pronase digestion.

When the pronase treatment was followed by RNase digestion, MRDV particles did not disappear completely, but left thin rings, about 40 nm in diameter. This suggests that the particles have a double capsid, composed of an outer shell susceptible to pronase, and a more stable inner capsid. Such a structure has been demonstrated for reoviruses (Mayor *et al.* 1965; Mayor & Jordan, 1968). This would add to the similarity between plant and animal viruses belonging to the group of double-stranded RNA viruses.

The autoradiographic experiments demonstrated that [H³]-uridine incorporation in virus-infected cells took place mainly in the viroplasmic masses rich in immature virus particles. Since no autoradiographic grains were visible over the mature virus particles free in the cytoplasm or ranged in tubular or crystal formations, it seems reasonable to suppose that the newly formed virus RNA gathers first in the viroplasm and needs a fairly long time (certainly more than 3 hr) to spread over the cytoplasm. It is impossible to state, on the basis of our experiments, whether such RNA is also synthesized in the viroplasm. However, the possibility that the marked labelling of the viroplasm is due to [H³]-uridine uptake by the ribosomes involved in the synthesis of viroplasm can be excluded, because no labelling was seen in the viroplasmic regions free of virus particles.
These findings are in agreement with the results of RNase digestion experiments, and give support to our hypothesis, that the viroplasm is the site of assembly of MRDV.

Experiments are now in progress, to see if and to what extent the host cell nucleus is involved in MRDV RNA synthesis.

REFERENCES


CONTI, M. (1966). Indagini sulla trasmissione del virus del nanismo ruvido del mais (MRDV) per mezzo di Laodelphax striatellus Fallén. Annales of the Faculty of Science and Agriculture, University, Torino 3, 337.


(Received 27 January 1972)