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Assembly and disassembly intermediates of maize streak geminivirus

Antonette Bennett[a], David Rodriguez[a,1], Samantha Lister[b,2], Margaret Boulton[b,3], Robert McKenna[a], Mavis Agbandje-McKenna[a,*]

[a] Department of Biochemistry and Molecular Biology, College of Medicine, Center for Structural Biology, McKnight Brain Institute, University of Florida, Gainesville, FL 32610-0245, United States

[b] John Innes Center, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK

A B S T R A C T

Maize streak virus (MSV) belongs to the *Geminiviridae*. Four forms of MSV coat protein (CP) assemblages were isolated from infected plants: geminate capsids, T = 1 icosahedral capsids, pentamers and decamers of CPs. Sequential exposure of geminate capsids to increasing pH, from 4.8 to 7.2 was used to monitor capsid disassembly. The capsids remain intact at pH4.8, disassemble to decamers and pentamers by pH6.4 and aggregate by pH7.2. Similarly, high salt and divalent cations cause disassembly. The disassembly process was reversed in low pH and low salt, but resulted in empty (no DNA) single and geminate capsid assemblies. This is likely due to disruption of CP-DNA interactions under acidic conditions and suggests a mechanism of capsid assembly in which the genome is packaged into preformed empty capsids. The pH assay developed in this study provides a method for characterizing the conditions that are the determinants of geminivirus assembly and disassembly.

1. Introduction

The *Geminiviridae* are pathogenic ssDNA plant viruses that cause significant economic loss due to infection of a number of agricultural crops worldwide, including beans, cassava, cotton, maize, squash, and tomatoes. They are divided into nine genera: *Becurtovirus, Begomovirus, Capulavirus, Curtovirus, Eragrovirus, Grablovirus, Mastrevirus, Topocuvirus*, and *Turncurtovirus* based on their genome organization, host range, and insect vector (Zerbini et al., 2017). The assembled geminate capsid is a quasi-isometric, pseudo T = 1 icosahedral shell assembled from 110 copies of the capsid protein (CP), and is a unique architecture among viruses. Presently, efforts aimed at geminivirus control include the generation of transgenic crops resistant to virus infection either by gene silencing, or encoding mutant viral replication (Rep, RepA) or movement (MP) proteins (reviewed in (Coursey et al., 2018; Reyes et al., 2013; Vanderschuren et al., 2007)). Expression of modified versions of these proteins reduce viral genome packaging and systemic spread, respectively. However, none of these approaches has been sufficiently successful for the long-term control of geminiviruses. For several members of other virus families, including *Papaya ringspot virus* (PRV) (genus *Potyvirus*, *Potyviridae)* and *Tobacco mosaic virus* (TMV) (genus *Tobamovirus*, *Virgaviridae*) understanding their mechanism of capsid assembly or disassembly was crucial to the successful production of transgenic plants that are resistant to viral infection (Hallan and Gafni, 2001; Osbourn et al., 1990; Tripathi et al., 2008; Vanderschuren et al., 2007; Yaakov et al., 2011).

Maize Streak Virus (MSV) is one of the most extensively studied geminivirus species (Bosque-Perez, 2000; Boulton et al., 1989). Infection by the Nigerian strain of MSV (MSV-A[NG1]) (genus *Mastrevirus)* has resulted in loss of maize, other cereals, and grasses in sub-Saharan Africa and India (Bosque-Perez, 2000). MSV-A[NG1] packages a genome of 2687 nucleotides (Mullineaux et al., 1984) which encodes four gene products, and binds a 75–80 oligodeoxyribonucleotide that serves as a primer for the synthesis of the complementary strand (Donson et al., 1984; Lazarowitz et al., 1989). Its assembled capsid consists of 110 copies of a 27 kDa (244 amino acid) CP (Boulton et al., 1989; Morris-Krsinich et al., 1985; Mullineaux et al., 1984). The CP plays a role in insect transmission (Liu et al., 1997), systemic infection (Boulton et al., 1989; Lazarowitz, 1988; Liu, 2008), binding of ss and dsDNA (Liu et al., 1997), and specific accumulation and encapsulation of genomic ssDNA (Boulton et al., 1989). The CP accumulates in the nucleus, where it is believed assembly occurs, and it facilitates nuclear and cell-to-cell transport of the genome, via an interaction with the MP (Liu et al., 1997, 2001).

The transfer of MSV from its insect vector, *Cicadulina mbila* (Order *Hemiptera*), via its feeding acquisition probe inserted into infected maize

* Corresponding author.
E-mail address: mckenna@ufl.edu (M. Agbandje-McKenna).
[1] Department of Orthopedic Surgery University of Texas Health Science Center 6400 Fannin Suites 1620, Houston, Texas 77030.
[2] SB, The Norwich BioScience Institutes, Norwich Research Park, Norwich, UK.
[3] MB, The Almonds, 21 South Green, Mattishall, Norfolk, England, NR20 3JT.

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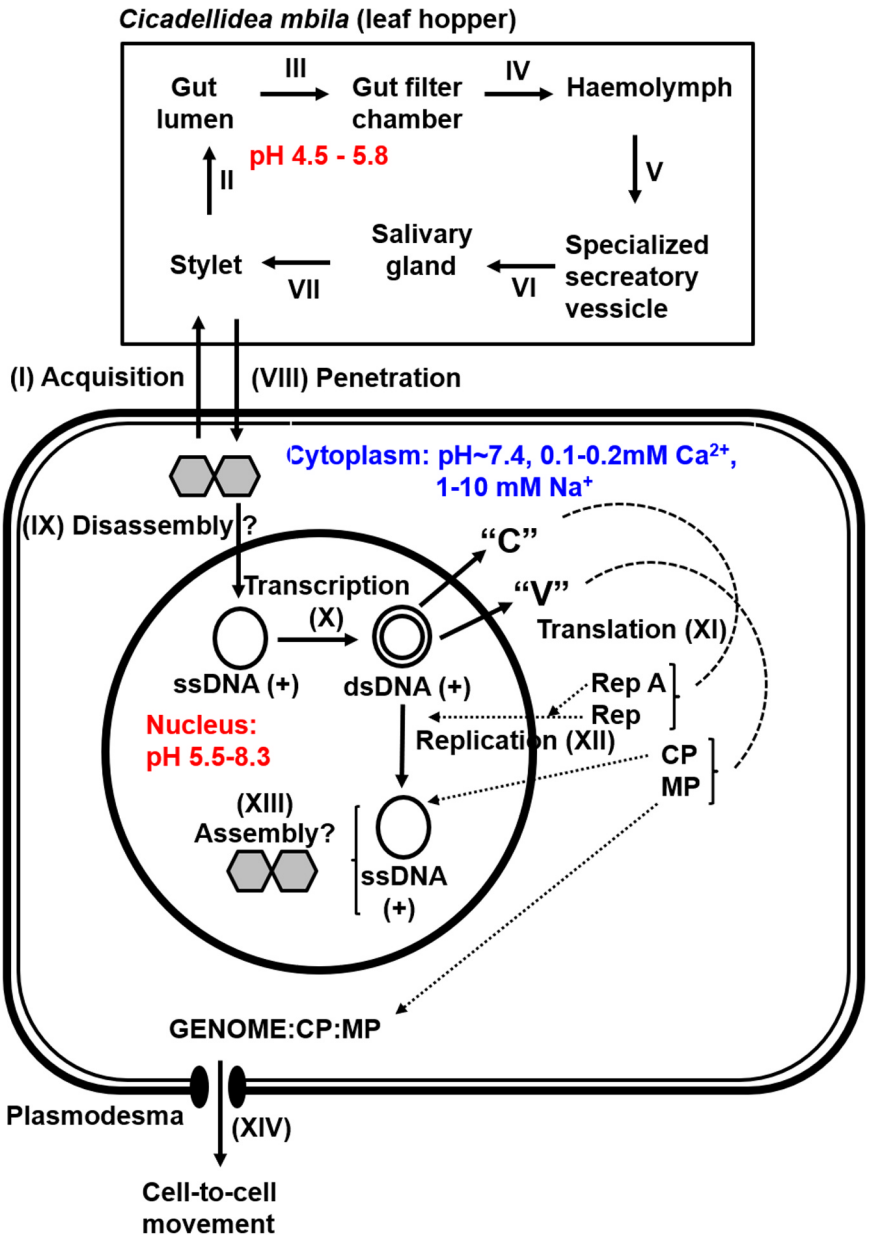

Fig. 1. Schematic of the infection cycle of MSV. (I) The virus is acquired by the vector, *Cicadulina mbila* following probing into the plant *meso*phyll of an infected maize plant. The virus is trafficked from the stylet, across the gut barrier and into the hemolymph (II – VII), and encounters acidity ranges in the gut region between pH 4.5–5.8. MSV is then transmitted from the vector to a healthy maize plant by penetration of the stylet (VIII) into the plant *meso*phyll during feeding. The virus is transported to the nucleus (IX) where it is replicated to produce dsDNA for transcription (X), it is not known if capsid disassembly occurs before or after nuclear import. The transcripts are transported to the cytoplasm where they are translated (XI). The translated virus replication proteins and CPs are then transported to the nucleus where viral (ssDNA) genome amplification (XII) occurs and virus particles are assembled (XIII). The cytoplasmic pH of the maize leaf is ~7.4 and the vacuolar pH ~4.5–6.0. The viral genome is transmitted from cell to cell by the interaction of the CP and the MP (XIV).

*meso*phyll, is illustrated in Fig. 1. The virus traffics through the digestive and circulatory tract of the vector and is subsequently reintroduced into the cytoplasm of phloem cells of an uninfected host plant during feeding (Bosque-Pérez and Buddenhagen, 1999). The geminate capsid travels within the phloem of its host plant (Carrington et al., 1996). Although the mechanism of capsid uncoating is unknown, the viral genome is delivered to the nucleus by the NLS within the CP (Liu et al., 1999). Replication is reported to occur in the nucleus by a rolling circle mechanism and utilizes a dsDNA replicative form and host enzymes (Davies et al., 1987; McGivern et al., 2005; Ruschhaupt et al., 2013). The dsDNA is bidirectionally transcribed to produce both viral and complementary strand transcripts (Morris-Krsinich et al., 1985; Mullineaux et al., 1984). These are translated to form CP and MP, and Rep and RepA, respectively, (Liu et al., 1997, 2001, 1999). The translated CP are transported to the nucleus to assemble the capsid (Liu et al., 1997, 2001, 1999). During the infection cycle, the geminate capsid experiences a wide range of pH, from 4.5 to 8.3 (Fig. 1) (Nation, 2016).

A cryo-electron microcopy and image reconstructed (cryo-EM) structure of the MSV-A[NG1] geminate capsid reported at 25 Å resolution and Ageratum yellow vein virus reported at 3.3 Å described three types of pentameric CPs (Hesketh et al., 2018; Zhang et al., 2001). These are apical (the two ends of the particle), peritoneal (adjacent to the apical capsomers), and equatorial (the midline of the virus). A conformation switch of the N-terminus of one of the CPs at the equatorial pentamer was proposed to occur to enable assembly of the unique geminate capsid. Significantly, in addition to the geminate capsid, complete T = 1 icosahedral capsids (singles) have also been reported (Casado et al., 2004). Until now there have no reports characterizing isolated pentameric intermediates.

There are no host proteins homologous to the geminivirus CP, and the CP:CP and CP: DNA interactions appear to be uniquely tailored to the capsid architecture (Hesketh et al., 2018). Therefore, understanding the nature of these CP interactions, identifying the intermediates of capsid assembly and disassembly, and elucidating the likely effects of the pH and cellular environment present in the vector and host on these processes during the virus transmission and infection cycle could aid development of methods of control. Within the vector, MSV-A[NG1], will encounter pH values between 4.5 and 5.8 in the digestive tract, while the pH of the circulatory system is not known. In contrast, the phloem sap of maize has a calculated pH range of 7.2–8.0 (Lohaus et al.,