Plant Pathol. J. 15(4) : 193-198 (1999)

The Plant Pathology Journal

Mini-Review

Cell-to-Cell Movement of Plant Viruses: Ultrastructural Aspects

Kyung Soo Kim*, Elizabeth M. Martin and Ki Woon Lee[1]

Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA

[1]Department of Agricultural Biology, Kyungpook National University, Taegu 702-701, Korea

(Received on August 14, 1999)

For any virus of either animal or plant to establish a successful infection in a susceptible host, progeny virus in the initially infected cells must be able to move to neighboring cells. In animal tissue, there are two general pathways for viruses to enter neighboring cells; by surface fusion or receptor-mediated endocytosis. In plants, however, these two pathways can not be utilized because of the presence of the cell wall which acts as a barrier to extracellular release and subsequent uptake. Consequently, plant viruses have evolved a distinct mechanism for cell-to-cell movement.

Through pioneering work on tobacco mosaic virus (TMV) followed by recent work on other viruses, it has been fully established that plant viruses have a gene that encodes a specific protein that facilitates cell-to-cell movement of the virus by modifying the structure and function of plasmodesmata. The 30 kDa, 58/48 kDa, and 38 kDa proteins encoded by TMV (Deom et al., 1987), cowpea mosaic comovirus (CPMV) (van Lent et al., 1990), and cauliflower mosaic caulimovirus (CaMV) (Linstead et al., 1988), respectively, are the examples of this protein which has been termed as movement protein, M-protein.

It has been well established that plant viruses induce cytopathological structures or inclusions characteristic of a particular virus or a group of related viruses, thus providing a rapid and reliable tool for virus diagnosis and classification (Martelli and Russo, 1977; Francki et al., 1985; Edwardson et al., 1993). Among a variety of these virus-inducing inclusions, some of them exhibit structural features manifestative of involving intra- as well as intercellular movement of viruses. These are tubular structures, containing virus particles, induced by a number of different virus groups such as como-, nepo- and caulimoviruses, and cylindrical inclusions induced by potyviruses. These structures are all closely associated with plant intercellular connection, the plasmodesmata. This paper reviews briefly the details of these virus-induced structures in relation to virus-encoded, movement proteins, and presents possible functions involved in cell-to-cell movement of plant viruses.

*Corresponding author.
Phone) +1-501-575-3189, Fax) +1-501-575-7601
E-mail) kskim@comp.uark.edu

Plasmodesmata

In plants, cells communicate with their neighbor cells through narrow strands of cytoplasm called plasmodesmata which pierce through the non-living cell wall. Plasmodesmata of higher plants consist of the plasmalemma-lined cylinder containing an endoplasmic reticulum-derived axial component, the desmotubule (Fig. 1) (Robards, 1990). The area between the desmotubule and cytoplasm sleeve is partially occluded with closely packed globular subunits that functionally divide the cytoplasmic sleeve into discrete channels (Fig. 2) (Citovsky, 1993) These plasmodesmata, together with the cytoplasm of individual cells, form a continuous three dimensional network, the symplast. No channels other than the plasmodesmata occur between cells in plant.

The particles of plant viruses are initially introduced into host cells through mechanical wounding or biological damage, via vectors thereby disrupting the integrity of the cell wall and the plasma membrane. If, following entry and replication, progeny viruses can not move into adjacent healthy cells, only a subliminal infection occurs, and the host appears to be resistant to the virus. If, however, virus

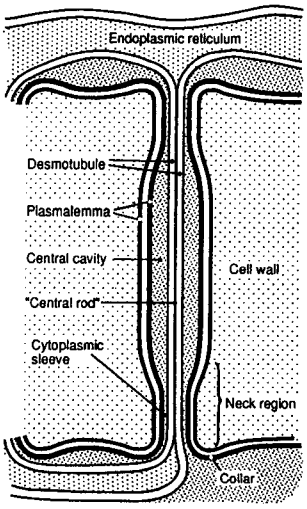

Fig. 1. Schematic diagram illustrating the component parts of a simple plasmodesma (Adapted from Robards and Lucas, 1990).

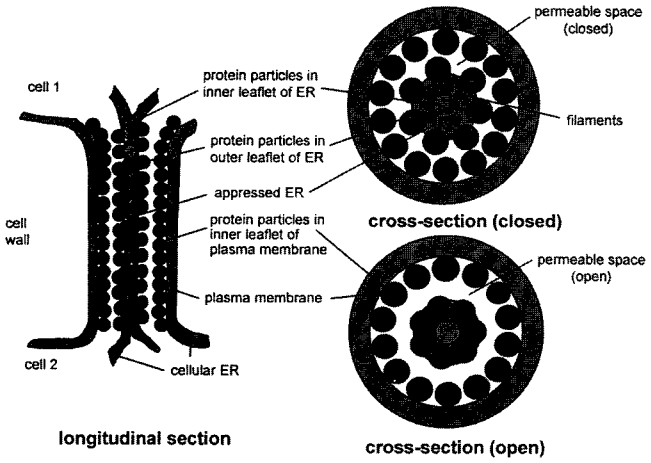

Fig. 2. Schematic model illustrating the details of the internal compartments of a simple plasmodesma. Diagrams of longitudinal view and of transverse views of a closed and an open plasmodesma (Adapted from Citovsky, 1993).

progeny are capable of spreading into neighboring cells, a progressive virus infection results. Although the specific virus-host interaction will ultimately define the nature of the infection, virus movement represents an important component in determining pathogenicity and virulence. (Deom et al., 1992).

There are various reasons that plant virologists have generally accepted that the plasmodesmata must be the route by which infectious viruses move from cell to cell. Firstly, kinetic data indicate that even small molecules such as sucrose are transported from mesophyll cells to the small vein parenchyma cells through symplastic routes, plasmodesmata. Thus, there appears to be no other reasonable route for much larger molecules such as virus particles. Secondly, the direct evidence that it is possible for virus to move through plasmodesmata comes from electron micrographs exhibiting the presence of various kinds of virus particles within the plasmodesmata (Kim and Fulton, 1971, 1975; Kim and Lee, 1992; Kitajima et al., 1969). However, because of the small size exclusion limits of plasmodesmata it is very difficult to conceive that such EM observations are an indication of the intact plasmodesmata for being a route for cell-to-cell movement of virus particles and viral nucleic acids. Under normal physiological conditions, plasmodesmata allow the passage of molecules having molecular weights up to 1.0 kDa (Goodwin 1983; Robards and Lucas 1990; Walf et al., 1989). High resolution electron micrographs have also revealed effective diameter of the plasmodesmal micro channels, through which most small molecules such as metabolites, hormones as well as ions diffuse, is about 2.5-3.0 nm (Ding et al., 1992). This small size exclusion limits of plasmodesmata relative to the large size of plant viruses ranging from 10-110 nm or free nucleic acid which has been estimated to have an average diameter of 10 nm indicate that plasmodesmata must be modified during virus infection for virus progeny to move from cell to cell.

Ultrastructure-involved cell-to-cell movement of plant viruses

With respect to the ultrastructural aspects of cell-to-cell movement, plant viruses can largely be divided into three categories, based on whether or not the cytopathological structures suggestive of cell-to-cell movement are induced, and the occurrence of virus particles in the plasmodesmata. Viruses in como-, nepo-, tospo- and caulimovirus groups (Kim and Fulton, 1971, 1973, 1975; Kim et al., 1974; Kitajima et al., 1969; Storms et al., 1995; Walkey and Weff, 1970) would be in one category of viruses which induce tubular structures that are often continuous with the plasmodesmata, containing linearly aligned virus particles. Viruses in the second category may include the groups of viruses that do not induce any structural features comparable to those induced by viruses in the first category. Viruses in the tobamo-, cucumo-, bromo-, and alfalfa mosaic virus (Francki et al., 1985) groups may belong to this category. The third category may include cytopathic structures similar to those induced by the viruses in the first category, but these structures are transient occurring only for a short period during a certain stage of infection. Some geminiviruses (Kim and Lee, 1992) appear to be a member of this category.

Most obvious cytopathological structures suggestive of virus movement induced by the viruses in the first category, would be tubules which are often continuous with the plasmodesmata containing virus particles. Tubules induced by comoviruses and nepoviruses are very similar. Since these tubules usually pass through in the middle of the plasmodesmata, it was hypothesized that virus-containing tubules are modified or extensions of the preexisting desmotubules (Hibino et al., 1977), implying that these tubules are solely host origin. Later studies by van Lent and his coworkers (Kasteel et al., 1996; van Lent et al., 1991), however, demonstrated that these tubules are also formed in CPMV-infected cowpea protoplasts from which the cell wall and plasmodesmata are absent and which are not involved in cell-to-cell contact, indicating that the formation of these tubules is not dependent upon preexisting cell wall or plasmodesmata, but formed in the cytoplasm independently encoded by the viral gene.

Thin-section electron microscopy revealed that the tubules in CPMV-infected protoplasts were formed at the surface of the protoplast extending outwardly with the plasma membrane around the tubules indicating the pres-

ence of functional polarity. This observation explains how the tubules with virus particles, surrounded by the plasmalemma, were common in the space between the plasmalemma and the cell wall in comovirus-infected leaf cells *in situ* (Kim and Fulton, 1971, 1975). The tubules apparently formed randomly at the periphery of the cytoplasm then were pushed outwardly, but the presence of rigid cell wall must have caused the tubules, along with the surrounding plasmalemma, to bend and run through the narrow space between the plasmalemma and the cell wall. When a tubule is formed at or near an opening of the plasmodesmata, it is assumed that it would be pushed through the channel of the plasmodesmata and reach into the cytoplasm of the adjacent cell. If this is the case, this process would involve structural modification of the plasmodesmata, in which a minimum modification necessary would be the rearrangement or removal of globular structures of regulatory proteins or desmotubule in the plasmodesmata thereby widening the cytoplasmic sleave (or permeable space) large enough for virus particles to pass through. Virus-coded M-protein may play a role in this modification.

Structural modification of the plasmodesmata can easily be detected in caulimovirus infections, partly because the particles of these viruses have a larger diameter (50 nm) than that of como- or nepoviruses which is 25-30 nm. The plasmodesmata containing virus particles in dahlia mosaic caulimovirus-infected zinnia leaf cells had consistently larger openings ranging 50-80 nm in diameter compared to 20-25 nm of those in uninfected normal plasmodesmata (Kitajima et al., 1969). No desmotubules were observed in these modified plasmodesmata containing virus particles, but a thin electron dense layer was present immediately next the plasmalemma within the plasmodesmata, suggesting that the desmotubule was replaced by these electron-dense tubules. Association of endoplasmic reticulum with the desmotubules, common in normal plasmodesmata, was also severed in modified or transformed plasmodesmata containing virus particles. In some cells, plasmodesmata, apparently half normal and half modified, occurred where one end of the plasmodesma was enlarged, containing virus particles, while the other end appeared normal and associated with endoplasmic reticulum.

Cowpea mosaic comovirus (CPMV), cauliflower mosaic caulimovirus (CaMV), and tomato spotted wilt tospovirus (TSWV), all of which induce the tubular structures, encode M-proteins; 58/48 kDa proteins for CPMV, 38 kDa protein for CaMV and 33.6 kDa protein for TSWV (Storms et al., 1995). Immunogold labeling, using specific antisera against these M-proteins, revealed that these proteins are specifically located in or on these tubules, indicating that the tubules are indeed involved in cell-to-cell movement of the viruses (van Lant et al., 1990).

The role of M-protein in the formation of virus-tubules has, however, not been fully understood. It is unknown whether the tubules are solely of virus origin or whether host components are also involved. Microtubules are the only host cell organelle which are similar in structure and size to the virus-containing tubules, and indeed, structural continuation of these two entities has been demonstrated in two comoviruses-infected tissues (Kim and Fulton, 1971, 1973). A striking feature of M-proteins of these viruses is their ability to induce tubules in protoplasts and cultured insect cells. In both cell systems (protoplast and insect cells) expression of M-protein results in tubular protrusions extending from the cell surface (Kasteel et al., 1993, 1996; Perbal et al., 1993; Storms et al., 1995; van Lent et al., 1991; Wellink et al., 1993). The plasmalemma is contiguous with the exterior of tubules visualized in plants that contain M-protein and, in case of infected protoplasts, are filled with virus particles aligned in a row. However, mature virions are not required for tubule formation. These protoplast data clearly indicate that plasmodesmata and/or cell wall are unnecessary for tubule development, but it is certainly possible that a plasmodesmal channel provides an opening to initiate tubule extension in intact tissue (Carrington et al., 1996). The fact that tubules can be induced in insect cells suggests that these M-proteins interact with cellular components that are conserved across the boundaries of the animal and plant kingdoms.

Based on the data accumulated for tubule-inducing viruses, it is speculated that virus-coded M-proteins are targeted to plasmodesmata, where they induce removal of the desmotubule, and are assembled into tubules possibly associated with the plasma membrane and host proteins. Extending tubules may be anchored by cytoskeletal structures such as microtubules. Virions assembled in the cytoplasm are escorted to tubular structures through interactions with M-protein. Virions are then transported through tubules via specific M-protein-capsid protein interactions and transported to the adjacent cell.

Potyviruses may also be included in the first category, although they do not induce tubular structures. Potyviruses, instead, induce cylindrical inclusions (CI) which appear in electron microscopy sections in various configurations such as pinwheels, bundles, tubules, and laminated aggregates, depending on the planes of sectioning (Edwardson et al., 1993). Some forms of CI, especially pinwheels and bundles, are closely associated with plasmodesmata in an early infection stage (Langenberg, 1986; Lawson and Hearon, 1971). Bundle inclusions, longitudinal sections of CI, usually appear perpendicular to the cell wall, with the ends of the bundles associated closely with the plasmodesmata (Weintraub et al., 1974). Pinwheels, on the other hand, which are the transverse sections of CI, were associated

with the plasmodesmata by converging their arms into the opening of the plasmodesmata (Lawson and Hearon, 1971). In sections cut transversely along the cell wall, the central core of the pinwheel was often directly over the plasmodesmal opening. It has been demonstrated with immunogold labeling that wheat streak mosaic potyvirus capsid protein or virions were located at bundle inclusions that are associated with plasmodesmata and also in the plasmodesmata themselves, suggesting that CI are indeed involved in the movement of virus particles from cell to cell (Langenburg, 1986).

The viruses in the first category have some additional properties that are common: 1) the cytopathic structures involved in cell-to-cell movement of viruses, such as tubules and CI, occur persistently throughout the infection stages, which coincide with the presence of movement proteins encoded by these viruses, 2) virus particles occur in the plasmodesmata, even though they are not associated with tubular structures, 3) the particles in the tubules appeared to be mature virions since they reacted specifically with antisera against viral coat protein. It is suggested, therefore, that the viral form involved in cell-to-cell movement is an encapsidated virion. This suggestion has been strengthened by the fact that, in case of some poty- and comoviruses, capsid protein is essential for cell-to-cell movement (Dolja et al., 1994, 1995).

Viruses in the second category are represented by tobacco mosaic tobamovirus (TMV) since it is the most thoroughly studied plant virus in virus movement. TMV induces tobamovirus-characteristic inclusion body, X-body, which is now know to be the viroplasm where TMV viral components are synthesized and assembled into virus particles (Matthews, 1991). X-body consists of a variety of structures including proteinaceous tubules (X-tubules), densely packed ribosomes, rough endoplasmic reticulum, virus particles and amorphous granular material (Mathews, 1991). However, no structural features suggestive of involving cell-to cell movement of virus, such as tubules or CI, were encountered. Despite massive accumulation of virus particles forming various orderly arranged crystalline aggregates, no TMV particles have been located in the plasmodesmata. In addition, no obvious structural modification of plasmodesmata has been noticed in infected cells.

As indicated, TMV encodes 30K M-protein (Deom et al., 1987, 1992). Transgenic plants expressing 30K M-protein gene complements a temperature-sensitive TMV mutant LS1 strain at the non-permissive temperature (Deom et al., 1987), as well as a mutant of TMV that contains a defective movement protein (Deom et al., 1991, Holt and Beachy, 1991). These experiments provide direct evidence that 30K M-protein acts on the plasmodesmata to "gate" the passage of TMV. Immunogold labeling studies demonstrated that 30K M-protein accumulated in plasmodesmata during viral infection, supporting the hypothesis that 30K M-protein modify the gating properties of plasmodesmata and directly facilitate the transfer of TMV to move from cell to cell (Ding et al., 1992; Tomenius et al., 1987). Subsequently, a defined activity of TMV M-protein in plasmodesmata was first demonstrated (Wolf et al., 1989) using microinjection techniques to introduce fluorescent dextrans of varying size. The size exclusion limit of plasmodesmata between mesophyll cells of transgenic plants expressing 30K M-protein was 10-fold higher than that of plasmodesmata of control plants. This study clearly indicated that M-protein mediates virus movement by increasing the plasmodesmal size exclusion limit without overt structural modification of plasmodesmata.

In addition to the lack of cytopathic structures associated with the plasmodesmata, viruses in the second category also have some additional properties that are in common. These are the M-proteins encoded by these viruses such as TMV, alfalfa mosaic and cucumoviruses appear to be transient, occurring in an early stage of infection, and become degraded as infection progresses (Lehto et al., 1990). Also, no virus particles of these viruses have been located in the plasmodesmata despite massive accumulations of virus particles in the cytoplasm of infected cells. It seems, therefore, that there is a correlation between the absence of plasmodesma-associated cytopathic structures (Tubules, CI, etc.) and the occurrence of virus particles in the plasmodesmata. Absence of virus particles in the plasmodesmata suggests that the form in which these viruses move from cell to cell is something other than assembled virus particles. In fact, it has been demonstrated that short distance cell-to-cell movement of TMV is not requiring the presence of viral coat protein (Dawson et al., 1988). There is now enough information that M-proteins of TMV and other viruses in the second category have nucleic acid-binding properties which result in the formation of M-protein/viral nucleic acid complexes in infected cells (Citovsky et al., 1990, 1992; Li and Palukaitis, 1996), and these complexes have been suggested to be the form involved in cell-to-cell movement in these viruses (Carrington et al., 1996).

There are some viruses that do not quite fit to either of the two categories mentioned above. Recent studies of a geminivirus appear to represent this case (Kim and Lee, 1992). Geminiviruses have unique particle morphology and are the only group of plant viruses with a single-stranded DNA genome. In all cases of geminiviruses studied, especially those transmitted by whitefly vector, *Bemicis tabaci*, cytopathic effects, including an accumulation of virus particles, are confined to the nuclei of infected cells (Kim and Flores, 1979; Kim and Fulton, 1984; Kim and Lee, 1992; Kim et al., 1978, 1986). The fact that virus particles are localized

only in the nuclei of infected cells raised a question as to how the virus particles are released from the nucleus into the cytoplasm to undergo a cell-to-cell spread. No structural features suggestive of inter- as well as intracellular movement of virus particles, such as tubules with virus particles which are common in como-, nepo-, and caulimoviruses, have been reported to occur in any of the known whitefly-transmitted geminiviruses.

Lately, however, Euphorbia mosaic geminivirus (EMV) has been reported to induce tubular structures containing paired particles in the cytoplasm of an experimental host, *Datura stramonium*. As those of como- and caulimoviruses, tubules containing paired virus particles are continuous with the plasmodesmata, suggesting that theses tubules are involved in cell-to-cell movement and the viral form involved in virus spread is the paired gemni particles. Based on the presence of virus-containing tubules alone, EMV probably belongs to the first category joining with the como-, nepo-, and caulimoviruses. However, the tubules induced by Euphorbia mosaic virus were somewhat different in that they occur transiently only in an early stage of infection in the inoculated leaves. The transient nature of the virus-containing tubules observed in Euphorbia mosaic geminivirus infection raises a few questions as to their absence in cells of advanced lesions, in systemically infected cells of the same host, and in any cells of the naturally infected original host. It may be that the cells in advanced lesions on inoculated leaves and those in systemically infected leaves were all in well-advanced stages of infection and no longer active in cell-to-cell movement of virus particles. The tubules formed in an earlier stage of infection might have been degraded or depolymerized into a non-structural form, as in the case of mitotic spindle microtubules. In the natural host of Euphorbia mosaic, *E. heterophylla*, the virus is phloem-limited like most whitefly-transmitted geminiviruses, whereas in *D. stramonium* used in this study, the virus is not phloem-limited and infects all cell types. It is possible that the mechanism of cell-to-cell spread between the two hosts may be different. If this is the case, then tubules containing virus particles may not be formed in the natural host in which the virus is phloem-limited.

It should be noted here that most whitefly-transmitted geminiviruses have been demonstrated to encode two M-proteins, BR1 and BL1, which act in a cooperative way to facilitate cell-to-cell movement (Carrington et al., 1996). BR1 provides a nuclear shuttle activity to deliver viral DNA to the cytoplasm, after which BL1 mediates trafficking of DNA to and through plasmodesmata. These data clearly indicated that the form of these viruses involved in cell-to-cell movement is M-protein/viral nucleic acid complexes rather than assembled virus particles which would assign these geminiviruses to the second category. It is of interest to find out whether Euphorbia mosaic virus collected from a natural host would also encode BR1 and BL1 M-Proteins.

References

Carrington, J. C., Kasschau, K. D., Mahajan, S. K. and Schaad, M. C. 1996. Cell-to-cell and long-distance transport of viruses in plant. *Plant Cell* 8:1669-1681.

Citovsky, V. 1993. Probing plasmodesmal transport with plant viruses. *Plant Physiol.* 102:1071-1076.

Citovsky, V., Knorr, D., Schuster, G. and Zambryski, P. 1990. The P30 movement protein of tobacco mosaic virus is a single-stranded nucleic acid binding protein. *Cell* 60:637-647.

Citovsky, V., Wong, M. L., Shaw, A. L., Prasad, B. M. V. and Zambryski, P. 1992. Visualization and characterization of tobacco mosaic virus movement protein binding to single-stranded nucleic acid. *Plant Cell* 4:397-411.

Dawson, W. O., Bubrick, P. and Grantham, G. L. 1988. Modifications of the tobacco mosaic virus coat protein gene affecting replication, movement and symptomatology. *Phytopathology* 78:783-789.

Deom, C. M., Lapidot, M. and Beachy, R. N. 1992. Plant virus movement proteins. *Cell* 69:221-224.

Deom, C. M., Oliver, M. J. and Beachy, R. N. 1987. The 30-kilodalton gene product of tobacco mosaic virus potentiates virus movement. *Science* 237:389-394.

Deom, C. M., Wolf, S., Holt, C. A., Lucas, W. J. and Beach, R. N. 1991. Altered function of the tobacco mosaic virus movement protein in a hypersensitive host. *Virology* 180:251-256.

Ding, D., Haudenshield, J. S., Hull, R. J., Wolf, S., Beachy, R. N. and Lucas, W. J. 1992. Secondary plasmodesmata are specific sites of localization of the tobacco mosaic virus movement protein in transgenic tobacco plants. *Plant Cell* 4:915-928.

Dolja, V. V., Haldeman, R., Robertson, N. L., Dougherty, W. G. and Carrington, J. C. 1994. Distinct functions of capsid protein in assembly and movement of tobacco etch potyvirus in plants. *EMBO J.* 13:1482-1491.

Dolja, V. V., Haldeman-Cahill, R., Montgomery, A. E., Vanderbosch, K. A. and Carrington, J. C. 1995. Capsid protein determinates involved in cell-to-cell and long distance movement of tobacco etch potyvirus. *Virology* 207:1007-1016.

Edwardson, J. R., Christie, R. G., Purcifull, W. E. and Petersen, M. A. 1993. Inclusions in diagnosing plant virus diseases. In: *Diagnosis of Plant Virus Diseases*, ed. by R. E. F. Matthews, pp. 101-128. CRC Press, Boca Raton, Fl. USA.

Francki, R. I. B., Milne, R. G. and Hatta, T. 1985. *Atlas of Plant Viruses*, Vol. I, II. CRC Press, Boca Raton, Fl. USA.

Goodwin, P. B. 1983. Molecular size limit for movement in the symplast of the *Elodea* leaf. *Planta* 157:124-130.

Hibino, H., Tsuchizaski, T., Usugi, T. and Saito, J. 1977. Fine structure and developmental process of tubules induced by mulberry ringspot virus and satsuma ringspot virus infection. *A. Phytopath. Soc. Jpn.* 43:255-264.

Holt, C. A. and Beachy, R. N. 1991. *In vivo* complementation of

infections transcripts from mutant tobacco mosaic virus cDNAs in transgenic plants. *Virology* 181:109-117.

Kasteel, D., Wellink, J., Verver, J., VanLent, J., Goldback, R. and van Kammen, A. 1993. The involvement of cowpea mosaic virus M RNA-encoded proteins in tubule formation. *J. Gen. Virol.* 74:1721-1724.

Kasteel, D. T. J., Perbal, M. C., Boyer, J. C., Ellink, J., Goldback, R. W., Maule, A. J. and van Lent, J. W. M. 1996. The movement proteins of cowpea mosaic virus and cauliflower mosaic virus induce tubular structures in plant and insect cells. *J. Gen. Virol.* 77:2857-2864.

Kim, K. S. 1979. Ultrastructure of plant cells infected with the beetle-transmitted comoviruses. *Fitopat. Braz.* 4:227-239.

Kim, K. S., Bird, J., Rodriguez, R. L., Martin, E. M. and Escudero, J. 1986. Ultrastructural studies of *Jatropha gossipifolia* infected with Jatropha mosaic virus, a whitefly-transmitted geminivirus. *Phytopathology* 76:80-85.

Kim, K. S. and Flores, E. M. 1979. Nuclear changes associated with Euphorbia mosaic virus transmitted by the whitefly. *Phytopathology* 69:980-984.

Kim, K. S. and Fulton, J. P. 1971. Tubules with virus-like particles in leaf cells infected with bean pod mottle virus. *Virology* 43:329-337.

Kim, K. S. and Fulton, J. P. 1973. Plan virus-induced cell wall overgrowth and associated membrane elaboration. *J. Ultrastruct. Res.* 45:328-342.

Kim, K. S. and Fulton, J. P. 1975. An association of plant cell microtubules and virus particles. *Virology* 64:560-565.

Kim, K. S., Fulton, J. P. and Scott, H. A. 1974. Osmiophylic globules and myclinic bodies in cells infected with two comoviruses. *J. Gen. Virol.* 25:445-452.

Kim, K. S. and Fulton, R. W. 1984. Ultrastructure of *Datura stramonium* infected with an Euphorbia virus suggestive of a whitefly-transmitted geminivirus. *Phytopathology* 74:236-241.

Kim, K. S. and Lee, K. W. 1992. Geminivirus-induced macrotubules and their suggested role in cell-to-cell movement. *Phytopathology* 82:664-669.

Kim, K. S., Shock, T. L. and Goodman, R. M. 1978. Infection of *Phaseolus vulgaris* by bean golden mosaic virus: Ultrastructural aspects. *Virology* 89:22-33.

Kitajima, E. W., Lauritis, J. A. and Swift, H. 1969. Fine structure of zinnia leaf tissues infected with dahlia mosaic virus. *Virology* 39: 240-249.

Langenberg, W. G. 1986. Virus protein associated with cylindrical inclusions of two viruses that infect wheat. *J. Gen. Virol.* 67:1161-1168.

Lawson, R. H. and Hearon, S. S. 1971. The association of pinwheel inclusions with plasmodesmata. *Virology* 44:454-456.

Lawson, R. H. and Hearon, S. S. 1973. Ultrastructure of carnation etched ring virus-infected *Saponaria vaccaria* and *Dianthus caryophyllus*. *J. Ultrastruct. Res.* 48:201-215.

Lehto, K., Bubrick, P. and Dawson, W. D. 1990. Time course of TMV 30K protein accumulation in intact leaves. *Virology* 174:290-293.

Li, Q. and Palukaitis, P. 1996. Comparison of the nucleic acid- and NTP-binding properties of the movement proteins of cucumber mosaic cucumovirus and tobacco mosaic tobamovirus. *Virology* 216:71-79.

Martelli, G. P. and Russo, M. 1977. Plant virus inclusion bodies. *Adv. Virus Res.* 21:175-266.

Matthews, R. E. F. 1991. *Plant Virology*, Third ed. Academic Press, New York, USA.

Perbal, M. C., Thomas, C. L. and Maule, A. J. 1993. Cauliflower mosaic virus gene product (PI) forms structures which extend from the surface of infected protoplasts. *Virology* 195:281-285.

Robards, A. W. and Lucas, W. J. 1990. Plasmodesmata. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:369-419.

Storms, M. M. H., Kormelink, R. Peters, D., van Lent, J. W. M. and Goldbach, R. W. 1995. The non-structural NSM protein of tomato-spotted wilt virus induces tubular structures in plants and insect cells. *Virology* 214:485-493.

Tomenius, K., Clapham, D. and Meshi, T. 1987. Localization by immunogold cytochemistry of the virus-encoded 30K protein in plasmodesmata of leaves infected with tobacco mosaic virus. *Virology* 160:363-371.

van Lent, J., Storms, M., Vander Meer, F., Wellink, J. and Goldbach, R. 1991. Tubular structures involved in movement of cowpea mosaic virus are also formed in infected cowpea protoplasts *J. Gen. Virol.* 72:2615-2623.

van Lent, J., Wellink, J. and Goldbach, R. 1990. Evidence for the involvement of the 58K and 48K proteins in the intercellular movement of cowpea mosaic virus. *J. Gen. Virol.* 71:219-223.

Walkey, D. G. A. and Webb, M. J. W. 1970. Tubular inclusion bodies in plants infected with viruses of the NEPO type. *J. Gen. Virol.* 7:159-166.

Weintrauf, M., Ragetli, H. W. J. and Lo, E. 1974. Potato virus Y particles in plasmodesmata of tobacco leaf cells. *Ultrastruct. Res.* 46:131-148.

Wellink, J. and Van Kammen, J. 1989. Cell-to-cell transport of cowpea mosaic virus requires both the 58K/48K proteins and the capsid proteins. *J. Gen. Virol.* 70:2279-2286.

Wolf, S., Deom, C. M., Beachy, R. N. and Lucas, W. J. 1989. Movement protein of tobacco mosaic virus modifies plasmodesmata size exclusion limit. *Science* 246:377-379.