Ultrastructure and Origin of Cytoplasmic Multivesicular Bodies Induced by Carnation Italian Ringspot Virus

By A. DI FRANCO, M. RUSSO AND G. P. MARTELLI*

Dipartimento di Patologia vegetale, Università di Bari and Centro Virosi Colture Mediterranea del CNR, Bari, Italy

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SUMMARY

Ultrastructural investigations of different hosts infected with the tombusvirus carnation Italian ringspot have shown that the multivesicular bodies typical of tombusvirus infections develop from modified mitochondria. Digestion with RNase in high- or low-salt medium demonstrated that multivesicular bodies contain RNA, some of which is double-stranded and, possibly, of viral origin. Cytochrome oxidase was identified in multivesicular bodies by cytochemical tests.

As with all definitive members of the tombusvirus group, infection by carnation Italian ringspot virus (CIRV) has been shown to induce the formation of membranous inclusions, known as multivesicular bodies (MVB), in the cytoplasm of infected cells (Martelli & Russo, 1972). In that report no account was given of the origin of MVB. However, subsequent work with other tombusviruses, i.e. Cymbidium ringspot (CyRSV) (Russo et al., 1983), tomato bushy stunt, artichoke mottled crinkle, eggplant mottled crinkle, petunia asteroid mosaic and pelargonium leaf curl viruses (M. Russo, A. Di Franco & G. P. Martelli, unpublished information) has shown that MVB arise by extensive modification of peroxisomes.

Re-examination of old micrographs of CIRV-infected Chenopodium quinoa plants suggested that mitochondria, rather than microbodies, might give rise to MVB. This prompted us to re-investigate the ultrastructure of CIRV infections.

The virus strain used was that obtained from Dr M. Hollings (Glasshouse Crops Research Institute, Littlehampton, U.K.) and used previously (Martelli & Russo, 1972). Ultrastructural observations were made on tissue from local lesions in leaves of C. quinoa, Gomphrena globosa and Datura stramonium and on systemically infected leaves of Nicotiana benthamiana and N. clevelandii.

Tissue samples were excised in a drop of 4% glutaraldehyde in 0.05M-sodium cacodylate buffer pH 7.0, and vacuum-infiltrated in the same fixative for 2 h at room temperature. The samples were post-fixed in 1% osmium tetroxide for 2 h at 4 °C, stained overnight in 0.5% aqueous uranyl acetate, dehydrated in ethanol and embedded in Spurr's medium. Thin sections were viewed with a Philips 201C electron microscope usually after double staining with uranyl acetate and lead citrate. Tissues from healthy plants of comparable age were processed similarly to serve as controls.

Enzyme digestion tests for single- and double-stranded RNA were done using 20 μg/ml pancreatic RNase A (Type III-A, Sigma) in 0.3M-sodium chloride and 0.3M-sodium citrate, pH 7-0 (high-salt) or a 1/200 dilution of this medium (low-salt), as described by Hatta & Francki (1978). Control samples were incubated without RNase in the same media. Tissue pieces were stained overnight in 0.5% aqueous uranyl acetate.

Cytochrome oxidase was detected by incubating glutaraldehyde-fixed tissue samples for 1 h at 37 °C in 0.1 m-phosphate buffer pH 7.2 containing 1 mg/ml 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Weintraub & Schroeder, 1979). Control samples were incubated with DAB in the presence of 1 mg/ml KCN, an inhibitor of cytochrome oxidase.

The results of enzyme digestion and DAB treatment were assessed by observing those cells cut...
open with a razor blade during aldehyde fixation and their immediate neighbours. Thin sections of treated tissues were observed either unstained or after staining with uranyl acetate and lead citrate.

Whereas no cytological abnormalities were seen in tissues of healthy control plants, the

Fig. 1. (a to d) Sections of mitochondria and mutivesicular bodies in CIRV-infected cells, showing the likely successive stages of the transformation of mitochondria into MVB. Many virus particles (V) are visible. Bar markers represent 200 nm.
Fig. 2. Effect of treatment with RNase in low- or high-salt medium on the fibrils present in the vesicles of the multivesicular bodies. Fibrils are still visible following treatment with high-salt medium alone (a), or containing RNase (b), but not in vesicles of samples treated with RNase in low-salt medium (c). Arrowheads point to some of the vesicles with fibrils. Bar markers represent 200 nm.

The general cytology of infected samples, regardless of the host, was typical of tombusvirus infections (for reviews, see Martelli et al., 1977; Martelli, 1981). Virus particles were plentiful in the cytoplasm and readily identified, although they had not aggregated in crystalline arrays (Fig. 1 a to d). Chloroplasts seemed slightly modified by infection whereas nuclei and peroxisomes appeared unaffected.

Mitochondria were the only organelles greatly changed by infection. Changes in shape and internal structure occurred in all cells containing virus. The main change was that the stroma became more electron-opaque and cristae became fewer and appeared either to be replaced progressively by membranous rings (Fig. 1 a), or to become dilated, forming electron-lucent vesicles in the stroma (Fig. 1 a, b). Peripheral vesiculation, however, was the most striking alteration of affected mitochondria. Fig. 1 (a to d) shows possible transitional stages of this process in which mitochondria become progressively transformed into a disorderly aggregate of vesicles intermingled with remnants of shredded stroma (Fig. 1 d).

Peripheral vesicles were formed from single membranes, were rounded or flask-shaped with diameters up to 200 nm and developed between the twoochondrial membranes (Fig. 1 a to c). Some vesicles, especially the flask-shaped ones, were connected by the neck with the outer membrane, from which they had probably developed by invagination. Individual vesicles were electron-lucent and apparently empty, or contained finely granular material of low electron opacity, or, in about 60% of cases, contained a network of fine fibrils (Fig. 2a).

Fibrils in about 1/3 of these vesicles were resistant to treatment with RNase in high-salt medium (Fig. 2b) although only 1/20 contained fibrils resistant to RNase in low-salt medium (Fig. 2c).

The DAB tests showed cytochrome oxidase activity in mitochondria of uninfected tissues (Fig. 3a) and in MVB in early or intermediate stages of development (Fig. 3b). No reaction was observed in MVB in which cristae were not recognizable or in MVB in a very advanced stage of vesiculation like that of Fig. 1(d). Incubating tissues in KCN inhibited the DAB reaction (Fig. 3c, d) suggesting that the reaction was specific.
The results show the following. (i) In CIRV infections of five host species and in both localized and systemic infections, MVB arise by a progressive modification of mitochondria. This conclusion, primarily drawn from visual evidence, is supported by the detection of cytochrome oxidase in MVB in early and intermediate stages of development. (ii) The membrane-bound vesicles of MVB contain RNA, some of which appears to be double-stranded. Although the nature of this dsRNA was not established experimentally, it may, by analogy with the MVB of CyRSV infection, consist of replicative forms and/or replicative intermediates of viral RNA (see discussion in Russo et al., 1983). It is striking that, despite their different origins, the MVB induced by infection with CIRV and CyRSV may prove to have similar functions. Thus, it is established that MVB induced by definitive members of the tombusvirus group arise from modifications of different cell organelles which, depending on the virus, may be peroxisomes (Russo et al., 1983) or mitochondria.

In this respect CIRV, although serologically related to tomato bushy stunt virus (Hollings et al., 1970), resembles the serologically unrelated turnip crinkle (TCV) and galinsoga mosaic (GMV) viruses which may not be tombusviruses at all but have recently been shown to induce peripheral vesiculation in mitochondria (Russo & Martelli, 1982; Hatta et al., 1983). However, comparable mitochondrial disorders have also been detected in cells infected with viruses unrelated to the tombusvirus group such as tobacco rattle (Harrison et al., 1970) and cucumber green mottle mosaic (Hatta & Ushiyama, 1973) as well as in begonia plants with filamentous virus-like particles (Petzold, 1972) and in Panicum sabulorum (Gill et al., 1981) and grapevine (Castellano et al., 1983) containing isometric virus-like particles. Therefore, the apparent similarity in cytopathology between CIRV, TCV and GMV may still not be sufficient evidence to support the inclusion of these viruses in the same taxonomic cluster.
REFERENCES


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