probably plays a role in core assembly. Moreover, although CA_{151} is monomeric at 1 mM concentrations, the protein self-associates at higher concentrations (Gitti et al., 1996). We therefore consider it possible that CA_{151}–CA_{151} interactions seen in the crystal may mimic functional in vivo interactions at the high concentrations (~6 mM) of CA present in the virion.

Within the crystal, CA_{151} molecules associate into planar strips that extend the length of the crystal and are completely isolated from all other CA_{151} molecules. All of the CA_{151} N-termini reside on the same side of the plane (toward the viewer in Figure 5A), and all of the C-termini reside on the opposite side of the plane. This mimics the arrangement expected for CA molecules on the viral core structure, where, barring dramatic reorientation after cleavage, the N-terminal side would face the viral membrane, and the C-terminal side would face the interior of the virus (see Figure 6).

The strips of CA_{151} seen in the crystal are formed by the association of CA molecules through two different interfaces, both of which exhibit 2-fold symmetry. The 2-fold local symmetry axes of interfaces 1 and 2 are parallel to one another and perpendicular to the plane of the strips. The strips of CA_{151} are further stabilized by interactions between molecules that are related by translational symmetry, with ~280 Å^2 of accessible surface area occluded from each molecule at this interface (Figure 5A). The interfaces seen in our structure are distinct from the interface seen between CA molecules in a CA–Fab cocrystal structure, where it appears that normal CA interactions are disrupted by dominant Fab interactions (Momany et al., 1996).

Interface 1 is stabilized by the C-terminal helix 7 of two CA_{151} molecules, which form a parallel coiled/coil-like interaction (Figure 5B). This interface appears to be quite weak, occluding only 350 Å^2 of accessible surface area on each CA_{151} molecule and exhibiting no apparent hydrogen bonding interactions. Nevertheless, this interface juxtaposes the C-termini of two CA_{151} molecules and may therefore be an extension of the C-terminal domain that mediates dimerization of full-length CA protein in solution (Gitti et al., 1996).

Interface 2 is significantly larger than interface 1 and is primarily mediated by helices 1 and 2, which form a 4-helix bundle with their symmetry-related pairs (Figure 5C). Additional interactions are observed between the N-terminal β-hairpin structures that project above these helices, although the CA_{151}-2 hairpin is poorly defined in the crystal structure. This interface occludes 800 Å^2 of accessible surface area on each CA_{151} molecule, and this value is 570 Å^2 even when the β hairpins are not included in the calculation. The core of the interface is formed by buried hydrophobic side chains and is surrounded by polar residues.

Model of Higher Order CA Assembly

We propose that the strips of CA_{151} molecules seen in the crystal contribute to the formation of the surface of the virion core through side-to-side interactions to form a two-dimensional surface (Figure 6B). This model has a number of attractive features. (1) All of the CA N-termini in the hypothetical surface would be facing "up" as if toward the MA protein and the viral membrane, and all of the C-termini would be pointing "down" as if toward the CA dimerization domain, NC protein, and RNA in the interior of the core. (2) Construction of this surface would use all of the CA–CA interactions seen in

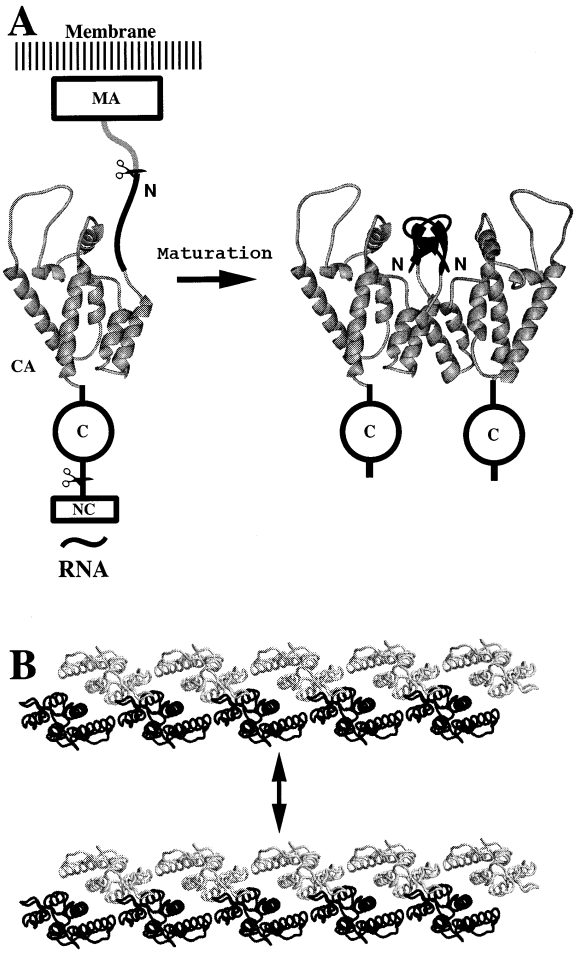

Figure 6. Model of CA_{151} Interactions in the HIV-1 Virion

(A) Left: schematic representation of Gag shows the MA, CA, and NC domains interacting with the viral membrane and RNA. The N-terminal domain of CA is shown as a ribbon diagram; the C-terminal domain is designated with a C. Right: two CA_{151} molecules associated via interface 2 as seen in the crystal. CA residues 1–13 are in bold to illustrate how formation of the N-terminal hairpin upon proteolysis (scissors) could stabilize this interface and thus trigger formation of the CA core following proteolysis.

(B) Model of higher order CA assembly to form a surface: the double-headed arrow indicates the proposed side-to-side interaction between two crystallographic strips (oriented as in Figure 5A, with CA_{151}-1, black, and CA_{151}-2, gray).

(*Figure 5 legend continued from previous page*)

(C) Interface 2 is created by intermolecular packing of helices 1 and 2 (below) and the N-terminal β hairpin (above). Residues that lose more than 20 Å^2 of accessible surface area upon complex formation are shown explicitly. Asn-121 also contacts the β hairpin of the adjacent CA_{151} molecule, although it is not shown in the figure.