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Cryo-electron tomography of Kaposi's sarcoma-associated herpesvirus capsids reveals dynamic scaffolding structures essential to capsid assembly and maturation

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Abstract

Kaposi's sarcoma-associated herpesvirus (KSHV) is a recently discovered DNA tumor virus that belongs to the γ-herpesvirus subfamily. Though numerous studies on KSHV and other herpesviruses, in general, have revealed much about their multilayered organization and capsid structure, the herpesvirus capsid assembly and maturation pathway remains poorly understood. Structural variability or irregularity of the capsid internal scaffolding core and the lack of adequate tools to study such structures have presented major hurdles to earlier investigations employing more traditional cryo-electron microscopy (cryoEM) single particle reconstruction. In this study, we used cryo-electron tomography (cryoET) to obtain 3D reconstructions of individual KSHV capsids, allowing direct visualization of the capsid internal structures and systematic comparison of the scaffolding cores for the first time. We show that B-capsids are not a structurally homogenous group; rather, they represent an ensemble of "B-capsid-like" particles whose inner scaffolding is highly variable, possibly representing different intermediates existing during the KSHV capsid assembly and maturation. This information, taken together with previous observations, has allowed us to propose a detailed pathway of herpesvirus capsid assembly and maturation.

Keywords: Cryo-electron tomography; Tumor herpesvirus; Kaposi's sarcoma-associated herpesvirus; Assembly; Scaffolding; Portal

1. Introduction

Kaposi's sarcoma-associated herpesvirus (KSHV) is a DNA tumor virus and the most recently discovered human pathogen in the *Herpesviridae* family (Chang et al., 1994). It is the causative agent of all forms of Kaposi's sarcoma and closely linked to AIDS-associated lymphomas, including primary effusion lymphoma and multicentric Castleman's disease (Ganem, 1998). KSHV and Epstein-Barr virus (EBV) are the two known human pathogens of the γ-herpesviruses subfamily of *herpesviradae* (Chang et al., 1994; Ganem, 1997; Damania et al., 1999, 2000; Mueller, 1999; Roizman et al., 2001; Carbone, 2003). Like other herpesviruses, the KSHV virion consists of a double-stranded (ds) DNA genome enclosed within an icosahedral capsid shell, a thick, proteinaceous tegument compartment, and a lipid bilayer envelope spiked with glycoproteins (Rixon, 1993; Steven and Spear, 1997; Liu and Zhou, 2007). Genetic and biochemical studies have suggested the existence of a bacteriophage-like DNA-packaging/ejection portal presumably located at one of the 12 vertices (Newcomb et al., 2001b), which has been confirmed recently by cryo-electron tomography (cryoET) studies of native KSHV capsids (Deng et al., 2007), although the exact

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orientation and radial placement of the portal remains controversial in herpes simplex virus (HSV-1) capsids (Trus et al., 2004; Cardone et al., 2007; Chang et al., 2007).

Since the 1970s, the scientific community has had biochemical evidence that herpesviruses form three distinct capsids during assembly in the host nucleus, designated A-, B- and C-capsids, which at the time were only known as different bands, conveniently labeled alphabetically as "A", "B" and "C", according to the order of their positions on a density gradient. These particles were later discovered via protein analysis and negative stain electron microscopy to be an empty capsid shell (A-capsid), or a capsid containing scaffolding protein (B-capsid), or a capsid containing the viral genome (C-capsid), respectively. In 1996, a fourth capsid category was discovered, termed the procapsid, that was different from A-, B- and C-capsids in that it was spherical, rather than angular, and porous, rather than sealed (Newcomb et al., 1996). Procapsids are assembled upon a skeleton comprised of large numbers of the internal scaffold protein, SCAF (assembly protein [AP] in HSV-1). The procapsid spontaneously angularizes shortly after it forms, but exactly when this angularization occurs and whether or not A- and B-capsids are both possibly dead-end products resulting from angularization at an inappropriate time is still under debate. At an indeterminate time following capsid assembly, the viral protease, PRO (Pr in HSV-1 and HCMV), cleaves the inner SCAF proteins, resulting in capsid angularization. Little is currently known about the roles of PRO and its cleavage of SCAF in KSHV assembly, partly due to the lack of any structural information regarding their organization inside KSHV capsids. The protease also disassembles the scaffolding protein complex within the capsid. When exactly this occurs is unclear, but Newcomb et al. found that HSV-1 procapsids contain more scaffolding protein than B-capsids (Newcomb et al., 2001a), indicating perhaps that some scaffolding is lost during angularization. Either concurrent with or immediately after the cleaved scaffolding proteins leave the capsid, the viral genome is packaged into the capsid. The mechanism by which this packaging occurs likewise remains unclear, though the linear double-stranded genome likely enters through the portal, a structurally unique vertex in the capsid (Newcomb et al., 2001b; Trus et al., 2004; Cardone et al., 2007; Chang et al., 2007; Deng et al., 2007).

With the advent of cryo-electron microscopy (cryoEM) in the late 1980s, it became possible to reconstruct the A-, B- and C-capsids and procapsids and in three dimensions via single particle reconstruction and icosahedral averaging, in which hundreds to thousands of particles are averaged together to overcome the low signal-to-noise ratio typical of cryoEM (Wu et al., 2000; Zhou et al., 2000; Trus et al., 2001; Heymann et al., 2003; Yu et al., 2003). Such studies have succeeded in generating clear reconstructions of the highly iterative and ordered capsid shell, but in the case of B-capsids, signal from the inner scaffolding was averaged out in reconstructions that failed to reveal any ordered internal structure, presumably due to either lack of icosahedral symmetry or variable numbers of scaffolding core structures among the individual capsids. γ-Herpesviruses have provided even more of a challenge as both EBV and KSHV are extremely difficult to grow in cell culture; KSHV, for example, requires chemical treatment to induce lytic replication (Renne et al., 1996). Nealon et al. were able to provide biochemical evidence that the *amount* of scaffolding protein in B-capsids seems to be variable, but there was still no definitive evidence clarifying the organization of scaffolding protein within the B-capsids (Nealon et al., 2001). In light of the above considerations, it became clear that cryoEM is inadequate for revealing the internal structures of the B-capsid, and thus we turned to cryoET.

Where cryoEM requires different particles for 3D reconstruction, cryoET can reveal 3D structures of an individual particle by reconstructing it using a tilt series, or a series of micrographs taken from the same sample tilted at different angles. The advantage cryoET holds over cryoEM is that it can extract structural information from even one single particle, structures unique to that particle will not get averaged out as they would by cryoEM after integrating signals from a population of particles analyzed. In this study, we used cryoET to reconstruct individual KSHV capsids, and show that B-capsids are actually a heterogeneous collection of B-capsids-like, or "B-type" capsids, with highly variable inner scaffolding formations that form hollow, spiky spheres. These spheres are sometimes situated in the center of the capsid, and sometimes adjacent to the inner wall opposite to the portal complex. Other times, the scaffolding appears disorganized and lacks any visible spherical shape. Integrating this information and previous structural and biochemical data, we provide an updated mechanistic model of herpesvirus capsid assembly and maturation.

2. Results and discussion

2.1. KSHV A-, B- and C-capsids

The difficulties in isolating human tumor herpesvirus capsids for structural studies have been well recognized (Wu et al., 2000; Nealon et al., 2001). In order to establish statistical significant observations, we collected a total of 33 cryoET tilt series containing 297 ice-embedded KSHV capsids. One example is provided Fig. 1. A total of 297 capsids were examined. 96 were A-capsids, 198 were B-capsids, and only 3 were C-capsids. Five high quality tomograms with 51 KSHV capsids were obtained using the marker-free alignment program developed by Winkler and Taylor (see details in Section 3) (Winkler and Taylor, 2006) as judged by the clear resolution of the unique portal complex in one of the 12 vertices (Fig. 1c). In addition, the 11 pentons and 150 hexons making up the capsid shell are clearly resolved and are similar to those obtained by cryoEM icoshaedral reconstruction (Fig. 1c) (Wu et al., 2000; Trus et al., 2001).