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Mahesh Kumar
Dr. Kalam Agricultural College (Bihar Agricultural University, Sabour), Kishanganj, Bihar, India

Md. Shamim
Dr. Kalam Agricultural College (Bihar Agricultural University, Sabour), Kishanganj, Bihar, India

Tushar Ranjan
Department of Molecular Biology & Genetic Engineering, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Santosh Kumar
Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Hari Om
Dr. Kalam Agricultural College (Bihar Agricultural University, Sabour), Kishanganj, Bihar, India

Ravi Ranjan Kumar
Department of Molecular Biology & Genetic Engineering, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Vinod Kumar
Department of Molecular Biology & Genetic Engineering, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Nimmy MS
NRC on Plant Biotechnology, IARI, Pusa Campus, New Delhi

Pankaj Kumar
Department of Molecular Biology & Genetic Engineering, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India

Corresponding Author:
Mahesh Kumar
Dr. Kalam Agricultural College (Bihar Agricultural University, Sabour), Kishanganj, Bihar, India

Recent development on plant virus movement and movement protein

Mahesh Kumar, Md. Shamim, Tushar Ranjan, Santosh Kumar, Hari Om, Ravi Ranjan Kumar, Vinod Kumar, Nimmy MS and Pankaj Kumar

Abstract
Plant viruses are major constraint in crop production and production and leading to severe economic losses. These losses mainly due poor growth and development of the plant after hijacking plant defense system by virus. Viruses are entering into the host cell through wound that cause by mechanically or insect. The movement of virus's starts from infected cell to healthy cell through plasmodesmata. The majority of viruses encode special types of proteins for their replication, movement and packaging of virion particle. Among them movement protein (MP), which is responsible for viral movement to neighboring cells after increasing the size exclusion limit of plasmodesmata by interacting with callose deposits.

Short-distance movement is promoted by the interactions of MP and/or coat protein (CP) with the host cellular cytoskeleton, which directs targeting of viral particles to the plasmodesmata. In this review; we mainly focused on the movement aspects of the different plant viruses and its recent development. To counteract the viral movement is important for agriculture by using vector engineering or recombinant products.

Keywords: movement, movement protein, plant viruses, plasmodesmata, callose

1. Introduction

Animal viruses can negotiate their entry into the host cell by manipulation of the host's array of receptor systems. But unlikely to the animal viruses, the plant viruses have to face the impervious barrier of the cell wall. For this reason, the primary infection of plant viruses sometimes remains confined to a single cell or a few cells only. This occurs after mechanical damage to the plant cell wall and plasma membrane by the vectors transmitting the virus, or by mechanical inoculation. The infection can be passed onto the adjacent cells with the help of specialized virus-encoded proteins called movement proteins (MPs). An interesting fact about viruses is that the specificity of a plant virus infection does not occur at the level of replication and plant viruses have the ability to replicate within non-host cells. However, the susceptibility is linked to the ability of the virus to gain access to the phloem tissues of the plant for long distance transport, and thereby spread a systemic infection.

In systemic infection, the virus moves from the source leaves to sink leaves in a passive mode along with the flow of photo-assimilates (Leisner and Howell, 1993) [1]. But in disparity, the cell-to-cell movement is an active process that involves the contact of virus and plasmodesmata (PD). Before going to the Movement protein let's describe the Plasmodesmata. Plasmodesmata serve as intercellular channels that maintain a plant wide simplistic domain and enable the entry and exit of viruses in different parts of the plant.

Plasmodesmata have been studied for several decades to delineate their mechanism and molecular organization. Some proteins have been described that seem to be characteristic of PD protein (Blackman *et al*., 1999) [2]. According to the ultra-structural analysis of plant tissues, there are several forms of plasmodesmata including primary plasmodesmata, which are formed during cytokinesis (Lucas *et al*., 1993 [3]; Ding, 1997 [4]; Crawford and Zambryski, 1999 [5]). The secondary plasmodesmata that are formed later during development through existing cell walls and are involved in the expansion of the cytoplasmic continuum (Vander Schoot and Rinne, 1999) [5]. Combined cytoplasm of all cells is interconnected by plasmodesmata, which enables communication throughout the plant.

Generally, plasmodesmata are narrow channel with a diameter of 20-30 nm, which cross the plant cell wall (Itaya *et al*., 1998) [6]. Plasmodesmata may consist of only one channel, linear or simple plasmodesmata or network of channels which are branched (Itaya*et al*., 1998) [6].

Desmotubule is a stretch of appressed endoplasmic reticulum present in the centre of the channel. It links the endomembrane systems of the neighboring cells. Some plasmodesmata contain a central cavity between the plasma membrane and desmotubule. Both the plasma membrane and the desmotubule are associated with protein globules called bridging proteins (Ding *et al*., 1995) [7]. These bridging proteins form the linkages between the plasma membrane and desmotubule globules across the central cavity. It is the space between the plasma membrane and desmotubule the cytoplasmic sleeves, through which the virus and other macromolecules are proposed to pass. The cytoplasmic sleeve, the space between the plasma membrane and desmotubule is subdivided into smaller microchannels, each with a diameter of 1.5-2.0 nm. Actin (White *et al*., 1994) [8] and myosin (Reichelt *et al*., 1999 [9]; Radford and White, (1998) [10] might be structural components of these microchannels.

Plasmodesmata are influenced by environmental (Cleland *et al*., 1994 [11]; Schulz, (1995) [12] and developmental signals (Duckett *et al*., 1994 [13]; Oparka *et al*., (1999) [14] and tissue specific features (Kempers and Van Bel, 1997) [15]. In general, with respect to metabolism, sink-source transition has been noticed. The source tissues produce excessive photo-assimilates which are transported through the phloem to the different sink tissues which are utilized subsequently for their growth and development. The transition of sink to source results in structural changes in PD's from linear to branched plasmodesmata (Oparka *et al*., 1999) [14]. Plasmodesmata act as gateways to local and systemic virus infection (Benitez-Alfonso *et al*., 2010) [16]. The structure of PD and the contribution of viral MPs to intercellular movement was explained in several reviews (Ueki and Citovsky, (2011) [17]; Benitez-Alfonso *et al*., 2010 [16]; Tilsner *et al*., 2011 [18]; Niehl and Heinlein, (2011) [19].

In the past, it was widely accepted that the cell-to-cell movement of the plant viruses occurs through a passive diffusion process. The first report opposing this idea came from the works of Nishiguchi *et al*., (1978) [20] and Nishiguchi *et al* (1980) [21]. He used temperature-sensitive Ls1 mutants of *Tobacco mosaic virus* (TMV). At restrictive temperatures, these mutants could replicate efficiently at the single cell level and form virus particles, but it was incapable of moving out of the primary infected cells and cause systemic infection. The Ls1 defect was mapped to the gene encoding 30 kDa protein in TMV (Doem *et al*., 1987) [22]. This 30 kDa protein was later named as movement protein (MP). In the past decades, it has been repeatedly proved that not only in TMV, but the MPs are present in most plant virus families and perform the function of intercellular viral transport.

2. Movement Protein

The MPs encoded by different virus families may complement each other or are functionally interchangeable (De jong and Ahlquist, 1992 [23]; Giesman-Cookmeyer *et al*., 1995 [24]; Fajardo *et al*., 2013) [25]. The movement defect of a virus strain in a particular host plant can be complimented by co-infection with another unrelated plant virus which is movement competent (Atabekov and Tilianisky, 1990) [26]. One such example is *Brome mosaic irus* (BMV) gains the ability to move through tomato plants when coinfected with TMV (Atabekov and Tilianisky, 1990) [26]. Sometimes cell type restriction can be overcome by movement function of heterologous viruses. For example, the blockage to the movement of *Potato leaf roll lutiovirus* into the mesophyll cells from phloem could be overcome by co infection with *Potato virus Y* (Barker, 1987) [27]. This exchangeability and complementation of movement function from unrelated virus families suggest that virus can move through common intercellular movement pathways or all movement proteins may have some common ancestors and some common mechanisms of transport within the host.

There are many functions of viral movement protein as mentioned below

1. Formation of viral replication factories
2. Formation of replication complex with host cellular factors promoting replication.
3. Movement of the virion particle or genetic material through plasmodesmata
4. Dissemination and virions assembling.
5. Direct movement of viral proteins via interactions with cytoskeleton
6. Direct and indirect expanding of plasmodesmata, etc.

So, the movement protein plays a important role in life establishment of any virus, because it is responsible for any kind of movement. MP of Tobacco mosaic virus plays a crucial role in virus spread throughout the plant. Point mutation in the middle cistron of MP impairs all kinds of viral movement and thus prevents disease spread (Boyko *et al*., 2000) [28]. Molecular weight of MP is 30 kD, so it is usually referred as 30K protein by Scholthof, (2005) [29].

MP of many plant viral species resembles each other by homologous nucleotide sequences, which possibly are key points in proper functioning of the protein. So, there are several families of MP, most common of which are double-block, triple-block, porline-rich tymovirus like MP, quintuple-block and 30K by Verchot-Lubicz *et al*. (2010) [30].These superfamilies are formed in order to facilitate classification of properties, features, molecular arrangement, and nucleotide sequences of MP derived from different plant viral studies (Melcher, 2000) [31]. Unique feature of 30K superfamily includes the ability to cooperatively bind to single-stranded nucleic acids to facilitate formation of non-virion ribonucleoprotein of viral genome, which is a carrier unit in many viral species. Examples of viruses bearing 30K superfamily MP are Cilevirus, Citrivirus, Ourmiavirus, Sadwavirus, Ophioviridae and Rhabdoviridae [32].

MP plays a crucial role in movement by its active role in regulating size exclusion limit of plasmodesmata. Some viral MP directly interacts with cellular cytoskeleton, actin microfilaments in particular, to enlarge plasmodesmata's diameter. In rare cases, MP self-interacts to form hollow tubular structures like cellular microfilaments to promote movement of viral particles from cytoplasm directly to plasmodesmata and otherwise (Volkmann *et al*. 2003) [33].

2.1. Classification of movement protein

The genetic constituent of viruses has a particular coding sequence for movement protein which is necessary for their cell- -cell as well as systemic movements. Movement proteins from plant viruses are classified under two super families (Carrington *et al*., 1996) [34]. First was exemplified by TMV movement protein, the MP aids the movement of the virus as a nucleoprotein complex through the plasmodesmata. Second was represented by *Cowpea Mosaic Virus* (CPMV) encoded MP, the MP forms tubular structures through which intact

virions move from cell-to-cell. These gross differences in the mechanism by which a virus would transport from cell-to-cell depends on whether the virus requires complete functional capsid protein for its intercellular translocation or not. Based on the requirement of the MP and CP for the movement of virion particle or infectious molecule from one cell to another cell, viruses are grouped under three categories (Scholthof, 2005 [29] and Niehl and Heinlein, (2011) [19] which are mentioned in table 1.

Table 1: Classification of the viruses based on the requirement of the MP and CP for cell to cell movement

Group I	**Only MP required for cell-to-cell movement of their RNA**	**Example Tobamovirus, Dianthovirus, and Umbravirus**
Group II	Multiple MPs and the CP required for both cell-to-cell and systemic trafficking of the viral RNA	Potyviruses, Hordeiviruses, and Potexviruses
Group III	MP and CP required for cell-to-cell and long distance movement	Comovirus and Closteroviruses,

For the Systemic movement of *Tobacco mosaic virus*, it also requires the CP and a component of the replication complex (Liu and Nelson, 2013) [35]. The role of the CP is to facilitate MP activity or to protect the genome. *Red clover necrotic mosaic virus* (RCNMV) capsid protein deletion mutants could move from inoculated cells to the neighboring cells, but were not capable of spreading to uninoculated leaves and were restricted only to the inoculated leaves (Liu and Nelson, 2013) [35]. On the other hand, some viruses do not require capsid protein for long distance movement. For example, when the capsid protein of *Tomato bushy stunt virus* (TBSV) was replaced with GUS (β-glucoronidase) gene, the genetically modified virus could still systemically infect the host Scholthof, (2005) [29]. It has been established that viruses for which capsid proteins are necessary for their systemic spread in the host tend to follow the microtubule mediated intracellular transport pathway, since complete viruses are incapable of negotiating the plasmodesmal size exclusion limit (SEL). On the other hand, viruses which can dispense their capsid for the intercellular spread tend to move as nucleoprotein complexes through the plasmodesmata following the TMV strategy. TMV MP increases the SEL of PD by microfilament severing activity (Su *et al*., 2010) [36].

2.2. Characteristics of movement proteins

Irrespective of the superfamily to which the MPs belong, they have some basic underlying similarities in their functional domains. Three domains are identified in most of the MP of RNA viruses. These are the domain for RNA binding, the domain for cooperative RNA binding and the domain for interaction with plasmodesmata. The plasmodesmata interacting domain may also be necessary for targeting the MP to the cell wall. Several groups of viruses which replicate in the cytoplasm encode MPs with structural and functional differences from those encoded by TMV like viruses. But the gross nucleic acid binding property remains a common and highly conserved feature. At least four groups of viruses have a triple gene block, which encodes a set of three MPs. The largest of the three protein products of the triple block gene block (open reading frame 2 proteins) and *Barley stripe mosaic* Hordivirus βb protein bind ssRNA cooperatively, have ATPase activity and a highly conserved helicase-like sequence motif (Donald *et al.*, 1995) [37]. The phosphorylation activities on replication and movement of viruses are still unclear (Tyulkina *et al*., 2010) [38]. Although function of helicase and the nature of interaction among the three MPs are not very clear, the RNA binding activity would be required for the formation of nucleoprotein complex, analogous to those formed by the TMV like MPs.

The MP (P1 protein) of *Cauliflower mosaic virus* (CaMV), a dsDNA- containing pararetrovirus also possesses ssRNA binding activity (Citovsky, *et al*., 1992 [39]. Citovsky *et al*. (1992) [39] hypothesized that the 35S RNA reverse transcription template is the entity that moves from cell-to-cell. Although the binding domain of P1 clearly overlaps with a large region required for intercellular movement of the virus, it remains an open question as to whether or not the RNA-binding function is involved directly in transport, because other evidences indicate that CaMV moves from cell-to-cell as icosahedral virions in which dsDNA is packaged. Furthermore, P1 protein induces cell wall spanning tubules through which virions are proposed to pass. It is possible that CaMV may actually use two distinct movement strategies at different stages of its multiplication cycle or the strategies may be different among tissues it is infecting (Citovsky, *et al*., 1992 [39].

2.3. Role of movement protein in inter- and intra-cellular transport of viruses

Viruses move throughout the plant via plant intercellular conduits the plasmodesmata (Esau 1948). Viral spread through these connections occurs in two distinct steps, local and systemic. In the initial phase of infection by mechanical or insect mediated inoculation, many plant viruses spread cell to cell through PD until they reach the vascular system. Later, the viruses are transported systemically through the vasculature. Virus particles and naked viral RNAs and viroids are too large to pass through PD by diffusion.

The movement protein of *Cucumber mosaic virus* forms tubular structure on the protoplasts surface (Canto and Palukaitis, 2005) [40]. The interactions of the MP with tubular structure were not determined. However, unlike all of the other tubule-forming viruses, these tubules are not necessary for CMV intercellular movement. The MP of TMV, CMV and AMV, members of the alpha virus super group localizes to ER (Huang and Zhang, 1999) [41]. Later it was proved that mutant MP did not localize to PD even after fractionation (Huang *et al*., 2001) [42]. Recently, the MP of *Prunus necrotic ringspot virus*, an ilarvirus who's MP can complement the function of the related AMV MP, was shown to contain a hydrophobic region that associated with a membrane, but did not span it (Martı´nez-Gil *et al*., 2009) [43]. The need of hydrophobic region for the cell-to-cell movement of the virus AMV RNA3 was proved through mutational analysis (Martinez-Gil *et al*., 2009) [43].

In the case of Ilarvirus proteins accumulate early in virus infection, bind RNA co-operatively, associate with the cell wall fraction, locate the plasmodesmatal region, and increase the plasmodesmatal size exclusion limit (SEL) (Canto and Palukaitis, (1999) [40]. Cell-to-cell movement of Bromoviridae requires the viral coat protein in most situations (kalpan *et al*., 1998) [44] and is associated with the formation of tubules

(Canto and Palukaitis, (1999) [40]; Sanchez-Navarro and Herranz, 2006) [45]. However, although cell-to-cell movement of *Cucumber mosaic virus* needs coat protein, the virus does not need to be encapsidated as virions (Kalpan *et al*., 1998) [44] The one of the well-studied movement protein is *Tobacco mosaic virus* (TMV) p30 with molecular weight of 30 kDa (Deom *et al*., 1987) [22].

TMV MP; Most of the research on this type of MPs was done using TMV and RCNMV. The biochemical nature of the virus encoded MP is well-characterized 30 kDa (Melcher, 2000) [31]. Based on the decade-long research, a model has been proposed. By experiments performed in late 1980s, Wolf *et al*., (1989) [46] has demonstrated that fluoresceins-isothiocyanate-labelled dextran with an average molecular mass of 9400 Da and an approximate stockes radius of 2.4 nm was able to move between cells of transgenic plants expressing the movement protein of TMV, whereas the size exclusion limit of normal plasmodesmata is 700-800 Da. They were also unable to visualize MP complex with ssDNA or ssRNA. These complexes were long, unfolded and very thin (1.5-2 nm) in diameter. Unlike TMV virions (diameter 18 nm), the complexes were thus compatible in size with the MP induced increase in plasmodesmatal permeability (2.4-3.1 nm), making them likely candidates for the structures involved in cell-to-cell movement of TMV. Thus after the MP is expressed, it performs two functions. First, it binds to viral RNA and unfolds the viral RNA from a random coil to a linear rod shaped structure so as to reduce its diameter. The RNA-MP complex is then translocated and targeted to the plasmodesmata, where the complex interacts with components of the plasmodesmata to increase its pore size or SEL (Wolf *et al.*, 1989) [46]. The increased pore size combined with the reduced molecular diameter of the viral RNA allows the ribonucleoprotein to move through the plasmodesmata to the next cell.

TMV MP binds ssRNA and ssDNA in a strong, highly cooperative and sequence non-specific manner. With the help of in-frame and double deletion mutation strategies, two independent nucleic acid binding domains were identified (amino acid 112-185 and amino acid 185-268). Another region spanning from amino acid 65 to 86 is required for correct folding of the MP (Citovsky *et al*., 1992 [39]).

RCNMV was also shown to follow the same strategy of cell-to-cell transport *in vitro* and *in vivo* as that of TMV. However, cooperative RNA binding was not necessary for cell-to-cell movement in vivo and only a fraction of the wild type RNA binding was shown to be required.

The association of TMV like MP with the interior of the plasmodesmata was shown by immunochemical electron microscopy and the central and C-terminal sequence of MP is shown to be necessary for plasmodesmal localization (Giesman- Cookmeyer and Lomel, (1993) [47]; McLean *et al*. (1995) [48]. It was shown that the MP of various viruses interacts with the plasmodesmal proteins to increase the SEL (Amari *et al.*, 2010) [49]. It has been proposed that MP is sequentially transported on microtubules and then on microfilaments towards the cell wall enroot to plasmodesmata. This is consistent with detection of actin in and around plasmodesmata (White *et al*., 1994) [8]. The cytoskeleton is not is not responsible for the movement of MP was also proved by treating with inhibitor Brefeldin A (Huang *et al*., 2001) [42].

It has also been shown that certain mutants of MP, that are defective in cell-to-cell transport of virus are unable to traffic through the plasmodesmata, but the exact point in the trafficking pathway where these altered proteins are arrested are not yet known. The fusion proteins consisting of TMV MP with β-glucoronidase (GUS) also traffic between cells, implying the presence of plasmodesmal transport signal in the movement proteins have shown Waigmann and Zembryski, 1995.

Thus, the three main steps for the passage of MP genome complex through the plasmodesmata are: binding at the plasmodesmatal surface, transit through the channel and release into the adjacent cells. Binding of the MP genome complex at and internalization into plasmodesmata may occur via a default pathway, which is used by cytoskeleton-associated elements involved in intracellular trafficking. Transit of MP-genome complex may be driven by an active mechanism in which both MP and genome components move via interconnections with a plasmodesmal transport apparatus. It is assumed that the apparatus comprises of resident escort proteins, chaperons and molecular motors. Whether genomes are transported as stable nucleoprotein complex or as a dynamic complex in which MP subunits cycle on and off the genome is not known. The mechanism governing the release of the transported complex into the adjacent cell is also not very clear. It can be assumed that like other cellular transport mechanisms, the plasmodesmal transport system is also energy dependent. It has been reported that both TMV and CMV MPs bind to GTP, which may be transferred to and hydrolyzed by plasmodesmata associated GTPase during transport (Li and Palukaitis, 1996) [50]. A conserved aspartate in the D motif is shown to be essential for GTP binding (Carvalho *et al*., 2004) [51].

Tobacco mosaic tobamovirus (TMV) has been used as model system to study the intra and inter cellular movement. For movement TMV, it uses endoplasmic reticulum (ER) network for replication and spread through plasmodesmata (PD), symplastic communication channels through cell walls between neighboring cells (Niehl and Heinlein, 2011 [19]; Liu and Nelson, 2013 [35]. The movement protein of the TMV is present in the viral replication complexes (VRC) associates with RNA and acts as a microtubule associated protein (Boyko *et al*., 2000 [43]; Niehl *et al*. (2012) [52]. The interaction of the protein with MTs plays a role during early infection when ER membrane-associated VRCs localize to local inter sections of MTs with the ER (Boyko *et al*., 2007) [53], reviewed in Peña and Heinlein (2013) [54]. These sites, recently termed "cortical MT-associated ER sites" (C-MERs), are proposed to function as specific platforms for the recruitment of host factors and membranes in order to facilitate the maturation of the VRCs into movement competent VRCs and, later, into virus factories (Peña and Heinlein, 2013) [54].

3. Summary

A huge numbers of the complete genome sequencing for various plant viruses had done in recent past and subsequently converted into the infectious clone. Among them, only a few species were reported as biologically active. These infectious clones were utilized for elucidation of viral replication mechanism, host protein interaction, cell to cell movement and systemic movement. After fusion of the Green fluorescent proteins (GFP), Yellow fluorescent proteins (YFP), Red fluorescent proteins (RFP) and Cyan fluorescent proteins (CFP) gene with the movement protein gene was greatly exploited cell molecular biology study. A numbers of the viral

proteins were discovered in the recently to studies that interaction with the cytoskeleton of the host cell using different fluorescent proteins such as GFP, YPF, CFP and RFP. However, importance of these interactions were unclear (Niehl and Heinlein, 2011 [19]; Harries *et al*., 2010 [55]; Peña and Heinlein, (2013) [54].

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