Morphogenesis of Festuca Leaf Streak Virus in Cowpea Protoplasts

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SUMMARY

Festuca leaf streak virus (FLSV), a non-sap-transmissible rhabdovirus which has only been found in Festuca gigantea (Gramineae), multiplied in cowpea protoplasts inoculated with virus suspensions containing polyethylene glycol. The morphogenesis of FLSV particles was studied by electron microscopy. FLSV seemed to be assembled in the cytoplasm by simultaneous coiling and envelopment of the nucleocapsid. However, particles detached from the host membrane were not observed. Similarities with the morphogenesis of particles of Sonchus yellow net virus in cowpea protoplasts are discussed.

Festuca leaf streak virus (FLSV) is considered to be a rhabdovirus because of its morphology and its cytopathology (Lundsgaard & Albrechtsen, 1976, 1979). Plant rhabdoviruses are assembled either on the inner nuclear membrane or on cytoplasmic membranes; only wheat streak mosaic virus is reported to be assembled at both sites (Lee, 1970; Vela & Lee, 1974). The infection by rhabdoviruses that assemble in the cytoplasm is often accompanied by the occurrence of so-called viroplasms (Wolanski & Chambers, 1971; Conti & Appiano, 1973; Toriyama, 1976). Although FLSV induces viroplasm-like structures in the cytoplasm of infected cells (Lundsgaard & Albrechtsen, 1979), there is no report of its assembly in the cytoplasm.

Since no vector of FLSV has been discovered yet (Lundsgaard, 1984) and, like all other rhabdoviruses infecting Gramineae (Jackson et al., 1981), FLSV is not sap-transmissible, its study has so far been limited to its natural host Festuca gigantea (L.) Vill.

Recently, protoplasts of cowpea (Vigna unguiculata) were infected with Sonchus yellow net virus (SYNV), a virus which is assembled on the inner nuclear membrane (Van Beek et al., 1985a, b). Infection of cowpea protoplasts by FLSV would enable the comparison of the morphogenesis of this virus with that of SYNV in the same host. Therefore, we have investigated the possibility of infecting cowpea protoplasts by inoculation with purified FLSV.

FLSV was purified from systemically infected leaves of F. gigantea using a method described for SYNV (Van Beek et al., 1985a) but using different buffers. Extraction was done in 0-1 M-glycine-NaOH pH 8-3, containing 0-001 M-MgCl₂ and 0-01 M-Na₂SO₄, and the virus particles were resuspended in 0-1 M-glycine-NaOH pH 7-4, containing 0-01 M-MgCl₂. The virus concentration was taken to be the same as the protein concentration, which was determined according to Lowry et al. (1951). V. unguiculata plants were grown and protoplasts were isolated essentially as described previously (Van Beek et al., 1985a). The pellet containing approximately 3 x 10⁶ freshly isolated protoplasts was suspended in 100 μl virus suspension (30 μl FLSV at 2 mg/ml in 0-1 M-glycine-NaOH pH 7-4, containing 0-01 M-MgCl₂, and 70 μl 0-8 M-mannitol) and kept on ice for about 10 min. Treatment with polyethylene glycol, dilution with 9 vol. mannitol, and washing as well as incubation of the inoculated protoplasts were done according to Van Beek et al. (1985a). Protoplasts were studied by electron microscopy after fixation, embedding, sectioning and staining as described by Rezelman et al. (1982), except that prefixation was in

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Fig. 1. Purified preparation of FLSV negatively stained with phosphotungstate, pH 7. Bar marker represents 200 nm.

Fig. 2. Viroplasm (V) with budding particles at its edge, 26 h after inoculation. Bar marker represents 250 nm.

Fig. 3. Two particles, the shorter being in the process of budding from rough endoplasmic reticulum membrane, 26 h after inoculation. Bar marker represents 200 nm.

Fig. 4. Particle budding from cytoplasmic membrane, 26 h after inoculation. Bar marker represents 200 nm.

Fig. 5. Particle budding from cytoplasmic membrane, 48 h after inoculation. Arrow indicates nucleocapsid material probably connected with the nucleocapsid core. Bar marker represents 100 nm.

Fig. 6. Cytoplasm with budding particles (arrows) and with particles attached to and engulfed by endoplasmic reticulum membranes, 51 h after inoculation. A viroplasm is not present in this section. Bar marker represents 500 nm.
Sections were made of protoplast samples taken at 10 intervals, the first at 10 h and the last 72 h after inoculation. The specimens were examined with a Siemens Elmiskop 101 or with a Zeiss 109 electron microscope operating at 60 and 50 kV, respectively.

Because it was not possible to use bioassays to measure the infectivity of protoplast extracts, we scored as infected those protoplasts in which virus particles were detected by electron microscopy. The number of protoplasts that contained virus particles varied from experiment to experiment and ranged approximately between 15 and 60% of the total number of protoplasts which could have supported replication (judged from their condition and integrity in the electron microscope).

In purified suspensions of FLSV, particles contrasted with phosphotungstate were \( 316 \pm 16 \) nm long and \( 61 \pm 2 \) nm wide. A large number of particles appeared to be damaged during purification or preparation for electron microscopy (Fig. 1). The dimensions of particles in thin sections were \( 247 (\pm 9) \) by \( 44 (\pm 2) \) nm. Structures resembling coiled nucleocapsid strands, further referred to as cores, were up to 285 nm long and 32 \( \pm 2 \) nm in diameter (Fig. 9).

Ultrastructural changes in infected protoplasts were first observed 26 h after inoculation, when small viroplasms were found and when particles were seen budding from cytoplasmic membranes (Fig. 2). Fig. 3 shows two particles, one 105 nm and the other 170 nm long. The shorter particle is considered to be in the stage of simultaneous coiling and envelopment. Such intermediate stages in the budding process were often observed (Fig. 4, 5, 6, 7). The length of such particles ranged from 45 to 215 nm. In Fig. 5 a structure that is suggestive of an unwound nucleocapsid strand is visible beyond the coiled and assembled part of a particle. Core structures that were partly enveloped and partly protruding into the cytoplasm beyond the membranes were never observed.

Viroplasms that appeared to consist of strands of granular material (Fig. 2, 10) were often located near nuclei. Serial sectioning showed that some were hollow. In one sectioned protoplast, a viroplasm was found enclosed within a membrane, a phenomenon also seen in tissue infected by barley yellow striate mosaic virus (Conti & Appiano, 1973). Budding particles were often observed at the edges of viroplasms (Fig. 2, 4, 6). Full-length particles were invariably found to be attached to host membranes so that envelopment apparently was not succeeded by detachment of the host membrane (Fig. 6, 7).

Cores were observed lying freely in the cytoplasm of protoplasts 34 h after inoculation. They seemed to have originated from nucleocapsid strands which had coiled at the edge of viroplasms (Fig. 8). These cores seemed to have an affinity for membranes. Most were found close to the tonoplast of chloroplast membranes and sometimes were seen between chloroplast and cytoplasmic membranes (Fig. 9).

At later stages of infection, viroplasms often occupied much of the cytoplasm (Fig. 10), but virus particles never became abundant. At no stage in infection were any ultrastructural changes in the nucleus observed.

Our results clearly show that FLSV replicates in protoplasts of the dicotyledon *V. unguiculata*. However, inocula of FLSV infected fewer cowpea protoplasts than did inocula of SYNV (Van Beek *et al.*, 1985b). It is possible that preparations of FLSV particles contained fewer infective particles than preparations of SYNV. Also, the number of FLSV particles formed in each infected protoplast was less than the number of SYNV particles formed in cowpea protoplasts (Van Beek *et al.*, 1985b) and less than the number of particles found in cells of *F. gigantea* leaves systemically infected with FLSV (Lundsgaard & Albrechtsen, 1979). The occurrence of massive amounts of viroplasm suggests that after the formation of nucleocapsid strands their assembly into virus particles is blocked to a greater extent. If so, this may be because FLSV is poorly adapted to cowpea as a host. It is known that vesicular stomatitis virus in non-host cells exhibits G protein deficiency or altered M protein phosphorylation which may lead to reduced multiplication and impaired assembly of these viruses in such hosts (Wyers *et al.*, 1980; Blondel *et al.*, 1983).

As we never observed coiled core structures in the stage of budding it is most likely that virus particles are assembled by simultaneous coiling and envelopment as are animal rhabdoviruses.
Fig. 7. Cytoplasm with a planarly sectioned budding particle attached to and engulfed by membranous material and cross-sectioned particles engulfed by membranes, 48 h after inoculation. Bar marker represents 100 nm.

Fig. 8. Three partially coiled cores close to a chloroplast (C), 42 h after inoculation. The edge of a viroplasm (V) is in the plane of section. Bar marker represents 200 nm.

Fig. 9. Cores located at the edge of a viroplasm (V) and close to chloroplast and cytoplasmic membranes, 42 h after inoculation. Bar marker represents 100 nm.

Fig. 10. A viroplasm surrounded by cores close to chloroplast membranes and two obliquely sectioned virus particles (arrows), 48 h after inoculation. N, Nucleus. Bar marker represents 1 μm.
Short communication

(Murphy & Harrison, 1979). Budding was observed to occur on endoplasmic reticulum membranes. Detachment of the virus particles from the membrane as a final step in the maturation process does not seem to take place since all particles are found to be attached to and engulfed by membranes. The viroplasms found in the cytoplasm of protoplasts infected with FLSV seem to be similar to the granular matrices reported to occur in the nucleus of protoplasts infected with SYNV (Van Beek et al., 1985b). Nucleocapsid strands coil into core-like structures which occur at the edges of both viroplasms and granular matrices. We assume that such coiled nucleocapsid strands fail to become enveloped, as it is most unlikely that FLSV utilizes two different pathways for its assembly. Moreover, Toriyama (1976) found cores in the cytoplasm of cells infected with northern cereal mosaic virus and concluded that these cores would not acquire an envelope.

In various reviews of plant rhabdoviruses, authors have stated that there are marked differences between the ultrastructure of cells infected with different rhabdoviruses (Francki, 1973; Brown & Crick, 1980; Jackson et al., 1981). In contrast, we have been struck by the similarities between FLSV and SYNV in their morphogenesis in infected cowpea protoplasts, except that SYNV is enveloped at the nuclear membrane and FLSV at cytoplasmic membranes and that the FLSV particles do not detach after the budding process.

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