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Cellular localization and interactions of nucleorhabdovirus proteins are conserved between insect and plant cells

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ABSTRACT

Maize mosaic virus (MMV), similar to other nucleorhabdoviruses, replicates in divergent hosts: plants and insects. To compare MMV protein localization and interactions, we visualized autofluorescent protein fusions in both cell types. Nucleoprotein (N) and glycoprotein (G) localized to the nucleus and cytoplasm, phosphoprotein (P) was only found in the nucleus, and 3 (movement) and matrix (M) were present in the cytoplasm. This localization pattern is consistent with the model of nucleorhabdoviral replication of N, P, L and viral RNA forming a complex in the nucleus and the subvirion associating with M and then G during budding into perinuclear space. The comparable localization patterns in both organisms indicates a similar replication cycle. Changes in localization when proteins were co-expressed suggested viral proteins interact thus altering organelle targeting. We documented a limited number of direct protein interactions indicating host factors play a role in the virus protein interactions during the infection cycle.

1. Introduction

Maize mosaic virus (MMV) was first characterized in Hawaii infecting corn (Kunkel, 1921) and since then has been found in many tropical and subtropical regions including Hawaii, Florida, India and the islands of the Western Indian Ocean (Autrey, 1983; Bradfute and Tsai, 1983; Kunkel, 1921; Paliwal et al., 1968). Virus infection causes dwarfing, chlorosis and grain yield loss in corn, sorghum and pearl millet and the virus is vectored by the corn planthopper, *Peregrinus maidis* (Herold and Munz, 1965). MMV is classified as a nucleorhabdovirus as it replicates and undergoes morphogenesis in the nucleus of infected cells (Ammar and Nault, 2002; McDaniel et al., 1985). The virus is transmitted in a persistent, propagative manner by *P. maidis* and virus persists in the vector throughout development (Ammar and Nault, 1985; Ammar and Hogenhout, 2008; Barandoc-Alviar et al., 2016) and plant tissues (McDaniel et al., 1985). Phylogenetic analysis of the nucleorhabdovirus polymerase proteins provides evidence for three nucleorhabdovirus clades. MMV groups in a clade with other nucleorhabdoviruses: maize Iranian mosaic virus (MIMV) and taro vein chlorosis virus (TaVCV) (Massah et al., 2008; Revill et al., 2005). The genomes of the three viruses within this clade include one accessory protein that is hypothesized to be the movement protein (3). A thorough discussion of the phylogenies of rhabdoviruses can be found in Dietzgen et al. (2017).

MMV is a negative-sense, single-stranded RNA virus containing six genes arranged 3′ to 5′: nucleoprotein (N), phosphoprotein (P), putative movement protein (3), matrix protein (M), glycoprotein (G) and polymerase protein (L) (Reed et al., 2005). The N, P and L proteins form a polymerase complex that is absolutely required for infection as the genomic RNA is not infectious (Jackson et al., 2005). The model for morphogenesis of this family of viruses includes the binding of N, P and L to the negative sense RNA to form a RNP core. This complex then associates with the M protein to condense the core. Occurring in the nucleus, this core then interacts with the host inner nuclear membrane embedded with viral glycoproteins to bud into the perinuclear space. The movement of the subvirion particle (i.e. the nucleocapsid core which may be associated with the matrix protein) within the cell to the cell membrane and from cell-to-cell has two alternative hypotheses depending on plant or insect cell type (Jackson et al., 2005; Min et al., 2010; Oksayan et al., 2012; Redinbaugh and Hogenhout, 2005). Characterizing the multitude of protein-protein interactions in plant and insect hosts is essential for understanding the virus infection cycle.

It is unclear how viruses move within the cell from the nucleus to the cell periphery in any cell type. Viruses travel the endomembrane system as either mature virions or as RNP cores until they reach the outer membranes for entry into the adjacent cell (Jackson et al., 2005). However, in insects, the mature virions of MMV are hypothesized to bud out of the perinuclear space to traffic through the cytoplasm as RNP

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cores to the outer membrane where they bud again from the outer membrane as mature virions to reach another cell. This is similar to what is seen in animal infecting rhabdoviruses such as vesicular stomatitis virus and rabies virus (Oksayan et al., 2012). In plant cells, the virus particles or nucleocapsids are directed to the plasmodesmata for movement to neighbouring cells, some evidence suggests this may be as RNP cores, however, it is not known if MMV-3 and -G are present as part of a mature virion or as accessory proteins that have some function during movement (Jackson et al., 2005; Min et al., 2010; Redinbaugh and Hogenhout, 2005).

Host cells that support replication of plant-infecting rhabdoviruses differ dramatically in morphology. Unlike plant cells, insect cells do not contain plasmodesmata, cell walls or a large central vacuole. One hypothesis is that there are differences in the localization of MMV proteins in insect cells compared to plant cells to accommodate the differences in cellular morphology. Protein localization maps have been generated for other nucleorhabdoviruses in plant cells, but the infection cycle in the insect vector is not well characterized. To fill this knowledge gap, we have localized the proteins of MMV in both plant and insect cells as individuals and in pairs to determine the conserved localizations between both hosts. This is the first comprehensive report of plant rhabdovirus protein localization in both plant and insect cells. Our goals were to further characterize MMV and the possible infection strategies utilized in both plant and insect cells by mapping both localization and co-localization of each of the proteins in model insect (*Drosophila melanogaster* S2 cells) and plant (*Nicotiana benthamiana*) cells. We were also interested in the viral protein interactions, and to test this, we utilized bimolecular fluorescence complementation (BiFC) in *Nicotiana benthamiana* cells. This study helps to answer fundamental questions in the study of plant rhabdoviruses about the mechanisms of successful infection in both the plant and insect hosts.

2. Results

2.1. Sequence features of MMV genes

We conducted *in silico* analyses of each open reading frame to predict the structural features of MMV proteins indicative of cellular localization. There are six proteins expressed from the MMV genome ranging in size from the smallest, at 235 amino acids (MMV-M) to the largest for MMV-L at 1922 amino acids (Table 1). Three of the MMV proteins, P, G and L, have predicted nuclear localization signals (NLSs) as determined by PSORT and a NLS for MMV-N has been reported previously based on sequence similarities to potato yellow dwarf virus N (Anderson et al., 2012). MMV-3 and -M proteins do not contain predicted canonical NLSs, and we expected that the localization of these proteins will not be nuclear. We then analyzed each ORF to determine the relationship of MMV genes to those of homologous genes of other nucleorhabdoviruses. The closest relative to MMV is TaVCV based on blastx matches for five of the six MMV genes (Table 1). The only exception is the glycoprotein, G, which has the highest match to MIMV. MMV-G is the only MMV protein to contain a transmembrane (TM) domain (Table 1) which is predicted to cross the membrane only once (a type I integral membrane protein). Overall, these protein features are similar to those of other plant rhabdoviruses, indicating that MMV is a typical member of the *Nucleorhabdovirus* genus.

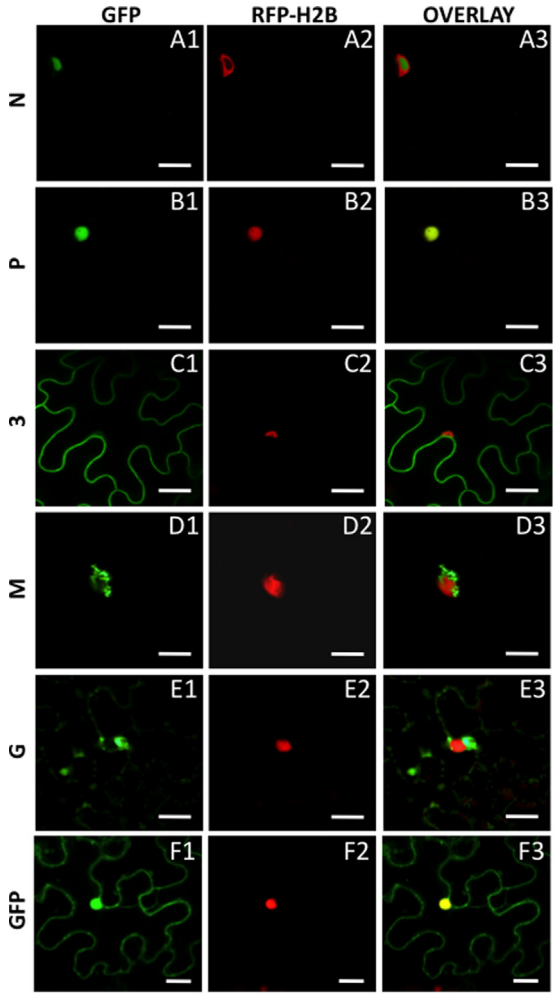

Fig. 1. Localization of MMV proteins expressed as fusions to green fluorescent protein (GFP) in leaf epidermal cells of transgenic *N. benthamiana.* Column 1, localization of individual MMV proteins fused to GFP. Column 2, localization of the transgenic plant marker, red fluorescent protein fused to histone 2B (RFP-H2B). Column 3, overlay of columns 1 and 2. The name of the row indicates the order of fusion, i.e. MMV-N-GFP is MMV-N expressed first followed by GFP. Rows are as follows: A, MMV-N-GFP, B, MMV-P-GFP, C, MMV-3-GFP, D, MMV-M-GFP, E, MMV-G-GFP, and F, unfused GFP. Scale bars are equal to 20 μm.

2.2. Protein localization in nuclear marker plants

MMV proteins were expressed in plant cells as fusions to green fluorescent protein (GFP) to validate our *in silico* analysis. This was done in transgenic *N. benthamiana* plants expressing a nuclear marker to provide a cellular context for the localization patterns observed

Table 1
Predicted features of MMV protein coding regions.

Protein	Size (aa)	Transmembrane Domain	Predicted Nuclear localization Signal	Closest viral match / E-Value (BLASTx)
N	447	None	438–444 PAPKKTR (Anderson et al., 2012)	Taro vein chlorosis virus-N/ 0.0
P	269	None	262–266 KRPR[a]	Taro vein chlorosis virus-P/ 2e−73
3	286	None	None	Taro vein chlorosis virus-P3/ 1e−82
M	235	None	None	Taro vein chlorosis virus-M/ 1e−69
G	591	551–573 aa	431–435 RKKP[a]	Maize Iranian mosaic virus-G/ 0.0
L	1922	None	374–391 KKNPRQSVLDEIRRQFK[a]	Taro vein chlorosis virus-L/ 0.0

[a] Determined by PSORT.