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Absconding with the Chaperone: Essential Cyclophilin–Gag Interaction in HIV-1 Virions

Minireview

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Retroviral virion assembly and uncoating are orchestrated by *gag*-encoded proteins, including the Capsid protein (CA) which forms the mature virion core. New genetic and structural data demonstrate that cyclophilin A (CyPA), a cytoplasmic protein best known as the receptor for the immunosuppressant cyclosporine A, forms a stable and essential complex with CA in HIV-1 virions. The most recent contribution to this evolving story, the solution of the three-dimensional structure of the HIV-1 CA–CyPA complex, is described in a highly informative paper in this issue of Cell (Gamble et al., 1996). These data illuminate aspects of the retroviral life cycle that had been relatively inaccessible to study and evoke questions about the function of the ubiquitous cyclophilin family of proteins.

The Many Faces of Gag

The myristylated Gag polyprotein of HIV-1 and other retroviruses is sufficient for the formation and release of enveloped virions. Gag recruits other viral components required for infectivity, including genomic RNA and Env glycoprotein, via direct interactions during assembly. The protease product of the *pol* gene cleaves the Gag polyprotein into several mature proteins: the matrix protein, which lines the virion envelope; the capsid protein (CA), which forms the virion core; and the nucleocapsid zinc-finger protein, which coats the genomic RNA. To initiate infection of a susceptible cell, extracellular virus binds to a cell surface receptor, fuses with the plasma membrane, and delivers the ribonucleoprotein core into the cytoplasm. In a poorly defined manner, *gag*-encoded proteins then dissociate from the complex (uncoating), or change conformation, allowing reverse transcription to begin. Finally, *gag* protein may participate in transport of the DNA copy of the viral genome to the nucleus, or in covalent linkage of viral DNA to host chromosomal DNA.

To identify cellular factors that might be coopted to help Gag fulfill its many roles, the HIV-1 Gag polyprotein was subjected to a yeast two-hybrid screen and found to interact with members of the CyP family (Luban et al., 1993). CyPs were originally discovered because of their affinity for the potent immunosuppressive drug cyclosporine A (Handschumacher et al., 1984); the CyP–cyclosporine A complex binds and inactivates the calcium-dependent phosphatase calcineurin, thus blocking signal transduction in activated T-cells (Schreiber and Crabtree, 1992). CyP proteins also catalyze the isomerization of peptidyl-prolyl bonds between the cis and trans states, though the biological significance of this activity has never been clearly demonstrated.

One Cell's Cyclophilin Is Another Cell's Ticket to Ruin

Does CyP play a role in HIV-1 replication? CyPA, the member of the CyP family that colocalizes with Gag in the cytoplasm, is specifically incorporated into HIV-1 virions via interaction with the Gag polyprotein (Braaten et al., 1996b; Franke et al., 1994; Thali et al., 1994). Gag binding to CyPA requires a proline-rich region located in the center of the CA domain, and mutation of a single proline, P90, or of the residue immediately preceding it, G89, disrupts CyPA incorporation into virions and precludes viral replication. Furthermore, cyclosporine A inhibits the production of replication-competent HIV-1 virions by disrupting the Gag–CyPA interaction (Braaten et al., 1996b; Thali et al., 1994).

Though CyPA is incorporated into HIV-1 virions it is not required for virion assembly per se: virions rendered CyPA-deficient by *gag* mutation, or by production in the presence of cyclosporine A, are produced at normal levels and are otherwise indistinguishable from wild-type virions by standard biochemical criteria (Braaten et al., 1996b; Franke et al., 1994; Thali et al., 1994). Nonetheless, disruption of CyPA incorporation causes a quantitative reduction in virion infectivity, with the block occuring early in the virus life cycle, after membrane fusion, but prior to the initiation of reverse transcription (Figure 1). Target cell CyPA is not required for these early events and cannot rescue CyPA-deficient virions.

Two genetic approaches were used to demonstrate that Gag not only packages CyPA into virions but that

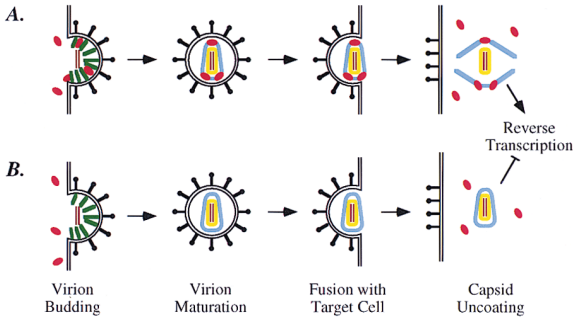

Figure 1. Model for the Role of Cyclophilin A in HIV-1 Replication

The HIV-1 Gag polyprotein (green) is shown forming a virion bud from the plasma membrane of an infected cell. Viral genomic RNA (brown) and envelope glycoprotein (black ball-and-stick figures) are incorporated into nascent virions via interactions with the Gag polyprotein. CyPA (red) from the infected cell cytoplasm is incorporated into virions by the wild-type Gag polyprotein (A), but not by Gag mutants G89A or P90A, nor by wild-type Gag expressed in the presence of cyclosporine A (B). At the time of virion release from the producer cell, viral protease is activated, and Gag is cleaved to form the CA (blue) and nucleocapsid (yellow) proteins, among other products. CyPA is associated with the CA protein in the core of wild-type virions. Virion membrane fuses with the plasma membrane of susceptible target cells, releasing the virion core into the cytoplasm of the new host cell. CyPA packaged in the wild-type virion then destabilizes the core, permitting the initiation of reverse transcription. CyPA-deficient virion cores cannot be rescued by CyPA present within the new host-cell cytoplasm.

a *gag*-encoded function requires CyPA. Cyclosporine A-resistant HIV-1 isolates were selected in tissue culture by passage of virus in the presence of the drug, and the drug-resistant phenotype was shown to be conferred by either of two *gag* missense mutations, A92E or G94D (Aberham et al., 1996). Although the amino acids altered by the drug resistance mutations are located in the immediate vicinity of residues G89 and P90, they have no effect on Gag's CyPA-binding properties (Braaten et al., 1996a), indicating that CyPA-binding and CsA-resistance are genetically separable properties in *gag*. CsA-resistance results because the mutations confer CyPA-independence upon HIV-1 *gag*; this was confirmed by the following experiment: the single mutant P90A is unable to replicate due to its inability to bind CyPA, but the double mutant P90A A92E is replication competent, despite its inability to package CyPA into virions.

The second approach that demonstrated that CyPA is required for a *gag*-encoded function involved the construction of a chimeric virus in which CA coding sequences of the SIV_{MAC239} DNA provirus were replaced with those from HIV-1 (Dorfman and Göttlinger, 1996). Unlike HIV-1, SIV_{MAC239} does not incorporate CyPA into virions and replication of the latter is not inhibited by cyclosporine A (Franke et al., 1994; Thali et al., 1994). Chimeric virions were capable of incorporating CyPA and their replication was inhibited by cyclosporine A.

Cyclophilin's Biochemical Role Gleaned from Structural Studies

The first high-resolution structural information for a retroviral CA has recently been published (Gamble et al., 1996; Gitti et al., 1996; Momany et al., 1996). Isolation of a soluble core domain by limited proteolysis of HIV-1 CA provided the technical breakthrough that led to the production of a protein preparation sufficiently well-behaved for structural analysis. The structure of the amino-terminal two-thirds of CA was solved by NMR (Gitti et al., 1996); the X-ray structure of a complex between this same CA domain and CyPA has been determined (Gamble et al., 1996), and information missing from a crystal structure of a CA–Fab antibody complex has been provided (Momany et al., 1996).

It had been widely suspected that retroviral CA would be an eight-stranded, antiparallel β-barrel, the structure common to the capsids of many viruses. CyPA is an eight-stranded, antiparallel β-barrel, and it was appealing to imagine the β-sheets of CA and CyPA packed in a regular array in virions. The actual results were not at all like the predictions: HIV-1 CA is a triangular pyramid composed almost entirely of α-helices (Figure 2A).

One of the few nonhelical elements in CA is an exposed loop between helices IV and V that is thought to be located on the surface of the virion core (Figure 2A). The exposed loop corresponds to the proline-rich domain required for CyPA-binding (Franke, Yuan et al., 1994), and is the only part of CA contacting CyPA in the cocrystal (Gamble et al., 1996). The critical G89 and P90 residues are prominently situated at the apex of the loop and are embedded deep in the hydrophobic pocket of CyPA.

Though it is tempting to propose that CyPA catalyzes cis-trans isomerization of the bond connecting G89 and P90, this appears not to be the case: the bond is primarily trans in the free protein (Gitti et al., 1996) and all trans

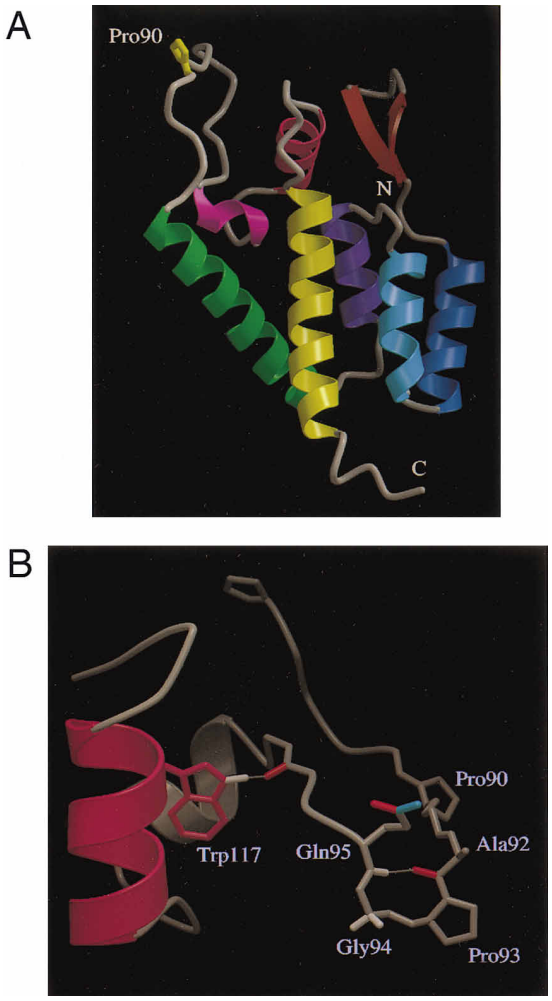

Figure 2. Structure of the HIV-1 CA Core Domain

(A) Ribbon representation of the first 151 amino acids of HIV-1 CA as determined by NMR spectroscopy. Helices I-VII are colored, the N-terminal β-hairpin is shown in brown, and disordered segments and loops are shown in grey. The position of the P90 side chain in the exposed loop between helices IV and V is shown.

(B) Detailed view of the exposed proline-rich loop. The position of the P90 side chain is shown, as is the type II turn formed by residues P93 and G94. Hydrogen bonds between A92 and Q95, and between R97 and W117 on helix VI, are indicated. Figure kindly provided by Rossitza K. Gitti, Brian M. Lee, and Michael F. Summers.

as a complex with CyPA (Gamble et al., 1996). The latter result is at odds with previous structural studies that had shown that model peptides will only fit within the CyPA hydrophobic pocket in the cis conformation. Gamble et al. (1996) suggest that the absence of a side chain at the residue preceding P90 allows CA to fit snugly within the pocket without distortion of the peptide backbone. Thus, CyPA must promote HIV-1 virion infectivity by forming a stable complex with CA, and not by catalyzing the peptidyl-prolyl isomerization of G89-P90.

CyPA: HIV-1 Core Destabilizer?

Cytoplasmic fractionation studies indicate that soon after entry HIV-1 CA dissociates from the viral nucleoprotein complex (Gallay et al., 1995). Biochemical analyses such as this are difficult to interpret since the percentage of infectious particles within any population of

retroviral virions is extremely small, and the biologically relevant fraction might not be detectable by biochemical methods; currently there are no methods for confirming that a given preparation of nucleoprotein complexes is capable of successfully completing an infection in vivo. Assuming that separation of CA from the viral nucleoprotein complex is required for productive infection, and given that CyPA is required for the initiation of reverse transcription (Braaten et al., 1996b), it would be reasonable to propose a model in which CyPA promotes HIV-1 CA uncoating (Figure 1) in a manner vaguely analogous to that of the chaperone Hsp70 in the uncoating of clathrin from coated vesicles (Rothman and Schmid, 1986).

Elucidation of the biochemical role of CyPA in virion uncoating would be aided by a detailed map of the CA–CA interactions required for formation of the virion core. CA–CA contacts involving two CA faces were observed in the cocrystal with CyPA (Gamble et al., 1996), and the authors propose that contacts made by the third face would involve a type II tight turn located on the proline-rich loop, adjacent to the CyPA-binding site (Figure 2B). Binding of CyPA to the proline-rich loop would directly interfere with CA–CA interactions that involve the tight turn, weaken assembly of CA complexes, and allow the core to more readily dissociate during uncoating. This model predicts that core assembly would not only tolerate disruption of some CA–CA interactions by CyPA, but that significant reduction of the CyPA:CA ratio in virions from the observed 1:10 (Franke et al., 1994; Thali et al., 1994) would shift the balance towards a core that was too inflexible to uncoat. Indeed, a graded decrease in virion infectivity is observed in response to decreases in the CyPA:CA ratio (Braaten et al., 1996b). The cyclosporine A-resistance of the A92E and G94D mutants is also explained by this model (Braaten et al., 1996a): by disrupting the type II tight turn, the mutants would destabilize CA–CA interactions and allow uncoating to proceed in the absence of CyPA. The development of HIV-1 virion core purification methods, as well as assays for the infectivity of the purified cores, would provide a means by which one could directly test if CyPA promotes HIV-1 uncoating by destabilizing CA–CA interactions.

CyPs: Specialized Protein Recognition Modules

Perhaps the greatest significance of these studies with HIV-1 CA is the contribution they make to our understanding of the role of CyPs. A growing collection of data indicates that a primary role of CyPs is to mediate protein–protein interactions. As a high-affinity complex with cyclosporine A, CyP creates a composite surface that inhibits calcineurin (Schreiber and Crabtree, 1992). The Drosophila CyP NinaA forms a stable complex with the major isoform of rhodopsin that is required for this photoreceptor protein to exit the endoplasmic reticulum (Baker et al., 1994). Now it has been demonstrated that HIV-1 replication requires the formation of a stable complex between CA and CyPA (Braaten et al., 1996b; Franke et al., 1994; Thali et al., 1994).

The first CyPs to be cloned were simple molecules consisting of a core CyP domain and, at most, terminal extensions that direct subcellular localization. Recently, CyP family members have been identified which are components of large macromolecular complexes and possess several well-characterized motifs. Nup358/RanBP2, the largest nuclear pore component, constitutes the most dramatic example; in addition to a cyclophilin-homologous domain, this protein possesses a leucine-rich region, Ran-binding sites, nucleoporin-characteristic repeats, and zinc-fingers (Wu et al., 1995; Yokohama et al., 1995). The identification of proteins such as this supports the contention that CyP functions as a modular protein–protein interaction cassette reminiscent of other protein subunits such as the Src homology domains.

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