Evidence for the involvement of the 58K and 48K proteins in the intercellular movement of cowpea mosaic virus

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Infection of cowpea cells with cowpea mosaic virus (CPMV) is accompanied by the appearance of tubular structures containing virus-like particles which protrude from or penetrate the cell wall. Immunogold labelling of sections of infected cells using antisera against a CPMV M RNA translation products, and Protein A-gold, showed that the 58K and/or 48K tentative transport proteins of CPMV were located in or on these tubular structures. Furthermore, these proteins were detected in small electron-dense areas near the tail-end of the tubules. The possible function of these structures in virus movement from cell to cell is discussed.

The bipartite genome of cowpea mosaic virus (CPMV) consists of two positive single-stranded RNA molecules (B and M RNA) that each contain only one large open reading frame and are translated into polyproteins which are cleaved by a B RNA-encoded protease into functional proteins (Goldbach & van Kammen, 1985; Vos et al., 1988). B RNA encodes all the functions necessary for replication of the RNAs (Goldbach et al., 1980; Eggen & van Kammen, 1988) and is able to replicate independently of the M RNA in cowpea protoplasts (Rezelman et al., 1982). The M RNA is translated in vitro into two overlapping polyproteins of Mr 105K and 95K (Vos et al., 1984), which are processed proteolytically to give the overlapping 58K and 48K non-structural proteins and, via a 60K precursor, the 37K and 23K coat proteins (Franssen et al., 1982; and Fig. 1). Rezelman et al. (1989) showed recently that these proteins are also produced in CPMV-infected cowpea protoplasts.

Although the B RNA can replicate independently of M RNA in protoplasts, M RNA is essential for infection of plants (Rezelman et al., 1982). Using infectious transcripts of the M RNA, Wellink & van Kammen (1989) showed that mutations and deletions in the coding regions of both 58K/48K proteins and the capsid proteins prevented systemic infection in cowpea plants. These results suggest that both the 58K/48K proteins and the coat proteins of CPMV are indispensable for cell-to-cell movement of the virus and also that CPMV is transported as particles and not as naked RNA.

To study the possible involvement of M RNA products in virus movement we detected these products in sections of infected cells by the binding of specific antisera to antigens as shown by its reaction with Protein A-gold.

To achieve a near-simultaneous infection of the cells in secondary leaves of cowpea plants (Vigna unguiculata cv. California Blackeye), plants were given a differential temperature treatment (DTT) after inoculation of the primary leaves with 1 mg/ml of purified CPMV in 0.01 M-phosphate buffer pH 7.0 (Dawson & Schlegel, 1976). This treatment results in considerable synchronization of

![Diagram](#)

Fig. 1. Model for the expression of CPMV M RNA. The open reading frame is represented by a speckled bar and the positions of the translational start and stop codons are indicated. The polyproteins 105K and 95K are processed by specific proteolytic cleavages at Gln-Met (\(\nabla\)) and Gln-Gly sites (\(\blacktriangle\)) into the 58K and 48K polypeptides and the two coat proteins. VPg is represented by a solid square (■).
virus replication in systemically infected leaves (Dawson & Schlegel, 1976; Dorokhov et al., 1981). The method, therefore, facilitates the observation of cytological changes early in the infection of cells and in relation to the infection time (i.e. period after the temperature shift). Samples from the petioloïde and leaf were taken 0, 12 and 24 h after transferring the plants to 25 °C. The samples were fixed with glutaraldehyde-paraformaldehyde and subsequently dehydrated and embedded at room temperature in LR White resin as described by van Lent & Verduin (1987). Alternatively, samples were fixed additionally with osmium tetroxide after aldehyde fixation. Leaf tissue from infected secondary leaves of cowpea plants at 14 days after conventional inoculation was also fixed and embedded as described above. Antiserum against CPMV was obtained by injecting a rabbit with purified CPMV components. Antiserum against the 58K and 48K proteins was prepared by injecting a synthetic peptide of 30 residues corresponding to the carboxy terminus of the overlapping 58K/48K proteins as described by Wellink et al. (1987). Gamma globulins (IgG) were purified from the antisera by affinity chromatography on columns of Protein A-Sepharose CL-4B. The purified suspensions contained 0.43 mg/ml (anti-58K/48K) and 0.9 mg/ml (anti-CPMV) gamma globulins in phosphate-buffered saline (PBS). For immunogold labelling the gamma globulin suspensions were diluted to a final concentration of 0.01 mg/ml in PBS-bovine serum albumin (BSA). The preparation of Protein A–gold complexes with 7 nm diameter gold particles and immunogold labelling were as described previously (van Lent & Verduin, 1986). Controls included sections of mock-inoculated plant tissue and sections of infected tissue treated with normal serum. Furthermore, the anti-58K/48K gamma globulin suspension was treated with the synthetic peptide against which the antiserum was raised. Ten µl of the gamma globulin suspension (0.43 mg/ml) was mixed with 100 µl of a 5 mg/ml suspension of synthetic peptide in PBS and the mixture was incubated for 2 h at 37 °C. The mixture was then diluted to a final gamma globulin concentration of 0.01 mg/ml and used for immunogold labelling.

In sections of aldehyde/osmium-fixed and LR White-embedded petioloïde and leaf tissue, treated with anti-CPMV and Protein A–gold at 24 h after DTT, viral coat protein was detected only in the cytoplasm. The density of gold labelling was low, suggesting that little virus was present in the cells (data not shown). No specific association was found between viral coat protein and (virus-induced) cell structures. When similar sections were treated with anti-58K/48K and Protein A–gold, specific gold labelling was seen over the tubular structures (Fig. 3 and 4). Gold label was located also on small areas of electron-dense material in the vicinity of these tubular structures (Fig. 3a and b). The large masses of electron-dense material typical of comovirus-infected cells were not labelled by the anti-58K/48K serum. It was shown previously that these structures contained the non-structural B RNA translation products (Wellink et al., 1988). Specificity of the labelling was demonstrated in serial sections (Fig. 4b and c) where gold label was found only on the tubular structures and associated electron-dense material. Furthermore, no labelling was observed on sections incubated with non-immune serum or with anti-58K/48K serum adsorbed with its homologous peptide. The limited labelling of only a part of the tubular structures displayed in the micrographs can be explained by the fact that only antigens exposed at the surface of the section are labelled. Therefore, it is difficult to establish whether the 58K and/or 48K proteins are associated with the tubules or with the virus-like particles included in the tubule. Tubules enclosing virus-like particles, protruding from or penetrating the cell wall through plasmodesmata, are seen often in plant cells infected with comoviruses (Van der Scheer & Groenewegen, 1971; Kim & Fulton, 1971, 1973), and also nepoviruses (Roberts & Harrison, 1970; Jones et al., 1973). Despite much speculation on their origin and function no conclusive data are available (for review see Martelli, 1980). Martelli (1980) argued that the tubules may not be modified desmotubules, as suggested by several researchers, but that they arise in the cytoplasm by an unknown mechanism. The occasional observation of tubules containing virus-like particles in CPMV-infected cowpea protoplasts (J. W. M. van Lent, unpublished results) supports this view as protoplasts lack plasmodesmata.

Our results provide the first evidence for the association of the 58K and/or 48K tentative transport proteins of CPMV with virus-induced tubules. The 58K and/or 48K proteins were also found in small electron-dense areas close to the end of the tubules, but not in the large

contrast, tubular structures were abundant in mesophyll cells 14 days after infection (Fig. 2). At these later stages of infection most of the tubular structures were sheathed with cell wall material, a process the initiation of which was visible early in the infection of cells.

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Fig. 2. Detail from aldehyde- and osmium-fixed infected mesophyll cells, 14 days after conventional inoculation. Tubular structures containing virus-like particles (shown by arrows) protrude from the cell wall and are sheathed with cell wall material. n, Nucleus; bar represents 1 μm.

Fig. 3. Tubular structures observed in infected cells of the bundle sheath of a cowpea petiolule at 24 h after DTT (a and b). The tubules (shown by arrows) penetrate the cell wall (cw). Electron-dense material (ed) is observed at the tail-end of the tubules. The sections were treated with anti-58K/48K serum and Protein A-gold. Gold label is present only over the tubule and associated electron-dense material. Bars represent 0.1 μm.
electron-dense areas which are made up of non-structural B RNA products (Wellink et al., 1988). These small electron-dense areas associated with tubules may be the site where virus-containing tubules are formed. We have not been able to establish whether the coat protein is also associated with the tubules or the enclosed virus-like particles. Wellink & van Kammen (1989) showed recently that both the 58K/48K proteins and coat proteins of CPMV are essential for virus spread, which suggests that the viral genome is transported from cell to cell in an encapsidated form. Hence, tubules containing virus particles observed in the plasmodesmata of cell walls may demonstrate the route for virus movement.

The appearance of these tubules early (24 h after DTT) in the infection of plant cells supports this hypothesis further.

These findings seem to conflict with the results of Malyshenko et al. (1988) who demonstrated that for another comovirus, red clover mottle virus, the movement of non-encapsidated B RNA can occur when host plants are co-infected with a tobamovirus. However, tobamoviruses are able to move from cell to cell as nucleic acid (Atabekov & Dorokhov, 1984; Takamatsu et al., 1987). It is thus plausible that in the experiments of Malyshenko et al. (1988), the movement of B RNA was achieved by a transport function that enabled the
movement of nucleic acid. This implies the existence of at least two different types of mechanism for virus movement from cell to cell; one type allows the movement of viral nucleic acid (e.g. the tobramovirus transport mechanism) and the other allows the movement of virus particles (e.g. the comovirus transport mechanism).

References


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