Virology 510 (2017) 216–223

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Cryo-EM maps reveal five-fold channel structures and their modification by gatekeeper mutations in the parvovirus minute virus of mice (MVM) capsid

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ARTICLE INFO

Keywords:
Parvovirus
Minute virus of mice
MVM, cryo-EM image reconstruction
Gatekeeper mutation
Five-fold channel
Mutant capsid
Low resolution cryo-EM density
Packaging single-stranded DNA
Entry/exit portal dynamics

ABSTRACT

In minute virus of mice (MVM) capsids, icosahedral five-fold channels serve as portals mediating genome packaging, genome release, and the phased extrusion of viral peptides. Previous studies suggest that residues L172 and V40 are essential for channel function. The structures of MVMi wildtype, and mutant L172T and V40A virus-like particles (VLPs) were solved from cryo-EM data. Two constriction points, termed the mid-gate and inner-gate, were observed in the channels of wildtype particles, involving residues L172 and V40 respectively. While the mid-gate of V40A VLPs appeared normal, in L172T adjacent channel walls were altered, and in both mutants there was major disruption of the inner-gate, demonstrating that direct L172:V40 bonding is essential for its structural integrity. In wildtype particles, residues from the N-termini of VP2 map into claw-like densities positioned below the channel opening, which become disordered in the mutants, implicating both L172 and V40 in the organization of VP2 N-termini.

1. Background

Minute Virus of Mice (MVM) is a member of genus *Protoparvovirus* in the family *Parvoviridae* (Cotmore et al., 2014). These viruses have small (~ 26 nm diameter) non-enveloped T = 1 icosahedral protein capsids (Agbandje-McKenna et al., 1998; Llamas-Saiz et al., 1997), containing a single copy of a linear single-stranded DNA genome of ~ 5 kb (Bourguignon et al., 1976). Capsids are assembled from a total of sixty VP1 (83 kDa) and VP2 (63 kDa) polypeptides, in an approximately 1:5 ratio (Tattersall et al., 1976), which are encoded by alternative splicing from a single structural gene, such that VP1 includes the entire sequence of VP2 plus an additional 142 amino acid N-terminal peptide (Pintel et al., 1983; Labieniec-Pintel and Pintel, 1986). Atomic structures derived by X-ray crystallography of wildtype and mutant MVM particles (Llamas-Saiz et al., 1997; Agbandje-McKenna et al., 1998; Kontou et al., 2005; Plevka et al., 2011) identified unique positions for the C-termini of these proteins (VP2 residues 39–585 in MVMi), but provided little information about the disposition of the VP N-termini. On the surface of the particle, raised cylinders or "towers" surround each icosahedral five-fold vertex (Tsao et al., 1991; Chapman and Rossmann, 1993; Llamas-Saiz et al., 1997; Agbandje-McKenna et al., 1998). As shown in Fig. 1, these towers are formed by the juxtaposition of antiparallel β-strand hairpins contributed by the five symmetry-related structural proteins, which enclose a channel that penetrates from the outer surface of the capsid to its inner core. Previous studies indicate that these channels serve as portals, mediating the inward and outward movement of virion components at multiple stages in the viral life cycle (reviewed in Cotmore and Tattersall, 2014).

One unique five-fold channel creates a packaging conduit, supporting the 3'-to-5' encapsidation of a linear single-stranded DNA genome into a preassembled empty capsid, driven by the helicase activity of NS1 (King et al., 2001; Farr and Tattersall, 2004; Cotmore and Tattersall, 2005; Plevka et al., 2011). Viral genomes are ultimately released from the intact capsid, also in a 3′-to-5′ direction, through a single five-fold channel, or “exit” portal, which initial analyses suggest

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http://dx.doi.org/10.1016/j.virol.2017.07.015
Received 5 April 2017; Received in revised form 11 July 2017; Accepted 12 July 2017

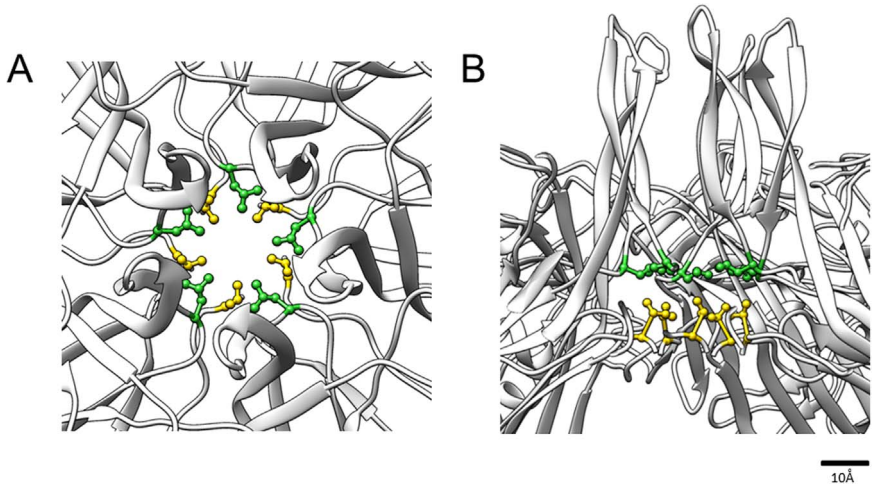

Fig. 1. The view down the icosahedral five-fold axis (A) and a cut-away side view (B) from the crystal structure of MVMp VP2-only VLPs (grey ribbon) (PDB ID 1Z14: Kontou et al., 2005) show that the five-fold channel is constructed from symmetry-related VP2 molecules. The positions of L172 (green) and V40 (yellow) are shown with their side chains rendered as ball and sticks.

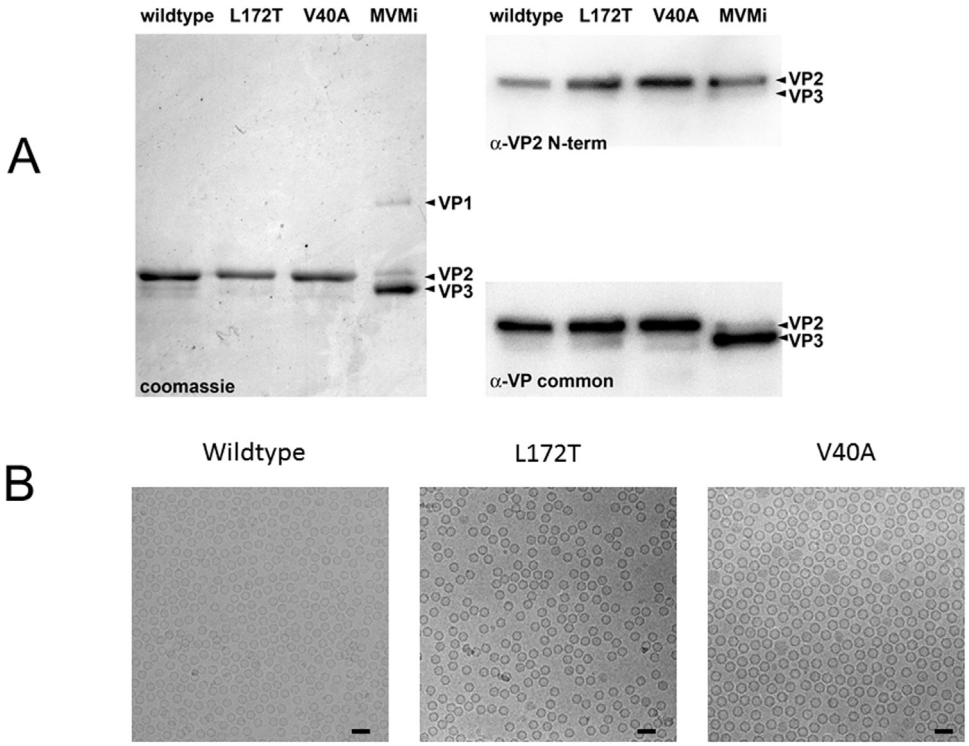

Fig. 2. (A) The left panel shows a Coomassie Brilliant Blue-stained SDS-PAGE of total protein present in samples of purified MVMi wildtype, L172T and V40A VP2-only VLPs, run in parallel with authentic MVMi virions. The latter show the positions of VP1 and VP2, and of VP3, the proteolytic cleavage product found in DNA-containing virions, which has lost ~25 amino acids from its N-terminus. The top right panel shows a western blot of the VP2/VP3 region of a parallel SDS-PAGE probed with α-VP2 N-term, a rabbit polyclonal antibody raised against a 25mer peptide with the sequence NH2 - M S D G T S Q P D G G N A V H S A A R V E R A A - COOH, corresponding to residues 1–24 of the VP2 sequence. The bottom right panel shows the same western blot probed with α-VP common, a rabbit polyclonal antibody raised against an 18mer oligopeptide with the sequence NH2 - Q G S R H G A T Q M E V N W V S K - COOH, located in the MVMi VP sequence from amino acid residues 453–469 of VP1 and 311–327 of VP2. (B) Cryo-EM micrographs of vitrified MVM show (left to right) assembled wildtype, L172T, and V40A VLPs (50 nm scalebar).

is different from the packaging portal (Cotmore et al., 2010; Cotmore and Tattersall, 2012). In MVM and other viruses from genus *Protoparvovirus,* the channels have further adapted to mediate a complex pattern of capsid protein dynamics, progressively delivering effector VP N-terminal peptide sequences to the particle surface at appropriate times during the life cycle. Thus, the five-fold channels of viruses from genus *Protoparvovirus* appear to have adapted to support a protracted program of nucleic acid and peptide extrusion and retraction during infectious entry, but currently we have little structural information to document how this critical process is controlled.

Here we report our use of cryo-EM to explore the structures of MVMi wildtype and mutant VLPs carrying L172T or V40A substitutions. These structures, obtained at resolutions ranging from 6.2 to 7.6 Å, allow us to re-examine the conformation of the five-fold cylinder and to identify disordered regions in the mutants. Changes occurred predominantly at constrictions we have named the mid-gate, which includes residue L172, and the inner-gate, which involves V40. In wildtype crystal structures the side chains of each symmetry-related L172 and V40 residue (shown in Fig. 1) are linked by hydrophobic bonding, and it has been suggested that this interaction might be