Structural Changes Accompanying Infection of Tobacco Protoplasts with Two Spherical Viruses

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Summary. Ultrastructural changes occurring in isolated tobacco leaf protoplasts following infection with cowpea chlorotic mottle virus and bromegrass mosaic virus variant 5, have been examined. Only the endoplasmic reticulum/nuclear membrane complex has shown a consistent series of changes in structure and distribution. Both viruses induce proliferation of the endoplasmic reticulum and its modification. The nuclear membrane gives rise to small cytoplasmic vacuoles probably containing nucleic acid by a process of blebbing involving both its inner and outer membrane. This is a prominent feature of CCMV infection. BMV5 infection is particularly characterised by the appearance of local organised arrays of assembled virus particles. The results are discussed in terms of possible nucleocytoplasmic interactions.

Introduction

Progress in many fields of plant virology has until recently been hampered by the lack of methods for obtaining efficient and synchronous infection. Fine structural studies have been limited for the most part to descriptions of heavily infected material, often with a view more to classification than the study of the infection process itself (Esau and Cronshaw, 1967; Kitajima et al., 1969; Paliwal, 1970). Early infection stages have been reported (Esau and Hoefert, 1972), sometimes using special techniques giving doubtful preservation of fine structure (Langenberg and Schlegel, 1969; Shalla and Amici, 1967).

The development of the isolated plant protoplast system has enabled synchronous infection to be achieved using tobacco mosaic virus (TMV) (Takebe and Otsuki, 1969), cowpea chlorotic mottle virus (CCMV) (Motoyoshi et al., 1973), cucumber mosaic virus (Otsuki and Takebe, 1973) and brome grass mosaic virus, variant five (BMV5) (this report). This system allows sequential sampling for electron microscopy and thus removes doubts which may exist in intact plant systems as to the stage of infection. The process of virus entry into protoplasts has been the subject of earlier papers (Burgess et al., 1973a, b). Here we report on the course of infection by two spherical viruses. We also present some details of features of the protoplast system which though abnormal are not directly associated with virus infection.
Materials and Methods

These have been fully described previously. Tobacco protoplasts were prepared and inoculated with CCMV in the presence of poly-L-ornithine exactly as described by Motoyoshi et al. (1973) BMV V5 was prepared as described by Bancroft and Lane (1973) and used at a final concentration of 50 μg/ml in the presence of 1 μg/ml poly-L-ornithine (PLO) at pH 5.2. Electron microscopic preparations were made as described by Burgess et al. (1973a). In addition to infected protoplasts, controls were examined which had been inoculated with CCMV or TMV in the absence of poly-L-ornithine, and subsequently incubated under the same conditions as infected protoplasts. Preparations were fixed at 6, 10, 18 and 24 h post inoculation (BMV5) or 7, 15, 24 and 48 h post inoculation (CCMV). Controls were fixed at 10, 24 and 48 h. The percentage of protoplasts which became infected was scored by fluorescent antibody staining. Typically in both CCMV and BMV5 experiments 55–65% of protoplasts were infected. It always appeared that the percentage of protoplasts infected was substantially higher when judged by electron microscopy than the figure given by fluorescent antibody staining. This difference in sensitivity of the two techniques has been commented upon by Shalla and Petersen (1973).

Results

A. General Observations

The experimental conditions of virus infection in the isolated protoplast system involve several traumas. The presence of poly-L-ornithine and a chelating buffer during the infection period, followed by incubation in a reduced salts medium containing antibiotics represent factors which might be expected to induce some structural abnormalities. The damaging effects of the inoculation conditions have been described previously (Burgess et al., 1973a). These appear to be only temporary, and in practice it is not possible to distinguish protoplasts which have been inoculated using PLO from those which have not after the lapse of more than a few hours.

Fig. 1. Section through a protoplast incubated for 24 h after inoculation with TMV but no poly-L-ornithine. Infection has not been achieved. The presence of small osmiophilic droplets on several membranes and the whorled ER are typical results of this experimental handling. × 16000

Fig. 2. A microbody within the cytoplasm of a freshly isolated uninfected protoplast. These organelles do not represent a response to infection or incubation. × 30000

Fig. 3. Section of a protoplast fixed 15 h after inoculation with CCMV and poly-L-ornithine. Groups of ER vacuoles have formed in the cytoplasm. Two extensions of the outer nuclear envelope are visible (arrows). × 16000

Fig. 4. A section through distended ER in the cytoplasm of a 15 h CCMV infected protoplast. The lumen of the ER contains vesicles, which may contain a central dot of electron dense material (arrows). × 90000