The type I human immunodeficiency virus (HIV-1) contains a conical capsid comprising ~1,500 CA protein subunits, which organizes the viral RNA genome for uncoating and replication in a new host cell. In vitro, CA spontaneously assembles into helical tubes and cones that resemble authentic viral capsids. Here we describe electron cryo-microscopy and image reconstructions of CA tubes from six different helical families. In spite of their polymorphism, all tubes are composed of hexameric rings of CA arranged with approximate local p6 lattice symmetry. Crystal structures of the two CA domains were ‘docked’ into the reconstructed density, which showed that the amino-terminal domains of the protein are essential for silencing in fission yeast. Nature Genet. 19, 192–195 (1998).

Although the HIV-1 capsid appears organized, its intrinsic conical asymmetry has prevented molecular structure determinations. Recombinant HIV-1 CA and related proteins encompassing the downstream nucleocapsid (NC) RNA binding domain can spontaneously assemble into both cones and long helical tubes (Fig. 1a). The cones and tubes seem to be closely related, as they form simultaneously and individual particles can be identified in...
which a tube turns into a cone at a point of disclination (data not shown). These in vitro assemblies appear to be analogues of authentic HIV-1 capsids, because both conical and tubular capsids are also observed in virions (at a ratio of ~20:1)\(^1\)-\(^10\). Structural analyses of the CA assemblies formed in vitro are therefore likely to be relevant for understanding the organization of the viral capsid.

We carried out electron cryo-microscopy (cryo-EM) and image reconstructions to determine the three-dimensional structures of the HIV-1 CA tubes. As the tubes were from a number of distinct helical families, we initially surveyed the range of structures formed by reconstructing nine tubes (from six different families) exhibiting data to at least 30 Å resolution (Table 1). Computed Fourier transforms and three-dimensional reconstructed images of tubes from four of the different helical families are shown in Fig. 1b–e. All of the helical projections appeared centrosymmetric, and the resulting phase residuals from twofold averaging were low in every case, implying that the surface lattice was based on at least p2 symmetry, and indicating that the data are of high quality to the reported resolution cut-offs.

Although the computed Fourier transforms differed significantly between the different families, the reconstructed images were highly related. In addition to the local p2 symmetry, each surface lattice appeared as a network of hexameric rings arrayed with approximate local p6 symmetry (also reflected in the p2 unit-cell parameters; Table 1). All subunits comprising the hexameric rings were radially elongated, bi-lobed structures, with their external lobes associating

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**Figure 2** Reconstructed density and molecular modelling of a CA tube. Reconstructions were created by averaging the two highest quality tube images from the (−12;11) family. a. View from the tube exterior, with the density contoured at 1.3σ. A single hexamer is highlighted for clarity. Scale bar, 100 Å. b. Stereo image of the CA hexamer viewed with the sixfold axis vertical. Crystal structures of the N- and C-terminal domains of HIV-1 CA were manually ‘docked’ into reconstructed density (1.0σ). c. View of the CA hexamer from the exterior of the tube. The N-terminal CA domains project towards the viewer. d. Molecular model for the hexameric ring formed by the N-terminal domain of the HIV-1 CA protein. Structures are coloured as follows: N-terminal β-hairpin, orange; helix 1, red; helix 2, yellow; helix 3, green; helix 4, cyan; helix 5, dark blue; helix 6, red; helix 7, pink. Arrow denotes a cyclophilin-A-binding loop.

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**Table 1 Reconstruction data for the HIV-1 CA tubes**

<table>
<thead>
<tr>
<th>Image</th>
<th>Underfocus (μm)</th>
<th>r(_{max}) (Å)</th>
<th>Helical family</th>
<th>Resolution cut-off (Å)</th>
<th>Number of layer lines</th>
<th>Phase residual(°)</th>
<th>(p2) unit-cell parameters ((a, b, \gamma))</th>
</tr>
</thead>
<tbody>
<tr>
<td>c421a</td>
<td>1.20</td>
<td>232</td>
<td>(−12,11)</td>
<td>20</td>
<td>46</td>
<td>25.9</td>
<td>106,108,114</td>
</tr>
<tr>
<td>c656b</td>
<td>0.55</td>
<td>240</td>
<td>(−12,11)</td>
<td>20</td>
<td>54</td>
<td>28.1</td>
<td>108,111,113</td>
</tr>
<tr>
<td>c661a2</td>
<td>2.22</td>
<td>227</td>
<td>(−12,11)</td>
<td>28</td>
<td>24</td>
<td>20.1</td>
<td>106,108,116</td>
</tr>
<tr>
<td>906b</td>
<td>1.47</td>
<td>206</td>
<td>(−11,10)</td>
<td>26</td>
<td>22</td>
<td>21.2</td>
<td>100,103,109</td>
</tr>
<tr>
<td>c140b</td>
<td>1.46</td>
<td>228</td>
<td>(−11,10)</td>
<td>30</td>
<td>25</td>
<td>17.1</td>
<td>108,110,111</td>
</tr>
<tr>
<td>c406b</td>
<td>2.37</td>
<td>263</td>
<td>(−7,9)</td>
<td>30</td>
<td>21</td>
<td>23.2</td>
<td>106,104,121</td>
</tr>
<tr>
<td>c419b</td>
<td>1.34</td>
<td>250</td>
<td>(−10,13)</td>
<td>22</td>
<td>37</td>
<td>28.2</td>
<td>115,105,113</td>
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<td>228</td>
<td>(−8,9)</td>
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<td>28.1</td>
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<tr>
<td>c661b</td>
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<td>(−6,8)</td>
<td>30</td>
<td>23</td>
<td>19.0</td>
<td>111,109,123</td>
</tr>
</tbody>
</table>

* Additional data are provided in Supplementary Information.
† Underfocus values were determined from the contrast transfer function using the sector-averaging method.
‡ Helical family designations follow the conventions in ref. 27.
§ Layer line data were extracted within the first zero of the contrast transfer function.
¶ Amplitude-weighted phase deviation from nearest 0° or 180°; \(R(\phi)\), amplitude of the Fourier term at radius \(R\) on layer line \(\phi\).
* Additional data are provided in Supplementary Information.
to form hexamers on the tube exterior. At a lower tube radius, the interior lobes projected down and away from the axes of the hexameric rings with a right-handed aspect, as viewed from the tube exterior, and their densities connected each hexameric ring to its six adjacent neighbours. Thus, the helical lattices appeared as networks of interconnected cogwheels (Fig. 2a).

We averaged the two highest quality images from tubes of the \((-12,11)\) helical family to produce the most reliable reconstruction. The averaging procedure lowered the twofold phase residual (19.6°) and improved the map quality significantly, although basic map features were not altered by any of the averaging steps. The averaged map exhibited high signal to noise and very clear local three- and sixfold symmetry axes (unimposed) (Fig. 2a). This map was therefore used in all subsequent modelling studies. The averaged \((-12,11)\) CA tube was 470 Å in diameter, and the protein shell was \(\sim72\) Å thick (corresponding to the longest axis of the individual CA subunits). The hexameric rings had exterior diameters of \(\sim100\) Å, central holes of \(\sim25\) Å, and inter-ring spacings of \(\sim107\) Å.

The HIV-1 CA protein comprises two domains separated by a flexible linker sequence. The N-terminal domain is essential for capsid formation, whereas the C-terminal dimerization domain is essential for forming both the immature particle and the mature capsid\(^{11}\). High-resolution structures of both domains have been determined\(^{12-16}\), allowing molecular modelling of the reconstructed CA helix (Fig. 2b–d). The N-terminal domain of CA is an asymmetric, arrowhead shape of about \(45 \times 35 \times 18\) Å. This structure fits well into the outer lobe of the reconstructed density, with the longest domain axis roughly perpendicular to the tube axis (Fig. 2b, c).

The N-terminal domains of CA form the hexameric rings, with helices 1 and 2 lining the inner holes of each ring. Intermolecular packing interactions between these two helices presumably stabilize the hexamer, and their polypeptide backbones approach within 8 Å at every interface in our model. The density connecting adjacent subunits is strongest towards the interior of the tube (near the C- and N-terminal ends of helices 1 and 2, respectively). The importance of this region for capsid assembly is also suggested by mutational analyses, which showed that residues in helices 1 and 2 are essential for tube formation in vitro, and for replication and capsid formation in cultured virus\(^{11}\) (U. K. von Schwedler, K. M. Stray and W.I.S., unpublished data; B. K. Ganser et al., personal communication). Hexamers have not been observed in any of the crystal structures of retroviral CA proteins, and the packing interactions (Fig. 2d) are therefore necessarily different from any previously described crystal lattice interactions. The reconstruction is also incompatible with a different hexamer model that was proposed on the basis of a dimeric lattice contact seen in crystals of the equine infectious anaemia viral CA protein\(^{17}\). The inner lobes of density that link each hexameric ring to its six nearest neighbours were assigned to the C-terminal domains of CA. The overall size and shape of the monomeric C-terminal CA domain (\(28 \times 28 \times 20\) Å) generally matched the experimental density (Fig. 2b), but unambiguous positioning of this domain will require a higher-resolution reconstruction.

Although structural polymorphism is not uncommon in macromolecular helical assemblies, the degree of polymorphism exhibited by the HIV-1 CA tubes is unprecedented. Three unit-cell vectors define both the p2 lattice of a tube’s radial projection and its helical family (see Supplementary Information). These vectors also describe the directions of the three helical lines formed by contiguous hexagons within the tube. Reconstructions of the six different families of CA tubes showed that the three unit-cell vectors could adopt essentially any orientation with respect to the helix axis (Fig. 3a). The different tubes also exhibited flexibility in their p2 unit-cell dimensions (100–115 Å), showing that the packing distance between hexagons can vary. These different types of tube polymorphism were all accommodated primarily by interdomain ‘hinge’ motions that allowed the C-terminal domains of CA to adopt many orientations with respect to the N-terminal hexameric rings. This flexibility is presumably allowed by the unstructured linker sequence that connects the two domains (CA residues 146–149)\(^{12-16}\).

Lentiviral capsids may be assembled on the principles of a fullerene cone\(^{8,17}\), a structure with underlying hexagonal lattice symmetry. The geometric constraints of the hexagonal lattice require that the cones adopt one of five allowed cone angles (19.2°, 38.9°, 60°, 83.6° or 112.9°), and this quantization has been verified experimentally for the CA-NC/RNA cones formed in vitro\(^{8}\). Our image reconstructions show that the CA tubes are also constructed from curved p6 lattices of hexameric rings. We therefore propose that CA cones and tubes are constructed on similar hexagonal nets and differ primarily in the orientations of their hexameric rings. In the tubes the hexamer planes lie parallel to the helix axis, whereas in the cones the hexamers are tilted with respect to the (cone) axis.

We constructed a model for the conical HIV-1 capsid as follows: (1) The body of the cone is a curved p6 surface lattice composed of hexameric CA rings derived from the helical image reconstructions. (2) The cone is selected to be the narrowest of the allowed fullerene

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**Figure 3** CA helical family polymorphism and cone formation. **a.** Orientations of the unit-cell vectors in the six reconstructed CA helical families drawn to scale and labelled with the corresponding helical start number of contiguous CA hexagons. Tube axes (\(z\)) are oriented vertically. Vectors representing the different helical families are coloured as follows: \((-12,11)\), black; \((-11,10)\), light green; \((-7,9)\), dark green; \((-10,13)\), dark blue; \((-8,5)\), red; \((-6,8)\), pink. Scale bar, 100 Å. **b.** Stereo view of a model of the HIV-1 capsid. Pentameric defects are shown in red, and a contiguous line of CA hexamers is highlighted in gold to illustrate how the spiral gradually changes diameter and pitch, and eventually reverses helical hand. Figure created by docking the molecular models shown in Fig. 2 into an idealized fullerene cone of 1,572 CA subunits\(^4\), and calculating a mask at 8 Å around the Cα atoms.
cones (19.2°). Although wider cones are occasionally observed in vitro, in SIV and possibly also in HIV-1 (ref. 10), narrow cones predominate in all cases. (3) The narrow and wide ends of the cone are allowed to close through the introduction of 5 and 7 pentagonal defects, respectively. These ‘pentons’ are distributed so that the model roughly mimics the shape of an authentic viral capsid, although alternative arrangements are possible at both ends, in analogy to triangulation in icosahedral viruses. (4) For simplicity, the pentons are constructed by removing a single subunit from a hexagon and closing the ring (in analogy to strict icosahedral quasi-equivalence), although alternative penton configurations are possible.

As shown in Fig. 3b, contiguous lines of hexagons within the body of the cone trace out spirals that can be described as helices in which the local helical parameters are constantly changing. This model therefore requires that the hexameric CA rings must have the flexibility to adopt many different orientations with respect to the direction and degree of curvature in the cone. This is exactly what is observed in the ensemble of reconstructed CA helices, where the loose packing of adjacent hexameric rings and the hinge between the two CA domains allows great flexibility in the unit-cell orientation and helical family (Fig. 3a). To our knowledge, the long 5,7 fullerene cones formed by the HIV-1 CA protein are unique to the lentiviruses, although aspects of CA assembly are reminiscent of other viral systems that assemble on hexagonal nets (for example, see refs 19–21).

Although many details of the viral capsid structure must await higher resolution studies, some important features are already evident. The CA lattice structure is quite open (Figs 2 and 3), consistent with the idea that the viral capsid primarily organizes, rather than protects, the RNA genome. The open lattice should also allow small macromolecules to diffuse in and out of the viral core, for example allowing nucleotide triphosphates to penetrate and be incorporated by reverse transcriptase in situ.

As described above, the CA subunits are orientated with their N-terminal domains forming the hexameric rings and the C-terminal dimerization domains linking adjacent hexamers. Hexamerization and dimerization might be regulated at different stages of proteolytic maturation and capsid formation. In particular, helix 1 shifts significantly upon proteolytic processing at the N-terminal end of CA, and this structural conversion may serve as a morphological switch by promoting hexamerization of the N-terminal domain during viral maturation (ref. 4; and T. L. Stemmler et al., personal communication).

The cellular prolyl isomerase, cyclophilin A (CypA), binds to the HIV-1 CA protein and is packaged within the viral particle at a CypA:CA ratio of about 1:10 (ref. 24). The radial disposition of the N-terminal domains in our model places the CypA-binding loop on the capsid exterior, away from any intermolecular CA interfaces (Fig. 2d). Isolated CypA molecules can be modelled on the capsid surface without steric clash, suggesting that, counter to an earlier proposal, low levels of CypA will not promote core disassembly. Saturation of adjacent CypA-binding sites appears sterically disallowed, consistent with the observation that CA tubes cannot assemble in the presence of low levels of CypA, but not at 1:1 stoichiometries.

Although the capsid model does not define the viral function of CypA, our observation that CA mutations that alleviate the viral requirement for CypA also facilitate CA assembly in vitro is consistent with a postulated role for the protein as a capsid assembly chaperone.

The interior surface of the capsid model is mainly defined by the highly conserved 20 amino-acid ‘major homology region’ (MHR) motif that lies at the ‘bottom’ of the C-terminal domain of CA. The precise orientation of this domain is not defined unambiguously, but the MHR motif does not make important intermolecular contacts in any of our models, leaving the polypeptide free to interact with other molecules. This observation is consistent with possible roles for the MHR in helping to organize genomic and/or reverse transcription complexes for early stages in the retroviral life cycle.

Although the HIV-1 capsid is highly organized, it is an intrinsically asymmetric object in which every subunit resides in a slightly different environment and the cone length is not uniquely defined. This lack of regularity may pose particular challenges for capsid assembly and may explain why the capsid is assembled through a temporally defined proteolytic pathway. Conversely, the ability to assemble capsids with a range of different volumes may provide the virus with added flexibility in packaging and replicating genomes of different lengths, thereby allowing retroviruses to incorporate cellular genes when advantageous.

Methods
Sample preparation and data collection
HIV-1 CA was expressed, purified and assembled by incubation of 0.4 mM CA in 1 M NaCl 50 mM Tris- HCl (pH 8.0) for 1 h at 37°C (ref. 4). Freshly purified CA spontaneously formed tubes under these conditions. The MHR motif does not make important intermolecular contacts with other CA molecules, but the MHR motif does not make important intermolecular contacts with other CA molecules, allowing nucleotide triphosphates to penetrate and be incorporated by reverse transcriptase in situ.

EM data were collected on Philips 420 or CM12 electron microscopes at 120 kV, under two dose conditions (~10 electrons per Å² with underfocus values of 1–2 μm). Images were recorded on Kodak SO163 film at nominal magnifications of ×34,000 and ×40,000.

Image analysis and reconstruction
We analysed EM images as described using programs developed by N. Unwin (personal communication). Micrographs were digitized using a Zeiss SCAI densitometer (7-μm step size), corresponding to 1.75 Å on the specimen (×40,000). Underfocus and astigmatism values for images were obtained using the sector-averaging method (assuming amplitude contrast of 7%). The repeat distance of each tube was estimated by correlating two separate stretches of tube along its length. Tubes were boxed using this axial repeat and the box stretched to 4.096 Å by interpolating the data in real space. After Fourier transforming (box size 4,096 × 2,048 pixels), this procedure placed all layer lines exactly on transform grids. Reciprocal lattices were then manually constructed on the computed Fourier transforms. Probable values for the Bessel orders (n) for each layer line were estimated. The possible indexing schemes were run in the programs HXLXS and SRCH, which gave a clearly lower residual for the correct scheme. Out of plane tilt angles (α) and x-shift corrections (SRCH output) were input into the program HXLXF, which extracted the layer line data to the resolution cut-off. Phase values were very close to 0° or 180°, indicating that the projected images were centroymmetric (in good agreement with Fourier filtered images of flattened tubes). We therefore used the program H2FOLD for twofold averaging, and the low phase residuals (below 29° in all cases) confirmed the validity of the averaging procedure.

Tubes that had averaged data were used to calculate final three-dimensional maps using the programs LTIG and HHROR. Intermediate maps all showed similar hexagonal surface lattices, which did not vary significantly with different equator weights (a weight of 0.3 was used). Absolute tilt angles (α) were determined for the images c56sb and c642a by measuring contrast transfer function values at both ends of each tube. The difference between these two values reflected the sense of the out-of-plane tilt angle of the tube (α), and was used to determine the absolute hand of the reconstructed helix. The local hand of the hexameric rings was the same in both structures, and the hands of the other tubes were adjusted accordingly. As an additional test of the validity of the reconstruction procedure, independent reconstructions of images 8960 and c642b were performed using the MRC helical program package. The resulting images agreed with those obtained as described above.

We occasionally identified CA (and CA-p2-NC/RNA) tubes that had flattened to produce extended two-dimensional crystals. Fourier filtering showed that both types of projected lattices were composed of six-membered rings of density arrayed with approximate, in-plane group symmetry, in excellent agreement with the three-dimensional reconstructions. Representative examples of the unit-cell dimensions: CA tube, a = 105 Å, b = 106 Å, β = 115°; CA-p2-NC/RNA tube, a = 90 Å, b = 90 Å, β = 120.5°.

Image averaging and model building
Using image c421a as a reference, the c65sb image was shifted to the same phase origin as c421a, and re-indexed using the program HREINDEX. The two data sets were then merged using HLXADD, and the data from each tube was weighted based upon differences in their repeat lengths. The overall twofold phase residual was 19.6° (34.8° in the 20–25 Å resolution shell). A total of 6,732 CA molecules were averaged to produce the final map. Crystal structures of HIV-1 CA were manually docked into the reconstructed density...
using the program O2. We used a single CA molecule as the docking unit; the linker between the N-terminal domain and C-terminal domain (residues 146–149) was allowed full flexibility within steric constraints.

Received 22 February; accepted 5 July 2000.


Correction

Signalling through CD30 protects against autoimmune diabetes mediated by CD8 T cells


It has come to our attention that the conclusions drawn in this paper need to be revised. We have found that the effects attributed to CD30 were the result of background gene(s) of 129 origin. Subsequent backcrossing of the CD30-deficient OT-I line by G. Davey in our laboratory segregated the capacity of low numbers of OT-I cells to cause disease from their capacity to express CD30. Our conclusion that signalling through CD30 protects against autoimmune diabetes must be reconsidered as an effect of a background gene(s) that we are currently mapping.

Erratum

How self-tolerance and the immunosuppressive drug FK506 prevent B-cell mitogenesis

Richard Glynne, Srinivas Akkaraju, James I. Healy, Jane Rayner, Christopher C. Goodnow & David H. Mack


The fourth sentence in the Methods section should have read ‘‘For stimulation experiments, HEL (Sigma) was used at 500 ng ml-1, goat anti-mu (Jackson Labs) at 10 μg ml-1, FK506 (gift from G. Crabtree laboratory) at 10 ng ml-1, PD98059 (NEB) at 20 μM unless otherwise stated, ionomycin (gift from G. Crabtree laboratory) at 1 μM and EGTA at 3 mM.’’