HIV-1 Gag: a Molecular Machine Driving Viral Particle Assembly and Release

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Introduction

HIV-1 and other primate lentiviruses assemble at the plasma membrane and are released by budding from the cell surface. Apart from offering a nonlytic pathway for virus egress, this mode of assembly leads to the acquisition of a host cell-derived lipid envelope that enwraps the nascent viral capsid and protects it from the environment. HIV-1 assembly is controlled primarily by the Gag protein, one of the three gene products that are encoded by all retroviruses. Gag orchestrates assembly by recruiting all the building blocks required for the formation of a fully infectious virion, which in the case of HIV-1 include both viral and cellular components. Furthermore, Gag provides the principal driving force for virus assembly, as illustrated by the fact that HIV-1 Gag can efficiently form virus-like particles even when expressed in the absence of other viral proteins (Gheysen *et al.*, 1989). Gag thus constitutes an autonomous molecular machine for particle assembly.

General features of Gag and its role in virus morphogenesis

Gag is often referred to as a precursor, because it is subject to cleavage by the viral protease (PR), which yields the internal structural proteins of the mature virion (Freed, 1998; Hunter, 1994; Swanstrom and Wills, 1997; Vogt, 1997; Wills and Craven, 1991). PR and other essential viral enzymes are brought into the virion as components of the Gag-pol polyprotein, which is produced by ribosomal frameshifting between the overlapping *gag* and *pol* genes. Three of the Gag cleavage products, matrix (MA), capsid (CA), and nucleocapsid (NC), are common to all retroviruses and are always arranged in this order within the Gag precursor, with MA being at the N-terminus. Additionally, the Gag precursor of HIV-1 possesses a C-terminal domain called p6 that is unique to primate lentiviruses, as well as two "spacer" regions which separate CA from NC, and NC from p6 (Henderson *et al.*, 1992; Mervis *et al.*, 1988) (see Figure 1).

The HIV-1 Gag precursor is synthesized on cytosolic ribosomes (Tritel and Resh, 2000) and becomes cotranslationally modified by the N-terminal attachment of a myristyl group, which increases its affinity for membranes (Bryant and Ratner, 1990; Gottlinger *et al.*, 1989). Myristylated Gag precursor molecules associate with the inner leaflet of the plasma membrane, where they coalesce into a patch, the first stage in the assembly process that can be visualized by electron microscopy (Swanstrom and Wills, 1997). Through the continuous lateral addition of extra Gag molecules, the electron-dense patch grows into a spherical structure that increasingly protrudes from the cell surface and eventually pinches off, releasing an immature virus particle into the extracellular environment. How Gag reaches the site of virus assembly is not known. There is evidence that Gag can form oligomeric complexes in the cytoplasm, but whether these represent assembly intermediates or dead-end products remains controversial (Lee *et al.*, 1999; Lee and Yu, 1998; Lingappa *et al.*, 1997; Tritel and Resh, 2000).

Immature HIV-1 particles are non-infectious (Gottlinger *et al.*, 1989; Kohl *et al.*, 1988), and it is thought that this is at least in part because the palisade-like shell of radially arranged, unprocessed Gag precursor molecules which forms the immature capsid is too stable to permit its disassembly. This

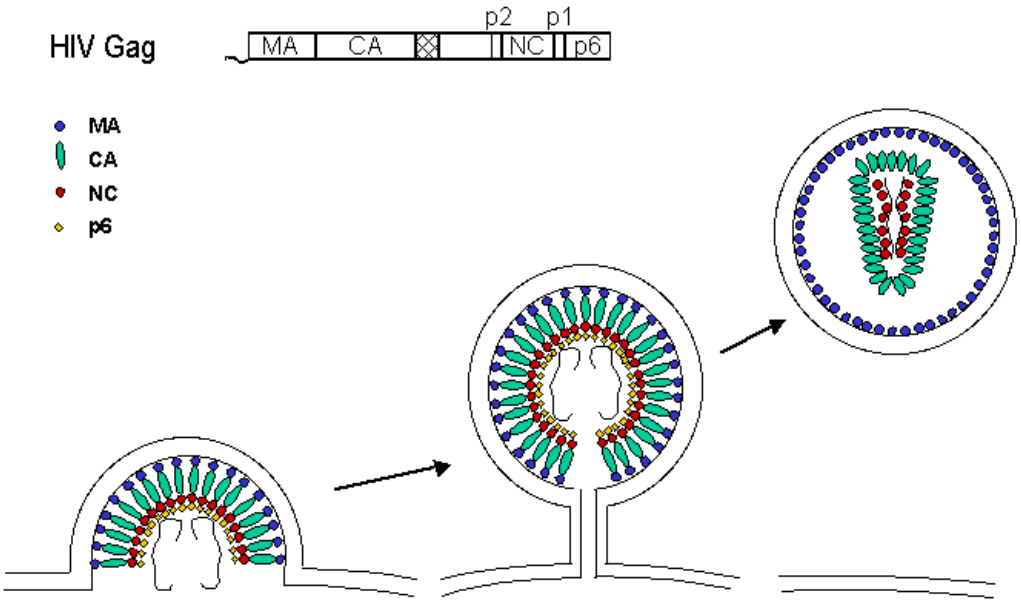

Figure 1. *Schematic illustration of Gag-driven particle production. Gag oligomerizes underneath the plasma membrane through protein-protein and protein-RNA interactions, with the MA domain oriented towards the lipid bilayer. Eventually, a spherical Gag protein shell is formed and a membrane fusion event at the neck of the bud becomes necessary to release the assembled particle. Subsequently, the Gag polyprotein is processed by the viral protease and the cleavage products are rearranged as indicated. The location of p6 in the mature particle remains uncertain.*

prevents uncoating after virus entry into a new target cell, which is essential to release the viral genome in a form that can be imported into the nucleus and integrated into the host genome. The immature capsid thus needs to undergo a maturation step that makes it metastable, and this process is initiated by the activation of PR. This leads to a series of proteolytic processing events at the domain boundaries within the Gag precursor, and is followed by a drastic rearrangement of the liberated Gag domains (Vogt, 1996). Only the MA domain remains associated with the viral lipid envelope, while the CA and NC domains condense around the viral genome, yielding the characteristic conical core of the mature HIV-1 virion. Virus maturation considerably reduces the stability of the viral capsid, as measured by its detergent resistance, presumably to make it sufficiently flexible for uncoating (Wang and Barklis, 1993).

While we do not yet precisely understand how Gag as a whole assembles into a spherical protein shell that is capable of extruding through the cell surface, much has been learned by dissecting the Gag domains which contribute to various stages of this process. Furthermore, structural information about individual domains of the HIV-1 Gag precursor has become available in recent years. This review attempts to integrate biochemical and structural data to convey an overview of our present understanding of the role of each of the HIV-1 Gag domains in virus morphogenesis.

Matrix

MA constitutes the N-terminal domain of the Gag precursor and remains intimately associated with the lipid envelope of the mature virion. During assembly, MA has well-established roles in the targeting of Gag to the plasma membrane and in the incorporation of the viral envelope (Env) glycoproteins into nascent particles. Additionally, MA has been implicated in post-assembly steps of the viral replication cycle, particularly in non-dividing cells.

The membrane-targeting function of MA is dependent on the N-terminal attachment of a myristic acid moiety (Bryant and Ratner, 1990; Gottlinger *et al.*, 1989; Spearman *et al.*, 1994). This hydrophobic modification is crucial for the stable membrane association of the Gag precursor, and is essential for extracellular particle formation and virus replication. Although the signal which directs the myristylation of Gag remains poorly understood, it is clear that the N-terminal six residues of the MA domain are sufficient (Lee and Linial, 1994). Remarkably, at least in certain cell lines, this N-terminal myristyl anchor is sufficient for the efficient production of extracellular HIV-1 particles in the absence of all other MA sequences (Lee and Linial, 1994; Reil *et al.*, 1998; Wang *et al.*, 1993). On the other hand, the binding energy contributed by a single myristyl moiety appears insufficient for the stable attachment of a protein to a cellular membrane (Blenis and Resh, 1993). Thus, the ability of the myristylation signal to fully substitute for the assembly function of MA suggests that Gag binds to membranes in a cooperative manner that relies on Gag oligomerization.

In addition to the hydrophobic myristyl anchor, a charged N-proximal region contributes to the membrane-targeting ability of MA (Yuan *et al.*, 1993; Zhou *et al.*, 1994). This region harbors a cluster of conserved basic residues that can bind to acidic phospholipids *in vitro* (Zhou *et al.*, 1994). In eukaryotic cells, anionic phospholipids are highly enriched on the cytoplasmic face the plasma membrane, which exhibits a considerable asymmetry in the distribution of phospholipids across the bilayer (Buckland and Wilton, 2000). Interactions between MA and the head groups of acidic phospholipids may thus enhance Gag membrane binding, and may also contribute to the selective targeting of Gag to the plasma membrane. This model is supported by the three-dimensional structure of HIV-1 MA, which shows a trimer that forms a large composite surface on which the conserved basic residues cluster (Hill *et al.*, 1996; Matthews *et al.*, 1995) (see Figure 2). The exposure of those cationic residues on the putative membrane-binding surface of MA is compatible with their proposed role in the targeting of Gag to the anionic cytoplasmic leaflet of the plasma membrane. Furthermore, a tendency to assemble into trimers with a positively charged face appears to be a conserved property of otherwise highly divergent retroviral MA proteins (Christensen *et al.*, 1996; Conte *et al.*, 1997; Matthews *et al.*, 1996; McDonnell *et al.*, 1998; Rao *et al.*, 1995). Interestingly, in the case of Rous sarcoma virus (RSV), the presence of a certain number of basic residues in MA is crucial for budding, but at least some of these residues can be repositioned without affecting particle release (Callahan and Wills, 2000). Thus, there appears to be a considerable degree of freedom in the way MA can interact with acidic phospholipids.

There are several examples which illustrate that the subcellular localization of a myristylated protein can be regulated through the exposure of the myristyl group. For instance, the membrane association of the small GTPase ARF1 is mediated by GTP binding, which induces a conformational change that makes its N-terminal myristyl group available for membrane insertion (Goldberg, 1998). Recoverin, another myristyl switch protein, sequesters the myristyl group in a deep pocket formed by five a-helices (Tanaka *et al.*, 1995), and calcium binding causes a dramatic rearrangement of the helices and allows the myristyl group to move out of the pocket (Ames *et al.*, 1997; Ames *et al.*, 1996). Although the three-dimensional structure of myristylated HIV-1 MA is not known, there is considerable genetic and biochemical evidence that the membrane association of MA is also regulated by a myristyl switch mechanism. The crystal structure of non-myristylated HIV-1 MA reveals a single globular domain that is composed of five a-helices (Hill *et al.*, 1996), and mutations within the helical regions often dramatically increase the membrane affinity of MA, as one would expect if the globular core is required to sequester the myristyl group (Ono and Freed, 1999; Paillart and Gottlinger, 1999; Spearman *et al.*, 1997; Zhou and Resh, 1996). Also, mutations near the N-terminus of MA can cause severe defects in membrane binding without affecting myristylation, and these defects are completely reversed by second-site mutations in the a-helical core of MA (Ono and Freed, 1999; Paillart and Gottlinger, 1999). These phenotypes are dependent on the presence of a myristyl group (Ono and Freed, 1999), which suggests that the mutations have opposing effects on its exposure, and that mutations in the globular core are dominant because they prevent the sequestration of the myristyl group. Interestingly, the compensatory mutations in the globular core of MA by themselves also significantly increase viral particle production (Paillart and Gottlinger, 1999). Furthermore, a similar increase in particle production is seen if MA is deleted and only the myristyl anchor is retained, consistent with the interpretation that particle production is increased

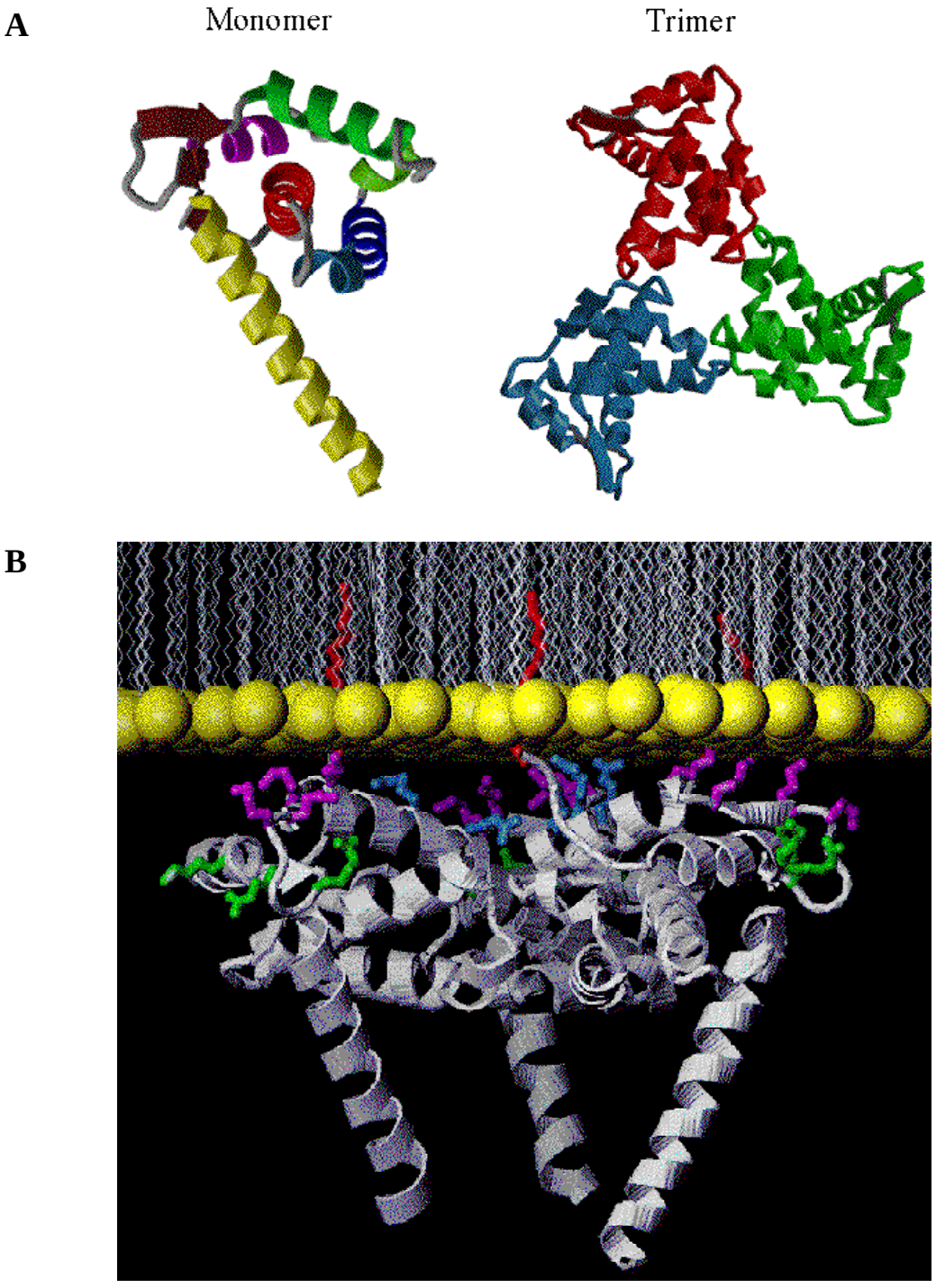

Figure 2. *(A) Three-dimensional structure of HIV-1 MA monomer and trimer as determined by X-ray crystallography. The putative membrane binding surface of the trimer is shown. (B) Model of membrane binding by MA. The MA myristyl groups are in red, basic MA residues critical for HIV-1 replication are in magenta, nonessential basic residues are in green, and other MA residues implicated in the membrane association of Gag (Cannon et al., 1997) are in blue. (Courtesy of Wesley Sundquist and reprinted with permission from Hill et al., 1996.)*

because the myristyl moiety can no longer be sequestered and is therefore constitutively exposed (Reil *et al.*, 1998).

It is also conceivable that the intact MA domain interferes with assembly unless a cellular cofactor is bound that triggers a conformational switch needed for efficient Gag polymerization. In this model, deleting the globular core of MA would increase extracellular particle production, at least in part, because the cellular cofactor is no longer required. It is noteworthy in this respect that HIV-1 assembly is blocked in murine cells (Bieniasz and Cullen, 2000; Mariani *et al.*, 2000), and recent work demonstrates that the MA domain is responsible for this defect (Chen *et al.*, 2001; Reed *et al.*, 2002), providing strong evidence for the existence of a specific host cofactor required for HIV-1 MA function. Also, MA appears to interfere with HIV-1 assembly in vitro, because a Gag protein lacking the globular core of MA formed spherical particles of the correct size, whereas particles produced in the presence of the intact MA domain were much smaller (Campbell and Rein, 1999; Gross *et al.*, 2000). Intriguingly, a recent study shows that particles of normal size are obtained in the presence of MA if inositol phosphates or phosphatidylinositol phosphates are added to the in vitro assembly reaction (Campbell *et al.*, 2001). These observations raise the possibility that a phosphorylated inositol lipid at the inner leaflet of the plasma membrane serves as a cofactor for HIV-1 assembly in vivo.

In principle, a conformational transition that is triggered by a plasma membrane-associated cofactor and results in the exposure of the myristylated N-terminus of MA could form the basis for the selective membrane targeting of Gag. One would then expect that mutations in MA which interfere with its ability to sequester the myristyl group should lead to non-selective membrane binding. Indeed, as predicted by this model, mutations that disrupt the globular core of MA cause massive budding into intracellular compartments (Facke *et al.*, 1993; Freed *et al.*, 1994). However, recent work reveals that Gag targeting can be altered by mutations in the basic domain of MA even if the overall affinity of Gag for membranes remains unchanged (Ono *et al.*, 2000). While a large deletion in MA caused promiscuous assembly at the most abundant cellular membrane, the endoplasmic reticulum (Facke *et al.*, 1993), certain point mutations in the basic domain directed Gag to a Golgi compartment (Ono *et al.*, 2000), indicating that MA retained some ability to discriminate between cellular membranes. In view of the reported affinity of the basic domain of MA for acidic phospholipids (Zhou *et al.*, 1994), it is conceivable that electrostatic interactions with anionic head groups help to target Gag to a unique phospholipid environment. Recent studies suggest that HIV-1 Gag specifically associates with raft-like microdomains in the plasma membrane (Lindwasser and Resh, 2001; Nguyen and Hildreth, 2000; Ono and Freed, 2001), which are rich in acidic phosphoinositide lipids, in addition to cholesterol and sphingolipids (Brown and London, 1998). However, whether Gag is targeted to preexisting rafts or induces the formation of a raft-like microenvironment following membrane binding remains to be determined.

In addition to its role in Gag membrane targeting, MA is essential for the incorporation of the Env glycoprotein spikes during virus assembly (Dorfman *et al.*, 1994b; Yu *et al.*, 1992b). The Env glycoprotein spikes, which mediate virus entry, are trimeric complexes of a heterodimer that consists of the surface glycoprotein (SU) and the transmembrane glycoprotein (TM). The TM of HIV-1 has a very long cytoplasmic domain, which contains endocytic signals that minimize the amount of free Env on the cell surface (Egan *et al.*, 1996; Rowell *et al.*, 1995), probably to limit the cytopathic effects of Env and to avoid targeting of infected cells by the immune system. Although the levels of HIV-1 Env on the cell surface are very low unless the cytoplasmic domain of TM is removed, intact Env is nevertheless efficiently incorporated into assembling particles. This suggests that Gag and Env may interact prior to their transport to the cell surface, and this notion is strongly supported by the observation that the localization of Env determines the site of HIV-1 budding in polarized cells (Owens *et al.*, 1991). In addition to a specific sorting signal in the cytoplasmic domain of TM, an intact MA domain is needed for polarized budding (Lodge *et al.*, 1994; Lodge *et al.*, 1997), which indicates that an intracellular interaction between MA and TM is required. This conjecture is supported by direct biochemical evidence for a stable interaction between the MA domain of HIV-1 Gag and the cytoplasmic domain of Env (Cosson, 1996; Wyma *et al.*, 2000).

Surprisingly, the cytoplasmic domain of TM is dispensable for Env incorporation if HIV-1 is expressed from transiently transfected proviral DNA (Wilk *et al.*, 1992). Moreover, whereas the

incorporation of full-length HIV-1 Env is highly dependent on the integrity of the globular domain of MA (Dorfman *et al.*, 1994b), HIV-1 Env that lacks the cytoplasmic domain can be efficiently incorporated even in the complete absence of MA (Reil *et al.*, 1998). Truncating the cytoplasmic domain of TM also allows the functional incorporation of HIV-1 Env into unrelated retroviruses that are unable to incorporate full-length HIV-1 Env (Mammano *et al.*, 1997; Schnierle *et al.*, 1997). Conversely, HIV-1 MA mutants that exhibit an absolute block in the incorporation of the full-length HIV-1 Env complex readily accept heterologous viral Env proteins that naturally have a short cytoplasmic domain (Freed and Martin, 1995; Mammano *et al.*, 1995). Taken together, these observations are most compatible with a model in which the role of HIV-1 MA in Env incorporation is primarily the accommodation of the long cytoplasmic tail of TM. However, a caveat is that these results were obtained with transfected viral DNA, of which multiple copies are usually taken up by individual cells. The requirements for Env incorporation may be more stringent in infected cells, which generally harbor only a single integrated provirus. Furthermore, the requirements may be cell-type dependent, as indicated by a recent study in which the removal of the cytoplasmic domain of TM reduced HIV-1 Env incorporation more than 10-fold in the majority of human T cell lines, but to a lesser extent in HeLa cells (Murakami and Freed, 2000).

During the early stages of HIV-1 replication, a fraction of MA is thought to remain associated with the viral preintegration complex (Bukrinsky *et al.*, 1993b; Miller *et al.*, 1997). In contrast to oncoretroviruses, HIV-1 can productively infect non-dividing cells because the preintegration complex is actively transported through the nucleopore (Bukrinsky *et al.*, 1992; Lewis *et al.*, 1992; Weinberg *et al.*, 1991). One of the viral components which confers nucleophilic properties to the HIV-1 preintegration complex appears to be MA, in which a putative nuclear localization signal (NLS) has been identified (Bukrinsky *et al.*, 1993a; Gallay *et al.*, 1995). Additionally, a nuclear export activity has been described for MA that counteracts the NLS during virus production, thus ensuring that Gag is available for virus assembly (Dupont *et al.*, 1999). The NLS in MA maps to the exposed basic patch on the globular head that has also been implicated in Gag membrane binding (Bukrinsky *et al.*, 1993a; Zhou *et al.*, 1994). It has been reported that HIV-1 mutants that carry substitutions in the MA NLS replicate efficiently in diving but not in growth-arrested cells or in terminally differentiated macrophages, consistent with a role of MA in nuclear import (Bukrinsky *et al.*, 1993a; Heinzinger *et al.*, 1994; von Schwedler *et al.*, 1994). However, this model has been challenged by others, who reported that the putative NLS in MA is not specifically required for the productive infection of terminally differentiated cells (Fouchier *et al.*, 1997; Freed *et al.*, 1995).

While the phenotypes of certain HIV-1 mutants suggest a post-assembly role for MA (Kiernan *et al.*, 1998; Yu *et al.*, 1992a), the analysis of mutants with large deletions has demonstrated that MA is not absolutely required for the early stages of the HIV-1 replication cycle (Reil *et al.*, 1998; Wang *et al.*, 1993). For instance, the globular domain of MA can be deleted with only minor effects on HIV-1 infectivity for a variety of cell types, provided that the Env incorporation defect is corrected by truncating the cytoplasmic domain of TM (Reil *et al.*, 1998). Under these circumstances, HIV-1 retains some infectivity even if the entire MA domain is replaced by a heterologous myristyl anchor (Reil *et al.*, 1998). Moreover, in cells where the cytoplasmic domain of TM is not required for virus propagation, MA-less HIV-1 could initiate a productive infection that spread rapidly after the acquisition of compensatory changes elsewhere in Gag (Reil *et al.*, 1998). These observations indicate that the mature HIV-1 core by itself is fully capable of initiating the retroviral replication cycle once it gains access into a permissive cell.

Capsid

CA, which directly follows upon MA in the context of the Gag precursor, has crucial roles in particle assembly and also following entry into a new target cell. However, the function of CA in the early phase of the replication cycle is not well understood. In the mature virion, CA forms the shell of the core, which is occasionally tubular but most often conical, a feature that distinguishes lentiviruses such as HIV-1 from most other retroviruses (Gelderblom, 1991). CA has two predominantly a-helical domains that are

connected through a flexible linker region (Gamble *et al.*, 1997; Gitti *et al.*, 1996; Momany *et al.*, 1996), and these domains appear to correspond to distinct densities in cyroelectron micrographs of immature virus particles (Fuller *et al.*, 1997; Wilk *et al.*, 2001; Yeager *et al.*, 1998). The two CA domains have different roles in virus morphogenesis. The N-terminal domain, which comprises two thirds of HIV-1 CA, is required for the formation of the mature core, but is dispensable for the assembly of immature virus particles (Borsetti *et al.*, 1998; Dorfman *et al.*, 1994a; Reicin *et al.*, 1996; Reicin *et al.*, 1995; Srinivasakumar *et al.*, 1995; Wang and Barklis, 1993). In contrast, the C-terminal CA domain is crucial both for particle assembly and for core formation (Dorfman *et al.*, 1994a; Mammano *et al.*, 1994; McDermott *et al.*, 1996; Reicin *et al.*, 1995). The central role of the C-terminal CA domain in assembly is illustrated by the finding that this region of HIV-1 Gag suffices for the efficient production of virus-like particles when combined with a minimal membrane anchor, a heterologous protein interaction domain, and a short peptide that promotes virus release (Accola *et al.*, 2000b).

The N-terminal domain of HIV-1 CA interacts with the human peptidyl-prolyl *cis-trans* isomerase cyclophilin A (CyPA), which leads to the specific incorporation of this ubiquitous cytosolic host protein into virions (Franke et al., 1994b; Luban et al., 1993; Thali et al., 1994). CyPA is perhaps best known as the intracellular receptor for the immunosuppressant cyclosporin A (CsA), which binds to the active site of CyPA with high affinity (Handschumacher et al., 1984). CsA and nonimmunosuppressive analogues such as SDZ NIM 811 inhibit the incorporation of CyPA into HIV-1 virions, and lowered levels of virion-associated cyclophilin A result in lowered levels of virion infectivity (Thali et al., 1994). Although virus assembly and maturation are unaffected by cyclosporins, virus replication is defective at an early step (Braaten et al., 1996a; Wiegers et al., 1999). As a consequence, CsA and SDZ NIM 811 potently inhibit the spread of HIV-1 in peripheral blood mononuclear cells (Rosenwirth et al., 1994). Virus replication is also decreased in a T cell line that lacks CyPA, and is not inhibited further by CsA, indicating that no other members of the cyclophilin family are involved in HIV-1 replication (Braaten and Luban, 2001). The primary CyPA-binding site centers around a critical proline residue in a region of CA that shows unusual variability between different lineages of primate lentiviruses (Braaten et al., 1996b; Colgan et al., 1996; Franke et al., 1994b). Indeed, HIV-1 is unique among these viruses in its ability to interact with CyPA, and cylosporins are inactive against other primate lentiviruses such as HIV-2 or simian immunodeficiency virus SIVmac (Billich et al., 1995; Braaten et al., 1996b; Thali et al., 1994). Interestingly, the transfer of the primary CyPA binding site from HIV-1 to the equivalent position in the CA domain of SIVmac resulted in the efficient incorporation of CyPA into SIVmac virions and also conferred an HIV-1-like sensitivity to cyclosporin (Bukovsky et al., 1997). Thus, CA not only provides a docking site for CyPA but also appears to be its functional target.

A crystal structure of CyPA bound to the N-terminal CA domain shows that a solvent-exposed CA loop which contains the critical proline residue identified by genetic studies binds in the active site of the rotamase (Gamble *et al.*, 1996). This CA loop emanates from the top of a largely a-helical domain that is tapered towards the C-terminal end, which may facilitate the formation of curvature during assembly (Gitti *et al.*, 1996). Within the CA-CyPA cocrystal, the CA molecules associated into planar strips, and this led to the suggestion that the surface of the mature virion core may be formed through the side by side association of such strips (Gamble *et al.*, 1996). Binding of CyPA would then be expected to interfere with the interaction between the strips and thus to lead to occasional defects in the core of the virion, where the CA:CyPA ratio is about 10:1. CyPA would thereby destabilize the virion core and facilitate uncoating (Gamble *et al.*, 1996), consistent with its reported role early in the viral life cycle (Braaten *et al.*, 1996a). While this hypothesis is attractive, other studies argue against the possibility that CyPA serves as an uncoating factor. First, reconstructed images of *in vitro* assembled CA tubes suggest a different model for the organization of the HIV-1 core in which CyPA can be docked onto the exterior without steric clashes between CA and CyPA residues (Li *et al.*, 2000). Thus, the presence of CyPA during core assembly would not necessarily result in a weakening of CA-CA interactions. Second, detergent stripping of HIV-1 virions with different CyPA contents revealed no difference in core stability (Wiegers *et al.*, 1999). Third, CyPA did not significantly destabilize *in vitro* assembled CA cylinders (Grattinger *et al.*, 1999). On the contrary, the presence of CyPA at the same molar ratio to CA as in the virion led to the formation of longer CA cylinders (Grattinger *et al.*, 1999), indicating that CyPA may act as chaperone that supports the rearrangement of CA during virus maturation.

CA forms dimers in solution, and it is principally the C-terminal domain that mediates dimerization (Gamble *et al.*, 1997). The crystal structure of the C-terminal domain shows a globular domain composed of four a-helices which presents a dimer interface that centers around a-helix 2 (Gamble *et al.*, 1997). A point mutation in a-helix 2 prevented both CA dimerization and HIV-1 replication, indicating that the crystallographically defined interface is essential for the viral life cycle (Gamble *et al.*, 1997). In the context of the Gag precursor, the functional dimerization domain may extend into the adjacent p2 "spacer" peptide, which separates CA from NC. Molecular modeling has led to the proposal that the C-terminus of CA and a conserved N-terminal region of p2 together form a continuous a-helix (Accola *et al.*, 1998). The ability of this region to adopt an a-helical structure appears to be crucial during assembly, because mutations that are predicted to disrupt the a-helical conformation lead to the formation of grossly aberrant budding structures (Accola *et al.*, 1998; Gottlinger *et al.*, 1989; Krausslich *et al.*, 1995; Morikawa *et al.*, 2000). These often consist of flat electron-dense patches at the cell membrane (Accola *et al.*, 1998; Krausslich *et al.*, 1995), or of tubular structures that protrude from the cell surface (Gottlinger *et al.*, 1989; Morikawa *et al.*, 2000). Collectively, these findings indicate that the CA-p2 boundary is primarily required for the induction of curvature rather than for assembly per se. Cleavage at the CA-p2 junction, which occurs late during virus maturation (Pettit *et al.*, 1994; Wiegers *et al.*, 1998), is essential for the formation of a cone-shaped core shell (Accola *et al.*, 1998; Wiegers *et al.*, 1998). If CA-p2 processing is selectively blocked by mutagenesis, CA-p2 becomes separated from MA but remains in a thin, roughly spherical layer near the viral membrane (Accola *et al.*, 1998; Wiegers *et al.*, 1998). These observations are consistent with the idea that the helix at the CA-p2 junction must be destroyed to weaken CA-CA interactions sufficiently to allow the collapse of the spherical CA shell into a cone.

The C-terminal CA domain also includes a stretch of 20 residues that has been called the major homology region (MHR) because of its conservation among unrelated retroviruses, which otherwise show little if any sequence homology in CA (Wills and Craven, 1991). The unique conservation of this region suggests a crucial function in the retroviral life cycle, and genetic analyses have shown that the MHR is essential for virus replication and likely has crucial roles both in assembly and at post-assembly stages (Alin and Goff, 1996; Cairns and Craven, 2001; Craven *et al.*, 1995; Mammano *et al.*, 1994; Strambio-de-Castillia and Hunter, 1992; Willems *et al.*, 1997). The C-terminal half of the MHR has an a-helical conformation, and the entire conserved region forms a compact strand-turn-helix motif that is stabilized by a complex network of hydrogen bonding interactions (Gamble *et al.*, 1997). In the X-ray structure of the C-terminal CA domain, the MHR did not contribute to the dimer interface, suggesting that it may serve as an interaction site for another viral component or for a cellular factor (Gamble *et al.*, 1997).

In vitro, the CA domain alone can assemble into hollow tubes that are reminiscent of the tubular cores seen in a fraction of mature HIV-1 virions (Gross *et al.*, 1997). However, tube formation was prevented by N-terminal extensions that consisted of as few as four MA residues (Gross *et al.*, 1998; von Schwedler *et al.*, 1998). Instead, spherical shells were observed which resembled immature virus particles but were smaller in size. This switch from tube to sphere formation supports the proposal that cleavage at the MA-CA junction leads to the refolding of the N-terminal end of CA, creating a new CA-CA interface in the mature core (Gitti *et al.*, 1996; Gross *et al.*, 1998; von Schwedler *et al.*, 1998). Specifically, the three-dimensional structure of the N-terminal CA domain suggests that the proteolytic liberation of the N-terminal proline of CA allows the formation of a salt bridge with a highly conserved aspartate residue, which stabilizes the refolded conformation (Gitti *et al.*, 1996). Mutagenesis of the conserved aspartate indicates that the postulated salt bridge is crucial for the formation CA tubes *in vitro* (von Schwedler *et al.*, 1998). However, a recent study shows that N-terminal extensions of CA do not always abolish tube formation, demonstrating that there is no absolute requirement for the salt bridge (Gross *et al.*, 2000). Interestingly, in the latter study, which used a Gag molecule that included NC in addition to N-terminally extended CA, the presence of the p2 spacer peptide between the CA and NC domains was essential for the formation of spheres. These *in vitro* results support the notion that p2 functions as a molecular switch region that controls the transition from sphere to cone formation.

In addition to tubes (Campbell and Vogt, 1995), purified CA-p2-NC can yield significant amounts of cones that appear strikingly similar to viral cores (Ganser *et al.*, 1999) (See Figure 3). While

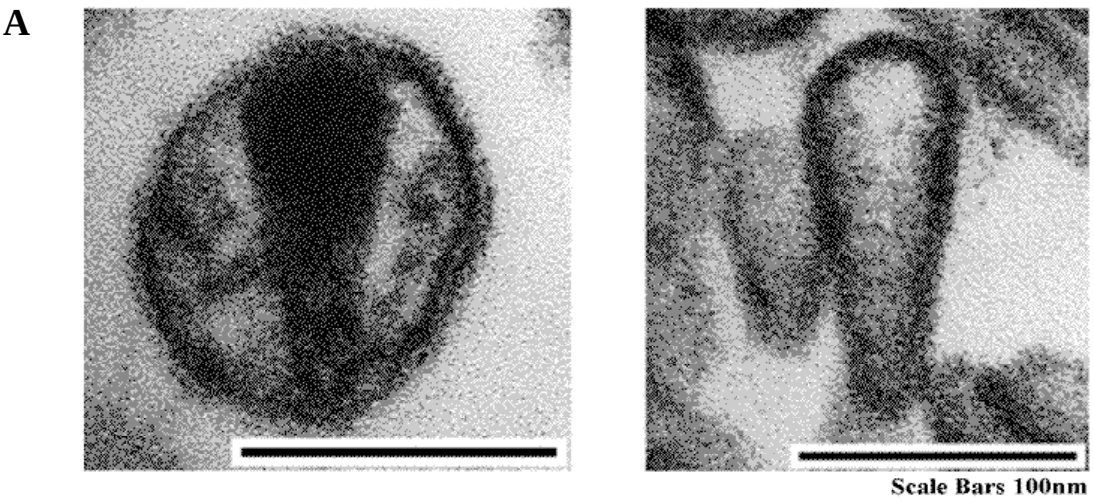

Figure 3. *(A) Transmission electron micrograph of an authentic HIV-1 virion (left) and of an in vitro assembled cone formed by HIV-1 CA-NC in the presence of RNA (right). (B) Hexagonal lattice model of the HIV-1 core. Structures of the CA hexamers are derived from cryo-EM image reconstructions of CA helices formed in vitro. Pentameric defects are indicated in red. (Courtesy of Wesley Sundquist and reprinted with permission from Ganser et al., 1999.)*

the presence of RNA promoted the *in vitro* assembly of both types of structures, an RNA template was not absolutely required, demonstrating that the ability to form tubes and cones is an intrinsic property of CA-p2-NC (Ganser *et al.*, 1999). Based on the regular appearance of the synthetic cones, Sundquist and coworkers propose that retroviral cores are composed of hexagonal lattices that are closed through the incorporation of a total of 12 pentagons (Ganser *et al.*, 1999). The position of the pentameric defects in the lattice would then determine whether the cores are spherical, tubular, or conical. This model allows five discrete cone angles, and all of the predicted angles were indeed observed in synthetic core preparations (Ganser *et al.*, 1999). The majority of the synthetic cores had cone angles of about 19 degrees, as expected if five pentagons were present at the narrow end. Authentic HIV-1 cores have recently been isolated (Accola *et al.*, 2000a; Kotov *et al.*, 1999; Welker *et al.*, 2000), and on average these exhibited a cone angle that was close to the predicted 19 degree value. However, individual cone angles varied considerably, indicating a significant degree of flexibility in the precise configuration of the core.

Reconstructed images of tubes assembled from purified HIV-1 CA show a surface of hexameric rings that are likely formed by the N-terminal domains of CA (Li *et al.*, 2000). These rings appear connected via the C-terminal CA domains, which project towards the interior of the tubes. The images indicate a high degree of structural polymorphism that was attributed to domain movements permitted by the flexible nature of the linker sequence between the two CA domains (Li *et al.*, 2000). This high degree of flexibility facilitated the construction of a model of the HIV-1 core that was obtained by docking CA hexamers into an idealized fullerene cone (Li *et al.*, 2000). This model suggests that the MHR is not involved in critical CA-CA contacts and may thus be available to interact with other components within the core. Another interesting feature of the model is that it suggests a rather open HIV-1 core structure that should be permeable for nucleotide triphosphates, which may allow reverse transcription of the viral genome to proceed within the intact core.

Nucleocapsid

The NC domain, which lies C-terminal to CA, harbors two copies of a CCHC-type zinc finger motif that is present at least once in the NC proteins of all retroviruses, except in those of spumaviruses. Each of the two conserved CCHC motifs in HIV-1 NC coordinates a zinc ion (Bess *et al.*, 1992; Summers *et al.*, 1992; Summers *et al.*, 1990), and both motifs are absolutely essential for virus replication (Aldovini and Young, 1990; Dorfman *et al.*, 1993; Gorelick *et al.*, 1993; Gorelick *et al.*, 1990). The NC domain is essential for the specific packaging of two copies of the genomic viral RNA into assembling particles (Berkowitz *et al.*, 1996). This function of NC involves interactions with stem loops upstream of the *gag* initiation codon, which are required in *cis* for the efficient encapsidation of the unspliced viral RNA. The highly basic NC protein also has a non-specific nucleic acid-binding activity and assists in various annealing reactions during the viral life cycle (Berkowitz *et al.*, 1996). These include the genomic placement of the tRNA primer, the maturation of the dimeric RNA genome into a more compact and thermostable form, and the minus- and plus-strand transfer during reverse transcription (Rein *et al.*, 1998). In the mature virion, NC is found within the core where it coats the viral RNA.

A role for NC in virus assembly was initially suggested by the observation that point mutations that disrupt both CCHC motifs simultaneously can significantly impair HIV-1 particle production in transiently transfected cells (Dorfman *et al.*, 1993). Subsequently, HIV-1 mutants with in-frame deletions in NC were shown to be highly defective for particle assembly (Dawson and Yu, 1998; Zhang and Barklis, 1997). While particle production is not completely blocked in the absence of NC, the small amounts that continue to be released band at a lower density than authentic HIV-1 virions (Sandefur *et al.*, 1998; Zhang and Barklis, 1997). Studies using the yeast two-hybrid system or in vitro binding assays to map Gag-Gag interactions support the view that NC plays a central role in Gag multimerization (Burniston *et al.*, 1999; Franke *et al.*, 1994a). The region of NC that mediates Gag-Gag interactions has been called the interaction (I) domain, and it has been suggested that the I domain mediates the tight packaging of Gag required for the assembly of particles of normal density (Bowzard *et al.*, 1998). However, the precise relationship

between the assembly function of NC and its role in determining virion density is still not clear. Although mutations in NC often affect both particle assembly and virion density, a recent study has revealed that the determinants which govern these two parameters are not identical (Cimarelli and Luban, 2000). There is ample evidence that basic residues within NC are required for I domain function (Bowzard *et al.*, 1998; Cimarelli and Luban, 2000; Cimarelli *et al.*, 2000; Sandefur *et al.*, 2000), but it appears that they are not key determinants for virion density (Cimarelli and Luban, 2000).

The importance of NC basic residues for I domain function suggests that there is a link between the ability of NC to promote particle assembly and its non-specific affinity for RNA. Consistent with this notion, NC and RNA were both required in an *in vitro* Gag-Gag interaction assay (Burniston *et al.*, 1999). If interactions between NC and RNA are critical for retroviral assembly, one would expect that all particles of a given size contain roughly similar amounts of nucleic acid, and this prediction has now been confirmed (Muriaux *et al.*, 2001). Although it has long been known that the presence of packageable genomic viral RNA is not required for efficient particle assembly, Rein and coworkers find that retroviral particles which lack viral RNA instead contain an equivalent amount of cellular mRNA (Muriaux *et al.*, 2001). These observations support a model in which RNA nucleates assembly by serving as a scaffold that concentrates Gag molecules, which then oligomerize via protein-protein interactions.

A central role of Gag-RNA interactions in retroviral morphogenesis is also suggested by the remarkable effects of nucleic acid on particle assembly in vitro. In a ground-breaking study, Campbell and Vogt showed that bacterially produced CA-NC protein can self-assemble in vitro, and that the presence of RNA dramatically increases the efficiency of assembly (Campbell and Vogt, 1995). The structures formed in the presence of nucleic acid are hollow cylinders whose average length is proportional to the length of the input RNA. These cylinders disassembled upon exposure to ribonuclease, indicating that protein-protein interactions alone are not sufficient to maintain the oligomeric complexes (Campbell and Vogt, 1995). RNase A treatment also disrupted the in vitro assembled spherical particles formed by a near full-length HIV-1 Gag protein, suggesting that protein-RNA interactions remain critical even in the presence of all the major Gag-Gag interaction domains (Campbell and Rein, 1999). Together these observations strongly suggested that RNA plays a critical structural role in retrovirus particles, and this was recently confirmed by the finding that immature retroviral particles assembled in vivo are also disrupted by ribonuclease (Muriaux *et al.*, 2001).

In remarkable accordance, the effect of nucleic acid on the *in vitro* assembly of recombinant Gag proteins depends on clusters of basic residues within NC that are also critical for viral particle assembly *in vivo* (Bowzard et al., 1998; Yu et al., 2001). However, the *in vitro* assembly reaction does not depend on the presence of a specific type of nucleic acid. It proceeds with equal efficiency whether or not the RNA that is added to the reaction contains an HIV-1 packaging sequence, indicating that the non-specific RNA binding activity of NC is sufficient to nucleate assembly (Campbell and Rein, 1999). Furthermore, RNA can be replaced by DNA and even by the unrelated polyanion heparin without compromising the efficiency of assembly (Campbell and Rein, 1999; Yu et al., 2001). Collectively, these observations suggest that the polymerization of Gag is initiated by electrostatic interactions between NC basic residues and the phosphate backbone of the viral RNA.

The *in vitro* assembly reaction can be triggered by DNA oligonucleotides, but these need to be of a minimal size in order to work efficiently (Campbell and Rein, 1999; Yu *et al.*, 2001). The size limit suggests that at least two Gag molecules need to be able to bind per molecule of nucleic acid. The primary role of nucleic acid in retroviral assembly may thus be to induce the formation of Gag dimers, which may then be able to form higher order oligomers through protein-protein interactions. Interestingly, the assembly function of NC *in vivo* can be fully replaced by heterologous dimerization domains, and the incorporation of RNA into assembling particles is then no longer necessary (Accola *et al.*, 2000b; Zhang *et al.*, 1998). Furthermore, by replacing NC with a domain that promotes homodimerization in the presence of a cell-permeable synthetic compound we recently obtained direct evidence that Gag dimerization triggers HIV-1 particle assembly *in vivo* (A. Calistri, G. Palu, and H Göttlinger, unpublished). These results strongly support the view that the basic assembly unit is a Gag dimer, and that the critical function of the NC-RNA interaction during assembly is to promote the dimerization of the Gag precursor.

p6

While MA, CA, and NC are common to all retroviruses, the presence of a p6 domain at the C-terminus of the Gag polyprotein is a characteristic feature of HIV-1 and other primate lentiviruses. Within the Gag precursor, the NC and p6 domains are separated by a peptide called p1 (Henderson *et al.*, 1992), whose N-terminus is encoded by a sequence that directs translational frameshifting into the overlapping pol gene (Jacks *et al.*, 1988; Wilson *et al.*, 1988). As a consequence of this arrangement, p6 is the only Gag domain that is absent from the Gag-pol polyprotein. The p6 domain exhibits little if any conventional secondary structure (Stys *et al.*, 1993), and appears to serve primarily as a flexible extension that provides docking sites for cellular factors. A recent study reveals that p6 is the major phosphoprotein in mature HIV-1 virions, but the functional relevance remains to be determined (Muller *et al.*, 2002). The p6 domain is essential for HIV-1 propagation in cell culture, but two thirds of the domain can be deleted from the C-terminus without completely blocking virus replication (Gottlinger *et al.*, 1991). Small deletions in HIV-1 p6 have recently been associated with nonprogressive infection in humans, attesting to the importance of this region for HIV-1 pathogenicity (Alexander *et al.*, 2000).

Among the Gag domains of different subtypes of HIV-1, the p6 domain is by far the most variable, both in length and in sequence. However, a P(T/S)APP motif near the N terminus of p6 and a LXXLF motif near the C-terminus of the domain stick out because they are exceptionally well conserved. The LXXLF motif is essential for the incorporation of the regulatory viral protein Vpr into assembling HIV-1 virions (Kondo and Gottlinger, 1996; Lu *et al.*, 1995). However, this does not fully explain its absolute conservation across all six currently known lineages of primate lentiviruses, because in certain simian immunodeficiency viruses the LXXLF motif is completely dispensable for the incorporation of Vpr or of the related Vpx protein (Accola *et al.*, 1999; Pancio and Ratner, 1998; Wu *et al.*, 1994). It thus appears likely that the LXXLF motif constitutes a binding site for another crucial factor, such as for a host protein that is engaged by all the primate lentiviruses.

The P(T/S)APP motif is conserved among five lineages of primate lentiviruses, the sole exception being a recently described novel lineage that is represented by SIVcol (Courgnaud *et al.*, 2001). Non-primate lentiviruses lack a p6 domain, but except for equine infectious anemia virus (EIAV) nevertheless have a P(T/S)APP motif at an equivalent position of the Gag precursor. In the p6 domains of some strains of HIV-1 or HIV-2, the P(T/S)APP motif is duplicated. Interestingly, a recent study reveals that duplications which involve all or part of the P(T/S)APP motif occur with high frequency in viruses from patients exposed to nucleoside analog reverse transcriptase inhibitors (Peters *et al.*, 2001). While the mechanism by which these duplications apparently contribute to drug resistance is not understood, a gene dosage-like effect may be involved because there is evidence that the P(T/S)APP motif controls the levels of reverse transcriptase that are present in mature virions (Dettenhofer and Yu, 1999; Peters *et al.*, 2001; Yu *et al.*, 1998).

A major function of p6 is to promote the detachment of assembled virions from the cell surface and/or from each other. In an initial study, it was observed that p6 is not required for HIV-1 particle assembly in mammalian cells (Gottlinger *et al.*, 1991). However, the assembled particles did not mature and for the most part remained attached to the plasma membrane via a thin membranous stalk, apparently because a final membrane fusion event needed to sever the stalk did not occur (Gottlinger *et al.*, 1991). This release defect is seen in adherent cell lines and in monocyte-derived macrophages (Demirov *et al.*, 2002b; Gottlinger *et al.*, 1991; Huang *et al.*, 1995). In contrast, in T cell lines and in primary lymphocytes the major defect of p6 mutants appears to be at the level of virion-virion detachment (Demirov *et al.*, 2002b). A defect in virus separation may also account for the observation that HIV-1 particles lacking p6 appear very large as assayed by sucrose gradient sedimentation (Garnier *et al.*, 1999; Garnier *et al.*, 1998b).

Within p6, the major determinant required for the virus detachment function is the conserved P(T/S)APP motif near the N-terminus of the domain (Gottlinger *et al.*, 1991; Huang *et al.*, 1995). Functionally equivalent regions have also been identified in the Gag polyproteins of other retroviruses (Parent *et al.*, 1995; Wills *et al.*, 1994; Xiang *et al.*, 1996; Yasuda and Hunter, 1998; Yuan *et al.*, 1999) and in the matrix proteins of rhabdo- and filoviruses (Craven *et al.*, 1999; Harty *et al.*, 2001; Harty *et al.*,

2000; Harty *et al.*, 1999; Jayakar *et al.*, 2000). These regions are now commonly called late assembly (L) domains (Garnier *et al.*, 1998a). In EIAV, which is unusual for a lentivirus because it lacks a P(T/S)APP motif, a region required for efficient virus release has been mapped to a YxxL motif in the unique C-terminal Gag domain of that virus (Puffer *et al.*, 1997). While the L domains of lentiviruses have a C-terminal position within Gag, those of oncoretroviruses are in the N-terminal half of the Gag precursor and contain a PPxY motif at their core (Xiang *et al.*, 1996; Yasuda and Hunter, 1998; Yuan *et al.*, 1999). Remarkably, although the L domains of HIV-1 and of oncoretroviruses such as RSV are unrelated at the primary sequence level, they are functionally exchangeable (Parent *et al.*, 1995; Yuan *et al.*, 2000). Furthermore, L domains behave like autonomous modules that function largely independent of position (Parent *et al.*, 1995; Yuan *et al.*, 2000), suggesting that they act by recruiting cellular factors to the site of virus assembly.

Retroviruses have long been known to contain free ubiquitin, a highly conserved 76-amino-acid polypeptide that often serves as a protein modifying group (Putterman et al., 1990). Intriguingly, a small fraction of p6 in mature HIV-1 virions is monoubiquitinated, as is the L domain-containing Gag cleavage product of mature Moloney murine leukemia virus (Ott et al., 1998). While the monoubiquitination of HIV-1 p6 is not necessary for efficient virus release or replication (Ott et al., 2000), these observations nevertheless indicate that L domains come into close contact with the enzymatic machinery that attaches ubiquitin to other proteins. This has recently been confirmed by a study which examined the minimal requirements for HIV-1 particle formation (Strack et al., 2000). Strack *et al.* observed that the unrelated L domains of HIV-1 and RSV both induced the ubiquitination of minimal Gag constructs, and that point mutations which abolished L domain activity prevented Gag ubiquitination. The ubiquitination of Gag and the enhancement of particle release were both maximal in the presence of the Ebola virus L domain, which contains overlapping P(T/S)APP and PPxY L domain core motifs (Strack et al., 2000). Since the L domains of RSV or Ebola virus lack lysine residues to which ubiquitin could be attached, these results imply that L domains serve as docking sites for the ubiquitination machinery.

A role for ubiquitin in L domain function is also implied by the finding that inhibitors of the proteasome interfere with the budding of both retroviruses and rhabdoviruses at a late stage, similar to what is seen in the absence of an L domain (Harty *et al.*, 2001; Patnaik *et al.*, 2000; Schubert *et al.*, 2000; Strack *et al.*, 2000). It is thought that this effect is indirect and results from the depletion of free ubiquitin levels in the absence of proteasome activity. Consistent with this notion, proteasome inhibitors reduce the ubiquitination of Gag (Strack *et al.*, 2000), and particle production can be rescued if extra free ubiquitin is provided (Patnaik *et al.*, 2000). Taken together, these findings strongly suggest that L domains engage the cellular ubiquitination machinery to promote the release of a diverse group of enveloped viruses. However, whether the functionally relevant target for ubiquitination is a viral or a cellular component at the site of assembly remains unknown.

Accumulating evidence suggests that L domains co-opt components of the endocytic machinery of the host cell to facilitate virus exocytosis. For instance, the critical YxxL motif in the L domain of EIAV has been reported to interact with adaptor complexes that regulate clathrin-mediated endocytosis (Puffer et al., 1998). Furthermore, it has recently become clear that tagging with ubiquitin is involved in the regulated sorting of proteins throughout the endosomal system (Hicke, 1999; Hicke, 2001). L domains with a PPxY motif interact *in vitro* and *in vivo* with WW domains of the E3 ubiquitin protein ligase Nedd4 (Harty et al., 2001; Harty et al., 2000; Harty et al., 1999; Kikonyogo et al., 2001; Pirozzi et al., 1997), which has a well-established role in ubiquitin-mediated endocytosis (Rotin et al., 2000). Leis and coworkers have recently shown that the WW domain region of one Nedd4 family member designated LDI-1 prevented RSV Gag budding in a dominant negative manner, supporting the view that a Nedd4-type ubiquitin ligase is involved in L domain function (Kikonyogo et al., 2001). However, in another study full-length forms of Nedd4 or LDI-1 with cysteine-to-alanine substitutions at the active site did not interfere with RSV L domain-mediated budding (Strack et al., 2000).

In the case of HIV-1 p6, yeast two-hybrid screens performed by two laboratories have yielded Tsg101, which functions in late endosomal trafficking (Garrus *et al.*, 2001; VerPlank *et al.*, 2001). Tsg101 is an apparently inactive homolog of E2 ubiquitin-conjugating enzymes that is required for the recognition and sorting of ubiquitinated cargo into vesicles that bud into multivesicular bodies/late

endosomes (Babst *et al.*, 2000; Bishop and Woodman, 2001). Intriguingly, the binding of Tsg101 to HIV-1 p6 depends on the P(T/S)APP L domain core motif and is enhanced if p6 is modified by ubiquitin (Garrus *et al.*, 2001; VerPlank *et al.*, 2001). Also, there is a perfect correlation between the effects of single amino acid substitutions in p6 on Tsg101 binding and on infectious virus release (Martin-Serrano *et al.*, 2001). Furthermore, HIV-1 budding is arrested at a late stage if cellular Tsg101 is depleted, and is rescued if Tsg101 expression is restored (Garrus *et al.*, 2001). In contrast, Tsg101 is not required for the release of MLV, which contains a PPxY L domain core motif (Garrus *et al.*, 2001). Remarkably, the HIV-1 L domain became dispensable when Tsg101 was fused directly to Gag, and only a C-terminal portion of Tsg101 was necessary in that context (Martin-Serrano *et al.*, 2001). It is known that this C-terminal region of Tsg101 interacts with human Vps28, another component of a 350 kDa endosomal sorting complex called ESCRT-1 that recognizes ubiquitinated cargo via the N-terminal ubiquitin-binding domain of Tsg101 (Bishop and Woodman, 2001; Katzmann *et al.*, 2001). Thus, Tsg101 may serve to recruit ESCRT-1 to the site of virus assembly, consistent with the recent observation that overexpression of the N-terminal E2-like domain of Tsg101 inhibits HIV-1 budding in an L domain-dependent manner (Demirov *et al.*, 2002a). Taken together, the picture that emerges is that the HIV-1 L domain makes use of the cellular machinery which controls the inward budding of vesicles into late endosomes, a process that is topologically equivalent to virus budding at the plasma membrane (see Figure 4).

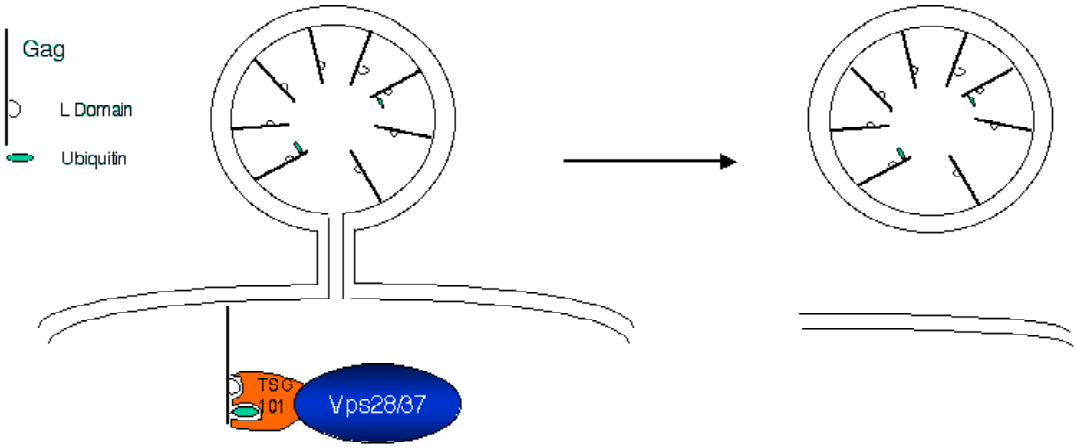

Figure 4. *Model for the function of the HIV-1 late assembly (L) domain. Recent studies indicate that the P(T/S)APP L domain core motif in p6 and Gag-bound ubiquitin cooperate to recruit Tsg101 via its ubiquitin binding domain. Tsg101 is a component of a complex called ESCRT-I that is essential for the invagination and budding of vesicles into the lumen of late endosomes.*

Conclusion

Considerable headway has been made in elucidating how Gag interacts with itself and with other viral components during assembly. The three-dimensional structures of individual Gag domains are now available, and the structural principles for the organization of the viral capsid are beginning to emerge. The development of *in vitro* assembly systems has helped to clarify the structural role of RNA and the importance of conformational switch mechanisms during virus morphogenesis. Some of these conformational transitions are likely to be modulated by cellular factors, as it is becoming clear that human cells contain essential cofactors for HIV-1 assembly (Bieniasz and Cullen, 2000; Mariani *et al.*, 2001; Mariani *et al.*, 2000). A recent flurry of exciting observations implicates specific cellular factors in virus detachment and infectivity, and it is hoped that these novel insights will lead to the development of small molecules with broad activity against HIV-1 and other enveloped RNA viruses.

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References

Accola, M.A., Bukovsky, A.A., Jones, M.S. and Gottlinger, H.G. (1999) A conserved dileucine-containing motif in p6(gag) governs the particle association of Vpx and Vpr of simian immunodeficiency viruses SIV(mac) and SIV(agm). *J Virol*, **73**, 9992-9999.

Accola, M.A., Hoglund, S. and Gottlinger, H.G. (1998) A putative alpha-helical structure which overlaps the capsid-p2 boundary in the human immunodeficiency virus type 1 Gag precursor is crucial for viral particle assembly. *J Virol*, **72**, 2072-2078.

Accola, M.A., Ohagen, A. and Gottlinger, H.G. (2000a) Isolation of human immunodeficiency virus type 1 cores: retention of Vpr in the absence of p6(gag). *J Virol*, **74**, 6198-6202.

Accola, M.A., Strack, B. and Gottlinger, H.G. (2000b) Efficient particle production by minimal gag constructs which retain the carboxy-terminal domain of human immunodeficiency virus type 1 capsid-p2 and a late assembly domain. *J Virol*, **74**, 5395-5402.

Aldovini, A. and Young, R.A. (1990) Mutations of RNA and protein sequences involved in human immunodeficiency virus type 1 packaging result in production of noninfectious virus. *J Virol*, **64**, 1920-1926.

Alexander, L., Weiskopf, E., Greenough, T.C., Gaddis, N.C., Auerbach, M.R., Malim, M.H., O'Brien, S.J., Walker, B.D., Sullivan, J.L. and Desrosiers, R.C. (2000) Unusual polymorphisms in human immunodeficiency virus type 1 associated with nonprogressive infection. *J Virol*, **74**, 4361-4376.

Alin, K. and Goff, S.P. (1996) Amino acid substitutions in the CA protein of Moloney murine leukemia virus that block early events in infection. *Virology*, **222**, 339-351.

Ames, J.B., Ishima, R., Tanaka, T., Gordon, J.I., Stryer, L. and Ikura, M. (1997) Molecular mechanics of calcium-myristoyl switches. *Nature*, **389**, 198-202.

Ames, J.B., Tanaka, T., Stryer, L. and Ikura, M. (1996) Portrait of a myristoyl switch protein. *Curr Opin Struct Biol*, **6**, 432-438.

Babst, M., Odorizzi, G., Estepa, E.J. and Emr, S.D. (2000) Mammalian tumor susceptibility gene 101

(TSG101) and the yeast homologue, Vps23p, both function in late endosomal trafficking. *Traffic*, **1**, 248-258.

Berkowitz, R., Fisher, J. and Goff, S.P. (1996) RNA packaging. In Krausslich, H.G. (ed.), *Current topics in microbiology and immunology: RNA packaging*. Springer Verlag, Berlin, Germany, Vol. 214, pp. 177-218.

Bess, J.W., Jr., Powell, P.J., Issaq, H.J., Schumack, L.J., Grimes, M.K., Henderson, L.E. and Arthur, L.O. (1992) Tightly bound zinc in human immunodeficiency virus type 1, human T-cell leukemia virus type I, and other retroviruses. *J Virol*, **66**, 840-847.

Bieniasz, P.D. and Cullen, B.R. (2000) Multiple blocks to human immunodeficiency virus type 1 replication in rodent cells. *J Virol*, **74**, 9868-9877.

Billich, A., Hammerschmid, F., Peichl, P., Wenger, R., Zenke, G., Quesniaux, V. and Rosenwirth, B. (1995) Mode of action of SDZ NIM 811, a nonimmunosuppressive cyclosporin A analog with activity against human immunodeficiency virus (HIV) type 1: interference with HIV protein-cyclophilin A interactions. *J Virol*, **69**, 2451-2461.

Bishop, N. and Woodman, P. (2001) TSG101/mammalian VPS23 and mammalian VPS28 interact directly and are recruited to VPS4-induced endosomes. *J Biol Chem*, **276**, 11735-11742.

Blenis, J. and Resh, M.D. (1993) Subcellular localization specified by protein acylation and phosphorylation. *Curr Opin Cell Biol*, **5**, 984-989.

Borsetti, A., Ohagen, A. and Gottlinger, H.G. (1998) The C-terminal half of the human immunodeficiency virus type 1 Gag precursor is sufficient for efficient particle assembly. *J Virol*, **72**, 9313-9317.

Bowzard, J.B., Bennett, R.P., Krishna, N.K., Ernst, S.M., Rein, A. and Wills, J.W. (1998) Importance of basic residues in the nucleocapsid sequence for retrovirus Gag assembly and complementation rescue. *J Virol*, **72**, 9034-9044.

Braaten, D., Franke, E.K. and Luban, J. (1996a) Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription. *J Virol*, **70**, 3551-3560.

Braaten, D., Franke, E.K. and Luban, J. (1996b) Cyclophilin A is required for the replication of group M human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus SIV(CPZ)GAB but not group O HIV-1 or other primate immunodeficiency viruses. *J Virol*, **70**, 4220-4227.

Braaten, D. and Luban, J. (2001) Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T cells. *Embo J*, **20**, 1300-1309.

Brown, D.A. and London, E. (1998) Functions of lipid rafts in biological membranes. *Annu Rev Cell Dev Biol*, **14**, 111-136.

Bryant, M. and Ratner, L. (1990) Myristoylation-dependent replication and assembly of human immunodeficiency virus 1. *Proc Natl Acad Sci U S A*, **87**, 523-527.

Buckland, A.G. and Wilton, D.C. (2000) Anionic phospholipids, interfacial binding and the regulation of cell functions. *Biochim Biophys Acta*, **1483**, 199-216.

Bukovsky, A.A., Weimann, A., Accola, M.A. and Gottlinger, H.G. (1997) Transfer of the HIV-1 cyclophilin-binding site to simian immunodeficiency virus from Macaca mulatta can confer both cyclosporin sensitivity and cyclosporin dependence. *Proc Natl Acad Sci U S A*, **94**, 10943-10948.

Bukrinsky, M.I., Haggerty, S., Dempsey, M.P., Sharova, N., Adzhubel, A., Spitz, L., Lewis, P., Goldfarb, D., Emerman, M. and Stevenson, M. (1993a) A nuclear localization signal within HIV-1 matrix protein that governs infection of non-dividing cells. *Nature*, **365**, 666-669.

Bukrinsky, M.I., Sharova, N., Dempsey, M.P., Stanwick, T.L., Bukrinskaya, A.G., Haggerty, S. and Stevenson, M. (1992) Active nuclear import of human immunodeficiency virus type 1 preintegration complexes. *Proc Natl Acad Sci U S A*, **89**, 6580-6584.

Bukrinsky, M.I., Sharova, N., McDonald, T.L., Pushkarskaya, T., Tarpley, W.G. and Stevenson, M. (1993b) Association of integrase, matrix, and reverse transcriptase antigens of human immunodeficiency virus type 1 with viral nucleic acids following acute infection. *Proc Natl Acad Sci U S A*, **90**, 6125-6129.

Burniston, M.T., Cimarelli, A., Colgan, J., Curtis, S.P. and Luban, J. (1999) Human immunodeficiency virus type 1 Gag polyprotein multimerization requires the nucleocapsid domain and RNA and is promoted by the capsid- dimer interface and the basic region of matrix protein. *J Virol*, **73**, 8527-8540.

Cairns, T.M. and Craven, R.C. (2001) Viral DNA synthesis defects in assembly-competent Rous sarcoma virus CA mutants. *J Virol*, **75**, 242-250.

Callahan, E.M. and Wills, J.W. (2000) Repositioning basic residues in the M domain of the Rous sarcoma virus gag protein. *J Virol*, **74**, 11222-11229.

Campbell, S., Fisher, R.J., Towler, E.M., Fox, S., Issaq, H.J., Wolfe, T., Phillips, L.R. and Rein, A. (2001) Modulation of HIV-like particle assembly in vitro by inositol phosphates. *Proc Natl Acad Sci U S A*, **98**, 10875-10879.

Campbell, S. and Rein, A. (1999) In vitro assembly properties of human immunodeficiency virus type 1 Gag protein lacking the p6 domain. *J Virol*, **73**, 2270-2279.

Campbell, S. and Vogt, V.M. (1995) Self-assembly in vitro of purified CA-NC proteins from Rous sarcoma virus and human immunodeficiency virus type 1. *J Virol*, **69**, 6487-6497.

Cannon, P.M., Matthews, S., Clark, N., Byles, E.D., Iourin, O., Hockley, D.J., Kingsman, S.M. and Kingsman, A.J. (1997) Structure-function studies of the human immunodeficiency virus type 1 matrix protein, p17. *J Virol*, **71**, 3474-3483.

Chen, B.K., Rousso, I., Shim, S. and Kim, P.S. (2001) Efficient assembly of an HIV-1/MLV Gag-chimeric virus in murine cells. *Proc Natl Acad Sci U S A*, **98**, 15239-15244.

Christensen, A.M., Massiah, M.A., Turner, B.G., Sundquist, W.I. and Summers, M.F. (1996) Three-dimensional structure of the HTLV-II matrix protein and comparative analysis of matrix proteins from the different classes of pathogenic human retroviruses. *J Mol Biol*, **264**, 1117-1131.

Cimarelli, A. and Luban, J. (2000) Human immunodeficiency virus type 1 virion density is not determined by nucleocapsid basic residues. *J Virol*, **74**, 6734-6740.

Cimarelli, A., Sandin, S., Hoglund, S. and Luban, J. (2000) Basic residues in human immunodeficiency virus type 1 nucleocapsid promote virion assembly via interaction with RNA. *J Virol*, **74**, 3046-3057.

Colgan, J., Yuan, H.E., Franke, E.K. and Luban, J. (1996) Binding of the human immunodeficiency virus type 1 Gag polyprotein to cyclophilin A is mediated by the central region of capsid and requires Gag dimerization. *J Virol*, **70**, 4299-4310.

Conte, M.R., Klikova, M., Hunter, E., Ruml, T. and Matthews, S. (1997) The three-dimensional solution structure of the matrix protein from the type D retrovirus, the Mason-Pfizer monkey virus, and implications for the morphology of retroviral assembly. *Embo J*, **16**, 5819-5826.

Cosson, P. (1996) Direct interaction between the envelope and matrix proteins of HIV-1. *Embo J*, **15**, 5783-5788.

Courgnaud, V., Pourrut, X., Bibollet-Ruche, F., Mpoudi-Ngole, E., Bourgeois, A., Delaporte, E. and

Peeters, M. (2001) Characterization of a novel simian immunodeficiency virus from guereza colobus monkeys (Colobus guereza) in Cameroon: a new lineage in the nonhuman primate lentivirus family. *J Virol*, **75**, 857-866.

Craven, R.C., Harty, R.N., Paragas, J., Palese, P. and Wills, J.W. (1999) Late domain function identified in the vesicular stomatitis virus M protein by use of rhabdovirus-retrovirus chimeras. *J Virol*, **73**, 3359-3365.

Craven, R.C., Leure-duPree, A.E., Weldon, R.A., Jr. and Wills, J.W. (1995) Genetic analysis of the major homology region of the Rous sarcoma virus Gag protein. *J Virol*, **69**, 4213-4227.

Dawson, L. and Yu, X.F. (1998) The role of nucleocapsid of HIV-1 in virus assembly. *Virology*, **251**, 141-157.

Demirov, D.G., Ono, A., Orenstein, J.M. and Freed, E.O. (2002a) Overexpression of the N-terminal domain of TSG101 inhibits HIV-1 budding by blocking late domain function. *Proc Natl Acad Sci U S A*, **99**, 955-960.

Demirov, D.G., Orenstein, J.M. and Freed, E.O. (2002b) The late domain of human immunodeficiency virus type 1 p6 promotes virus release in a cell type-dependent manner. *J Virol*, **76**, 105-117.

Dettenhofer, M. and Yu, X.F. (1999) Proline residues in human immunodeficiency virus type 1 p6(Gag) exert a cell type-dependent effect on viral replication and virion incorporation of Pol proteins. *J Virol*, **73**, 4696-4704.

Dorfman, T., Bukovsky, A., Ohagen, A., Hoglund, S. and Gottlinger, H.G. (1994a) Functional domains of the capsid protein of human immunodeficiency virus type 1. *J Virol*, **68**, 8180-8187.

Dorfman, T., Luban, J., Goff, S.P., Haseltine, W.A. and Gottlinger, H.G. (1993) Mapping of functionally important residues of a cysteine-histidine box in the human immunodeficiency virus type 1 nucleocapsid protein. *J Virol*, **67**, 6159-6169.

Dorfman, T., Mammano, F., Haseltine, W.A. and Gottlinger, H.G. (1994b) Role of the matrix protein in the virion association of the human immunodeficiency virus type 1 envelope glycoprotein. *J Virol*, **68**, 1689-1696.

Dupont, S., Sharova, N., DeHoratius, C., Virbasius, C.M., Zhu, X., Bukrinskaya, A.G., Stevenson, M. and Green, M.R. (1999) A novel nuclear export activity in HIV-1 matrix protein required for viral replication. *Nature*, **402**, 681-685.

Egan, M.A., Carruth, L.M., Rowell, J.F., Yu, X. and Siliciano, R.F. (1996) Human immunodeficiency virus type 1 envelope protein endocytosis mediated by a highly conserved intrinsic internalization signal in the cytoplasmic domain of gp41 is suppressed in the presence of the Pr55gag precursor protein. *J Virol*, **70**, 6547-6556.

Facke, M., Janetzko, A., Shoeman, R.L. and Krausslich, H.G. (1993) A large deletion in the matrix domain of the human immunodeficiency virus gag gene redirects virus particle assembly from the plasma membrane to the endoplasmic reticulum. *J Virol*, **67**, 4972-4980.

Fouchier, R.A., Meyer, B.E., Simon, J.H., Fischer, U. and Malim, M.H. (1997) HIV-1 infection of non-dividing cells: evidence that the amino-terminal basic region of the viral matrix protein is important for Gag processing but not for post-entry nuclear import. *Embo J*, **16**, 4531-4539.

Franke, E.K., Yuan, H.E., Bossolt, K.L., Goff, S.P. and Luban, J. (1994a) Specificity and sequence requirements for interactions between various retroviral Gag proteins. *J Virol*, **68**, 5300-5305.

Franke, E.K., Yuan, H.E. and Luban, J. (1994b) Specific incorporation of cyclophilin A into HIV-1 virions. *Nature*, **372**, 359-362.

Freed, E.O. (1998) HIV-1 gag proteins: diverse functions in the virus life cycle. *Virology*, **251**, 1-15.

Freed, E.O., Englund, G. and Martin, M.A. (1995) Role of the basic domain of human immunodeficiency virus type 1 matrix in macrophage infection. *J Virol*, **69**, 3949-3954.

Freed, E.O. and Martin, M.A. (1995) Virion incorporation of envelope glycoproteins with long but not short cytoplasmic tails is blocked by specific, single amino acid substitutions in the human immunodeficiency virus type 1 matrix. *J Virol*, **69**, 1984-1989.

Freed, E.O., Orenstein, J.M., Buckler-White, A.J. and Martin, M.A. (1994) Single amino acid changes in the human immunodeficiency virus type 1 matrix protein block virus particle production. *J Virol*, **68**, 5311-5320.

Fuller, S.D., Wilk, T., Gowen, B.E., Krausslich, H.G. and Vogt, V.M. (1997) Cryo-electron microscopy reveals ordered domains in the immature HIV-1 particle. *Curr Biol*, **7**, 729-738.

Gallay, P., Swingler, S., Aiken, C. and Trono, D. (1995) HIV-1 infection of nondividing cells: C-terminal tyrosine phosphorylation of the viral matrix protein is a key regulator. *Cell*, **80**, 379-388.

Gamble, T.R., Vajdos, F.F., Yoo, S., Worthylake, D.K., Houseweart, M., Sundquist, W.I. and Hill, C.P. (1996) Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. *Cell*, **87**, 1285-1294.

Gamble, T.R., Yoo, S., Vajdos, F.F., von Schwedler, U.K., Worthylake, D.K., Wang, H., McCutcheon, J.P., Sundquist, W.I. and Hill, C.P. (1997) Structure of the carboxyl-terminal dimerization domain of the HIV-1 capsid protein. *Science*, **278**, 849-853.

Ganser, B.K., Li, S., Klishko, V.Y., Finch, J.T. and Sundquist, W.I. (1999) Assembly and analysis of conical models for the HIV-1 core. *Science*, **283**, 80-83.

Garnier, L., Bowzard, J.B. and Wills, J.W. (1998a) Recent advances and remaining problems in HIV assembly. *Aids*, **12**, S5-16.

Garnier, L., Parent, L.J., Rovinski, B., Cao, S.X. and Wills, J.W. (1999) Identification of retroviral late domains as determinants of particle size. *J Virol*, **73**, 2309-2320.

Garnier, L., Ratner, L., Rovinski, B., Cao, S.X. and Wills, J.W. (1998b) Particle size determinants in the human immunodeficiency virus type 1 Gag protein. *J Virol*, **72**, 4667-4677.

Garrus, J.E., von Schwedler, U.K., Pornillos, O.W., Morham, S.G., Zavitz, K.H., Wang, H.E., Wettstein, D.A., Stray, K.M., Cote, M., Rich, R.L., Myszka, D.G. and Sundquist, W.I. (2001) Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell*, **107**, 55-65.

Gelderblom, H.R. (1991) Assembly and morphology of HIV: potential effect of structure on viral function. *Aids*, **5**, 617-637.

Gheysen, D., Jacobs, E., de Foresta, F., Thiriart, C., Francotte, M., Thines, D. and De Wilde, M. (1989) Assembly and release of HIV-1 precursor Pr55gag virus-like particles from recombinant baculovirus-infected insect cells. *Cell*, **59**, 103-112.

Gitti, R.K., Lee, B.M., Walker, J., Summers, M.F., Yoo, S. and Sundquist, W.I. (1996) Structure of the amino-terminal core domain of the HIV-1 capsid protein. *Science*, **273**, 231-235.

Goldberg, J. (1998) Structural basis for activation of ARF GTPase: mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. *Cell*, **95**, 237-248.

Gorelick, R.J., Chabot, D.J., Rein, A., Henderson, L.E. and Arthur, L.O. (1993) The two zinc fingers in the human immunodeficiency virus type 1 nucleocapsid protein are not functionally equivalent. *J Virol*, **67**, 4027-4036.

Gorelick, R.J., Nigida, S.M., Jr., Bess, J.W., Jr., Arthur, L.O., Henderson, L.E. and Rein, A. (1990) Noninfectious human immunodeficiency virus type 1 mutants deficient in genomic RNA. *J Virol*, **64**, 3207-3211.

Gottlinger, H.G., Dorfman, T., Sodroski, J.G. and Haseltine, W.A. (1991) Effect of mutations affecting the p6 gag protein on human immunodeficiency virus particle release. *Proc Natl Acad Sci U S A*, **88**, 3195-3199.

Gottlinger, H.G., Sodroski, J.G. and Haseltine, W.A. (1989) Role of capsid precursor processing and myristoylation in morphogenesis and infectivity of human immunodeficiency virus type 1. *Proc Natl Acad Sci U S A*, **86**, 5781-5785.

Grattinger, M., Hohenberg, H., Thomas, D., Wilk, T., Muller, B. and Krausslich, H.G. (1999) In vitro assembly properties of wild-type and cyclophilin-binding defective human immunodeficiency virus capsid proteins in the presence and absence of cyclophilin A. *Virology*, **257**, 247-260.

Gross, I., Hohenberg, H., Huckhagel, C. and Krausslich, H.G. (1998) N-Terminal extension of human immunodeficiency virus capsid protein converts the in vitro assembly phenotype from tubular to spherical particles. *J Virol*, **72**, 4798-4810.

Gross, I., Hohenberg, H. and Krausslich, H.G. (1997) In vitro assembly properties of purified bacterially expressed capsid proteins of human immunodeficiency virus. *Eur J Biochem*, **249**, 592-600.

Gross, I., Hohenberg, H., Wilk, T., Wiegers, K., Grattinger, M., Muller, B., Fuller, S. and Krausslich, H.G. (2000) A conformational switch controlling HIV-1 morphogenesis. *Embo J*, **19**, 103-113.

Handschumacher, R.E., Harding, M.W., Rice, J., Drugge, R.J. and Speicher, D.W. (1984) Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science*, **226**, 544-547.

Harty, R.N., Brown, M.E., McGettigan, J.P., Wang, G., Jayakar, H.R., Huibregtse, J.M., Whitt, M.A. and Schnell, M.J. (2001) Rhabdoviruses and the cellular ubiquitin-proteasome system: a budding interaction. *J Virol*, **75**, 10623-10629.

Harty, R.N., Brown, M.E., Wang, G., Huibregtse, J. and Hayes, F.P. (2000) A PPxY motif within the VP40 protein of Ebola virus interacts physically and functionally with a ubiquitin ligase: implications for filovirus budding. *Proc Natl Acad Sci U S A*, **97**, 13871-13876.

Harty, R.N., Paragas, J., Sudol, M. and Palese, P. (1999) A proline-rich motif within the matrix protein of vesicular stomatitis virus and rabies virus interacts with WW domains of cellular proteins: implications for viral budding. *J Virol*, **73**, 2921-2929.

Heinzinger, N.K., Bukinsky, M.I., Haggerty, S.A., Ragland, A.M., Kewalramani, V., Lee, M.A., Gendelman, H.E., Ratner, L., Stevenson, M. and Emerman, M. (1994) The Vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. *Proc Natl Acad Sci U S A*, **91**, 7311-7315.

Henderson, L.E., Bowers, M.A., Sowder, R.C., 2nd, Serabyn, S.A., Johnson, D.G., Bess, J.W., Jr., Arthur, L.O., Bryant, D.K. and Fenselau, C. (1992) Gag proteins of the highly replicative MN strain of human immunodeficiency virus type 1: posttranslational modifications, proteolytic processings, and complete amino acid sequences. *J Virol*, **66**, 1856-1865.

Hicke, L. (1999) Gettin' down with ubiquitin: turning off cell-surface receptors, transporters and channels. *Trends Cell Biol*, **9**, 107-112.

Hicke, L. (2001) A new ticket for entry into budding vesicles-ubiquitin. *Cell*, **106**, 527-530.

Hill, C.P., Worthylake, D., Bancroft, D.P., Christensen, A.M. and Sundquist, W.I. (1996) Crystal structures of the trimeric human immunodeficiency virus type 1 matrix protein: implications for membrane association and assembly. *Proc Natl Acad Sci U S A*, **93**, 3099-3104.

Huang, M., Orenstein, J.M., Martin, M.A. and Freed, E.O. (1995) p6Gag is required for particle production from full-length human immunodeficiency virus type 1 molecular clones expressing protease. *J Virol*, **69**, 6810-6818.

Hunter, E. (1994) Macromolecular interactions in the assembly of HIV and other retroviruses. *Semin Virol*, **5**, 71-83.

Jacks, T., Power, M.D., Masiarz, F.R., Luciw, P.A., Barr, P.J. and Varmus, H.E. (1988) Characterization of ribosomal frameshifting in HIV-1 gag-pol expression. *Nature*, **331**, 280-283.

Jayakar, H.R., Murti, K.G. and Whitt, M.A. (2000) Mutations in the PPPY motif of vesicular stomatitis virus matrix protein reduce virus budding by inhibiting a late step in virion release [In Process Citation]. *J Virol*, **74**, 9818-9827.

Katzmann, D.J., Babst, M. and Emr, S.D. (2001) Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell*, **106**, 145-155.

Kiernan, R.E., Ono, A., Englund, G. and Freed, E.O. (1998) Role of matrix in an early postentry step in the human immunodeficiency virus type 1 life cycle. *J Virol*, **72**, 4116-4126.

Kikonyogo, A., Bouamr, F., Vana, M.L., Xiang, Y., Aiyar, A., Carter, C. and Leis, J. (2001) Proteins related to the Nedd4 family of ubiquitin protein ligases interact with the L domain of Rous sarcoma virus and are required for gag budding from cells. *Proc Natl Acad Sci U S A*, **98**, 11199-11204.

Kohl, N.E., Emini, E.A., Schleif, W.A., Davis, L.J., Heimbach, J.C., Dixon, R.A., Scolnick, E.M. and Sigal, I.S. (1988) Active human immunodeficiency virus protease is required for viral infectivity. *Proc Natl Acad Sci U S A*, **85**, 4686-4690.

Kondo, E. and Gottlinger, H.G. (1996) A conserved LXXLF sequence is the major determinant in p6gag required for the incorporation of human immunodeficiency virus type 1 Vpr. *J Virol*, **70**, 159-164.

Kotov, A., Zhou, J., Flicker, P. and Aiken, C. (1999) Association of Nef with the human immunodeficiency virus type 1 core. *J Virol*, **73**, 8824-8830.

Krausslich, H.G., Facke, M., Heuser, A.M., Konvalinka, J. and Zentgraf, H. (1995) The spacer peptide between human immunodeficiency virus capsid and nucleocapsid proteins is essential for ordered assembly and viral infectivity. *J Virol*, **69**, 3407-3419.

Lee, P.P. and Linial, M.L. (1994) Efficient particle formation can occur if the matrix domain of human immunodeficiency virus type 1 Gag is substituted by a myristylation signal. *J Virol*, **68**, 6644-6654.

Lee, Y.M., Liu, B. and Yu, X.F. (1999) Formation of virus assembly intermediate complexes in the cytoplasm by wild-type and assembly-defective mutant human immunodeficiency virus type 1 and their association with membranes. *J Virol*, **73**, 5654-5662.

Lee, Y.M. and Yu, X.F. (1998) Identification and characterization of virus assembly intermediate complexes in HIV-1-infected CD4+ T cells. *Virology*, **243**, 78-93.

Lewis, P., Hensel, M. and Emerman, M. (1992) Human immunodeficiency virus infection of cells arrested in the cell cycle. *Embo J*, **11**, 3053-3058.

Li, S., Hill, C.P., Sundquist, W.I. and Finch, J.T. (2000) Image reconstructions of helical assemblies of the HIV-1 CA protein. *Nature*, **407**, 409-413.

Lindwasser, O.W. and Resh, M.D. (2001) Multimerization of human immunodeficiency virus type 1 Gag promotes its localization to barges, raft-like membrane microdomains. *J Virol*, **75**, 7913-7924.

Lingappa, J.R., Hill, R.L., Wong, M.L. and Hegde, R.S. (1997) A multistep, ATP-dependent pathway for assembly of human immunodeficiency virus capsids in a cell-free system. *J Cell Biol*, **136**, 567-581.

Lodge, R., Gottlinger, H., Gabuzda, D., Cohen, E.A. and Lemay, G. (1994) The intracytoplasmic domain of gp41 mediates polarized budding of human immunodeficiency virus type 1 in MDCK cells. *J Virol*, **68**, 4857-4861.

Lodge, R., Lalonde, J.P., Lemay, G. and Cohen, E.A. (1997) The membrane-proximal intracytoplasmic tyrosine residue of HIV-1 envelope glycoprotein is critical for basolateral targeting of viral budding in MDCK cells. *Embo J*, **16**, 695-705.

Lu, Y.L., Bennett, R.P., Wills, J.W., Gorelick, R. and Ratner, L. (1995) A leucine triplet repeat sequence (LXX)4 in p6gag is important for Vpr incorporation into human immunodeficiency virus type 1 particles. *J Virol*, **69**, 6873-6879.

Luban, J., Bossolt, K.L., Franke, E.K., Kalpana, G.V. and Goff, S.P. (1993) Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell*, **73**, 1067-1078.

Mammano, F., Kondo, E., Sodroski, J., Bukovsky, A. and Gottlinger, H.G. (1995) Rescue of human immunodeficiency virus type 1 matrix protein mutants by envelope glycoproteins with short cytoplasmic domains. *J Virol*, **69**, 3824-3830.

Mammano, F., Ohagen, A., Hoglund, S. and Gottlinger, H.G. (1994) Role of the major homology region of human immunodeficiency virus type 1 in virion morphogenesis. *J Virol*, **68**, 4927-4936.

Mammano, F., Salvatori, F., Indraccolo, S., De Rossi, A., Chieco-Bianchi, L. and Gottlinger, H.G. (1997) Truncation of the human immunodeficiency virus type 1 envelope glycoprotein allows efficient pseudotyping of Moloney murine leukemia virus particles and gene transfer into CD4+ cells. *J Virol*, **71**, 3341-3345.

Mariani, R., Rasala, B.A., Rutter, G., Wiegers, K., Brandt, S.M., Krausslich, H.G. and Landau, N.R. (2001) Mouse-human heterokaryons support efficient human immunodeficiency virus type 1 assembly. *J Virol*, **75**, 3141-3151.

Mariani, R., Rutter, G., Harris, M.E., Hope, T.J., Krausslich, H.G. and Landau, N.R. (2000) A block to human immunodeficiency virus type 1 assembly in murine cells. *J Virol*, **74**, 3859-3870.

Martin-Serrano, J., Zang, T. and Bieniasz, P.D. (2001) HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nat Med*, **7**, 1313-1319.

Matthews, S., Barlow, P., Clark, N., Kingsman, S., Kingsman, A. and Campbell, I. (1995) Refined solution structure of p17, the HIV matrix protein. *Biochem Soc Trans*, **23**, 725-729.

Matthews, S., Mikhailov, M., Burny, A. and Roy, P. (1996) The solution structure of the bovine leukaemia virus matrix protein and similarity with lentiviral matrix proteins. *Embo J*, **15**, 3267-3274.

McDermott, J., Farrell, L., Ross, R. and Barklis, E. (1996) Structural analysis of human immunodeficiency virus type 1 Gag protein interactions, using cysteine-specific reagents. *J Virol*, **70**, 5106-5114.

McDonnell, J.M., Fushman, D., Cahill, S.M., Zhou, W., Wolven, A., Wilson, C.B., Nelle, T.D., Resh, M.D., Wills, J. and Cowburn, D. (1998) Solution structure and dynamics of the bioactive retroviral M domain from Rous sarcoma virus. *J Mol Biol*, **279**, 921-928.

Mervis, R.J., Ahmad, N., Lillehoj, E.P., Raum, M.G., Salazar, F.H., Chan, H.W. and Venkatesan, S. (1988) The gag gene products of human immunodeficiency virus type 1: alignment within the gag open reading frame, identification of posttranslational modifications, and evidence for alternative gag precursors. *J Virol*, **62**, 3993-4002.

Miller, M.D., Farnet, C.M. and Bushman, F.D. (1997) Human immunodeficiency virus type 1 preintegration complexes: studies of organization and composition. *J Virol*, **71**, 5382-5390.

Momany, C., Kovari, L.C., Prongay, A.J., Keller, W., Gitti, R.K., Lee, B.M., Gorbalenya, A.E., Tong, L., McClure, J., Ehrlich, L.S., Summers, M.F., Carter, C. and Rossmann, M.G. (1996) Crystal structure of dimeric HIV-1 capsid protein. *Nat Struct Biol*, **3**, 763-770.

Morikawa, Y., Hockley, D.J., Nermut, M.V. and Jones, I.M. (2000) Roles of matrix, p2, and N-terminal myristoylation in human immunodeficiency virus type 1 Gag assembly. *J Virol*, **74**, 16-23.

Muller, B., Patschinsky, T. and Krausslich, H.G. (2002) The Late-Domain-Containing Protein p6 Is the Predominant Phosphoprotein of Human Immunodeficiency Virus Type 1 Particles. *J Virol*, **76**, 1015-1024.

Murakami, T. and Freed, E.O. (2000) The long cytoplasmic tail of gp41 is required in a cell type-dependent manner for HIV-1 envelope glycoprotein incorporation into virions. *Proc Natl Acad Sci U S A*, **97**, 343-348.

Muriaux, D., Mirro, J., Harvin, D. and Rein, A. (2001) RNA is a structural element in retrovirus particles. *Proc Natl Acad Sci U S A*, **98**, 5246-5251.

Nguyen, D.H. and Hildreth, J.E. (2000) Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts. *J Virol*, **74**, 3264-3272.

Ono, A. and Freed, E.O. (1999) Binding of human immunodeficiency virus type 1 Gag to membrane: role of the matrix amino terminus. *J Virol*, **73**, 4136-4144.

Ono, A. and Freed, E.O. (2001) Plasma membrane rafts play a critical role in HIV-1 assembly and release. *Proc Natl Acad Sci U S A*, **98**, 13925-13930.

Ono, A., Orenstein, J.M. and Freed, E.O. (2000) Role of the Gag matrix domain in targeting human immunodeficiency virus type 1 assembly. *J Virol*, **74**, 2855-2866.

Ott, D.E., Coren, L.V., Chertova, E.N., Gagliardi, T.D. and Schubert, U. (2000) Ubiquitination of HIV-1 and MuLV Gag. *Virology*, **278**, 111-121.

Ott, D.E., Coren, L.V., Copeland, T.D., Kane, B.P., Johnson, D.G., Sowder, R.C., 2nd, Yoshinaka, Y., Oroszlan, S., Arthur, L.O. and Henderson, L.E. (1998) Ubiquitin is covalently attached to the p6Gag proteins of human immunodeficiency virus type 1 and simian immunodeficiency virus and to the p12Gag protein of Moloney murine leukemia virus. *J Virol*, **72**, 2962-2968.

Owens, R.J., Dubay, J.W., Hunter, E. and Compans, R.W. (1991) Human immunodeficiency virus envelope protein determines the site of virus release in polarized epithelial cells. *Proc Natl Acad Sci U S A*, **88**, 3987-3991.

Paillart, J.C. and Gottlinger, H.G. (1999) Opposing effects of human immunodeficiency virus type 1 matrix mutations support a myristyl switch model of gag membrane targeting. *J Virol*, **73**, 2604-2612.

Pancio, H.A. and Ratner, L. (1998) Human immunodeficiency virus type 2 Vpx-Gag interaction. *J Virol*, **72**, 5271-5275.

Parent, L.J., Bennett, R.P., Craven, R.C., Nelle, T.D., Krishna, N.K., Bowzard, J.B., Wilson, C.B., Puffer, B.A., Montelaro, R.C. and Wills, J.W. (1995) Positionally independent and exchangeable late budding functions of the Rous sarcoma virus and human immunodeficiency virus Gag proteins. *J Virol*, **69**, 5455-5460.

Patnaik, A., Chau, V. and Wills, J.W. (2000) Ubiquitin is part of the retrovirus budding machinery. *Proc Natl Acad Sci U S A*, **97**, 13069-13074.

Peters, S., Munoz, M., Yerly, S., Sanchez-Merino, V., Lopez-Galindez, C., Perrin, L., Larder, B., Cmarko, D., Fakan, S., Meylan, P. and Telenti, A. (2001) Resistance to nucleoside analog reverse transcriptase inhibitors mediated by human immunodeficiency virus type 1 p6 protein. *J Virol*, **75**, 9644-9653.

Pettit, S.C., Moody, M.D., Wehbie, R.S., Kaplan, A.H., Nantermet, P.V., Klein, C.A. and Swanstrom, R. (1994) The p2 domain of human immunodeficiency virus type 1 Gag regulates sequential

proteolytic processing and is required to produce fully infectious virions. *J Virol*, **68**, 8017-8027.

Pirozzi, G., McConnell, S.J., Uveges, A.J., Carter, J.M., Sparks, A.B., Kay, B.K. and Fowlkes, D.M. (1997) Identification of novel human WW domain-containing proteins by cloning of ligand targets. *J Biol Chem*, **272**, 14611-14616.

Puffer, B.A., Parent, L.J., Wills, J.W. and Montelaro, R.C. (1997) Equine infectious anemia virus utilizes a YXXL motif within the late assembly domain of the Gag p9 protein. *J Virol*, **71**, 6541-6546.

Puffer, B.A., Watkins, S.C. and Montelaro, R.C. (1998) Equine infectious anemia virus Gag polyprotein late domain specifically recruits cellular AP-2 adapter protein complexes during virion assembly. *J Virol*, **72**, 10218-10221.

Putterman, D., Pepinsky, R.B. and Vogt, V.M. (1990) Ubiquitin in avian leukosis virus particles. *Virology*, **176**, 633-637.

Rao, Z., Belyaev, A.S., Fry, E., Roy, P., Jones, I.M. and Stuart, D.I. (1995) Crystal structure of SIV matrix antigen and implications for virus assembly. *Nature*, **378**, 743-747.

Reed, M., Mariani, R., Sheppard, L., Pekrun, K., Landau, N.R. and Soong, N.W. (2002) Chimeric human immunodeficiency virus type 1 containing murine leukemia virus matrix assembles in murine cells. *J Virol*, **76**, 436-443.

Reicin, A.S., Ohagen, A., Yin, L., Hoglund, S. and Goff, S.P. (1996) The role of Gag in human immunodeficiency virus type 1 virion morphogenesis and early steps of the viral life cycle. *J Virol*, **70**, 8645-8652.

Reicin, A.S., Paik, S., Berkowitz, R.D., Luban, J., Lowy, I. and Goff, S.P. (1995) Linker insertion mutations in the human immunodeficiency virus type 1 gag gene: effects on virion particle assembly, release, and infectivity. *J Virol*, **69**, 642-650.

Reil, H., Bukovsky, A.A., Gelderblom, H.R. and Gottlinger, H.G. (1998) Efficient HIV-1 replication can occur in the absence of the viral matrix protein. *Embo J*, **17**, 2699-2708.

Rein, A., Henderson, L.E. and Levin, J.G. (1998) Nucleic-acid-chaperone activity of retroviral nucleocapsid proteins: significance for viral replication. *Trends Biochem Sci*, **23**, 297-301.

Rosenwirth, B., Billich, A., Datema, R., Donatsch, P., Hammerschmid, F., Harrison, R., Hiestand, P., Jaksche, H., Mayer, P., Peichl, P. and *et al.* (1994) Inhibition of human immunodeficiency virus type 1 replication by SDZ NIM 811, a nonimmunosuppressive cyclosporine analog. *Antimicrob Agents Chemother*, **38**, 1763-1772.

Rotin, D., Staub, O. and Haguenauer-Tsapis, R. (2000) Ubiquitination and endocytosis of plasma membrane proteins: role of Nedd4/Rsp5p family of ubiquitin-protein ligases. *J Membr Biol*, **176**, 1-17.

Rowell, J.F., Stanhope, P.E. and Siliciano, R.F. (1995) Endocytosis of endogenously synthesized HIV-1 envelope protein. Mechanism and role in processing for association with class II MHC. *J Immunol*, **155**, 473-488.

Sandefur, S., Smith, R.M., Varthakavi, V. and Spearman, P. (2000) Mapping and characterization of the N-terminal I domain of human immunodeficiency virus type 1 Pr55(Gag). *J Virol*, **74**, 7238-7249.

Sandefur, S., Varthakavi, V. and Spearman, P. (1998) The I domain is required for efficient plasma membrane binding of human immunodeficiency virus type 1 Pr55Gag. *J Virol*, **72**, 2723-2732.

Schnierle, B.S., Stitz, J., Bosch, V., Nocken, F., Merget-Millitzer, H., Engelstadter, M., Kurth, R., Groner, B. and Cichutek, K. (1997) Pseudotyping of murine leukemia virus with the envelope glycoproteins of HIV generates a retroviral vector with specificity of infection for CD4-expressing cells. *Proc Natl Acad Sci U S A*, **94**, 8640-8645.

Schubert, U., Ott, D.E., Chertova, E.N., Welker, R., Tessmer, U., Princiotta, M.F., Bennink, J.R., Krausslich, H.G. and Yewdell, J.W. (2000) Proteasome inhibition interferes with gag polyprotein processing, release, and maturation of HIV-1 and HIV-2. *Proc Natl Acad Sci U S A*, **97**, 13057-13062.

Spearman, P., Horton, R., Ratner, L. and Kuli-Zade, I. (1997) Membrane binding of human immunodeficiency virus type 1 matrix protein in vivo supports a conformational myristyl switch mechanism. *J Virol*, **71**, 6582-6592.

Spearman, P., Wang, J.J., Vander Heyden, N. and Ratner, L. (1994) Identification of human immunodeficiency virus type 1 Gag protein domains essential to membrane binding and particle assembly. *J Virol*, **68**, 3232-3242.

Srinivasakumar, N., Hammarskjold, M.L. and Rekosh, D. (1995) Characterization of deletion mutations in the capsid region of human immunodeficiency virus type 1 that affect particle formation and Gag-Pol precursor incorporation. *J Virol*, **69**, 6106-6114.

Strack, B., Calistri, A., Accola, M.A., Palu, G. and Gottlinger, H.G. (2000) A role for ubiquitin ligase recruitment in retrovirus release. *Proc Natl Acad Sci U S A*, **97**, 13063-13068.

Strambio-de-Castillia, C. and Hunter, E. (1992) Mutational analysis of the major homology region of Mason-Pfizer monkey virus by use of saturation mutagenesis. *J Virol*, **66**, 7021-7032.

Stys, D., Blaha, I. and Strop, P. (1993) Structural and functional studies in vitro on the p6 protein from the HIV-1 gag open reading frame. *Biochim Biophys Acta*, **1182**, 157-161.

Summers, M.F., Henderson, L.E., Chance, M.R., Bess, J.W., Jr., South, T.L., Blake, P.R., Sagi, I., Perez-Alvarado, G., Sowder, R.C., 3rd, Hare, D.R. and *et al.* (1992) Nucleocapsid zinc fingers detected in retroviruses: EXAFS studies of intact viruses and the solution-state structure of the nucleocapsid protein from HIV-1. *Protein Sci*, **1**, 563-574.

Summers, M.F., South, T.L., Kim, B. and Hare, D.R. (1990) High-resolution structure of an HIV zinc fingerlike domain via a new NMR-based distance geometry approach. *Biochemistry*, **29**, 329-340.

Swanstrom, R. and Wills, J.W. (1997) Synthesis, assembly, and processing of viral proteins. In Coffin, J.M., Hughes, S.H. and Varmus, H.E. (eds.), *Retroviruses*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 263-334.

Tanaka, T., Ames, J.B., Harvey, T.S., Stryer, L. and Ikura, M. (1995) Sequestration of the membrane-targeting myristoyl group of recoverin in the calcium-free state. *Nature*, **376**, 444-447.

Thali, M., Bukovsky, A., Kondo, E., Rosenwirth, B., Walsh, C.T., Sodroski, J. and Gottlinger, H.G. (1994) Functional association of cyclophilin A with HIV-1 virions. *Nature*, **372**, 363-365.

Tritel, M. and Resh, M.D. (2000) Kinetic analysis of human immunodeficiency virus type 1 assembly reveals the presence of sequential intermediates. *J Virol*, **74**, 5845-5855.

VerPlank, L., Bouamr, F., LaGrassa, T.J., Agresta, B., Kikonyogo, A., Leis, J. and Carter, C.A. (2001) Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55(Gag). *Proc Natl Acad Sci U S A*, **98**, 7724-7729.

Vogt, V.M. (1996) Proteolytic processing and particle maturation. In Krausslich, H.G. (ed.), *Current topics in microbiology and immunology: morphogenesis and maturation of retroviruses*. Springer Verlag, Berlin, Germany, Vol. 214, pp. 95-131.

Vogt, V.M. (1997) Retroviral virions and genomes. In Coffin, J.M., Hughes, S.H. and Varmus, H.E. (eds.), *Retroviruses*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 27-69.

von Schwedler, U., Kornbluth, R.S. and Trono, D. (1994) The nuclear localization signal of the matrix protein of human immunodeficiency virus type 1 allows the establishment of infection in macrophages and quiescent T lymphocytes. *Proc Natl Acad Sci U S A*, **91**, 6992-6996.

von Schwedler, U.K., Stemmler, T.L., Klishko, V.Y., Li, S., Albertine, K.H., Davis, D.R. and Sundquist, W.I. (1998) Proteolytic refolding of the HIV-1 capsid protein amino-terminus facilitates viral core assembly. *Embo J*, **17**, 1555-1568.

Wang, C.T. and Barklis, E. (1993) Assembly, processing, and infectivity of human immunodeficiency virus type 1 gag mutants. *J Virol*, **67**, 4264-4273.

Wang, C.T., Zhang, Y., McDermott, J. and Barklis, E. (1993) Conditional infectivity of a human immunodeficiency virus matrix domain deletion mutant. *J Virol*, **67**, 7067-7076.

Weinberg, J.B., Matthews, T.J., Cullen, B.R. and Malim, M.H. (1991) Productive human immunodeficiency virus type 1 (HIV-1) infection of nonproliferating human monocytes. *J Exp Med*, **174**, 1477-1482.

Welker, R., Hohenberg, H., Tessmer, U., Huckhagel, C. and Krausslich, H.G. (2000) Biochemical and structural analysis of isolated mature cores of human immunodeficiency virus type 1. *J Virol*, **74**, 1168-1177.

Wiegers, K., Rutter, G., Kottler, H., Tessmer, U., Hohenberg, H. and Krausslich, H.G. (1998) Sequential steps in human immunodeficiency virus particle maturation revealed by alterations of individual Gag polyprotein cleavage sites. *J Virol*, **72**, 2846-2854.

Wiegers, K., Rutter, G., Schubert, U., Grattinger, M. and Krausslich, H.G. (1999) Cyclophilin A incorporation is not required for human immunodeficiency virus type 1 particle maturation and does not destabilize the mature capsid. *Virology*, **257**, 261-274.

Wilk, T., Gross, I., Gowen, B.E., Rutten, T., de Haas, F., Welker, R., Krausslich, H.G., Boulanger, P. and Fuller, S.D. (2001) Organization of immature human immunodeficiency virus type 1. *J Virol*, **75**, 759-771.

Wilk, T., Pfeiffer, T. and Bosch, V. (1992) Retained in vitro infectivity and cytopathogenicity of HIV-1 despite truncation of the C-terminal tail of the env gene product. *Virology*, **189**, 167-177.

Willems, L., Kerkhofs, P., Attenelle, L., Burny, A., Portetelle, D. and Kettmann, R. (1997) The major homology region of bovine leukaemia virus p24gag is required for virus infectivity in vivo. *J Gen Virol*, **78**, 637-640.

Wills, J.W., Cameron, C.E., Wilson, C.B., Xiang, Y., Bennett, R.P. and Leis, J. (1994) An assembly domain of the Rous sarcoma virus Gag protein required late in budding. *J Virol*, **68**, 6605-6618.

Wills, J.W. and Craven, R.C. (1991) Form, function, and use of retroviral gag proteins [editorial]. *Aids*, **5**, 639-654.

Wilson, W., Braddock, M., Adams, S.E., Rathjen, P.D., Kingsman, S.M. and Kingsman, A.J. (1988) HIV expression strategies: ribosomal frameshifting is directed by a short sequence in both mammalian and yeast systems. *Cell*, **55**, 1159-1169.

Wu, X., Conway, J.A., Kim, J. and Kappes, J.C. (1994) Localization of the Vpx packaging signal within the C terminus of the human immunodeficiency virus type 2 Gag precursor protein. *J Virol*, **68**, 6161-6169.

Wyma, D.J., Kotov, A. and Aiken, C. (2000) Evidence for a stable interaction of gp41 with Pr55(Gag) in immature human immunodeficiency virus type 1 particles. *J Virol*, **74**, 9381-9387.

Xiang, Y., Cameron, C.E., Wills, J.W. and Leis, J. (1996) Fine mapping and characterization of the Rous sarcoma virus Pr76gag late assembly domain. *J Virol*, **70**, 5695-5700.

Yasuda, J. and Hunter, E. (1998) A proline-rich motif (PPPY) in the Gag polyprotein of Mason-Pfizer monkey virus plays a maturation-independent role in virion release. *J Virol*, **72**, 4095-4103.

Yeager, M., Wilson-Kubalek, E.M., Weiner, S.G., Brown, P.O. and Rein, A. (1998) 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*, **95**, 7299-7304.

Yu, F., Joshi, S.M., Ma, Y.M., Kingston, R.L., Simon, M.N. and Vogt, V.M. (2001) Characterization of Rous sarcoma virus Gag particles assembled in vitro. *J Virol*, **75**, 2753-2764.

Yu, X., Yu, Q.C., Lee, T.H. and Essex, M. (1992a) The C terminus of human immunodeficiency virus type 1 matrix protein is involved in early steps of the virus life cycle. *J Virol*, **66**, 5667-5670.

Yu, X., Yuan, X., Matsuda, Z., Lee, T.H. and Essex, M. (1992b) The matrix protein of human immunodeficiency virus type 1 is required for incorporation of viral envelope protein into mature virions. *J Virol*, **66**, 4966-4971.

Yu, X.F., Dawson, L., Tian, C.J., Flexner, C. and Dettenhofer, M. (1998) Mutations of the human immunodeficiency virus type 1 p6Gag domain result in reduced retention of Pol proteins during virus assembly. *J Virol*, **72**, 3412-3417.

Yuan, B., Campbell, S., Bacharach, E., Rein, A. and Goff, S.P. (2000) Infectivity of Moloney murine leukemia virus defective in late assembly events is restored by late assembly domains of other retroviruses. *J Virol*, **74**, 7250-7260.

Yuan, B., Li, X. and Goff, S.P. (1999) Mutations altering the moloney murine leukemia virus p12 Gag protein affect virion production and early events of the virus life cycle. *Embo J*, **18**, 4700-4710.

Yuan, X., Yu, X., Lee, T.H. and Essex, M. (1993) Mutations in the N-terminal region of human immunodeficiency virus type 1 matrix protein block intracellular transport of the Gag precursor. *J Virol*, **67**, 6387-6394.

Zhang, Y. and Barklis, E. (1997) Effects of nucleocapsid mutations on human immunodeficiency virus assembly and RNA encapsidation. *J Virol*, **71**, 6765-6776.

Zhang, Y., Qian, H., Love, Z. and Barklis, E. (1998) Analysis of the assembly function of the human immunodeficiency virus type 1 gag protein nucleocapsid domain. *J Virol*, **72**, 1782-1789.

Zhou, W., Parent, L.J., Wills, J.W. and Resh, M.D. (1994) Identification of a membrane-binding domain within the amino-terminal region of human immunodeficiency virus type 1 Gag protein which interacts with acidic phospholipids. *J Virol*, **68**, 2556-2569.

Zhou, W. and Resh, M.D. (1996) Differential membrane binding of the human immunodeficiency virus type 1 matrix protein. *J Virol*, **70**, 8540-8548.