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Brief Communication

Structural insights into the architecture of the hyperthermophilic *Fusellovirus* SSV1

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ABSTRACT

The structure and assembly of many icosahedral and helical viruses are well-characterized. However, the molecular basis for the unique spindle-shaped morphology of many viruses that infect *Archaea* remains unknown. To understand the architecture and assembly of these viruses, the spindle-shaped virus SSV1 was examined using cryo-EM, providing the first 3D-structure of a spindle-shaped virus as well as insight into SSV1 biology, assembly and evolution. Furthermore, a geometric framework underlying the distinct spindle-shaped structure is proposed.

Introduction

The morphological diversity of viruses that infect hyperthermophilic *Archaea* vastly exceeds that of other known prokaryotic viruses (Prangishvili, 2003; Prangishvili et al., 2006). Indeed, unique features of these viruses have necessitated the introduction of 10 novel virus families: e.g. filamentous *Lipothrixviridae*, rod-shaped *Rudiviridae*, droplet-shaped *Guttaviridae*, and spindle-shaped *Fuselloviridae*. There are also numerous unclassified archaeal spindle-shaped viruses (Prangishvili, 2013). This diversity may reflect ancestral diversity of viral morphotypes present in hot environments during the prebiotic phase of evolution (Balter, 2000; Prangishvili, 2003; Prangishvili et al., 2006). Furthermore, because hyperthermophilic *Archaea* possess metabolisms well-suited for primordial hot anaerobic conditions, it has also been suggested that hyperthermophilic viruses may have played an important role at the earliest stages of evolution (Prangishvili, 2003).

Despite the unusual morphologies of archaeal viruses, studies on their genome organization, mechanism of replication, and regulation of gene expression indicate a distant evolutionary relationship between some of these viruses and viruses of mesophilic bacteria and eukaryotes (Blum et al., 2001; Iyer et al., 2006; Klein et al., 2002; Peng et al., 2001; Pfister et al., 1998; Prangishvili, 2003; Tang et al., 2004; Tang et al., 2002). Verification of this hypothesis by sequence comparison is challenging because the rapid evolution of viral genes can preclude detection of relationships over large evolutionary distances (Pagel, 1999). However, structural similarity often persists during evolution in spite of vanishing sequence similarity. For example, capsid proteins adopting either the jelly-roll or HK97 fold have been observed in icosahedral viruses infecting each of the three domains of life, suggesting that the ancestors of viruses utilizing these capsid protein folds predates the last universal common (cellular) ancestor (LUCA) (Fokine et al., 2004; Jiang et al., 2003; Khayat and Johnson, 2011; Morais et al., 2005; Pietila et al., 2013; Wikoff et al., 2000). Thus, the structural information regarding the morphologically divergent viruses infecting hyperthermophilic *Archaea* might provide insights into virus origin and the evolution of viruses and cells. Here, cryo-EM image analysis and reconstruction

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was used to structurally characterize the prototypical Sulfolobus fusellovirus SSV1 (Contursi et al., 2014), providing insight into the underlying geometry and assembly of the spindle-shaped capsid. In addition to a ~15 kbp positively supercoiled dsDNA genome, purified SSV1 virions are composed of; a major capsid protein, VP1; a minor capsid protein with very similar sequence, VP3; a DNA binding protein, VP2; and smaller amounts of the products of ORFs C792 and D244 (Menon et al., 2008; Reiter et al., 1987).

Results and discussion

Although no symmetry was initially assumed, preliminary cycles of the iterative refinement procedure indicated that D1 symmetry (two-fold symmetry perpendicular to the long-axis of the virus) could be applied to the virus capsid and six-fold symmetry applied to the tail. Upon convergence of refinement, the resolution of the reconstruction was ~32 Å as estimated using a Fourier shell correlation cutoff of 0.5 between independent half-data sets. Resolution was likely limited by particle size, lack of global symmetry, structural heterogeneity, and the relatively small number of particles included in the reconstruction, although ~doubling the number of particles did not result in any measurable improvement in the map. At the current resolution, there is no indication of an internal or external membrane despite that SSVs are apparently released by budding and have a low buoyant density (1.27 g/mL) (Martin et al., 1984). Moreover density for the packaged DNA is more diffuse than is observed for dsDNA bacteriophages, possibly due to the relatively small size (~15 kbp) of the SSV1 genome and/or its unusual positively supercoiled topology (Nadal et al., 1986; Palm et al., 1991). The SSV1 reconstruction (Fig. 1B–D) indicated that the capsid is ~750 Å long, and ~430 Å wide at the equator, and that the tail is ~120 Å long by 120 Å wide. The long dimension is consistent with TEM analysis of negatively stained SSV1 virions at ~1000 Å, however the narrow dimension, reported as 600 Å less so (Martin et al., 1984); the wider dimensions observed in negative stain are likely due to flattening of the particles during grid preparation, a common

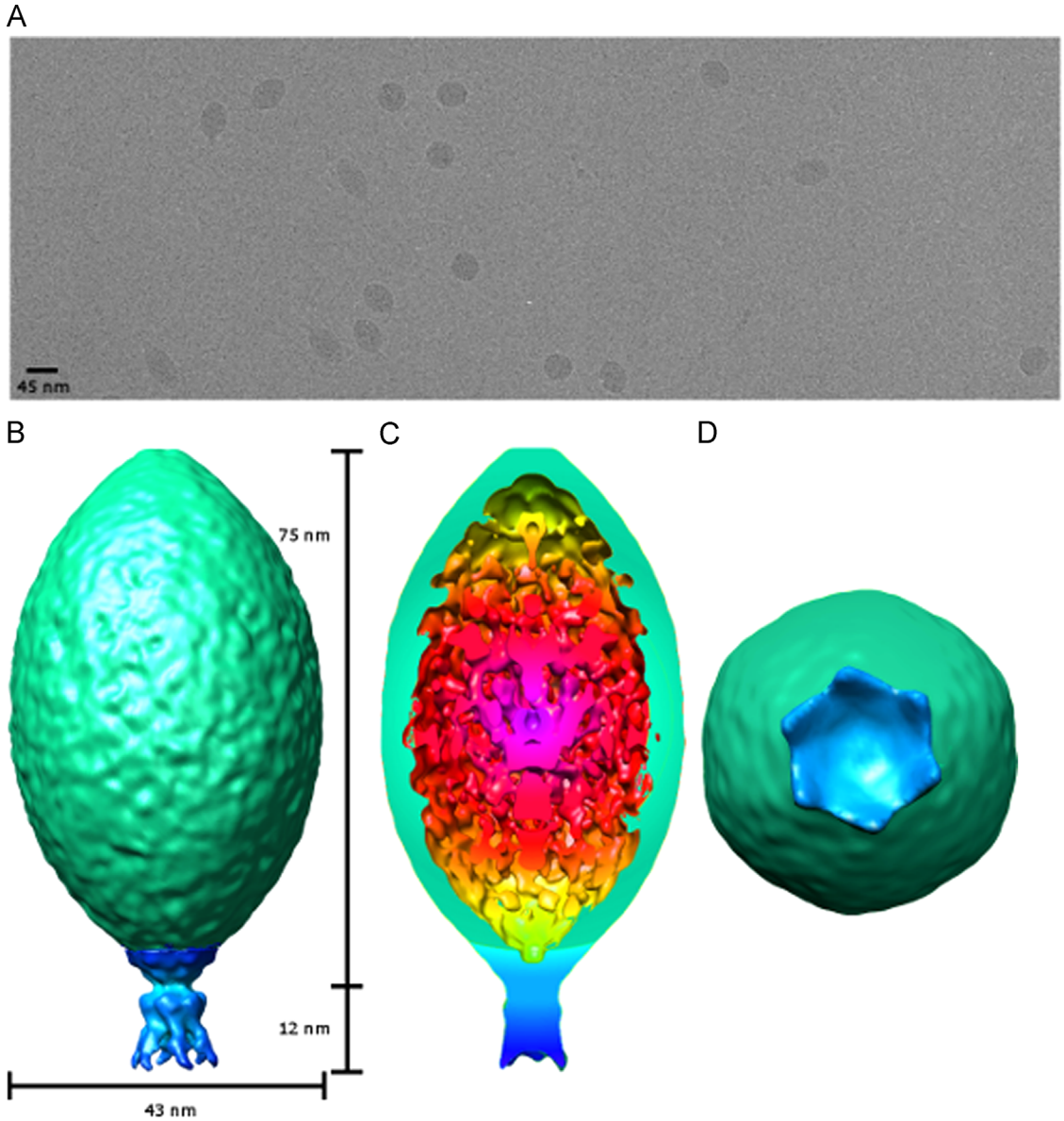

Fig. 1. Cryo-EM analysis of SSV1 particles. A) Typical field of particles. Three-dimensional reconstruction of an SSV1 particle from a side-view (A), side-view cross-section (B), and end-on view looking at the tail (C). In all panels, the capsid is colored green, and the approximate density corresponding to the is colored tail blue; in panel (C), density within the capsid, presumably corresponding to the viral genome, is colored from magenta to yellow according to radial distance from the center of the particle.