Ultrastructural Changes in Differentiated Leaf Cells Infected with Cherry Leaf Roll Virus

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Tubular structures containing virus-like particles were found by Walkey & Webb (1968) in squashes of meristems of Nicotiana rustica L. systemically infected with cherry leaf roll virus (CLRv), a nepovirus. These tubules occurred in the cytoplasm of undifferentiated meristematic cells of N. rustica, but were not found in differentiated leaf or stem cells (Walkey & Webb, 1970). We have examined differentiated leaf cells of Nicotiana clevelandii Gray infected with four strains of CLRv and here report that tubules occur often in the cell walls of these cells, but rarely in their cytoplasm. We also describe ultrastructural changes induced by CLRv that resemble those produced by bean pod mottle and cowpea mosaic viruses.

N. clevelandii leaves systemically infected with CLRv were used. The effects of four strains were compared: the elm mosaic strain (E; Varney & Moore, 1952; Jones & Murant, 1971), the golden elderberry strain (G; Hansen & Stace-Smith, 1971; Jones & Murant, 1971), a strain from cherry (C; Cropley, 1961) and a strain from rhubarb (R; Tomlinson & Walkey, 1967). The viruses were transmitted by inoculation of sap to young plants maintained in glasshouses kept at 18 to 25 °C. Young leaves (about 20 to 25 mm long) showing systemic necrotic motting were sampled 7 days after the plants were inoculated, but some samples were also taken 5 and 10 days after inoculation. Pieces of leaf tissue about 4 × 1 mm were double fixed using glutaraldehyde followed by osmium tetroxide, dehydrated through a graded alcohol series, and stained with uranyl acetate at the 100 % alcohol stage. After transfer to propylene oxide, they were embedded in Araldite and polymerized for 72 h at 68 °C. Sections cut with an LKB Ultratome I and showing silver/gold interference colours were post-stained in bulk with uranyl acetate followed by lead citrate using the grid-frame technique (Robertson & Roberts, 1972). Sections were cut from three different areas of each of four to six samples of each treatment and were examined in a Siemens Elmiskop I at 80 kV using a 50 μm objective aperture.

All four strains of CLRv induced similar symptoms in N. clevelandii, although strain R was the most virulent, symptoms in systemically infected leaves usually developing 1 or 2 days earlier than in plants infected with the other strains. The general ultrastructural changes induced by each of the four strains were also similar, but some differences in detail were noted. Seven days after infection, all strains induced inclusion bodies in palisade leaf cells, seen as the apparent amassing of membranous structures, endoplasmic reticulum and ribosomes. These inclusion bodies were invariably associated with the cell nucleus (Fig. 1). They resembled the inclusion bodies observed by Roberts & Harrison (1970) in Chenopodium amaranticolor Coste & Reyn. plants infected with strawberry latent ringspot virus (another nepovirus), but unlike these were devoid of tubular structures.

Palisade leaf cells infected with any of the four CLRv strains showed cell-wall abnormalities, notably thickening, and protrusions of the cell wall into the cytoplasm (Fig. 1). Such protrusions have not previously been reported for cells infected with nepoviruses, but were observed in Cherokee Wax bean systemically infected with another virus inducing the formation of tubules, bean pod mottle (Kim & Fulton, 1971). Like the cell wall protrusions observed by Kim & Fulton (1971), those induced by CLRv frequently contained rows of
virus-like particles which were penetrated by the stain and contained within narrow tubules (Fig. 2a, b). The particles, about 26 to 27 nm in diameter (a value similar to that obtained for purified virus (Jones & Mayo, 1972)) were closely packed together in a linear arrangement (Fig. 2a, b). Up to 44 such particles were observed within a single tubule. The tubules were similar to those observed by Walkey & Webb (1970) in N. rustica infected with CLRV and those observed by Kim & Fulton (1971) in bean infected with bean pod mottle virus. They were predominantly flexuous, about 48 nm in diameter and consisted of a unit mem-
brane. Serial sections indicated that the tubule membrane was continuous with the plasma-lemma. Unlike the tubules observed in plants infected with strawberry latent ringspot virus (Roberts & Harrison, 1970) and cowpea mosaic virus (Van der Scheer & Groenewegen, 1971), the tubules did not have an outer sheath.

With all four strains, tubules containing virus-like particles were found commonly within the cell wall and cell-wall projections but infrequently in the cytoplasm. Indeed tubules were occasionally seen only in the cytoplasm of cells infected with strains E and R, but not in cells infected with strains C and G, although these were not examined exhaustively.

A few tubules induced by strains E and G contained more than one row of virus-like particles; up to five rows were found but two or three rows were commoner (Fig. 2c). Further work would be needed to find whether this is a feature of these particular CLRV strains. Although this multiplicity of rows of particles within tubules has not been noted for other
viruses inducing typical tubules in plants, Fig. 2c of Van der Scheer & Groenewegen (1971) apparently shows two rows of particles in a tubule induced by cowpea mosaic virus in *Vigna unguiculata* (L.). Multiple rows of virus-like particles were also found within invaginations of the tonoplast induced by radish mosaic virus in *Raphanus sativus* L. (Honda & Matsui, 1972).

At least seven other isometric plant viruses are known to induce cell-wall projections into the cytoplasm. They are maize rough dwarf (Gerola & Bassi, 1966), dahlia mosaic (Kitajima & Lauritis, 1969), bean pod mottle (Kim & Fulton, 1971), cowpea mosaic (Van der Scheer & Groenewegen, 1971), radish mosaic (Honda & Matsui, 1972), cauliflower mosaic (Conti et al. 1972) and carrot mottle (Murant & Roberts, 1970) viruses. The significance of these protrusions is, however, not known.

Finally, the cell-wall abnormalities and general ultrastructure of CLRV-infected cells we have described here closely resemble those observed by Kim & Fulton (1971) for bean pod mottle virus. This further emphasizes the already noted similarly between nepoviruses and comoviruses (Walters, 1969; Kim & Fulton, 1971).

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REFERENCES


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