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Alteration of intersubunit acid–base pair interactions at the quasi-threefold axis of symmetry of *Cucumber mosaic virus* disrupts aphid vector transmission

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ABSTRACT

In the atomic model of Cucumber mosaic virus (CMV), six amino acid residues form stabilizing salt bridges between subunits of the asymmetric unit at the quasi-threefold axis of symmetry. To evaluate the effects of these positions on virion stability and aphid vector transmissibility, six charged amino acid residues were individually mutated to alanine. All of the six engineered viruses were viable and exhibited near wild type levels of virion stability in the presence of urea. Aphid vector transmissibility was nearly or completely eliminated in the case of four of the mutants; two mutants demonstrated intermediate aphid transmissibility. For the majority of the engineered mutants, second-site mutations were observed following aphid transmission and/or mechanical passaging, and one restored transmission rates to that of the wild type. CMV capsids tolerate disruption of acid–base pairing interactions at the quasi-threefold axis of symmetry, but these interactions are essential for maintaining aphid vector transmissibility.

Introduction

Cucumber mosaic virus (CMV) is one of the most geographically widespread plant virus species. Three properties responsible for its global distribution are its exceptionally wide host range, its movement in and transmission via seed, and its effective transmission by many different species of aphids (Palukaitis and Garcia-Arenal, 2003a, 2003b). Most plant viruses have evolved relationships with vectors to facilitate their transmission, including insects, nematodes, mites, fungi, and protist vectors. Among these, insects are the most important and aphids, in particular, are the most common of the vectors. The relationship between viruses and insect vectors are characterized with regard to whether the virus crosses membrane barriers and circulates within the insect (circulative vs. non-circulative), with regard to whether the virus replicates in the insect (propagative versus non-propagative), and temporally with regard to the time required for efficient acquisition and delivery of virus (persistent and nonpersistent) (Nault, 1997).

CMV is transmitted by aphid vectors in a non-circulative, nonpersistent manner (Ng and Perry, 2004). The relationship between the CMV and the vector is transient. The insect can acquire virions from, or transmit them to, a plant in seconds to minutes, and the virus does not cross membrane boundaries or circulate in the aphid. The transmission properties of the CMV and related members of the genus *Cucumovirus* are determined solely by the capsid protein (Chen and Francki, 1990; Gera et al., 1979). This stands in contrast to other nonpersistently, aphid transmitted viruses for which a nonstructural, viral-encoded 'helper component' is required in concert with the capsid protein to mediate aphid transmission. In both scenarios, the most compelling mechanistic model involves the binding of virions directly or indirectly (via helper component) to a ligand in the mouthparts of the insect (Pirone and Perry, 2002). The aphid ligand is likely to be concentrated at the tip of the stylet, and salivation is postulated to facilitate the release of virions from bound sites during the process of feeding and the delivery of virus into plant cells (Fereres, 2007; Martin et al., 1997; Wang et al., 1996).

A virus-centric approach to better understand mechanisms of vector transmission has been to combine structural studies of virions or viral proteins with mutational analysis to identify structural domains that are determinants of transmission. A limiting factor in this approach has been the limited availability of atomic structures for the virions and proteins of interest. For example, elegant studies have identified essential regions of helper components of potyviruses and Cauliflower mosaic virus (Moreno et al., 2005; Syller, 2005), but structural information on these viruses is only available in low resolution models. Modeling studies of the structure of geminiviruses have resulted in predictions of the capsid protein structure based on cryoelectron microscopic reconstructions (Bottcher et al., 2004; Zhang et al., 2001). Complementary studies in which mutants defective in transmission contained

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mutations localized to specific regions of the capsid protein have identified domains that are likely involved in vector transmission (Caciagli et al., 2009; Hohnle et al., 2001; Kheyr-Pour et al., 2000; Noris et al., 1998; Soto et al., 2005). In members of the genus *Nepovirus* that are transmitted by nematodes, the capsid protein has been shown to be a sole determinant of both vector transmission and vector specificity (Andret-Link et al., 2004; Marmonier et al., 2010). There is an atomic model for nepoviruses (Chandrasekar and Johnson, 1998), and domains of the capsid protein involved in transmission have been identified (Schellenberger et al., 2011). In studies on the fungal transmitted *Cucumber necrosis virus* (CNV), the capsid protein has been shown to mediate the binding of virions to zoospores during the process of transmission (Kakani et al., 2001). In this system, the molecular basis for this vector transmission is thought to require dynamic changes in virion structure (Kakani et al., 2004).

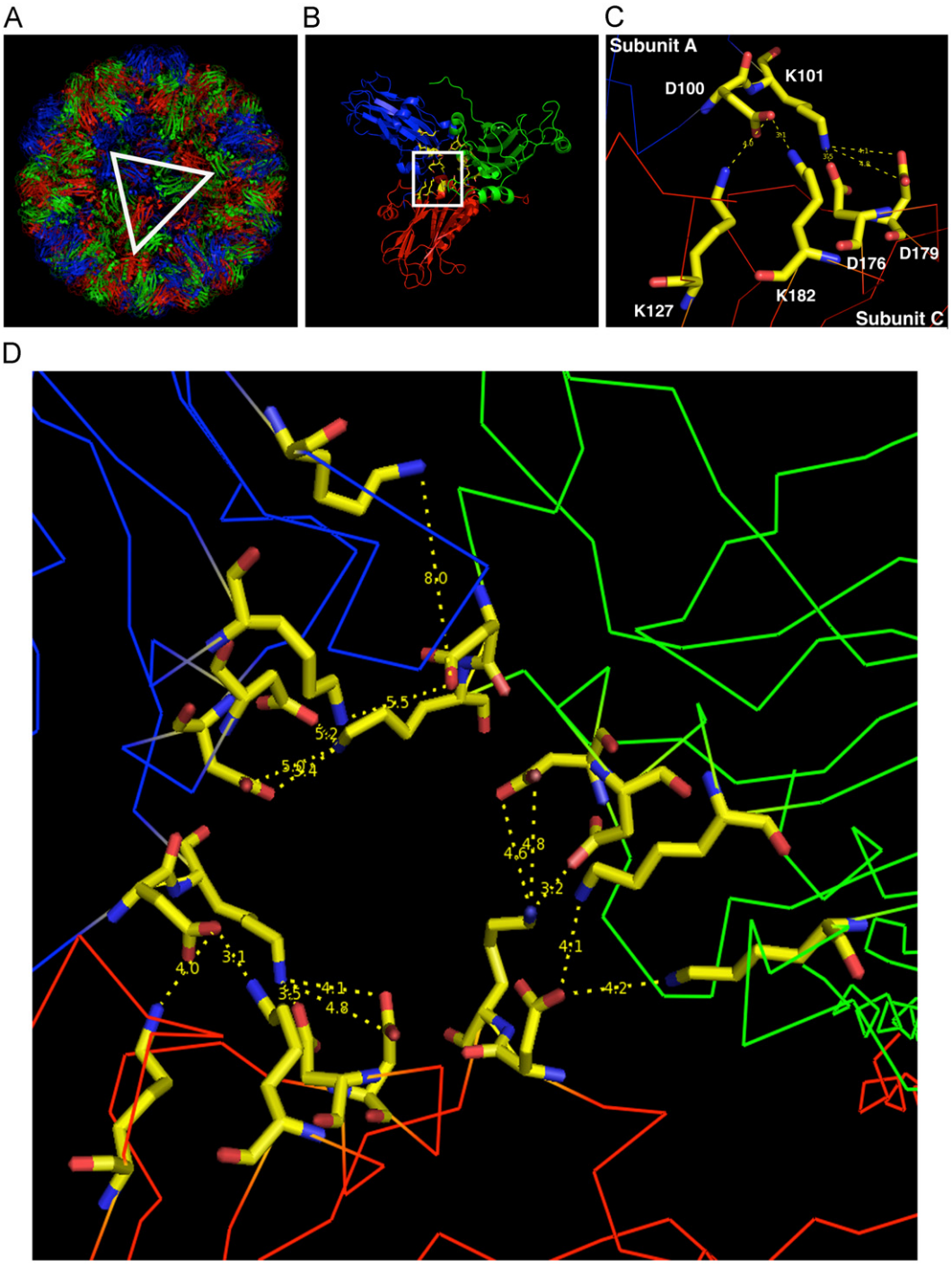

Fig. 1. Telescoping view of the quasi-threefold axes of symmetry and acid–base pair interactions in virions of *Cucumber mosaic virus* (CMV). (A) Virion of CMV with individual capsid protein subunits pictured as ribbon models and colored; subunit A is blue, subunit B is green, and subunit C is red. The triangle highlights an asymmetric unit at the quasi-threefold axes of symmetry. (B) An enlargement of the highlighted area in the previous panel, one of the 60 quasi-threefold axes of symmetry. (C) An enlargement of the interface between subunits A and C showing the amino acid side chains forming acid–base pairs with distances between pairs measured in Angstroms. (D) An enlargement of the interfacial amino acids at the quasi-threefold axis of symmetry, showing acid–base pair amino acid side chain interactions and their distances in Angstroms at the A–B, B–C, and C–A subunit interfaces.