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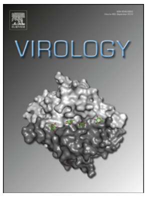

Insight into the three-dimensional structure of *maize chlorotic mottle virus* revealed by Cryo-EM single particle analysis

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ABSTRACT

Maize chlorotic mottle virus (MCMV) is the only member of the *Machlomovirus* genus in the family *Tombusviridae*. Here, we obtained the Cryo-EM structure of MCMV by single particle analysis with most local resolution at approximately 4 Å. The Cα backbone was built based on residues with bulky side chains. The resolved C-terminus of the capsid protein subunit and obvious openings at the 2-fold axis demonstrated the compactness of the asymmetric unit, which indicates an important role in the stability of MCMV. The Asp116 residue from each subunit around the 5-fold and 3-fold axes contributed to the negative charges in the centers of the pentamers and hexamers, which might serve as a solid barrier against the leakage of genomic RNA. Finally, the loops most exposed on the surface were analyzed and are proposed to be potential functional sites related to MCMV transmission.

Introduction

Maize chlorotic mottle virus (MCMV) is an exclusive pathogen of maize, which produces symptoms, typically including mild mosaic, severe stunting and leaf necrosis (Castillo and Hebert, 1974; Uyemoto et al., 1981). It was first reported in Peru in 1974 (Castillo and Hebert, 1974) and was then found in the United States and Mexico (Niblett and Clafin, 1978; Carrera-Martínez et al, 1989). In 2011, MCMV was detected in China and was shown to have a high sequence similarity with the virus previously reported (Nutter et al., 1989; Xie et al., 2011). MCMV is a high level of concern since the outbreak of the corn lethal necrosis (CLN) in Kansas (Uyemoto et al., 1980), which resulted in serious yield losses in maize. The following studies further demonstrate that the CLN disease results from the synergistic infection of the MCMV with other plant viruses in maize (Uyemoto et al., 1981; Stenger et al., 2007). Under experimental conditions, MCMV is found to be transmitted by six chrysomelid beetles (*Chrysomela populi*) (Nault et al., 1978), while recent reports suggest that MCMV can also be transmitted by corn thrips (*Frankliniella williamsi*) (Cabanas et al., 2013) and flower thrips (*Frankliniella occidentalis*) (Zhao et al., 2014). Nowadays, MCMV is widespread (Xie et al., 2011; Cabanas et al., 2013; Lukanda et al., 2014; Wang et al., 2014a; Zhao et al., 2014). However, the determinants of the MCMV transmission by these insect vectors have not been found yet.

The icosahedral virion of MCMV is composed of 180 copies of chemically identical capsid protein approximately 38 kD in size, and one copy of genomic RNA with a length of 4.4 kb (King et al., 2011). Despite of the similarity of MCMV with some members of the *Carmovirus* genus, the MCMV capsid protein shows less identity with the viruses in the *Carmovirus* genus and thus is grouped into a new genus, *Machlomovirus* in the family *Tombusviridae* (King et al., 2011). Based on structures registered in the PDB (protein data bank), viruses in the family *Tombusviridae* can be divided into two groups: one group is characterized by obvious protrusions on the surface while the other has a rather smooth surface. The first group includes *Tomato bushy stunt virus* (TBSV) (Olson et al., 1983), *Carnation mottle virus* (CarMV) (Morgunova et al., 1994), *Turnip crinkle virus* (TCV) (Hogle et al., 1986), *Melon necrotic spot virus* (MNSV) (Wada et al., 2008) and *Cucumber necrosis virus* (CNV) (Li et al., 2013). The second group is represented by *Panicum mosaic virus* (PMV) (Makino et al., 2013) and *Tobacco necrosis virus* A (TNV-A) (Oda et al., 2000). The common feature of these viruses is that all of the subunits possess the canonical jellyroll fold, which is composed with eight anti-paralleled β-sheets defined as a β-barrel structure (Stirk et al., 1992). For the viruses in the first group, the subunit is composed of two β−barrel structures: one contributes to the surface domain

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and the other to the protruding domain (Olson et al., 1983; Hogle et al., 1986; Morgunova et al., 1994; Wada et al., 2008; Li et al., 2013). For the viruses in the second group, the subunit is composed of only one β-barrel structure that contributes to the surface domain (Oda et al., 2000; Makino et al., 2013). The exposed residues on the viral surface most likely are the potential target of their transmission vectors (Liu et al., 2002; Andret-Link and Fuchs, 2005; Schellenberger et al., 2010). Thus, it is of great significance to understand the surface features prior to initiating an investigation into MCMV transmission by the insect vector.

Here, we present the three-dimensional structure of MCMV by cryo-electron microscopy (Cryo-EM) single particle analysis and reveal the features located on the surface of the virus. Our results will provide an important structural basis for further studies on MCMV transmission.

Results and discussion

Cryo-EM structure of MCMV

Approximately 53, 600 particles in total were selected from more than 1000 Cryo-EM micrographs with the best qualities for reconstruction (Fig. 1A, B and Fig. S1). The density map of the final reconstruction demonstrated that MCMV is a typical icosahedral virus with a diameter of ~30 nm (Fig. 1C and D), similar to other members of the family *Tombusviridae* [PMV (PDB ID: 4FY1)(Makino et al., 2013) and TNV (PDB ID: 1C8N) (Oda et al., 2000)]. No obvious protruding domain was found on the surface of MCMV. Instead, obvious slits were observed at the 2-fold axis from the surface view of the reconstructions (Fig. 1C). Based on the resolution of the density map, the subunits composing pentamers and hexamers showed

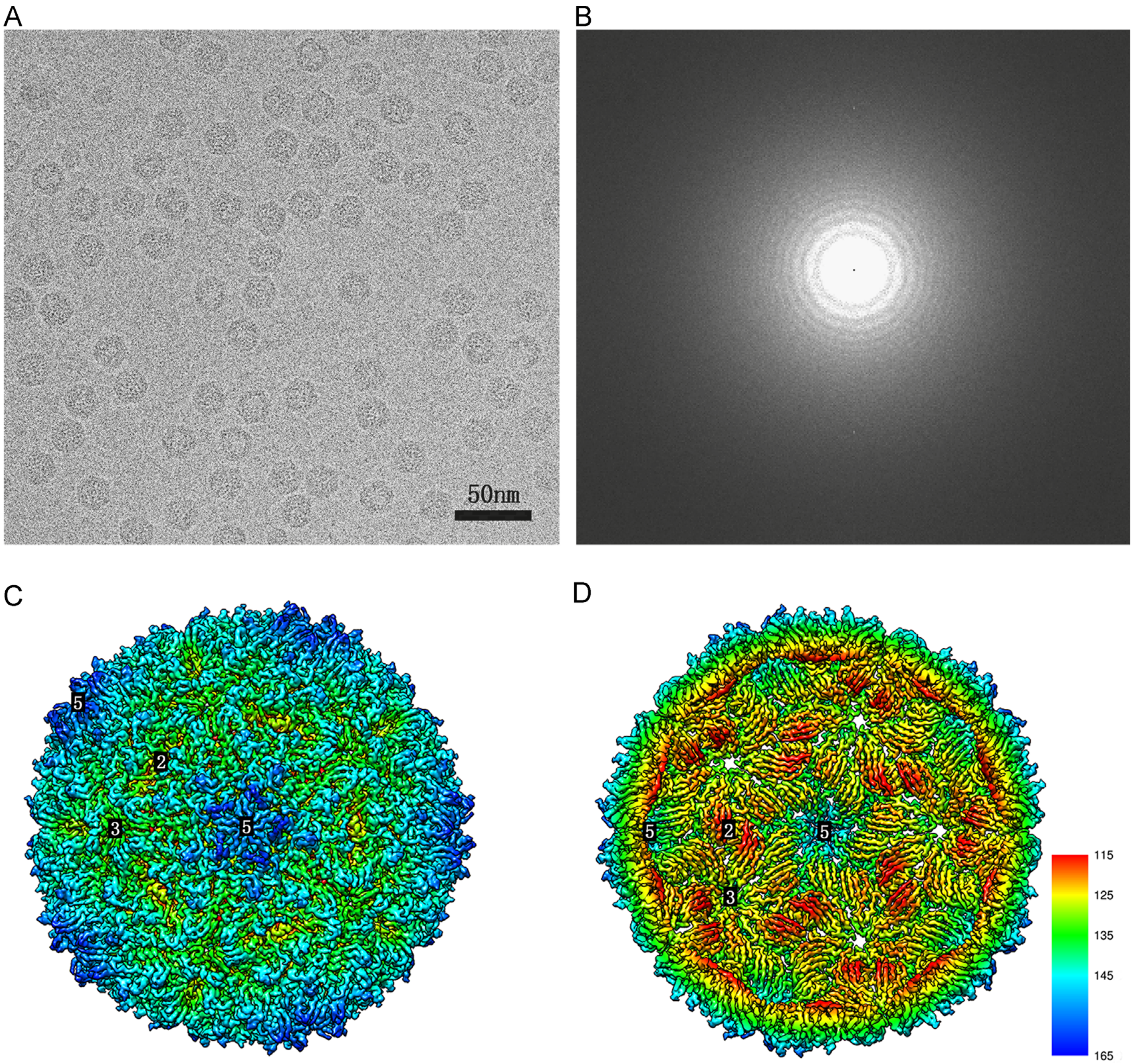

Fig. 1. Cryo-EM image and reconstruction of MCMV. (A) A two-dimensional Cryo-EM image of the MCMV particles shows that the purity and integrity of the particles are in good quality, (B) the power spectrum of the Cryo-EM image reveals that the image has no astigmatism, drift and vibration, (C) surface view of the reconstruction shows that MCMV has rather smooth surface, (D) inside view of the reconstruction demonstrates the separated β-sheets composing the surface domain of MCMV. The density map is colored by radius, and the locations of the 2-fold axis, 3-fold axis and 5-fold axis are indicated. The color key ranges from 115 Å to 165 Å, with the colors varying from red to blue.