Virology 516 (2018) 239–245

Contents lists available at ScienceDirect

Virology

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

Cryo-EM structure of a *Marseilleviridae* virus particle reveals a large internal microassembly

Kenta Okamoto[a,*,1], Naoyuki Miyazaki[b,1], Hemanth K.N. Reddy[a], Max F. Hantke[a], Filipe R.N.C. Maia[a], Daniel S.D. Larsson[a], Chantal Abergel[c], Jean-Michel Claverie[c,d], Janos Hajdu[a,e], Kazuyoshi Murata[b,*], Martin Svenda[a]

[a] Laboratory of Molecular Biophysics, Department of Cell and Molecular Biology, Uppsala University, Husargatan 3 (Box 596), SE-75124 Uppsala, Sweden
[b] National Institute for Physiological Sciences (NIPS), Okazaki, Aichi, 444-8585 Japan
[c] Structural and Genomic Information Laboratory, UMR 7256 (IMM FR 3479) Centre National de la Recherche Scientifique & Aix-Marseille University, Marseille 13288, France
[d] Assistance Publique des Hôpitaux de Marseille, La Timone, 13005 Marseille, France
[e] Institute of Physics AS CR, v.v.i., Na Slovance 2, 18221 Prague 8, Czech Republic

ARTICLE INFO

Keywords:
Cryo-electron microscopy
Tomography
Melbournevirus
Marseilleviridae
NCLDV
Virus
Structure
Capsid
Protein complex
Amoeba

ABSTRACT

Nucleocytoplasmic large DNA viruses (NCLDVs) blur the line between viruses and cells. Melbournevirus (MelV, family *Marseilleviridae*) belongs to a new family of NCLDVs. Here we present an electron cryo-microscopy structure of the MelV particle, with the large triangulation number T = 309 constructed by 3080 pseudo-hexagonal capsomers. The most distinct feature of the particle is a large and dense body (LDB) consistently found inside all particles. Electron cryo-tomography of 147 particles shows that the LDB is preferentially located in proximity to the probable lipid bilayer. The LDB is 30 nm in size and its density matches that of a genome/protein complex. The observed LDB reinforces the structural complexity of MelV, setting it apart from other NCLDVs.

1. Introduction

Nucleocytoplasmic large DNA viruses (NCLDVs) share genetic and structural traits (Iyer et al., 2001). Comparative genomics of NCLDVs has evoked speculations on the origin of DNA viruses as a distinct domain of life and their role in the evolution of cellular organisms (Abergel et al., 2015; Claverie and Abergel, 2013).

A large variety of icosahedral NCLDVs has to date been isolated from unicellular eukaryotes such as algae and amoeba (Claverie et al., 2009; Dornas et al., 2014; La Scola et al., 2003; Monier et al., 2008; Reteno et al., 2015; Saadi et al., 2013; Santini et al., 2013; Van Etten et al., 1982; Yan et al., 2005). *Marseilleviridae*, including Melbournevirus (MelV) reported in this study, is a recently established family among the large amoebal NCLDVs (Colson et al., 2013; Doutre et al., 2014). They form capsids that vary in size from 190 to 250 nm. The particle architecture and the protein-encoding genes places them taxonomically in a unique family separated from other NCLDVs (Aherfi et al., 2014; Doutre et al., 2015, 2014; Thomas et al., 2011). All these NCLDVs seem to share an evolutionary relationship, albeit complex, based on a core set of conserved genes such as the major capsid protein, the DNA polymerase and other common enzymes, and their virion structures (Iyer et al., 2001; Koonin and Yutin, 2010; Yutin and Koonin, 2012).

Structural studies of the NCLDV particles are important for understanding their assembly, mechanism of cell entry, and evolution. Electron cryo-microscopy (cryo-EM) structures of viral particles of NCLDVs have shown that some of the large capsids adopt regular icosahedral lattices with large triangulation numbers (T-number) built up by an evolutionary related major capsid protein (MCP) (Andreani et al., 2017; Ang and Schaposnik, 2017; Klose et al., 2010, 2016; Kuznetsov et al., 2010; Sinkovits and Baker, 2010; Xiao et al., 2005, 2017, 2009; Yan et al., 2005, 2000). The MCP consists of two jellyroll motifs, similar to many other dsDNA viruses, and the pseudo-hexametric capsomer unit is composed of a trimer of MCPs. The capsids of these large viruses tend to disintegrate into pentagonal and triangular units (penta-symmetrons and trisymmetrons) where the triangular units do not

* Corresponding authors.
E-mail addresses: kenta.okamoto@icm.uu.se (K. Okamoto), kazum@nips.ac.jp (K. Murata).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.virol.2018.01.021
Received 17 November 2017; Received in revised form 24 January 2018; Accepted 25 January 2018

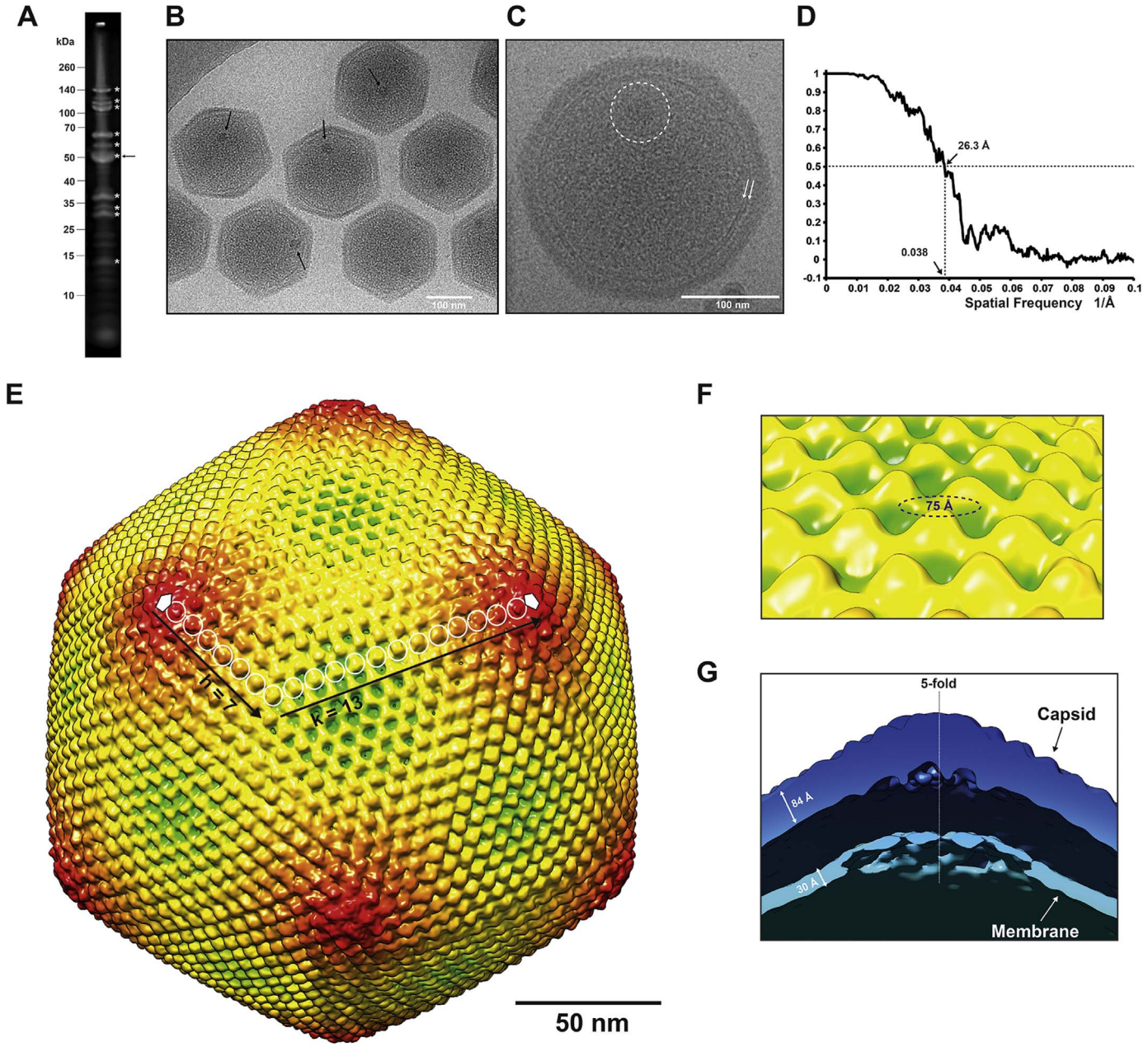

Fig. 1. Images of cryo-frozen MelV particles and single particle 3D reconstruction. A) A SDS-page gel of the purified virions used for the single particle analysis. The arrow indicates the band of the conserved MCP. White asterisks indicate 10 abundant major proteins. B, C) Raw images of the MelV. Black arrows in B) and white dotted circle in C) indicate LDBs. White arrows in C) indicate the double-layered membrane. D) Fourier shell correlation between two independently reconstructed half sets of images with an inner mask applied. The resolution estimated by a 0.5 cutoff is 26.3 Å. E) The T = 309 capsid lattice of the MelV. Surface plot of the reconstructed 3D structure at an isodensity contour level of 2.0 σ coloured according to the distance from the center of the virus particle (green < 1050 Å, yellow < 1070 Å, red < 1200 Å. The T-number was determined by counting the number of the protrusions in the lattice (black arrows and white circles). Each protrusion represents a capsomer. With h = 7 and k = 13 it corresponds to T = 309. F) Close-up view of the surface protrusions that are the tips of the pseudo-hexagonal capsomers. G) A cross-section of the 3D density perpendicular to a 5-fold axis. The capsid and membrane structures were rendered at an isodensity contour level of 1.0 σ using a cryo-EM model at the resolution of 35.0 Å without applying any inner mask.

necessarily correspond to the triangular facets of the approximately icosahedral capsid (Nandhagopal et al., 2002; Wrigley, 1969). In addition to these regular capsid traits of the NCLDVs, some NCLDVs possesses unique features such as the stargate, long fibers and internal multi-layered membranes of the Mimivirus particle (Abergel et al., 2015; Arslan et al., 2011; La Scola et al., 2003; Schrad et al., 2017; Xiao et al., 2005, 2009), the unique vertex of the *Paramecium bursaria* Chlorella virus 1 (PBCV-1) particle (Cherrier et al., 2009), and the double-layered capsids of Faustovirus (Klose et al., 2016). Here we present a cryo-EM structure of the MelV particle, displaying unique structural features that set it apart from other NCLDVs.

2. Results

2.1. Identification of structural proteins of the MelV particle

The MelV genome encodes 403 open reading frames. Purified virions contain at least 10 major (abundant) proteins and many minor proteins (Fig. 1A). The most intense band of the SDS-PAGE appears at around 50 kDa (black arrow in Fig. 1A) and is thought to correspond to the MCP (predicted molecular weight of 52.4 kDa). The purified virus particles were also analyzed by tandem mass spectrometry (MS)-based proteomics analysis after 9 M urea treatment (Supplementary Table S1 and S1-2). The MCP was identified as well as histone-like or histone-