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RSV Capsid Polymorphism Correlates with Polymerization Efficiency and Envelope Glycoprotein Content: Implications that Nucleation Controls Morphogenesis

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Abstract

We used cryo-electron tomography to visualize Rous Sarcoma Virus, the prototypic alpharetrovirus. Its polyprotein Gag assembles into spherical procapsids, concomitant with budding. In maturation, Gag is dissected into its matrix (MA), capsid (CA) and nucleocapsid (NC) moieties. CA reassembles into cores housing the viral RNA and replication enzymes. Evidence suggests that a correctly formed core is essential for infectivity. The virions in our data set range from ~ 105 to ~ 175 nm in diameter. Their cores are highly polymorphic. We observe angular cores, including some that are distinctively “coffin-shaped” for which we propose a novel fullerene geometry; cores with continuous curvature including, rarely, fullerene cones; and tubular cores. Angular cores are the most voluminous and densely packed; tubes and some curved cores contain less material, suggesting incomplete packaging. From the tomograms, we measured the surface areas of cores and hence their contents of CA subunits. From the virion diameters, we estimated their original complements of Gag. We find that RSV virions, like HIV, contain unassembled CA subunits and the fraction of CA that is assembled correlates with core type; angular cores incorporate ~ 80% of the available subunits and open-ended tubes, ~ 30%. The number of glycoprotein spikes is variable (~ 0 to 118) and also correlates with core type; virions with angular cores average 82 spikes, whereas those with tubular cores, 14 spikes. These observations imply that initiation of capsid assembly, in which interactions of spike endodomains with the Gag layer play a role, is a critical determinant of core morphology.

Keywords

virus maturation; nucleation of assembly; critical concentration; capsid assembly; cryo-electron tomography

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Introduction

Retrovirus research, long a focus of interest since the discoveries of their oncogenicity and retrotranscription activity, received a major additional stimulus upon the demonstration that a retrovirus, human immunodeficiency virus (HIV), is responsible for the AIDS pandemic. Although there tends to be little conservation among different retroviruses at the level of gene and protein sequences, they have similar genome organizations and assembly pathways¹. Assembly involves co-polymerization of the precursor polyprotein Gag with a minor amount of the Gag-Pol fusion (Pol encodes the viral enzymes, reverse transcriptase and integrase) to form a round shell enclosing two copies of the RNA genome - a linear, single-stranded, negative-sense molecule^{1; 2}. Concomitantly or subsequently, the nascent procapsid acquires an envelope containing the viral glycoprotein Env by budding through the host cell plasma membrane. After budding, the provirion undergoes maturation, a process marked by dramatic structural changes.

Maturation starts with activation of the viral protease which is incorporated into the provirion as part of the Gag (as in RSV) or Gag-Pol (as in HIV) polyproteins. The protease dissects Gag and Gag-Pol into their constituent moieties. Although structural transitions induced by proteolysis are a feature of maturation in many virus families³, retroviruses are unique in that their CA and NC subunits appear to reassemble *de novo* within the confines of the maturing virion (the MA moieties remain in a layer lining the inner surface of the envelope)^{4; 5; 6}. CA assembles into the surface shell of a ribonucleoprotein complex called the viral core that contains the genomes with associated NC protein, tRNA, and the viral enzymes.

Capsid assembly and maturation have been studied intensively for HIV and appear to follow a broadly similar course for other retroviruses⁷. In the provirion, the elongated Gag molecules pack laterally in a spherical shell with their long axes directed radially so that their MA domains contact the envelope and their other domains are positioned in order at lower radii^{8; 9}. Lateral contacts produce locally ordered hexagonal patches of Gag molecules but they do not conform to a global symmetry. Virions vary in diameter and their size is dictated primarily by CA^{10; 11}. Although the CA subunits are redistributed in maturation, virion size is maintained¹². *In vitro*, CA subunits assemble into tubular foldings of a hexagonal lattice and a strong case has been made that the predominant core morphology in virions is fullerene cones¹³, i.e. asymmetric closures of the same hexagonal lattice with five pentameric vertices at the narrow end and seven vertices at the wider end. Various sizes of fullerene cores are observed as well as other forms (e.g. tubes) and some virions contain more than one core⁴. Of particular note, up to two thirds of the total CA complement remain unassembled^{6; 14; 15}. A correctly formed core is thought to be essential for infectivity, as several studies have correlated CA mutations that alter the core with reduced infectivity^{16; 17; 18; 19}. However, the requirements for what constitutes a correctly formed core have yet to be fully defined.

In this study, we have used cryo-electron tomography (cryo-ET)²⁰ to investigate the structural variability of RSV, a well studied avian retrovirus. Cryo-ET allows visualization of the 3D structures of individual macromolecular particles in a native state - a major advantage when dealing with pleiomorphic viruses^{6; 21; 22; 23}. RSV virions are also round and variable in size and, on average, slightly smaller than HIV¹². RSV-Gag differs from HIV-Gag in having an extended linker (p10) between MA and CA; in lacking an N-terminal meristylation; and in having the protease constitutively appended at its C-terminus^{1; 7}. Notwithstanding, the structure of its CA subunit is virtually superimposable on that of HIV^{24; 25; 26}. Moreover, RSV-CA readily assembles *in vitro* into a hexagonal lattice^{26; 27} while, in the presence of RNA, the CA-NC fusion assembles into tubes²⁸. As with HIV (above), RSV infectivity is thought to depend on proper core assembly, and mutations in CA can disrupt core formation. A set of rather subtle substitutions in the most conserved CA segment has been shown to

compromise core stability and impair reverse transcription²⁹. More radical substitutions in this region abrogated viral assembly and budding, presumably resulting from disruption of normal Gag structure.

As outlined above, RSV has many features in common with HIV but has markedly different core structures that have been described as essentially isometric in a cryo-EM study³⁰. In plastic sections, virions exhibit a strongly staining central region, presumably containing the genome and associated proteins, that is sometimes observed to be surrounded by a slightly angular shell that may represent the capsid¹. A primary goal of the present study was to characterize the three-dimensional structures of well preserved RSV cores *in situ*. To explore assembly mechanisms, we sought to ascertain how core structures might correlate with other properties of the virions, as revealed in the tomograms. This investigation was motivated both by interest in RSV structure *per se* and the aim of contributing to a clearer understanding of how retrovirus assembly in general is regulated.

Results

Cryo-tomography

The Prague C strain of RSV was propagated in turkey embryo fibroblasts and virions isolated by centrifugation through a sucrose cushion. Specimens were vitrified for cryogenic observation and, after suitable areas were identified, tilt series were recorded and the corresponding tomograms reconstructed. Six tomograms, containing a total of 95 virions, were selected for detailed analysis. The cumulative electron dose was $\sim 60 \text{ e}^-/\text{\AA}^2$ and the in-plane resolution achieved was 5 – 6 nm. Other parameters are given in Table I.

Virion morphology

The virions are roughly spherical, with diameters mostly ranging between 110 and 145 nm (e.g. Fig. 1). (Here, as in other recent retrovirus studies, diameter is measured at the outer margin of the viral membrane; the spikes extend further). Their mean diameter was 125 ± 10 (s.d.) nm, similar to the values previously reported in cryo-EM studies of mature (127 ± 10 nm³⁰) and immature (129 ± 23 nm¹²) RSV virions.

Around the periphery of each virion is a continuous layer of density, ~ 7 nm thick, that we take to be the viral envelope, consisting of a lipid bilayer lined with a closely apposed layer of MA protein. Protruding from the surface of the bilayer are the ectodomain spikes of the Env glycoprotein. RSV spikes are oblate in shape. The number of spikes per virion is quite variable (see below).

The majority of virions ($\sim 80\%$) contain cores, clearly defined by a continuous layer of density ~ 5 nm thick. RSV cores are highly polymorphic. We assigned them to three predominant morphological classes: “angular”, “continuous curvature”, and tubular (Table II). The remainder either lacked a well-defined core or contained nested cores or double cores. Fig. 2 shows an RSV virion with an angular core, alternatively represented as serial grayscale sections (Fig. 2A) and after segmentation (Fig. 2B).

Capsid polymorphism

(i) Angular cores—These cores have faceted polyhedral structures (e.g. Fig. 3A). In sections, they typically present as somewhat irregular pentagons or hexagons, i.e. with five or six linear segments connecting at sharply defined angles. A distinctive subclass of angular cores ($n=6$) are “coffin-shaped” (e.g. Fig. 2), i.e. they appear in longitudinal sections as pentagons with a linear base, an apical chevron, and approximate mirror symmetry about the central axis.

Angular cores tend to be larger than cores with other morphologies and occupy greater portions of the space inside the envelope.

(ii) Continuous curvature cores—These cores also have closed shells but, compared to angular cores, they exhibit a more continuous curvature (e.g. Fig. 3B; cf. Kingston *et al.* ³⁰). This class includes, as rare members (n=3), conical cores of the kind common in HIV (e.g. Figs. 3B & 3F), and “lozenges” (n=6) with regular oval profiles (e.g. Fig. 3B). Some cores of this kind are closely associated with the surrounding membrane (e.g. Fig. 3F) which departs from the usual roundness and conforms to the shape of the adjacent capsid, as if the core had been forcibly excluded from the virion center, distorting the envelope.

(iii) Tubular cores—Many virions have tubular, i.e. cylindrical, cores. Most tubes are open at both ends (e.g. Fig. 3C) but some are capped at one end (e.g. Fig. 3D) and a few are capped at both ends (e.g. Fig. 3E). End caps tend to be partly or wholly flat, in the plane perpendicular to the tube axis. (The lozenges described above may be considered as closed tubes with non-planar caps). Most tubular cores are centrally positioned and extend across the full width of the virion interior. They vary markedly in diameter: cores open at both ends have diameters from 32 to 43 nm, mean: 37 nm. Tubes with one end open tend to be slightly wider (mean diameter, 39 nm).

(iv) Other core-related observations—Of the remaining virions, the majority have no sizable core-like internal structures (e.g. Fig. 1 & Fig. 3H). Nested cores with a second shell wrapped around an inner core have been reported for HIV ⁶. We observed three such cores in RSV virions (e.g. Fig. 3G), inferring that the second layer is also a lattice of CA subunits because it has the same thickness as the shell of single-layered cores. The other two virions have complete inner cores that are respectively tubular and triangular in cross-section. We also observed two virions with double cores (e.g. Fig. 3I), which are common in HIV, at ~ 33% ⁴, but rare in RSV.

Internal structures

The virion interior has two compartments: one inside the core and one between the core and the matrix layer. Inside angular cores and some continuous curvature cores, the density is, on average, greater than in the outer compartment (e.g. Fig. 3A), whereas we observed no such distinction with tubular cores whose internal density is typically no higher, and often lower, than in the outer compartment (cf. Figs. 3C & 3D). Closed conical cores appear to fall into both categories: they may be well filled (e.g. Fig. 3G) or have low-density interiors (e.g. Fig. 3B). In low-density cores, we suspect that the complex of genome(s) plus associated proteins may not be encapsidated or may be incompletely encapsidated. The relative sizes of these two compartments also vary with core type: the external compartment accounts for most of the internal volume of a virion with a curved or tubular core and markedly smaller fractions in the case of angular cores (Fig. 4B).

Inside eight cores of various morphologies, we detected tube-like structures with a diameter of 13 nm, a wall thickness of ~ 5 nm and mean length of ~ 17 nm (e.g. Fig. 3B, column 5). There are up to four such rings in a single core. Their molecular composition and functional significance are currently unclear.

Glycoprotein spikes

The tomograms allow counting of the spikes on individual virions. Their numbers vary widely, ranging from ~ 0 to ~ 118 (mean, 36). In general, spikes are distributed irregularly over the virion surface. The arrowed particle in Fig 1, which has an angular core, exemplifies a relatively

densely spiked virion. Spikes tend to be spread unevenly over the whole envelope; for example, the virion in Fig. 2 has bare patches as well as sizable clusters.

The large majority of spikes have a diameter of ~ 9 nm and are ~ 5 nm thick and are connected to the membrane by a narrow stalk, ~ 3 nm long. In transverse section, a triangular profile is often discernible (data not shown). We infer that they represent Env glycoprotein trimers. More detailed analysis of their substructure is ongoing and will be reported at a later date. However, we also observed a few envelope protrusions with markedly different structures (e.g. arrowheads, Fig 2A, row 3 column 2 and row 4, column 1), that are likely to be membrane proteins from the host cell.

Correlation of spike content with core morphology

Visual appraisal of the tomograms gave the impression that virions with angular cores tend to have more spikes. To take a more quantitative approach, we compiled a plot of spike number versus virion diameter in which the virions are classified according to core morphology (Fig. 4A). If spike acquisition were to be an entirely random event, we would expect to observe a trend whereby the number of spikes increases as a function of virion diameter squared. However, we observed no evident correlation of spike number with virion diameter. In fact, spike numbers vary in a more extreme manner than may be accounted for by such a dependence. Virions with angular cores do indeed have more spikes than virions with “continuous curvature” cores (Table II). Virions with tubular cores have the fewest spikes, particularly those with open-ended tubular cores (0 to 15; mean 5). Those whose tubes have one end closed have somewhat more (6 to 30; mean 14), while the two virions with tubular cores closed at both ends (Fig. 3E) had 48 and 50 spikes - as many as virions with other kinds of closed cores.

Correlation of core size with morphology

As noted above, angular cores appear to occupy relatively large fractions of their virions' interiors, and tubular cores, relatively small fractions (Fig. 4B). While it is difficult, in this context, to meaningfully compare the diameters of differently shaped objects, surface area is a more robust parameter and the tomograms allowed us to measure it for each core. These data are plotted in Fig. 4C *versus* virion diameter, again with different core morphologies color-coded accordingly. Angular cores have the largest surface areas, while tubular cores have the smallest surface areas (Table II).

Copy numbers of assembled CA subunits in cores and of Gag subunits in the provirions

Evidence has been presented that HIV virions contain two populations of CA molecules: respectively, assembled and unassembled. The unassembled fraction is estimated to account for about two thirds ⁶; 14 or half ¹⁵ of the total. These figures were based on ensemble averages over populations of virions that vary in size and core morphology. Individual measurements of diameter and core surface area allow one to estimate the number of Gag molecules originally incorporated into each provirion and the number of CA subunits assembled in its mature core ⁶. These data are plotted in Fig. 5. The number of unassembled CA subunits is given in each case by the difference between these two numbers (see arrows in Fig. 5).

This procedure was based on the following assumptions. We suppose that a mature virion has the same diameter as its immature precursor, as implied by the cryo-EM data cited above and as reported for HIV ¹². Gag copy numbers for the corresponding immature virions were calculated according to Briggs *et al.* ¹². Gag molecules are oriented radially, with their long axes perpendicular to the membrane. The CA domains are assumed to be located ~ 24 nm from the outer surface of the membrane and hexagonally packed with a center-to-center distance of ~ 7.8 nm. For each virion, we calculated the number of unit cells by dividing the surface area of a sphere of reduced diameter (by 48 nm) by the unit cell area, and assigned six Gag subunits

per unit cell (see also Wright *et al.*³¹). To estimate the number of CA subunits in a given core, we assume they are packed on a hexagonal lattice with a repeat of 9.5 nm (see below), with six monomers per unit cell. The number of unit cells is given by the core surface area measured from the tomogram divided by the unit cell area.

RSV-CA subunits form hexagonal lattices with a 9.5 nm repeat²⁷. This spacing is very similar to that of HIV-CA lattices³². It is also consistent with cryo-electron micrographs of isolated RSV cores on which we observe regular serrations along the edges of some cores (Figs. 6A & 6B; see also Kingston *et al.*³⁰). Our measurements of their spacings varied between 4.1 and 4.7 nm. We infer that they relate to the (2,0) and (1,1) reflections of a hexagonal lattice with a 9.5 nm repeat. In support of this interpretation, the distribution of mass in RSV-CA lattices is such that in their diffraction patterns, the (1,0) reflection is weak and the (1,1) and (2,0) reflections are strong (Fig. 3 of Mayo *et al.*²⁷).

Correlation of CA assembly efficiency with core morphology

The data in Fig. 4 & Fig 5 indicate that angular cores not only have the largest surface areas and enclosed volumes, but also that they leave the smallest percentages of residual, unassembled, CA subunits. On average, 80% of their CA subunits enter an ordered state (Table II). At the other extreme, tubular cores are the least efficient: typically, only 30–35% of the available CA subunits become assembled. Overall, taking into account the coreless particles, we estimate that 52% of the CA protein in this population of virions remained unassembled.

Core-envelope linkages

In most virions, particularly those with angular cores, we observed connections linking the envelope to the core (e.g. arrows in Fig. 2A; Fig. 3B; Fig. 6C). They occur both singly and in clusters, and are ~ 4 nm wide and, on average, 16 nm long, measured from the center of the envelope to the center of the capsid wall, corresponding to a 10 nm connection. Of 28 clearly defined linkers connecting to angular cores, 9 emanated from a vertex and 8 were aligned with a spike on the other side of the envelope. For reference, spikes cover ~ 10% of the surface of the envelope and vertices account for ~ 4% of the capsid surface for the average virion with an angular core. As discussed below, the linkers may represent unprocessed or partially processed Gag molecules.

Discussion

In this study, we investigated the three-dimensional structures of many individual RSV virions which are spherical in shape but variable in size and other respects. We designate them as both pleiomorphic, i.e. continuously variable, with reference to their envelopes and distributions of internal components, and polymorphic with respect to their capsids, where the latter term denotes a discreet range of variation. Mature core shells (capsids) appear to encompass many foldings of the same underlying hexagonal lattice. The perceived requirement for a correctly formed core to yield an infectious virion raises the questions of: what forms are, in fact, assembled? how is their assembly regulated? and of those assembled, which are likely to be compatible with infectivity?

Fullerene geometry of "coffins", tubular capsids, and lozenges

Ganser *et al.*¹³ derived a plausible model for HIV capsids as fullerene cones formed from a hexagonal lattice of CA subunits³². These cones have five 5-fold sites inserted in the lattice at their narrow end and seven at the wide end: in this way, closure is achieved while maintaining continuity of the lattice lines, i.e. repetition of the same quasi-equivalent interactions. It has been further proposed^{33; 34} that alternative foldings of the same hexagonal lattice are possible: for instance, open-ended tubular capsids are cylindrical closures.

In line with this idea, we propose a specific arrangement for the coffin-like cores (e.g. Fig. 7), i.e. their apical cap should be that of a regular icosahedron with a non-skew T-number (T_1) and the base, a hexagon made up of six equilateral triangles with a smaller non-skew T-number (T_2). Between the icosahedral cap and the base, there is a variable number (N_r) of rows of unit cells. Closed forms of this kind are defined by three parameters: T_1 , T_2 and N_r . They allow for the observed tapering in the mid-section between the cap and the base. (The tapering gives rise to a seam of dislocations that should be accommodated by local perturbations of the lattice). Coffins resemble cones in being tapered, but their convergence angle may be smaller: for instance, it is 2.6° for a ($T_1=5$, $T_2=4$, $N_r=8$) coffin, as compared to 19.2° for a minimally tapering cone³².

Unlike cones, fullerene coffins have six 5-fold sites at both ends distributed with a conventional icosahedral (5+1) configuration in the cap while the base has a (6+0) configuration if it is flat or a (4+2) configuration with the slightly more curved structure shown in Fig. 7. Thus, the mid-section of a regular coffin should have approximate 5-fold symmetry towards its wide end and approximate 6-fold symmetry towards its narrow end. Most coffins appear to be less regular than this design and we imagine that they represent irregular polyhedra whose vertices are distributed less evenly than in a regular coffin.

Although the tomograms do not visualize the basic lattice that we infer to be folded into cores, they allow the positions of vertices to be localized, which supports modeling the geometries of individual cores (Heymann *et al.*, Ms. in preparation). For instance, "lozenges" may also have six vertices at each end, distributed in a (3+3) arrangement.

Copy numbers of Gag and CA

Our calculations are internally consistent in that the number of assembled CA subunits in a mature virion never exceeds the number of Gag subunits inferred to be present in its precursor (Fig. 5). This accounting assumes that the provirion shell is complete, and there is not an additional disordered population inside the shell. It is consistent with other data. Based on STEM mass measurements of *in vitro*-assembled RSV Gag particles, Yu *et al.*³⁵ concluded that they contain, on average, 1200 Gag molecules. When the difference in size between these particles and provirions, measured at the radii of the inner CA domain, is taken into account, an average copy number of 1850 is estimated for provirions - in reasonable agreement with the average value of ~ 2110 copies that we calculated. The agreement improves further if one allows for the possibility of gaps in some Gag shells, as observed by cryo-EM of RSV Gag particles³⁵ and *in situ* by cryo-ET of HIV provirions³¹.

Analogy with bacteriophage T4 capsid assembly

Although the T4 major capsid protein gp23 has a different fold³⁶ from that of RSV-CA²⁶, the polymorphism exhibited by RSV cores is strikingly reminiscent of similar phenomena documented in the T4 system³⁷. This analogy has implications for how the assembly of distinct classes of RSV capsids may be regulated.

The wild-type T4 capsid is a prolate icosahedron, extended along a 5-fold axis of symmetry^{38; 39}. Although we have yet to encounter this geometry in intraviral RSV capsids, an isolated capsid imaged by Kingston *et al.*³⁰ (their Fig. 5A) looks to be a good candidate and this geometry is related to that which we propose for "coffins". T4 capsid assembly is an elaborate process with numerous participants. However, for present purposes, its essentials may be summarized as follows: the capsid first assembles as a procapsid via a pathway involving co-polymerization of gp23 and the scaffolding protein gp22, seeded from an initiation complex that contains the portal protein gp20 and gp22. In an infected *E. coli* cell, synthesis of T4 capsid proteins proceeds until a critical concentration of gp23 is reached whereafter procapsids are

produced at an approximately linear rate, with the pool of unassembled gp23 remaining at a constant level⁴⁰. In the absence of gp20, tubular particles called polyheads are formed instead of procapsids. Polyheads are cylindrical foldings of the same hexagonal gp23 lattice that is otherwise closed into icosahedral procapsids. Their assembly is delayed relative to procapsid assembly⁴⁰, reflecting a different initiation mechanism with a threefold higher critical concentration. In the absence of gp22, initiation is more severely affected: the predominant phenotype is multilayered polyheads, i.e. nested tubes, in which the inner layer templates assembly of the second layer and so on^{41; 42}.

These precedents suggest an explanation for the observed correlation between the incidence of tubular RSV capsids and relatively large fractions of unassembled CA subunits in virions containing them: i.e. failure to initiate assembly in a way that leads to a closed angular or continuous curvature core. Similarly, angular and curved cores - which tend to be accompanied by differently sized pools of unassembled CA subunits (Fig. 5) - may represent the outcomes of subtler distinctions in the initiation process. Furthermore, nested cores may denote virions whose maturation somehow led to the accumulation of relatively high concentrations of CA subunits (e.g. smaller procapsids, with higher area : volume ratios) in an "initiation-reluctant" context.

Possible involvement of Env endodomains in initiating core assembly

Virions with open-ended tubular cores have few spikes (Table II). In light of the foregoing discussion of T4 polymorphism, this observation raises the possibility that Env endodomains, interacting directly with the Gag layer, may play a role in initiating the assembly of closed cores. Thus a trimeric Env molecule or a cluster of Env molecules might coordinate one or several unprocessed or partially processed Gag (or Gag-Pol) molecules into a complex capable of nucleating CA assembly at a relatively low critical concentration, leading to formation of an angular or curved core. With few spikes, such an initiator is less likely to form and a tubular core is more likely to be produced. If the putative nucleating Gag molecules were intact, the NC moieties at their distal tips could recruit the genome(s) into the assembling core. The observed dearth of ribonucleoprotein inside tubular cores (Figs. 3C & 3D) is consistent with a packaging deficiency. It is also possible that Env, rather than being the actual initiator, is a marker for another membrane constituent that is present in particular microdomains of the membrane in which Env resides.

In this hypothesis, we invoke unprocessed or partially processed Gag or Gag-Pol molecules as initiators because they provide a straightforward means of associating the Env protein with assembly of CA subunits, i.e. via Gag's MA moieties. Interactions of Env with MA are consistent with the observation that self-assembled Gag particles are smaller than the Gag shells in provirions³⁵, presumably because they do not have to overcome the membrane's resistance to bending. Consistent with this idea, we have observed some elongated envelope-associated molecules inside mature core-containing virions (Fig. 6C). These molecules have the right dimensions, orientation, and placement for Gag, although we caution that direct evidence for their identification is lacking and a variety of host molecules may be incorporated into retroviral particles⁴³. Finally, the putative RSV initiation complex would still be susceptible to proteolysis and, after fulfilling its role in core initiation, may be processed and consequently no longer present in (some) mature virions.

Nucleated assembly of conical cores

From an appraisal of conical cores seen in tomograms of mature HIV virions, Briggs *et al.*⁴⁴ proposed that their assembly is nucleated in an envelope-associated event at the narrow end and propagates radially outwards to close when the wide end reaches the opposite side of the virion. These authors also reported densities linking the narrow end with the envelope in some

particles. (In this context, a 20 nm “core-envelope linker” was reported to be present at the narrow end of HIV cores in an earlier tomographic study of virions embedded in stained plastic sections⁴⁵). This scenario has some features in common with what we observe in the RSV system, although conical cores are rare with RSV and at least one of the few that we detected does not extend fully across the interior of its virion (Fig. 3B, column 1). The core-envelope linkers that we observe (Fig. 6C) are longer than the densities designated by Briggs *et al.*⁴⁴. Nevertheless, one idea common to both proposals is that the nucleation complexes may be responsible for inserting 5-fold sites in the nascent CA lattice.

The observation that closed RSV cores correlate with larger complements of Env spikes suggests that Env endodomains have a role in nucleating core assembly. Whether there may be similar involvement in HIV is unclear as these virions tend to have few spikes⁴⁶, typically no more than are present on RSV virions with tubular cores which we exclude from Env-associated core nucleation.

While there is no direct evidence as to the molecular identity of the nucleating complex, an attractive candidate is Gag or Gag-Pol in an unprocessed or partly processed state. Gag is an elongated molecule; its CA moiety could be inserted into the CA lattice, potentially promoting and guiding outwards growth; and NC and/or Pol could participate in packaging the viral RNA by securing it on the appropriate side of the assembling capsid, i.e. in what will become its interior.

What is a functional retrovirus core?

Several studies indicate that a correctly assembled core is essential for an infectious virion (see Introduction) in that mutations in CA that affect core assembly also impair infectivity. However, with retrovirions, as with many other enveloped viruses, specific infectivities, i.e. ratios of plaque-forming units to particles tend to be low, and there are reasons other than aberrant core structure why a virus particle may fail to elicit a productive infection; for instance, it may enter a non-productive pathway or be intercepted by host defense mechanisms. These considerations, compounded with evidently wide-ranging core polymorphism – for instance, in our data set of 95 virions, no two cores were superimposably alike – underscore that the question of what is a correctly assembled core is a difficult one. Taking previous as well as the present observations into account, we consider it likely that viable cores do not have a uniquely specified structure; rather, a range of capsid structures may be acceptable. Among the observed capsids, we distinguish primarily between closed and open shells; and between well filled and relatively empty cores. The open category includes most of the tubular cores, which also appear to be deficient in contents compared to most closed cores, and unassembled cores. Moreover, the contents of open cores would be exposed to nucleases, proteases, etc in the infected cell. We tentatively posit that a viable core may be one with a closed shell that has successfully packaged at least one genome and the enzymes, RT and integrase. About 30% of the particles in our data set have closed capsids with enough internal density to suggest that they meet these criteria. With RSV, the morphologies of putatively viable cores are angular or have continuous curvature; with HIV, the predominant morphology is fullerene cones. These cores represent the products of assembly pathways that are based on similar principles but differ substantively in detailed execution.

Materials & Methods

Preparation of virions

Infectious virions of the Prague C strain of RSV were obtained from stably infected cultures of turkey embryo fibroblasts by centrifugation of particles from the culture medium through a cushion of 25% sucrose in water and resuspended in a low salt buffer (10 mM Tris

hydrochloride, pH 7.6, 5 mM NaCl). For some experiments, the particles were further purified by centrifugation through a gradient of 20 to 50% sucrose.

Cryo-electron tomography

Virus was mixed with a suspension of 10-nm colloidal gold particles (Ted Pella Inc., Redding, CA) to provide fiducial markers. 4 μ l drops were applied to holey carbon films, thinned by blotting, and vitrified in liquid ethane before transfer into a cryogenic specimen holder (model 626 - Gatan, Warrendale, PA). A Tecnai-12 electron microscope (FEI, Hillsboro, OR) was used to record single-axis tilt series at 1° steps. The microscope was equipped with an energy filter (Gatan) that was operated in the zero-energy-loss mode with a slit width of 20 eV. Images were recorded on a 2,048 $\times$ 2,048-pixel CCD camera (Gatan) at 38,000x magnification (0.78 nm pixels). Data were recorded using SerialEM⁴⁷ to conduct automatic tilting, tracking, focusing and image recording. Cryoelectron microscopy was performed as described⁴⁸.

Image analysis

Projections in a tilt series were mutually aligned by using the gold particles as fiducial markers and tomograms were reconstructed using IMOD⁴⁹. Individual virions were extracted from the calculated tomograms and denoised by nonlinear anisotropic diffusion as implemented in Bsoft⁵⁰. The virion shown in Fig. 2B and virion capsids were manually segmented using the Amira Software package (Mercury Computer System, Inc). Capsid surface areas and volumes were also estimated with Amira. The tomogram in Fig. 1 was denoised by bilateral filtering⁵¹. The resolution was estimated by the NLOO-2D method⁵². For each tomogram, resolution was assessed as the average of three particles at the threshold 0.3.

To determine the repeat of the striations observed at the edges of the released RSV capsids, several capsid edges were extracted from the micrographs, and padded into 1024² boxes. The padded images were Fourier transformed and converted to power spectra using Bsoft.

Polyhedron construction

A custom module was written in Chimera⁵³ to generate a hexagonal planar lattice made up of equilateral triangles, edit it with the “Volume path tracer” tool of Chimera, wrap it as desired in three dimensions, and close the seams. The resulting network was then regularized with bpoly, a new program in Bsoft. The honeycomb polyhedron representing the geometrical dual of the triangular net-based construct was calculated with bpoly, yielding a polyhedron with pentagonal and hexagonal faces. (The dual of a polyhedron replaces the faces with vertices and *vice versa*).

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References

1. Vogt, VM. Retroviral Virions and Genomes. In: Coffin, JM.; Hughes, SH.; Varmus, H., editors. Retroviruses. Woodbury, NY: Cold Spring Harbor Laboratory; 1997. p. 27-70.
2. D'Souza V, Summers MF. How retroviruses select their genomes. Nat. Rev. Microbiol 2005;3:643–655. [PubMed: 16064056]
3. Steven AC, Heymann JB, Cheng N, Trus BL, Conway JF. Virus maturation: dynamics and mechanism of a stabilizing structural transition that leads to infectivity. Curr. Opin. Struct. Biol 2005;15:227–236. [PubMed: 15837183]

4. Briggs JA, Wilk T, Welker R, Kräusslich HG, Fuller SD. Structural organization of authentic, mature HIV-1 virions and cores. *EMBO J* 2003;22:1707–1715. [PubMed: 12660176]
5. Briggs JA, Watson BE, Gowen BE, Fuller SD. Cryoelectron microscopy of mouse mammary tumor virus. *J. Virol* 2004;78:2606–2608. [PubMed: 14963166]
6. Benjamin J, Ganser-Pornillos BK, Tivol WF, Sundquist WI, Jensen GJ. Three-dimensional structure of HIV-1 virus-like particles by electron cryotomography. *J. Mol. Biol* 2005;346:577–588. [PubMed: 15670606]
7. Swanstrom, R.; Wills, JW. Synthesis, Assembly, and Processing of Viral Proteins. In: Coffin, JM.; Hughes, SH.; Varmus, H., editors. *Retroviruses*. Cold Spring Harbor Laboratory Press: Woodbury, NY; 1997. p. 263-334.
8. Yeager M, Wilson-Kubalek EM, Weiner SG, Brown PO, Rein A. Supramolecular organization of immature and mature murine leukemia virus revealed by electron cryo-microscopy: implications for retroviral assembly mechanisms. *Proc. Natl Acad. Sci. U S A* 1998;95:7299–7304. [PubMed: 9636143]
9. Wilk T, Gross I, Gowen BE, Rutten T, de Haas F, Welker R, Kräusslich HG, Boulanger P, Fuller SD. Organization of immature human immunodeficiency virus type 1. *J. Virol* 2001;75:759–771. [PubMed: 11134289]
10. Krishna NK, Campbell S, Vogt VM, Wills JW. Genetic determinants of Rous sarcoma virus particle size. *J. Virol* 1998;72:564–577. [PubMed: 9420260]
11. Ako-Adjei D, Johnson MC, Vogt VM. The retroviral capsid domain dictates virion size, morphology, and coassembly of gag into virus-like particles. *J. Virol* 2005;79:13463–13472. [PubMed: 16227267]
12. Briggs JA, Johnson MC, Simon MN, Fuller SD, Vogt VM. Cryo-electron microscopy reveals conserved and divergent features of gag packing in immature particles of Rous sarcoma virus and human immunodeficiency virus. *J. Mol. Biol* 2006;355:157–168. [PubMed: 16289202]
13. Ganser BK, Li S, Klishko VY, Finch JT, Sundquist WI. Assembly and analysis of conical models for the HIV-1 core. *Science* 1999;283:80–83. [PubMed: 9872746]
14. Briggs JA, Simon MN, Gross I, Kräusslich HG, Fuller SD, Vogt VM, Johnson MC. The stoichiometry of Gag protein in HIV-1. *Nature Struct. Mol. Biol* 2004;11:672–675. [PubMed: 15208690]
15. Lanman J, Lam TT, Emmett MR, Marshall AG, Sakalian M, Prevelige PE Jr. Key interactions in HIV-1 maturation identified by hydrogen/deuterium exchange. *Nature Struct. Mol. Biol* 2004;11:676–677. [PubMed: 15208693]
16. Tang S, Murakami T, Agresta BE, Campbell S, Freed EO, Levin JG. Human immunodeficiency virus type 1 N-terminal capsid mutants that exhibit aberrant core morphology and are blocked in initiation of reverse transcription in infected cells. *J. Virol* 2001;75:9357–9366. [PubMed: 11533199]
17. Forshey BM, von Schwedler U, Sundquist WI, Aiken C. Formation of a human immunodeficiency virus type 1 core of optimal stability is crucial for viral replication. *J. Virol* 2002;76:5667–5677. [PubMed: 11991995]
18. Tang S, Murakami T, Cheng N, Steven AC, Freed EO, Levin JG. Human immunodeficiency virus type 1 N-terminal capsid mutants containing cores with abnormally high levels of capsid protein and virtually no reverse transcriptase. *J. Virol* 2003;77:12592–12602. [PubMed: 14610182]
19. von Schwedler UK, Stray KM, Garrus JE, Sundquist WI. Functional surfaces of the human immunodeficiency virus type 1 capsid protein. *J. Virol* 2003;77:5439–5450. [PubMed: 12692245]
20. Lucic V, Forster F, Baumeister W. Structural studies by electron tomography: from cells to molecules. *Annu. Rev. Biochem* 2005;74:833–865. [PubMed: 15952904]
21. Grünwald K, Desai P, Winkler DC, Heymann JB, Belnap DM, Baumeister W, Steven AC. Three-dimensional structure of herpes simplex virus from cryo-electron tomography. *Science* 2003;302:1396–1398. [PubMed: 14631040]
22. Cyrklaff M, Risco C, Fernandez JJ, Jimenez MV, Esteban M, Baumeister W, Carrascosa JL. Cryo-electron tomography of vaccinia virus. *Proc. Natl Acad. Sci. U S A* 2005;102:2772–2777. [PubMed: 15699328]
23. Harris A, Cardone G, Winkler DC, Heymann JB, Brecher M, White JM, Steven AC. Influenza virus pleiomorphy characterized by cryoelectron tomography. *Proc. Natl Acad. Sci. U S A* 2006;103:19123–19127. [PubMed: 17146053]

24. Momany C, Kovari LC, Prongay AJ, Keller W, Gitti RK, Lee BM, Gorbalenya AE, Tong L, McClure J, Ehrlich LS, Summers MF, Carter C, Rossmann MG. Crystal structure of dimeric HIV-1 capsid protein. *Nature Struct. Biol* 1996;3:763–770. [PubMed: 8784350]
25. Campos-Olivas R, Newman JL, Summers MF. Solution structure and dynamics of the Rous sarcoma virus capsid protein and comparison with capsid proteins of other retroviruses. *J. Mol. Biol* 2000;296:633–649. [PubMed: 10669613]
26. Kingston RL, Fitzon-Ostendorf T, Eisenmesser EZ, Schatz GW, Vogt VM, Post CB, Rossmann MG. Structure and self-association of the Rous sarcoma virus capsid protein. *Structure* 2000;8:617–628. [PubMed: 10873863]
27. Mayo K, Vana ML, McDermott J, Huseby D, Leis J, Barklis E. Analysis of Rous sarcoma virus capsid protein variants assembled on lipid monolayers. *J. Mol. Biol* 2002;316:667–678. [PubMed: 11866525]
28. Campbell S, Vogt VM. Self-assembly in vitro of purified CA-NC proteins from Rous sarcoma virus and human immunodeficiency virus type 1. *J. Virol* 1995;69:6487–6497. [PubMed: 7666550]
29. Cairns TM, Craven RC. Viral DNA synthesis defects in assembly-competent Rous sarcoma virus CA mutants. *J. Virol* 2001;75:242–250. [PubMed: 11119594]
30. Kingston RL, Olson NH, Vogt VM. The organization of mature Rous sarcoma virus as studied by cryoelectron microscopy. *J. Struct. Biol* 2001;136:67–80. [PubMed: 11858708]
31. Wright ER, Schooler JB, Ding HJ, Kieffer C, Fillmore C, Sundquist WI, Jensen GJ. Electron cryotomography of immature HIV-1 virions reveals the structure of the CA and SP1 Gag shells. *EMBO J* 2007;26:2218–2226. [PubMed: 17396149]
32. Li S, Hill CP, Sundquist WI, Finch JT. Image reconstructions of helical assemblies of the HIV-1 CA protein. *Nature* 2000;407:409–413. [PubMed: 11014200]
33. Ganser-Pornillos BK, von Schwedler UK, Stray KM, Aiken C, Sundquist WI. Assembly properties of the human immunodeficiency virus type 1 CA protein. *J. Virol* 2004;78:2545–2552. [PubMed: 14963157]
34. Ganser BK, Cheng A, Sundquist WI, Yeager M. Three-dimensional structure of the M-MuLV CA protein on a lipid monolayer: a general model for retroviral capsid assembly. *EMBO J* 2003;22:2886–2892. [PubMed: 12805204]
35. Yu F, Joshi SM, Ma YM, Kingston RL, Simon MN, Vogt VM. Characterization of Rous sarcoma virus Gag particles assembled in vitro. *J. Virol* 2001;75:2753–2764. [PubMed: 11222698]
36. Fokine A, Leiman PG, Shneider MM, Ahvazi B, Boeshans KM, Steven AC, Black LW, Mesyanzhinov VV, Rossmann MG. Structural and functional similarities between the capsid proteins of bacteriophages T4 and HK97 point to a common ancestry. *Proc. Natl Acad. Sci. USA* 2005;102:7163–7168. [PubMed: 15878991]
37. Black, LW.; Showe, MK.; Steven, AC. Morphogenesis of the T4 head. In: Karam, J., editor. *Molecular Biology of Bacteriophage T4*. Washington, D.C.: Am. Soc. Microbiol.; 1994. p. 218-258.
38. Moody MF. The shape of the T-even bacteriophage head. *Virology* 1965;26:567–576. [PubMed: 5833315]
39. Aebi U, Bijlenga R, v.d.Broek R, Eiserling F, Kellenberger C, Kellenberger E, Mesyanzhinov V, Muller L, Showe M, Smith R, Steven A. The transformation of tau particles into T4 heads. II. Transformations of the surface lattice and related observations on form determination. *J. Supramol. Struct* 1974;2:253–275. [PubMed: 4612249]
40. Laemmli UK, Eiserling FA. Studies on the morphopoiesis of the head of phage T-even. V. The formation of polyheads. *Mol. Gen. Genet* 1968;101:333–345. [PubMed: 5684818]
41. Yanagida M, Boy de la Tour E, Alff-Steinberger C, Kellenberger E. Studies on the morphopoiesis of the head of bacteriophage T- even. 8. Multilayered polyheads. *J. Mol. Biol* 1970;50:35–58. [PubMed: 4196078]
42. Steven AC, Aebi U, Showe MK. Folding and capsomere morphology of the P23 surface shell of bacteriophage T4 polyheads from mutants in five different head genes. *J. Mol. Biol* 1976;102:373–400. [PubMed: 775106]
43. Cantin R, Methot S, Tremblay MJ. Plunder and stowaways: incorporation of cellular proteins by enveloped viruses. *J. Virol* 2005;79:6577–6587. [PubMed: 15890896]

44. Briggs JA, Grünewald K, Glass B, Forster F, Kräusslich HG, Fuller SD. The mechanism of HIV-1 core assembly: insights from three-dimensional reconstructions of authentic virions. *Structure* 2006;14:15–20. [PubMed: 16407061]
45. Hoglund S, Ofverstedt LG, Nilsson A, Lundquist P, Gelderblom H, Ozel M, Skoglund U. Spatial visualization of the maturing HIV-1 core and its linkage to the envelope. *AIDS Res. Hum. Retroviruses* 1992;8:1–7. [PubMed: 1736937]
46. Zhu P, Chertova E, Bess J Jr, Lifson JD, Arthur LO, Liu J, Taylor KA, Roux KH. Electron tomography analysis of envelope glycoprotein trimers on HIV and simian immunodeficiency virus virions. *Proc. Natl Acad. Sci. U S A* 2003;100:15812–15817. [PubMed: 14668432]
47. Mastronarde DN. Automated electron microscope tomography using robust prediction of specimen movements. *J. Struct. Biol* 2005;152:36–51. [PubMed: 16182563]
48. Cheng N, Conway JF, Watts NR, Hainfeld JF, Joshi V, Powell RD, Stahl SJ, Wingfield PE, Steven AC. Tetrairidium, a four-atom cluster, is readily visible as a density label in three-dimensional cryo-EM maps of proteins at 10–25 Å resolution. *J. Struct. Biol* 1999;127:169–176. [PubMed: 10527906]
49. Kremer JR, Mastronarde DN, McIntosh JR. Computer visualization of three-dimensional image data using IMOD. *J. Struct. Biol* 1996;116:71–76. [PubMed: 8742726]
50. Heymann JB, Belnap DM. Bsoft: Image processing and molecular modeling for electron microscopy. *J. Struct. Biol* 2007;157:3–18. [PubMed: 17011211]
51. Jiang W, Baker ML, Wu Q, Bajaj C, Chiu W. Applications of a bilateral denoising filter in biological electron microscopy. *J. Struct. Biol* 2003;144:114–122. [PubMed: 14643214]
52. Cardone G, Grünewald K, Steven AC. A resolution criterion for electron tomography based on cross-validation. *J. Struct. Biol* 2005;151:117–129. [PubMed: 15964766]
53. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem* 2004;25:1605–1612. [PubMed: 15264254]

Fig. 1.
Central section through a denoised tomogram, showing RSV virions of different sizes with
cores of various kinds. Bar = 100 nm.

Fig. 2.

(A) Serial sections through a virion with an angular “coffin-like” core; and (B) Segmented surface renderings of the virion, showing both the outer surface studded with glycoprotein spikes (top) and the virion interior (bottom), with the capsid (red), internal material (orange) and material between the capsid and envelope (gray). Some densities connecting the capsid to the inner surface of the envelope are visible (e.g. arrows in A, row 2, column 2; row 3 column 3). In addition to the structurally homogeneous majority of surface protrusions representing Env spikes (see, e.g. A, row 3, column 1), the envelope contains a few morphologically distinct, presumably host-derived, protrusions (e.g. arrowheads A, row 3, column 2 and row 4, column 1; blow-ups in corners). The sections are 7.8 nm apart and 3.9 nm thick. Bar (A) = 50 nm.

Fig. 3.

(A–I) Gallery of central sections through RSV virions with various kinds of cores. Bar (bottom right) = 50 nm. The panels in A and B are complemented with contrast-inverted versions of the same images containing surface renderings of the capsid surface (yellow). (A) Some virions with angular cores. Densities connecting the capsid to the envelope are marked with arrows (panels 2 and 5). (B) Some virions with “continuous curvature” cores. On average, these cores are smaller than angular cores and less densely packed. The core in panel 4 and perhaps also that in column 1 is a fullerene core (white asterisks). (C–E) Some virions with tubular cores, where the tubes may be open at both ends (C), at one end (D), or at neither end (E). Tubular cores tend not to be densely packed. (F–I) Virions with unusual, minority classes of cores. (F)

A continuous curvature core appears to have been pressed tightly into the envelope, distorting its usually round shape. (G) Fullerene cone core, relatively densely packed and with a partial second layer capsid overlaid. (H) Virion lacking a well formed core. (I) Virion with two cores.

Fig. 4.

(A) Plot of number of spikes *versus* virion diameter. (B) Capsid internal volume expressed as a fraction of virion internal volume plotted *versus* virion diameter. (C) Plot of capsid surface area *versus* virion diameter. Each data point represents a virion, color-coded according to core type. One outlier with a larger diameter of 176 nm is not included.

Fig. 5.

Plot of number of assembled CA molecules assembled in a core versus the number of Gag molecules initially assembled into the provirion. If assembly were to be 100% efficient, these numbers would be equal, i.e. the data points would lie on the central diagonal. The difference between these numbers, ($\#Gag - \#CA$), represents the amount of unassembled CA in that virion. The arrowed lines at top give the estimated partition between assembled and unassembled CA for that virion. As in Fig. 4, each data point represents a virion, color-coded by core type. The colored dashed lines through the origin represent the average assembly efficiency for each core type. These data show that assembly efficiency varies systematically according to core type.

Fig. 6.

(A) Cryo-electron micrographs of isolated (incomplete) RSV capsids showing peripheral serrations with spacings of 4.1 nm (left) and 4.7 nm (right). (B) Diffraction patterns of the bracketed portions of (A), with the corresponding reflections marked (arrow). We infer that the serrations represent harmonics of a hexagonal lattice with 9.5 nm repeat (Results). The resolution (Table I) is insufficient to detect these spacings in intraviral capsids and the contrast of the 9.5 nm repeat is too low to be detected at prevailing noise levels. (C) Examples of tomographic sections through virions in which core-envelope linkers are visible (red arrows). Some of them underlie spike densities (yellow arrows). Bars (A, C) = 50 nm.

Fig. 7.

(A) Honeycomb model of an RSV capsid with the geometry of a fullerene coffin, shown in side view, top view and bottom view. This coffin has parameters ($T_1=16$; $T_2=9,13$; $N_r=6$). The cap (top) is that of a $T=16$ icosahedron; whereas the bottom has a (4+2) configuration of 5-fold vertices and pseudo-6-fold symmetry, corresponding to a mixture of $T=9$ and $T=13$ configurations. (B) Simulated density cross-sections, longitudinal and through the mid-section, at both ends.

Fig. 8.

Hypothetical model of proteolysis-induced maturation of the RSV provirion, including initiation of capsid assembly and genome packaging. (A) In the provirion, Gag polyproteins contact the host cell membrane where budding takes place (top) via their MA domains. Some may also interact with the less numerous Env spikes (bottom). (B) The protease, activated by being released from the polyprotein, processes the Gag molecules at their inter-domain cleavage sites. MA remains membrane-associated. The other domains enter soluble (or at least, disordered) pools in the virion interior (at right). (C) The putative initiation complex, containing of Env, Gag, and the viral RNA. The Gag shown here has lost its PR moiety but there is no reason to favor that model over unprocessed Gag. The NC domain binds RNA (and may already have done so in the provirion) to initiate packaging. The CA domains, possibly in a conformationally altered state or with other cofactor(s) bound, initiates assembly of CA subunits, leading to formation of a closed angular or curved capsid. The MA domains line the membrane. Absent an appropriate Env-Gag complex, polymerization of CA is eventually nucleated by a different mechanism with a higher critical concentration of CA subunits, producing a tubular capsid with low probability of successful packaging. The numbers of Env and Gag molecules in the putative initiation complex remain undefined. Alternatively, the nucleating principle may be Gag-Pol.

TABLE 1

Parameters of tomographic tilt series

Tilt series	Defocus - μm ^a (1 st zero - nm)	Tilt-range	No. of virions	Resolution ^b (nm)	Ice thickness (nm) ^c
1	-8 (5.2)	-60° to 65°	36	6.2	180
2	-8 (5.2)	-70° to 61°	16	6.1	195
3	-8 (5.2)	-70° to 65°	11	6.2	170
4	-6 (4.5)	-70° to 70°	14	5.7	175
5	-4 (3.7)	-65° to 70°	10	4.5	150
6	-4 (3.7)	-58° to 56°	8	4.9	170

^{a)}The nominal defocus setting is given in μm . The spacing corresponding to the position of the first zero of the contrast transfer function is given in nm (parenthesis).

^{b)}In-plane resolution of the tomograms was measured according to NLOO2D criterion (Cardone *et al.*, 2005) with a threshold of 0.3, was measured as an average over three particles selected from each tomogram.

^{c)}Measured directly from each tomogram.

TABLE II

Properties of RSV Virions with Different Core Types

Core type	n	Diameter (nm)	No. of spikes	Core surface area ($\times 10^3 \text{ nm}^2$)	Enclosed volume ($\times 10^4 \text{ nm}^3$)	% CA assembled
Angular	15	115 – 140 (122)	47 – 116 (82)	14 – 29 (20.4)	14 – 32 (22.4)	60 – 95 (80)
Continuous curvature	38	105 – 151 (124)	4 – 91 (34)	8.5 – 17 (13.0)	5.6 – 19 (11.0)	30 – 80 (48)
Tubular	21	112 – 176 (150)	0 – 50 (14)	5.5 – 17 (9.9)	4.7 – 23.9 (9.8)	17 – 48 (33)
Coreless	21	106 – 148 (127)	2 – 118 (28)	n/a	n/a	0

In each case, the observed range is given with the mean in parenthesis.

On average, angular capsids have 1583 CA subunits; continuously curved capsids, 1000; closed tubes 783; and open-ended tubes, 746.