Synthesis and Migration of Maize Rough Dwarf Virus in the Host Cell: an Autoradiographic study

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SUMMARY

Maize cells infected with maize rough dwarf virus were exposed to [3H]-uridine for various times and later examined by autoradiography. The results suggest that virus RNA is synthesized and the virus particles are assembled in the viroplasms, from which the particles slowly pass into other parts of the cytoplasm.

In previous autoradiographic studies (Bassi & Favali, 1972) made to investigate the sites of maize rough dwarf virus RNA (MRDV-RNA) synthesis in maize leaf tumours, it was found that after a 3 h incubation in [3H]-uridine the label was localized only in the virus particles embedded in the viroplasm, while the particles free in the cytoplasm or aggregated in crystals were not labelled. It was concluded that the newly synthesized virus RNA collects first in the viroplasm, which therefore can be considered the place of MRDV particle assembly.

In an attempt to find out if not only the assembly but also the replication of MRDV takes place in the viroplasm, or if the host cell nucleus is involved in the early stages of this process, we made further autoradiographic experiments using a much shorter incubation time in the radioactive solution. In addition, in other experiments we used long periods in the chase medium to investigate the fate of the newly synthesized virus particles.

No attempt was made to block nuclear RNA synthesis by actinomycin D (AMD), because preliminary experiments had shown that the absorption of AMD by the leaf tumours is very irregular and haphazard. Moreover, the value of the use of AMD to block RNA synthesis in virus-infected plant tissues has been questioned (Ralph & Wojcik, 1969).

Leaf veins and root turnouts were excised from maize plants (Zeama ys L. cv Wisconsin 641 AA') experimentally infected with MRDV (Conti, 1966). The plants were sampled 2 months after inoculation, because it is at this stage that the symptoms become evident also in roots.

Some tumours were incubated for 20 min in sterilized water containing 500 μg/ml[3H]-uridine (uridine-5-T, sp. act. 28.4 Ci/mmol), and then washed for 20 min in water containing unlabelled uridine (250 μg/ml). Some others were incubated for 3 h in the labelling solution and then washed for 15, 29 and 48 h, respectively, in the chase medium.

All the samples were then fixed in phosphate-buffered 3% glutaraldehyde, washed in buffer overnight, post-fixed in osmium tetroxide, dehydrated in ethanol and embedded in Epon-Araldite.

Ultrathin sections were mounted on gold grids, double-stained with uranyl acetate and lead citrate, coated with a thin carbon film and covered with Ilford L 4 emulsion following 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 1A.

The results obtained with either leaf or root tumours were the same. After 20 min incubation the [3H]-uridine uptake was very scanty. The label was found mainly in the viroplasmic
Fig. 1. Autoradiograph of a MRDV-induced root tumour cell after 20 min [³H]-uridine uptake. The label is localized only in the viroplasm containing immature virus particles (V), while the nucleus (N) and the mature particles as seen in the cytoplasm or in the crystalline aggregate (Cr) are not labelled.

Fig. 2 and 3. Autoradiographs of leaf vein tumour cells after 3 h [³H]-uridine uptake and 48 h washing in the chase medium. The labelling is visible both on the viroplasm (V) and on the mature particles, either scattered in the cytoplasm (Fig. 2) or aggregated in crystals (Fig. 2 and 3).
regions containing incomplete virus particles, while most of the nuclei were unlabelled (Fig. 1). The complete virus particles that can be seen outside the viroplasm were always unlabelled.

After 3 h incubation the labelling was much greater. After washing periods in the chase medium of 15 and 29 h, respectively, the silver grains were still found over the incomplete virus particles embedded in the viroplasm. After a washing period of 48 h a consistent amount of label was found in the complete particles in the cytoplasm either separate or aggregated in crystals (Fig. 2, 3). In the same cells, the labelling was minimal in the cytoplasmic regions devoid of virus particles.

Our results seem to suggest that MRDV particles are not only assembled but also replicated in the viroplasm. In fact, after an incubation time which was just enough to allow the minimum absorption necessary for the label to become visible, the label was detected more often and in greater amount in the viroplasm containing particles than in the nuclei. Moreover, when both the nucleus and viroplasm could be observed in the same cell, it was generally the viroplasm which appeared to be labelled and not the nucleus. It seems therefore that MRDV behaves like other viruses with double-stranded RNA genomes (Wood, 1973), performing its whole replicative cycle in the cytoplasm of the host-cell.

The migration of the complete virus particles from the viroplasm to the cytoplasm seems to be a rather slow process, because it takes more than 29 h. Even 48 h after the removal of the label many silver grains were still over the viroplasm. The labelled particles that by this time had moved to other regions in the cell were found in about equal proportions scattered in the cytoplasm or aggregated in crystals. It seems therefore that after they have moved out of the viroplasm, they can remain scattered at random, align inside and along cytoplasmic tubules, or become aggregated in crystals, without a pre-ordered time-sequence.

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REFERENCES


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