Electron Microscopy of Intracellular Radish Mosaic Virus

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ABSTRACT

Masses of radish mosaic virus particles occurred on the interface between the cytoplasm and vacuole or in the vacuole. In these masses, some virus particles aligned in crystal rows. Presumably, the virus was formed within the cytoplasm and liberated into the vacuole. The most prominent modification was invagination of the tonoplast into the cytoplasm. The virus particles attached to the tonoplast in the vacuole were incorporated into the tonoplastic channel during invagination of the tonoplast into the cytoplasm. Within the tonoplastic channels, the virus particles were aligned in multi-rows. The virus particles were seen in the plasmodesmata across the cell wall. Because the above-mentioned profiles of the virus particles were rather scarce, it is likely that most virus particles scatter randomly throughout the cytoplasm.

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Radish enation mosaic virus (REMV) was isolated in 1968 by Tochihara (9). The virus is spherical and about 25 nm in diam. REMV is similar to radish mosaic virus (RaMV) in physical properties, host range, symptoms, and type of vector (1, 9). On the basis of serological tests, REMV was recognized as an isolate of RaMV (1). The latter, isolated in 1939 by Tompkins (10), is a member of the cowpea mosaic virus group (4). The viruses belonging to the cowpea mosaic virus group characteristically consist of three different components: top, middle, and bottom. The middle and bottom components, both containing nucleic acid, are needed for efficient infection (7, 11). However, little is known about intracellular appearance of these viruses. Recently, Kim & Fulton (6) reported that tubules with bean pod mottle virus, a member of the cowpea mosaic virus group, were found between the plasmalemma and the cell wall or in the cell wall.

We found by thin sectioning that the intracellular appearance of RaMV was distinct from that of bean pod mottle virus.

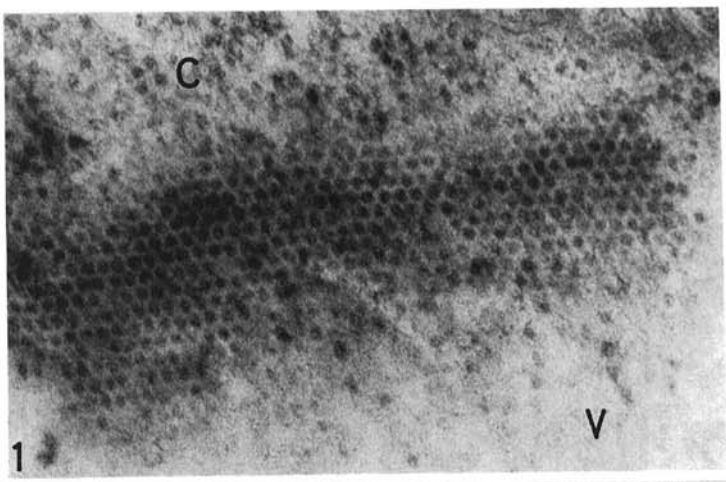
MATERIALS AND METHODS.–RaMV (9) was mechanically inoculated to the lower leaves of seedlings of *Raphanus sativus* L. 'Kameido'. About 20 days after inoculation, small portions of systemically infected leaves were fixed with 4% formaldehyde and 5% glutaraldehyde at 5 C for 1.5 hr, rinsed in Milloning's phosphate buffer (pH 7.5), and postfixed with 2% osmium tetroxide at 5 C for 5 hr. They were dehydrated and embedded in Epon 812. Thin sections were cut with a Porter-Blum ultramicrotome MT-1, stained with uranyl acetate and lead citrate, and examined in a JEM-T7S electron microscope (JEOL Co., Ltd.). Healthy tissues were also examined in the same way.

RESULTS AND DISCUSSION.–Intracellular RaMV particles were divided into the following profiles:

1) RaMV in the vacuole.–Masses of the spherical particles were observed between the cytoplasm and vacuole (Fig. 1), or in the vacuole (Fig. 2). Within some masses, the individual particles aligned in crystal rows. As the spherical particles within the masses were about 25 nm in diam and they could not be detected in healthy leaf cells, these spherical particles were believed to be RaMV particles. Presumably, after formation of RaMV particles within the cytoplasm, they accumulated on the interface between the cytoplasm and vacuole, and released into the vacuole. In this area, the tonoplast was apt to be indiscernible. In our previous paper (5), a similar conclusion on the origin of masses of cucumber mosaic virus particles in the vacuole was derived from observations on tobacco leaf cells infected with cucumber and tobacco mosaic viruses.

2) RaMV in the tonoplastic channel.–A pair of membranes in which the spherical particles aligned in a row was encountered within the cytoplasm (Fig. 3, 4). Some pairs of membranes appeared as filamentous (Fig. 3); others were circular (Fig. 4). Since diameters of the spherical particles within a pair of membranes were about 25 nm and were not observed in healthy leaf cells, the particles were assumed to be RaMV. Spherical virus particles aligned in a row within real tubules have been observed in the cells of citrus infectious variegation virus-, bean pod mottle virus-, and some NEPO viruses-infected plants (2, 3, 6, 8, 12), though their origin and nature are not yet clear. Some profiles of pairs of membranes with RaMV particles resembled real tubules (2, 3, 6, 8, 12). However, ends of these pairs of membranes in which

Fig. 1-5. Electron micrographs of radish leaf cells infected with radish mosaic virus (RaMV). **1)** Crystalline mass of RaMV particles between the cytoplasm (C) and vacuole (V) (X 80,000). **2)** Crystal of RaMV particles in the vacuole (V) (X 120,000). **3, 4)** Filamentous and circular pairs of membranes with RaMV particles within the cytoplasm (C). Ends of these pairs of membranes continued to the tonoplast (T) (arrows) (X 38,000 and 50,000, respectively). **5)** Details of a pair of membranes continued to the tonoplast (T) (arrows). C = cytoplasm and V = vacuole (X 120,000).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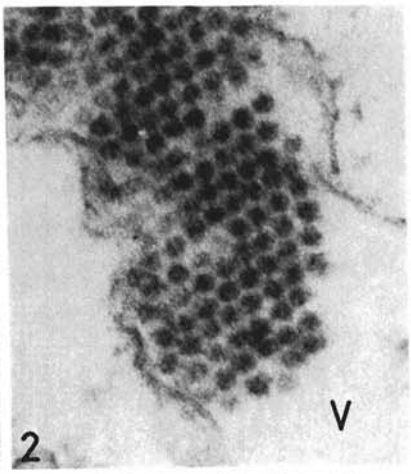
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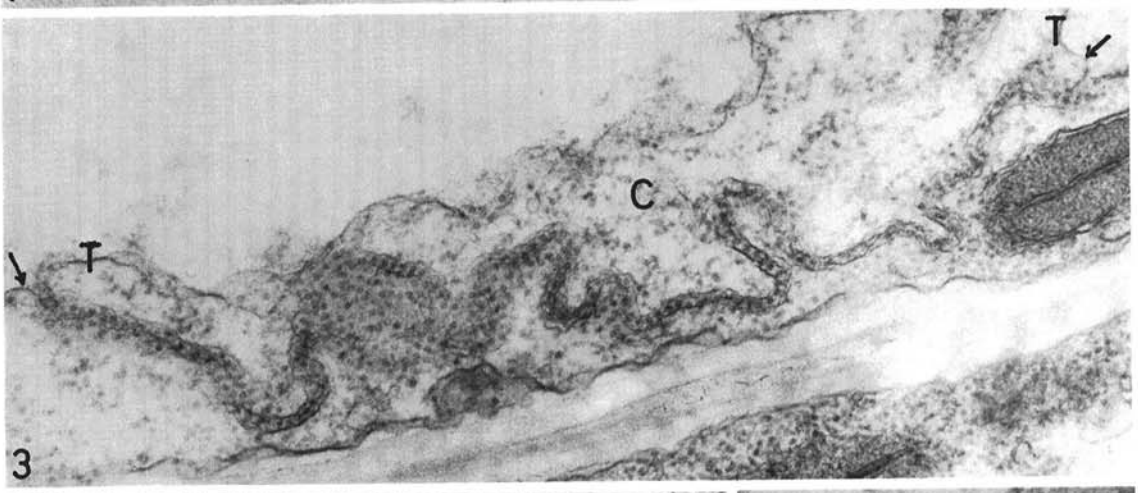
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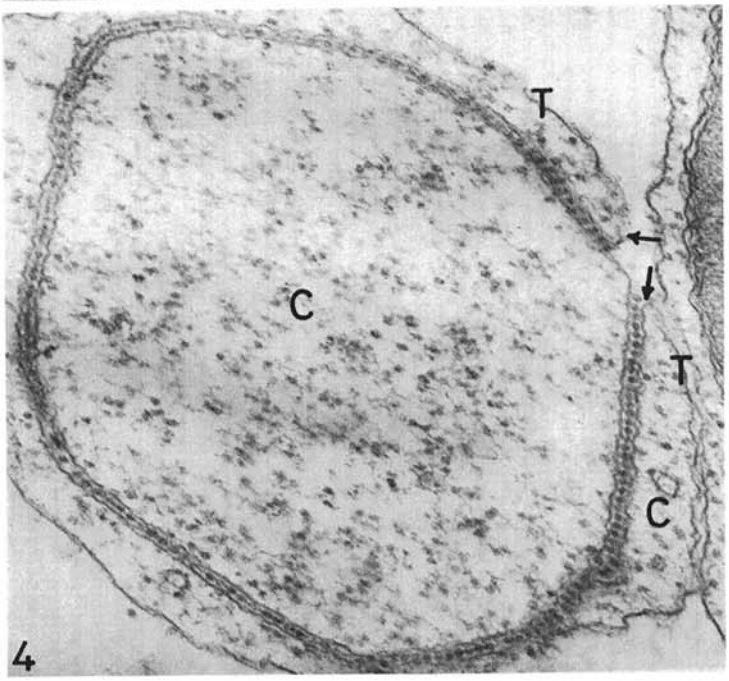
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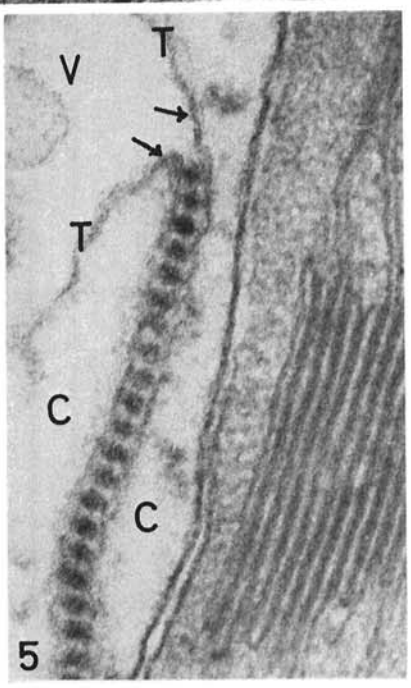
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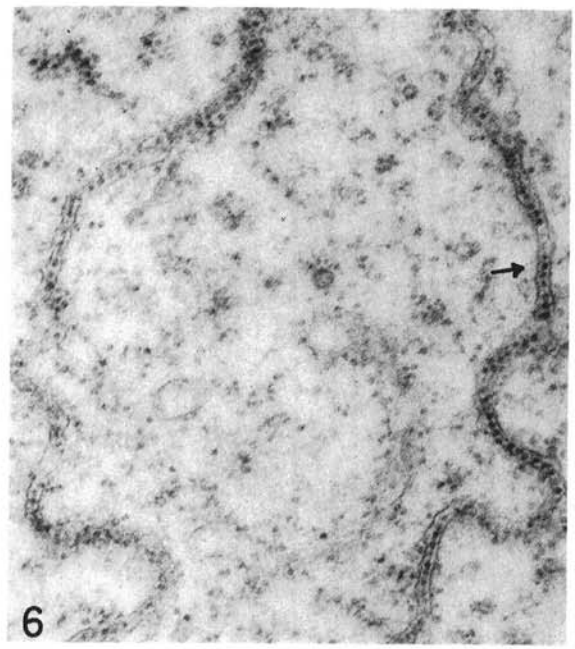
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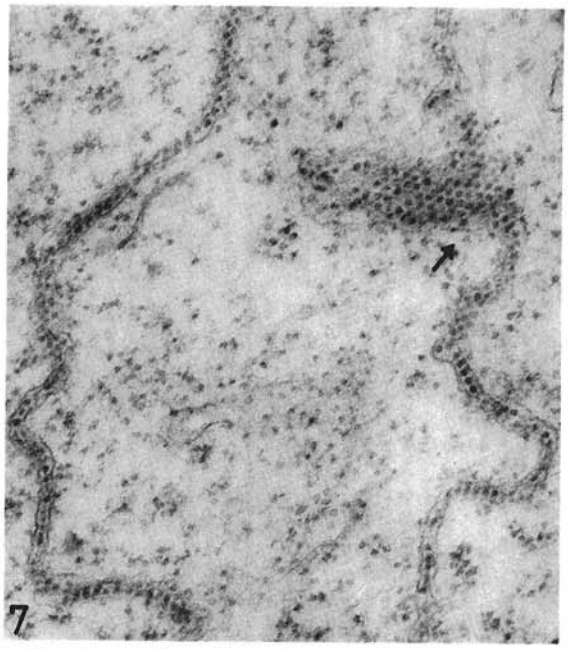
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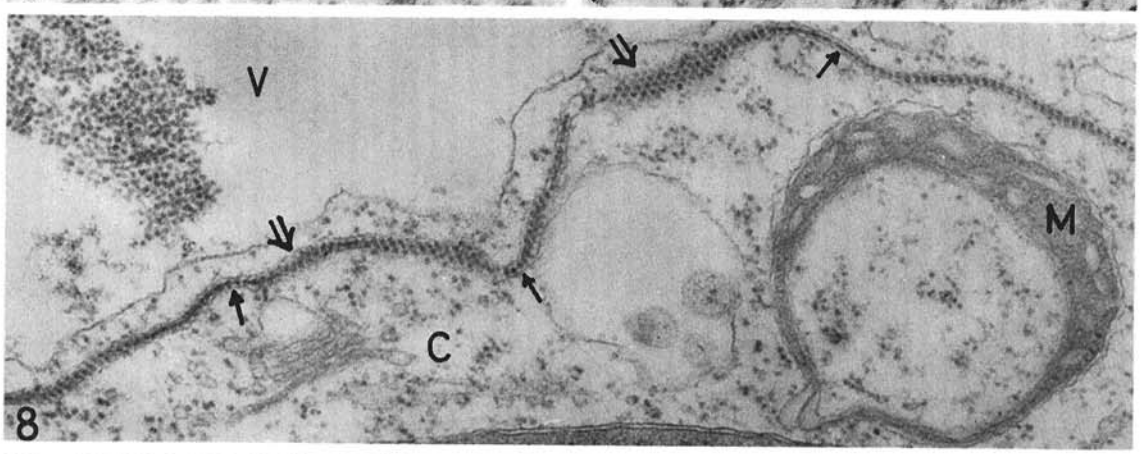
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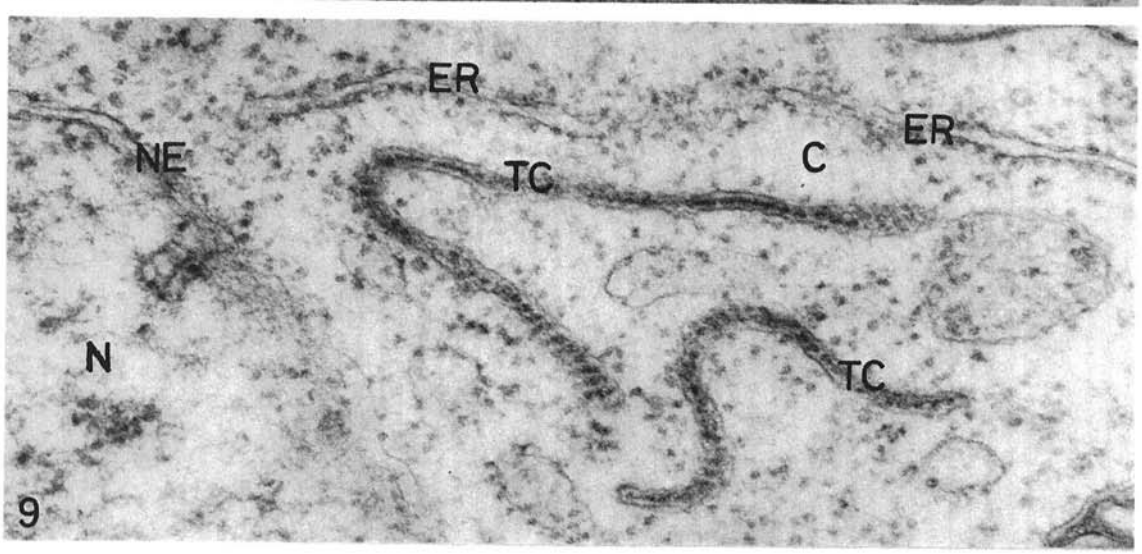
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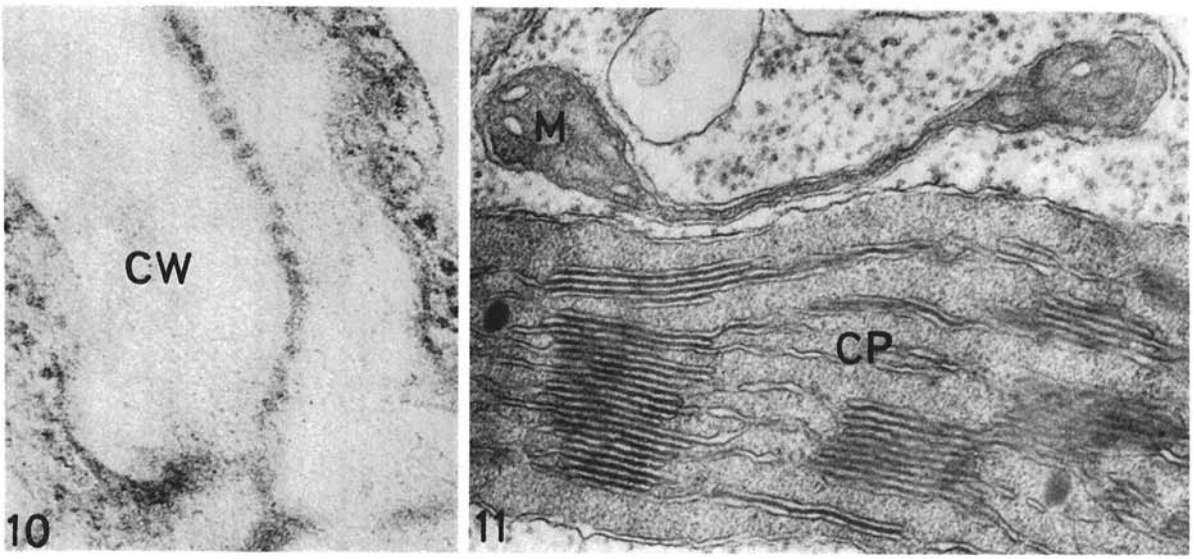

Fig. 10-11. Electron micrographs of radish leaf cells infected with radish mosaic virus (RaMV). **10)** RaMV particles in the plasmodesmata across the cell wall (CW) (X 85,000). **11)** The intact chloroplast (CP) and abnormal mitochondrion (M) in the cytoplasm (X 45,000).

RaMV particles aligned continued occasionally to the tonoplast (Fig. 3, 4, 5). Furthermore, serial sections of RaMV-infected leaf cells indicated that the tridimensional structures of pairs of membranes were not real tubules, and RaMV particles did not align in a row, but aligned in multi-rows (Fig. 6, 7). Thus, it is clear that intracellular appearance of RaMV particles is essentially different from the viruses within the real tubules reported previously (2, 3, 6, 8, 12). It is considered that a pair of membranes observed in thin sections corresponds to the tonoplastic channel invaginated into the cytoplasm, and RaMV particles in the vacuole or attached to the tonoplast are accompanied with invagination of the tonoplast into the cytoplasm. A pair of membranes was clearly observed at a portion of RaMV particles aligned in a row (Fig. 8), whereas the membranes were indiscernible in a portion of crystalline masses of RaMV particles (Fig. 8). It is likely that the former reveals a profile of the tonoplastic channel cut perpendicularly to the membrane surface; and the latter, a profile cut tangentially. The tonoplastic channels appear to be out of relation to RaMV formation. The tonoplastic channels without RaMV particles were also observed. No RaMV particles within the endoplasmic reticulum and the nuclear envelopes were observed (Fig. 9).

3) RaMV particles in the plasmodesmata.—RaMV particles were encountered in the plasmodesmata across the cell wall (Fig. 10). It is likely that cell to cell movement of RaMV particles is caused by the movement of complete virus particles through the plasmodesmata.

No ultrastructural changes were observed within the chloroplast (Fig. 11). The modified mitochondria were easily encountered. The mitochondrion with the large cytoplasmic protrusion (Fig. 8) and the unusually long dumbbell-shaped mitochondrion were observed (Fig. 11).

RaMV concentration in diseased plant sap is high (9). Although about 1,000 different cells were observed in the present study, the above-mentioned profiles of intracellular RaMV particles were encountered in about 5% of the cells. Presumably, most RaMV particles scattered randomly throughout the cytoplasm. Under an electron microscope, it is rather difficult to differentiate such small spherical viruses from the ribosomes.

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Fig. 6-9. Electron micrographs of radish leaf cells infected with radish mosaic virus (RaMV). **6, 7)** Two different cutting planes of the same tonoplastic channels with RaMV particles. Note the different arrangement of RaMV particles on the same areas of the two sections (arrows) (X 40,000 and 40,000, respectively). **8)** Channel of the tonoplast invaginated into the cytoplasm (C). At the positions indicated by a single-shafted arrow, a pair of membranes is clear and RaMV particles align in a row. In contrast, the membranes are obscure at a portion of RaMV particles aligned in multi-rows (double-shafted arrows). Modified mitochondrion (M) is observed. A mass of RaMV particles is in the vacuole (V) (X 35,000). **9)** No RaMV particles are seen within the endoplasmic reticulum (ER), the nuclear envelopes (NE), or the nucleus (N). Parts of the tonoplastic channels (TC) with RaMV particles are observed within the cytoplasm (C) (X 59,000).

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