in developing countries could be partly due to differences in luminal bacteria that colonize infants early in life. Transmissibility of IBD in humans has not been observed, perhaps because humans are more selective than mice in what they consume, and antibiotics have had mixed success in the treatment of IBD. Nevertheless, future metagenomic analyses, perhaps combined with therapeutic manipulations, may allow us to gain insights into the complex interactions in the intestinal microenvironment in human disease.

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How to Assemble a Capsid

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Retroviral capsids are composed of hexagonal arrays of the viral CA protein. In this issue of *Cell*, Ganser-Pornillos et al. (2007) provide a molecular model of the hexagonal HIV-1 CA lattice obtained from a new electron cryomicroscopic reconstruction. This study reveals the three principal stabilizing interfaces in the capsid lattice and explains how two different classes of inhibitors can block capsid assembly.

Like all retroviruses, HIV-1 is composed of a series of protein shells that organize the virion and help to facilitate the early stages of viral replication. The virion exterior comprises a lipid bilayer that is studded with transmembrane envelope protein spikes and coated on its internal surface by a matrix shell composed of the viral MA protein. Inside the matrix is a conical capsid composed of CA protein subunits that surround the viral nucleocapsid—a ribonucleoprotein complex composed of the RNA genome, NC protein, and viral enzymes. The HIV capsid has become a major focus of research owing to its intriguing conical morphology, its potential as a drug target, its critical functions at early stages of viral replication, and its interactions with innate immunity factors.

Studies of the HIV capsid have used a complementary array of different structural methods, each with its own strengths and limitations. Direct imaging of HIV CA protein assemblies using electron microscopy (EM) revealed that capsids belong to a family of closed geometric objects called fullerene cones that are composed primarily of hexagonal arrays of hexameric CA protein rings, with an additional twelve pentameric rings that allow the cones to close at both ends (Ganser et al., 1999; Li et al., 2000). These studies established the global capsid architecture but lacked the resolution necessary to visualize details of subunit packing interactions. In contrast, NMR and X-ray crystallography have produced high-resolution structures of the two domains of CA, the N-terminal domain (NTD, helices 1–7) and the C-terminal domain (CTD, helices 8–11), but have failed to provide a comprehensive picture of the subunit interactions in the hexagonal CA lattice (Gamble et al., 1997; Gitti et al., 1996). Fortunately, a new study in this issue by Ganser-Pornillos et al. (2007) bridges this gap in understanding by revealing the structure of the mature HIV CA protein in its native hexagonal packing environment.

Ganser-Pornillos and colleagues (2007) used electron cryomicroscopy (cryo-EM) and image reconstruction to visualize double-layered 2D crystals of hexamers formed by a mutant HIV-1 CA protein with particularly favorable assembly properties. The reconstruction confirms and extends

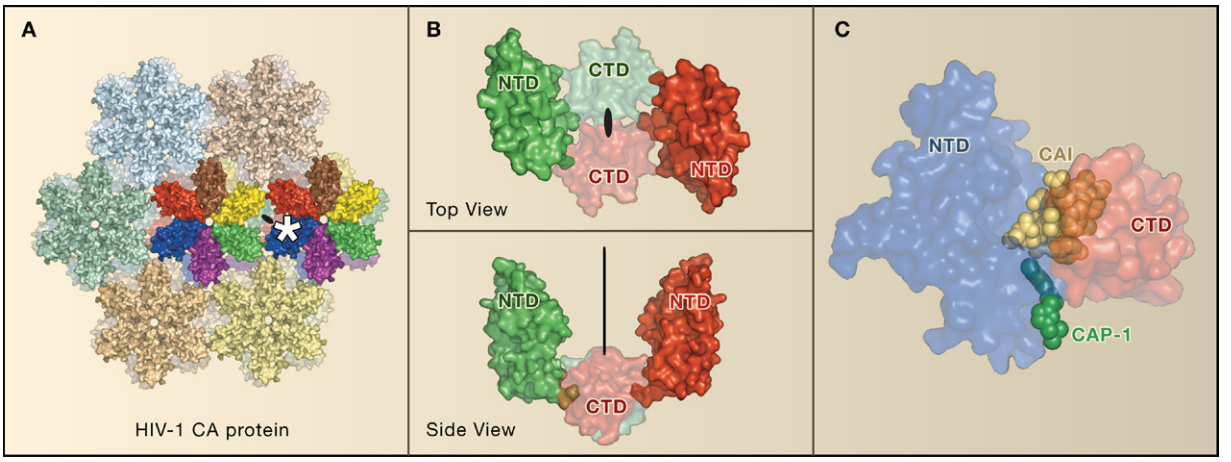

Figure 1. Hexagonal Lattice of the HIV-1 CA Protein

(A) Overview of the hexagonal CA lattice, as viewed from the capsid exterior. Seven CA hexamers are shown, and the subunits are colored individually in two of the hexamers. Note that the CA N-terminal domains (NTD, bright shades) define the capsid exterior whereas the C-terminal domains (CTD, light shades) define the capsid interior. The CTD dimer (black dyad) and NTD-CTD (white asterisk) interfaces are highlighted here and are shown in expanded views in (B) and (C), respectively.

(B) CA dimer viewed parallel (upper) and perpendicular (lower) to the two-fold axis of the CTD-CTD interface that connects adjacent hexamers.

(C) NTD-CTD interface formed between adjacent subunits in each CA hexamer. Structures of bound CAI (yellow) (Ternois et al., 2005) and CAP-1 (green) (Kelly et al., 2007) illustrate how these inhibitors could disrupt interface formation and thereby block capsid assembly.

Figure images created by Brian Kelly using coordinates from Ganser-Pornillos et al. (2007) and the program PyMOL.

several structural features that had been widely assumed but not fully verified. In particular, the structure demonstrates that CA NTD forms hexameric rings that are stabilized primarily by intermolecular interactions between helices 1–3 (Figure 1A). This arrangement was seen previously in an X-ray crystal structure of the CA NTD from the distantly related murine leukemia virus (Mortuza et al., 2004). Therefore, all retroviral capsids appear to be composed of similar hexameric building blocks, even though their CA proteins have diverged considerably in primary sequence and their capsids can adopt different shapes (spheres, rods, or cones). The new structure also shows that the CA CTD forms homodimeric interactions that connect neighboring hexamers through an interface that was previously observed in an HIV-1 CA CTD crystal structure (Figure 1B), although this interaction may adjust slightly to accommodate the constraints of the hexameric lattice (Ganser-Pornillos et al., 2007).

Most importantly, the new structure provides the first picture of the elusive third interface that is required to form a stable hexagonal CA lattice. This interface is created by the intermolecular packing of one CA CTD against the NTD of an adjacent subunit within the hexamer, with the termini of NTD helices 4 and 7 packing into a groove created by CTD helices 8, 9, and 11. This nicely explains previous genetic and biochemical data that indicated that the CA NTD and CTD interact within the viral capsid. For example, Craven and colleagues showed that a mutation within helix 9 of the Rous sarcoma viral CA CTD that inhibited capsid assembly could be suppressed by second site mutation within NTD helix 4 (Bowzard et al., 2001). Similarly, Prevelige and colleagues showed that HIV-1 capsid assembly brings the CA NTD and CTD close enough to allow crosslinking of lysine residues in helices 4 and 9, and they mapped interacting patches on the two domains using deuterium exchange protection (Lanman et al., 2003). The excellent agreement between genetic, biochemical, and structural studies leaves little doubt as to the biological authenticity of the new structure.

The newly visualized CA NTD-CTD interaction also explains the mechanism of action of two capsid assembly inhibitors that have recently been characterized structurally; a helical peptide inhibitor (CAI) that binds between CA CTD helices 8 and 11 (Ternois et al., 2005), and a small molecule inhibitor (CAP-1) that binds in a pocket formed at the junction between helices 1, 2, 4 and 7 in the CA NTD (Kelly et al., 2007). As illustrated in Figure 1C, these inhibitors bind within or adjacent to the third interface and probably disrupt this interaction thereby inhibiting capsid assembly. The expanding problem of HIV drug resistance continues to fuel the search for new viral inhibitors, and the functional importance of the viral capsid makes CA an attractive new drug target. Several major challenges must be overcome in developing CA inhibitors, however, including the presence of thousands of CA molecules within each virion and the need to inhibit protein-protein interactions, which typically do not possess the deep binding grooves of enzyme active sites. Nevertheless, these challenges may be mitigated by the highly cooperative nature of capsid assembly, which likely restricts the range of interface stabilities that can support both capsid assembly and disassembly, and by the possibility that inhibiting even a small subset of the CA interfaces may "poison" capsid lattice growth. The development and

characterization of even better small molecule inhibitors of HIV-1 capsid assembly will undoubtedly be aided by our improved understanding of the different protein-protein interactions within the CA lattice.

Despite this progress, a full understanding of HIV-1 capsid structure and function will require further advances, including: (1) learning how the flat hexagonal CA lattice adjusts to form the gradually curving body of the conical capsid; (2) visualizing the capsid lattice structure at a resolution sufficient to define sidechain interactions and guide inhibitor development; (3) characterizing the pentameric CA assemblies that are required to close the conical capsid; (4) establishing how sequential proteolytic processing of the Gag precursor protein drives capsid assembly during viral maturation; and (5) most importantly, learning the detailed fates of capsids as they enter the cytoplasm, interact with host factors, and support the early stages of viral replication. These outstanding issues ensure that capsids will remain at the forefront of retrovirology for some time to come.

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Dynamic Regulation of the INAD Signaling Scaffold Becomes Crystal Clear

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PDZ domains are common building blocks of scaffold proteins that enhance specificity and speed in signal transduction cascades. Although PDZ modules are often viewed as passive participants, Mishra et al. (2007) now show that a PDZ domain in INAD, a scaffold protein in photoreceptor cells of the fruit fly, undergoes a light-dependent conformational change, which has important consequences for signaling and animal behavior.

The organization of signaling molecules into macromolecular assemblies is essential for achieving specificity in signaling, as it is common for proteins functioning in one signal transduction cascade to be employed by others. Signaling complexes are also crucial to cascades that operate over very short time scales, such as during phototransduction in the fruit fly *Drosophila*. Without these macromolecular assemblies, the speed of signaling would be limited by stochastic collisions between activated molecules and downstream effectors. Signaling complexes are nucleated by a variety of scaffold proteins, and among the most common are those with multiple copies of ~90 amino acid PDZ domains. In many cases, interactions with PDZ domains are regulated by dynamic modifications of the target proteins. In contrast, the PDZ domains are often considered passive components during signaling. However, this concept needs to be reevaluated in light of a study reported in this issue by Mishra and colleagues. They demonstrate that one of the PDZ domains in INAD, a molecular scaffold in *Drosophila* photoreceptor cells, undergoes a light-dependent conformational change. The dynamic structural transformation disrupts the surface groove important for PDZ/target protein interactions and has important physiological effects on the light response.