AIDS Rev 2002;4:104-111

Assembly of the HIV-1 Core Particle

Andrew H. Kaplan

Department of Medicine, Microbiology & Immunology, University of North Carolina, Chapel Hill, North Carolina, USA

Abstract

The core particle of HIV-1 assembles at the membrane of the host cell as the virus buds from the surface. The structural proteins and enzymes that comprise the core are translated as part of two polyprotein precursors, Gag and GagPol. The Gag precursor contains the structural proteins of the core and is both necessary and sufficient for directing particle assembly and budding. Over the past few years, significant progress has been made in our understanding of the interactions that drive particle assembly. Specifically, determinants within the Gag precursor that direct membrane association, Gag-Gag interactions and particle budding have been identified and partially characterized. Subdomains of the host cell membrane that favor particle assembly and budding have also been described. Finally, a potential role for cellular processes in mediating the final stages in particle release has recently been proposed and a cellular protein that appears to bind directly to the Gag precursor has been identified. Each of these observations helps to clarify previously obscure aspects of viral replication and points towards potential targets for the design of novel therapies.

Key words

HIV-1. Gag precursor. Viral assembly. Membrane rafts. Tsg101. Vacuolar protein sorting.

Overview

Viral assembly is the process by which all of the components of the viral particle come together at the end of the life cycle to form a replication-competent virus. For the human immunodeficiency virus type 1 (HIV-1), as is the case for all C-type retroviruses, this occurs largely at the membrane of the infected cell and involves interactions between plasma membranes, the intracellular tracking apparatus and viral proteins. The infectious viral particle that ultimately arises from this complicated, ordered process is enveloped and approximately 80-100 nM in diameter[1]. It contains two identical positive-stranded copies of the viral genomic RNA within a cone-shaped, electron-dense core structure[1].

As an enveloped virus, HIV-1 buds from the surface of the infected cell, in the process acquiring the cellular membranes that surround the mature viral particle. Associated with the membrane are two viral envelope proteins, the transmembrane gp41 and the surface glycoprotein, gp120[1]. These glycoproteins are translated as a precursor of approximately 160 kDa that transits through the endoplasmic reticulum (ER), following the same pathway used by other membrane-associated proteins[2]. In the ER, the envelope polyproteins are glycosylated and associated into what are most likely trimers[3-5]. Upon transit into the Golgi apparatus, a cellular protease, either furin or a protein or pro-

Correspondence to:
Andrew H. Kaplan
Departments of Medicine and
Microbiology & Immunology
547 Burnett Womack, C.B. 7030
UNC School of Medicine
Chapel Hill, NC 27599-7030
Phone: (919) 966-2536
Fax: (919) 966-6714
Email: akaplan@med.unc.edu

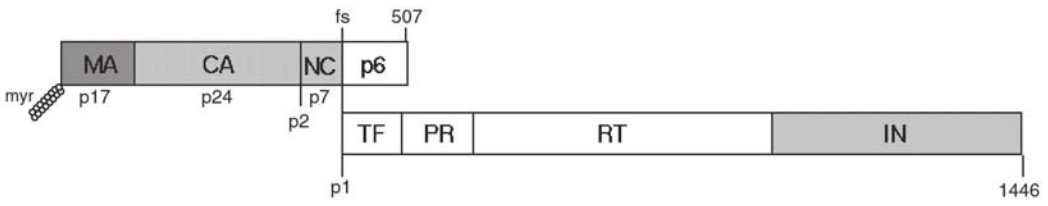

Figure 1A. *Distribution of core protein coding domains within the gag and pol reading frames. The structural proteins of the core (MA – matrix, CA – capsid, and NC – nucleocapsid) are contained within the gag reading frame. The core enzymes (PR – protease, RT – reverse transcriptase, and IN – integrase) are encoded within pol. There are several additional coding domains/protease cleavage products (p2, p1 and TF – transframe) whose role in virus replication is uncertain. p6 appears to play a role in the late steps of particle release of the particle from the surface of the host cell.*

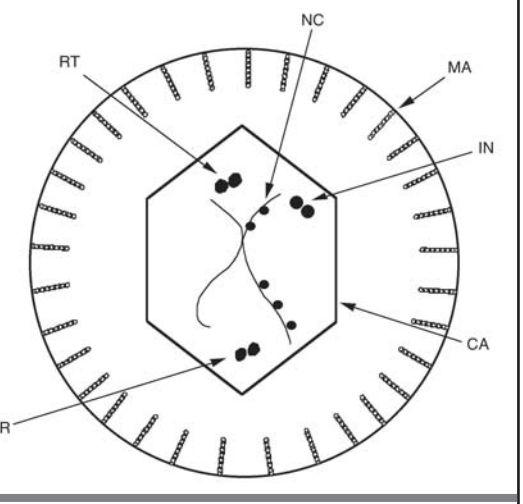

Figure 1B. *Distribution of Gag and GagPol proteins within a mature, fully assembled core particle.*

teins with a related activity, cleave the envelope precursor[6].

The structural proteins and enzymes that comprise the core particle are translated as part of the two polyprotein precursors, Gag and GagPol (Fig. 1 a,b)[7,8]. The *gag* and *pol* genes overlap near the 3' end of *gag* and the 5' end of *pol*; the *pol* reading frame is shifted-1 relative to that of Gag. GagPol is a fusion of the protein products of the *gag* and *pol* genes and is translated at a level about 5% that of Gag. This larger precursor arises from a -1 ribosomal frameshift. The structural proteins associated with the viral core are encoded by the Gag precursor. These include the matrix protein (MA) that lines the inner face of the viral membrane, the capsid protein (CA) that makes up the cone-shaped core particle, the nucleocapsid protein (NC) that interacts with the viral single-stranded RNA genome and p6, a protein that appears to be involved both in the incorporation of the virion-associated protein VPR and in the final steps in release of the budding viral particle from the membrane of the infected cell. Pol encodes several viral enzymes including the viral protease (PR) as well as the reverse transcriptase (RT) and integrase (IN).

These precursors are translated on free ribosomes in the cytoplasm and move to the membrane where they assemble with each other and interact with the viral glycoproteins through a series of processes that are only now being elucidated. The nascent viral particle then buds from the surface of the cell. At around the time of budding, the Gag and GagPol precursors are processed by the viral protease that is contained within GagPol[1,9].

What becomes clear from even this cursory overview is that the successful assembly of a viral particle requires the resolution of a number of complicated problems. Conceptually, these problems may be grouped into those related to the interaction of viral proteins with each other, and the interaction of viral proteins with normal cellular processes. Although the complete characterization of viral assembly is an endeavor that appears to be in its infancy, recent insights from studies in both *in vitro* and *in vivo* assembly systems have shed new light on critical features of assembly. These include the identification of domains within the viral core precursors that are involved in important protein-protein interactions, as well as potential cellular partners that appear to play crucial roles in the process of assembly. Overall, these findings deepen our understanding of this intricately choreographed process and raise the promise of alternate targets for drug therapies. This review will focus on recent advances in our understanding of the assembly of the core particle of HIV-1. Several excellent summaries have recently reviewed the structure and function of the viral envelope proteins[10] and packaging of the viral genomic RNA[11].

Assembly Domains within the Gag Precursor

For all retroviruses studied, the determinants of capsid assembly are contained within the Gag precursor itself; expression of the Gag precursor in the absence of other viral proteins results in the budding of enveloped viral-like particles (VLPs) from cells[7,8]. In fact, several groups have designed and expressed minimal Gag constructs that are competent for VLP production. In studies in which only native HIV sequences are used, the 55-kDa precursor may be replaced by a deleted 28-kDa protein[12]. By using heterologous sequences in the place of some functional sequences, a precursor as small as 16-kDa can produce VLPs[13]. Expression and characterization of the assembly properties of these so-called deleted "mini-precursors" has allowed investigators to map important assembly determinants.

Assembly of the Gag precursor into particles may be conceptualized as a series of steps: interaction of the Gag precursors with the cell membrane, interaction of the precursors with each other, and release of the particle from the cell surface. Recently, each of these steps in capsid assembly has been mapped to specific domains within Gag (Fig. 2).

M-domain. Membrane association of the precursor is, to a large part, determined by the M-domain, which is comprised of the first 32 residues in MA and the myristate that is added to the N-terminal glycine[7,8]. The M-domain contains a stretch of basic amino acids between residues 15 and 31[14,15]. Substitution of these basic residues impairs membrane binding of Gag; structural studies of the mature MA protein suggest that these residues form a charged patch in a globular domain of the protein that stabilizes the association of the membrane and precursor by interacting with membrane phospholipids[15]. Of note, comparative studies of other retroviruses suggest that anchoring of the Gag precursor to the membrane through interactions between basic amino acids in MA and membrane phospholipids may be a conserved feature of retroviral assembly[16,17]. However, these studies also indicate that membrane targeting of the precursor is, at least in some cases, species specific. For example, mouse cells are unable to support replication of HIV, even when species-specific blocks to early steps in the viral life cycle are bypassed[18-20]. Inappropriate membrane targeting of the precursor has been noted in a number of murine cell lines. By substituting MA sequences from the Moloney murine leukemia virus (M-MuLV), this block to appropriate membrane targeting could be overcome[21].

The role of the myristate moiety is more complex. It is clear that myristoylation of the N-terminus of the precursor promotes efficient association of the precursor with the membrane[1]. However, once the viral PR cleaves the MA from the precursor, this association is considerably weakened[22]. It has been suggested that this is consistent with a myristyl switch model[23]. In this scenario, cleavage of the precursor produces a conformational change in MA that reduces the exposure of the hydrophobic myristate[14,24-26]. Sequestration of the myristate, in turn, decreases the affinity of the mature matrix protein for the membrane. Such a model would help explain the proposed requirement that MA disengages from the membrane upon infection of susceptible cells to participate in early steps in viral replication, including nuclear import of the viral genome (for review, see 27). Evidence that the precursor interacts preferentially with specific regions of the membrane is reviewed below.

I-Domain. A portion of the Gag precursor that is important for Gag-Gag interactions has been mapped to two discontinuous regions stretching from the C-terminal portion of the CA protein into the viral NC protein. This has been termed the "I" or "Interaction" domain[28-30]. Equilibrium density measurements demonstrate that retroviral particles have a density of about 1.16 g/ml and mutational analyses suggest that residues in this region play a role in determining particle density[28-30]. Although there is a consensus that residues involved in this domain do not overlap with the amino acids that comprise the Zn-finger motifs of the NC protein, mapping studies have yielded somewhat conflicting results regarding the role of basic residues within NC[31].

Several studies of viral particles assembling *in vitro* suggest that viral RNA may play a role in Gag-Gag interaction[32-37]. Expression of HIV-1 CA-NC constructs in *E. coli* result in the generation of cylindrical protein aggregates. Additional findings include the observation that RNA is required for the production of these cylinders and that the length of the cylinders is dependent on the length of the RNA. Further, it was demonstrated that any RNA, and not necessarily viral RNA, is sufficient for cylinder production[32]. Finally, expression of either Gag or Gag/Pol in COS7 cells produces complexes that are pelletable in the presence of 1% Triton X-100[38]. These complexes are destabilized when exposed to RNase. Interestingly, this requirement for the presence of RNA is independent of HIV-1 RNA; non-viral RNA appears to be adequate to support precursor multimerization[38]. Overall, these studies suggest a model in which RNA acts as a scaffold that promotes Gag multimerization.

L-Domain. The presence of a domain within Gag that plays a role late in viral assembly was suggested by early deletion studies. Gag constructs lacking the C-terminal p6 protein produced unusual-looking

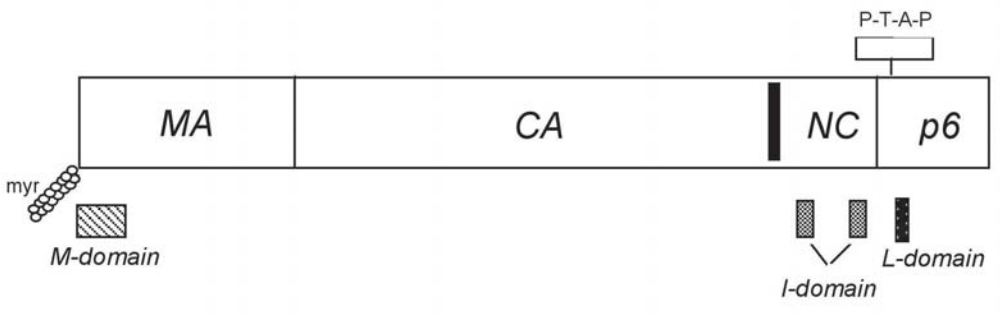

Figure 2. *Location of assembly domains within the Gag precursor. The location of the membrane association (M), interaction (I) and late (L) domains as well as the myristate (myr) moiety and PTAP (Pro-Thr-Ala-Pro) motif is noted.*

viral particles that remained attached to producer cells by what appeared to be thin cytoplasmic extensions or "stalks"[39]. Partially assembled viral particles could also be seen accumulating at the plasma membranes of the transfected cells. It appeared from EM studies that the final stages of assembly in which the cellular membrane "pinches off" and the viral particle is released were, in some way, defective for these mutants. Since that time, the nature of this domain and the mechanism of particle release have been the subject of intense scrutiny. The precise mechanism by which the assembling particle separates from the cell surface remains obscure, but several features of particle separation and the L-domain have been characterized.

Firstly, separation of the particle involves fusion of the membranes that ultimately surround the viral particle and is likely to be an energetically unfavorable process. As expected, VLP production is dramatically decreased in Gag-producing cells that are pharmacologically depleted of ATP[40]. Further, viral assembly intermediates stalled late in the assembly process were identified by density centrifugation[40]. Additional evidence supporting this conclusion comes from EM studies that reveal incompletely budded particles lining the surface of these ATP-depleted cells[40].

Secondly, the critical residues within the C-terminal p6 protein have been more completely characterized. It appears that a proline-rich sequence (Pro-Thr-Ala-Pro) the N-terminus of the protein is critical for this late assembly function[41,42]. Deletion of sequences downstream of this so-called "PTAP" motif appeared to have no effect on this late assembly function.

Finally, late domains with similar activities have been identified in other retroviruses. These include a proline-rich region of the p2b protein (PPPY) of Rous sarcoma virus (RSV)[41], an identical sequence in the Moloney murine leukemia virus (M-MuLV)[43] and the p9 protein (YPDL) of the equine infectious anemia virus (EIAV)[44]. Particularly intriguing are the observations that, not only can these L-domains from diverse viruses replace one another, but that they may function in a positionally independent manner. For example, it has been demonstrated that deletion of the YPDL late domain sequence from EIAV has dramatic effects on both viral assembly and infectivity[44]. However, if one replaces the native late domain of EIAV (YPDL) with the HIV PTAP or RSV PPPY sequences, both particle assembly and infectivity are restored to levels similar to that seen with the wild-type virus[45]. Further, insertion of the HIV or RSV domains at another site within the EIAV Gag precursor (in this case, the matrix protein) rescues the assembly defect but not the infectivity defect seen in L-domain-deleted variants[45]. Similar results have been obtained with constructs in which a heterologous L-domain from HIV replaced the RSV or M-MuLV L-domains[46]. The EIAV L-domain sequence was able to rescue RSV variants with a deleted L-domain[45].

Therefore, not only does the presence of this domain appear to be conserved across diverse retroviruses, but it also seems likely that the L-domains mediate distinct functions related to assembly and infectivity.

Other Gag Domains. Although much work has focused on these three regions of the Gag precursor, it is important to note that several other regions of Gag have been implicated in genetic studies as assembly determinants. For example, several studies have identified mutations within the HIV CA protein that lie outside of the recognized assembly domains, yet still have profound effects on particle assembly[47-51]. These include substitutions within and just C-terminal to the major homology region (MHR) of CA[52]. As its name suggests, the MHR is a conserved sequence found in the Gag precursor of all retroviruses. Genetic studies suggest that the MHR plays a role in assembly and infectivity[52]; biochemical studies in *in vitro* model systems indicate that Gag precursors lacking the MHR bind less efficiently to membranes[53]. *In vitro* studies have also demonstrated that Gag-Gag interactions are impaired for purified precursors lacking the MHR[54]. In addition, structural studies of the HIV capsid have identified a dimerization domain that is critical for particle assembly *in vitro*[55].

Another part of the Gag precursor that is important in directing assembly is the p2 spacer peptide that is present at the C-terminus of CA. These small peptides are a common feature of the Gag precursors in many retroviruses. Mutations in this region diminish particle release and produce aberrantly assembled, non-infectious particles[56].

Finally, the protease-mediated processing of Gag also plays a role in the appropriate particle assembly in addition to the myristyl switch mechanism proposed above. Although unprocessed Gag precursors may produce VLPs, the particles formed lack the condensed, electron-dense core seen in mature particles. Inhibition of the viral protease produces particles with a similar morphology[57]. Assembly of fully infectious particles appears to be exquisitely sensitive to protease inhibition; even minimal inhibition of the protease produces profound deficits in infectivity[57]. Studies of *in vitro* particle assembly also suggest a role for the protease. Although expression of CA alone can result in the production of hollow, cylindrical particles, a short N-terminal extension of capsid into the matrix protein converts these cylinders into spherical particles[33]. This suggests that cleavage of the precursor between MA and CA may be important in directing appropriate core assembly.

Interaction Between Particle Assembly and Cellular Processes

It has been recognized for some time that the efficient assembly of viral particles requires an active role for a number of cellular processes. For example, a number of investigators have determined that HIV Gag binds to cellular actin and it has been suggested that this interaction may be important in transport of the precursor to the cell membrane[58,59].

This is supported by the observation that actin appears to be concentrated within the particle itself[60]. Perhaps the most compelling evidence for a necessary "human factor" in HIV assembly comes from studies of mouse-human heterokaryons. In these experiments, the block to proper assembly of HIV particles in murine fibroblasts was overcome by fusing the murine cells with transformed human T-cell lines[61].

The cell membrane is the site at which the final steps in HIV particle assembly occur. Although membrane-based particle assembly would seem to require active processes with a relatively high degree of specificity, the available data present a somewhat complicated picture. Despite the complicated nature of the choreography that must occur between retroviral core precursors, viral core and envelope proteins and the membrane itself, studies indicate that many aspects of the Gag-membrane and Gag-envelope interaction are relatively non-specific. These include the observation that foreign glycoproteins can be readily incorporated into viral particles and that incorporation of the native envelope glycoproteins is independent of the length of the envelope's cytoplasmic tail[1].

In contrast, several lines of evidence suggest that the Gag precursor is targeted to a specific region of the membrane. First, EM studies of budding particles reveal that budding appears to occur in non-uniformly distributed patches of the cell membrane[1]. In addition, biophysical studies indicate that the lipid composition of the viral membrane does not reflect the cells in which they were produced[62,63]. Further support for a specific interaction between precursor and envelope proteins and the cellular membrane comes from experiments in which viral proteins are expressed in polarized epithelial cells. When the Gag precursor is the only viral protein expressed in these cells, VLPs are seen budding from both the basolateral and apical surfaces of the monolayer[64]. In contrast, expression of viral envelope proteins alone is restricted to the basolateral surface of the cells. Co-expression of viral envelope and Gag proteins produces particles that bud from the basolateral surface[64]. As expected, expression of the complete retroviral genome in polarized cells results in budding particles restricted to the basolateral surface of the polarized cell monolayer[65]. Overall, these results suggest that there is some specific interaction between Gag and Env that directs particle formation to specific sites in the plasma membrane.

Role of Membrane Rafts in Particle Assembly

Early studies of RSV demonstrated that the phospholipid composition of the viral particle differed from that of the producer cell membranes[62]. It was in part these findings that prompted investigators to examine the lipid composition of the HIV membrane. The earliest studies indicated that the phospholipids found in the viral membrane were similar to those found in other viruses[66]. It was also reported that the ratio of cholesterol to phospholipid (C/P ratio) was unexpectedly high and that these membranes were extremely ordered when evaluated by electron spin resonance[66,67]. Direct comparisons between viral membranes and the membranes of their host cells indicated that the viral membranes had a C/P ratio that was 2.5 times that of the host cell and that the membranes of the viral particles were significantly more ordered than the host cell membranes[63].

Several conclusions were suggested by this non-uniform distribution of lipids between producer cells and viral particles. Firstly, these observations suggest that there exists a mechanism that directs the viral precursors towards regions of the membrane rich in these particular components. Secondly, this arrangement of lipids in the host cell membrane must either somehow promote budding of the newly formed viral particle or in some other way be beneficial to viral replication. Finally, since host cell proteins are often incorporated into viral particles, it seems possible that the membrane-associated host cell proteins found in these regions may also play a role in promoting viral replication.

The observation that the cell-derived membrane of HIV is rich in cholesterol and sphingolipids was made coincident with the evolution of the notion that membranes may be organized into structures known as "rafts" (for review, see 68, 69). It has been recognized for some time that, despite the relatively fluid nature of the membrane bilayer, different lipid species are non-uniformly distributed. Membrane fractions that are insoluble in the presence of the detergent Triton X-100 were identified and were characterized as being composed of interacting sphingolipids and cholesterol components[70]. The sphingolipid-cholesterol interactions serve to exclude glycerophospholipids; specific proteins are excluded from these microdomains as well. In addition, membrane rafts are enriched for several protein families, including glycophosphotidylinositol (GPI)-linked proteins and the doubly acylated tyrosine kinases of the Src family[71-73]. Evidence has accumulated that these membrane rafts and their associated proteins play an important role as cell surface platforms involved in signaling[68]. Of particular interest, rafts appear to be a membrane assembly focal point for a diverse group of enveloped viruses including measles, influenza and Sendai viruses[69].

Given the high C/P ratio found in the viral membrane and its detergent insolubility, it is not surprising that a number of studies have implicated membrane rafts as a preferential location for the assembly of HIV particles[74-76]. Kinetic studies have demonstrated that the Gag precursor associates with rafts after binding the membrane and that binding is enhanced following Gag-Gag interactions[75,76]. In one series of experiments, high concentrations of multimerized Gag precursor were localized within subdomains of the rafts themselves. These raft subdomains had a much higher density than that reported for standard rafts; such collections of

membranes and oligomeric Gag-gag assembly complexes have imaginatively been termed "barges"[75]. As expected, raft association appears to be mediated by the Gag M-domain and binding is depressed by treating the host cell with cholesterol depleting agents[76]. Further, CD45, a membrane phosphatase that is relatively under-represented in the membrane of viral particles, is excluded from both viral particles and plasma rafts[74].

It is tempting to speculate on the role that these membrane microdomains play in viral replication. Interventions that redirect Gag from binding to rafts decrease particle production[75,76]. Expression of a Gag fused to the N-terminus of Fyn, a raft-associated member of the Src family, increased particle production[75]. Taken together, these studies suggest that this association is critical in allowing the particle to bud from the surface of the cell. Additionally, it is plausible that, by targeting assembly and budding to a specific region of the cell, particular proteins are incorporated into the viral membrane. These membrane-associated proteins may play a role in subsequent steps in viral replication. It has also been reported that the viral Nef protein interacts with membrane rafts and that association with rafts allows Nef to interact with signaling proteins[77]. Therefore, in this case it appears that association of this viral protein with membrane rafts promotes priming of T-cells.

Particle Assembly and the Vacuolar Protein Sorting Pathway

The recent focus on the final steps in virus assembly in general, and L-domain function in particular, has led to a search for cellular proteins that interact with the PTAP sequence in the HIV-1 p6 protein. As release of the assembled viral particle from the host cell membrane requires membrane fission, and membrane fission is not a known property of any HIV protein, it seemed likely that a cellular partner was involved. In addition, since mutations within the PTAP motif block these membrane fission-associated final stages in particle release, it also seemed likely that this motif might represent the docking site for such cellular partner(s). Recently, using the yeast two-hybrid screen with either the PTAP sequence or the entire p6 protein as bait, two groups have independently isolated the same cellular protein, human tumor susceptibility gene 101 (Tsg101)[78,79]. In a series of subsequent experiments, these investigators demonstrated that Tsg101 interacts directly with the PTAP motif, that depletion of Tsg101 blocks budding at a late step in virus assembly and that re-introduction of Tsg101 rescues budding. Finally, mutations within p6 that inhibit particle release, also block binding of Tsg10142 and overexpression of the N-terminal domain blocks HIV particle formation at a late step[80].

A number of functions have been ascribed to Tsg101, including an important role in vacuolar protein sorting (VPS). In this process, Tsg101 appears to help select membrane-associated proteins for ubiquitination and sorting into the lysosomal degradation pathway[81]. Ubiquinated proteins enter the pathway via endocytosis into multivesicular bodies (MVB). It has been suggested that the Gag precursor "hijacks" this normal cellular process and results in the exocytosis of the viral particle through the cellular membrane rather than endocytosis into the MVB. In support of this hypothesis, inhibition of VPS through the expression of a dominant negative mutant of a protein in the pathway resulted in arrest of particle assembly[79].

Conclusions

The assembly of HIV-1 particles reflects a complex interaction between viral proteins and cellular processes. It has become clear that a successful response to the challenge of assembly requires both carefully controlled interactions between viral proteins as well as co-opted normal cellular processes. Genetic, biophysical and functional/biological studies have been used to identify domains within the viral Gag precursor that direct each of the required steps of assembly. Cellular partners that interact directly with viral proteins and support viral assembly have recently been identified. Given the indispensable nature of these domains for viral assembly, as well as the evolving structural information that is becoming available, these interactions should make attractive targets for the development of novel therapeutics.

References

1. Swanstrom R, Wills J. Synthesis, assembly and processing of viral proteins. In "Retroviruses" (J.Coffin, S.Hughes, and H.Varmus, Eds). pp. 263-344. Cold Spring Harbor Laboratory Press. Plainview, NY 1997.
2. Doms R, Lamb R, Rose J, Helenius A. Folding and assembly of viral membrane proteins. Virology 1993;193:545-62.
3. Doms R, Earl P, Chakrabarti S, Moss B. Human immunodeficiency viruses types 1 and 2 possess a functionally conserved assembly domain. J. Virol 1990;64:7374-82.
4. Shugars D, Wild C, Greenwell T, Mathews T. Biophysical characterization of recombinant proteins expressing the leucine zipper-like domain of the human immunodeficiency virus type 1 transmembrane protein gp41. J Virol 1996;70:2982-91.
5. Chan D, Fass D, Berger J, Kim P. Core structure of gp41 from the HIV envelope glycoprotein. Cell 1997;89:263-73.
6. Decroly E, Vandenbranden M, Ruysschaert J, et al. The convertases furin and PC1 can both cleave the human immunodeficiency virus (HIV)-1 envelope glycoprotein gp160 into gp120 (HIV-1 SU) and gp41 (HIV-I TM). J Biol Chem 1994;269:12240-7.
7. Freed E. HIV-1 Gag proteins: diverse functions in the virus life cycle. Virology 1998;251:1-15.
8. Garnier L, Bowzard J, Wills J. Recent advances and remaining problems in HIV assembly. AIDS 1998;12(Suppl A)S5-S16.
9. Kaplan A, Manchester M, Swanstrom R. The activity of the protease of the human immunodeficiency virus type 1 is initiated at the membrane of infected cells before the release of viral proteins and is required for release to occur with maximum efficiency. J Virol 1994;68:6782-6.
10. Hunter E. Macromolecular interactions in the assembly of HIV and other retroviruses. Semin Virol 1994;5:71-83.

11. Berkowitz R, Fisher J, Goff S. RNA packaging. Curr Top Microbiol Immunol 1996;214:177-218.
12. Wang C, Lai H-Y, Li J. Analysis of minimal human immunodeficiency virus type 1 gag coding sequences capable of virus-like particle assembly and release J Virol 1998;72:7950-9.
13. Accola M, Strack B, Gottliinger H. Efficient particle formation 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 2000;74:5395-402.
14. Zhou W, Parent L, Wills J, Resh M. 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 1994;68:2556-69.
15. Hill C, Worthylake D, Bancroft D, Christensen A, Sundquist W. Crystal structures of the trimeric human immunodeficiency virus type 1 matrix protein: implications for membrane association and assembly. Proc Natl Acad Sci USA 1996;93:3099-104.
16. Verderame M, Nelle T, Wills J. The membrane-binding domain of the Rous sarcoma virus Gag protein. J Virol 1996;70:2664-8.
17. Rao Z, Belyaev A, Fry E, Roy P, Jones I, Stuart D. Crystal structure of SIV matrix antigen and implications for virus assembly Nature 1995;378:743-7.
18. Bienasz P, Cullen B. Multiple blocks to human immunodeficiency virus type 1 replication in rodent cells. J Virol 2000;74:9868-77.
19. Mariani R, Rasala A, Rutter G, et al. A block to human immunodeficiency virus type 1 assembly in murine cells. J Virol 2000;74:3859-70.
20. Moosmayer D, Reil H, Ausmeier M, et al. Expression and frameshifting but extremely inefficient proteolytic processing of the HIV-1 gag and pol gene products in stably transfected rodent cell lines. Virology 1991;183:215-24.
21. Reed M, Mariani R, Sheppard L, Pekrun K, Landau N, Soong N-W. Chimeric human immunodeficiency virus type 1 containing murine leukemia virus matrix assembles in murine cells. J Virol 2002;76:436-43.
22. Platt E, Haffar O. Characterization of human immunodeficiency virus type 1 Pr55 Gag membrane association in a cell-free system: requirement for a C-terminal domain. Proc Natl Acad Sci USA 1994;91:4594-8.
23. Ono A, Demirov D, Freed E. Relationship between human immunodeficiency virus type 1 Gag multimerization and membrane binding. J Virol 2000;74:5142-50.
24. Hermida-Matsumoto L, Resh M. Human immunodeficiency virus type 1 protease triggers a myristoyl switch that modulates membrane binding of Pr55 (Gag) and p17 MA. J Virol 1999;73:1902-8.
25. Spearman P, Horton R, Ratner L, Kuli-Zadne I. Membrane binding of human immunodeficiency virus type 1 matrix protein *in vivo* supports a conformational myristyl switch mechanism. J Virol 1997;71:6582-92.
26. Zhou W, Resh M. Differential membrane binding of the human immunodeficiency virus type 1matrix protein. J Virol 1996; 70:8540-8.
27. Stevenson M. HIV nuclear import: what's the flap? Nature Medicine 2000;6:626-8.
28. Bennet R, Nelle T, Wills J. Functional chimeras of Rous sarcoma virus and human immunodeficiency virus type 1 Gag proteins. J Virol 1993;67:6487-98.
29. Bowzard J, Bennet R, Krishna N, Ernst S, Rein A, Wills J. Importance of basic residues in the nucleocapsid sequence for retrovirus Gag assembly and complementation rescue. J Virol 1998;72:9034-44.
30. Sandefur S, Varthakavi V, Spearman P. The I domain is required for efficient plasma membrane binding of human immunodeficiency virus type 1 Pr55 Gag. J Virol 1998;72:2723-2.
31. Cimarelli A, Luban J. Human immunodeficiency virus type 1 virion density is not determined by nucleocapsid basic residues. J Virol 2000;74:6734-40.
32. Campbell S, Vogt V. Self-assembly *in vitro* of purified CA-NC proteins from Rous sarcoma virus and human immunodeficiency virus type 1. J Virol 1995;69:6487-97.
33. Gross I, Hohenberg H, Huckhagel C, Krausslich H-G. N-terminal extension of human immunodeficiency virus capsid protein converts the *in vitro* assembly phenotype from tubular to spherical particles. J Virol 1998;72:4798-810.
34. Gross I, Hohenberg H, Krausslich H-G. *In vitro* assembly properties of purified bacterially expressed capsid proteins of human immunodeficiency virus. Eur J Biochem 1997;249:592-600.
35. Ehrlich L, Liu T, Scarlata S, Chu B, Carter C. HIV-1 capsid protein forms spherical and tubular particles *in vitro*: structure switching by pH-induced conformational changes. Biophysical J 2001;81:586-94.
36. Singh A, Hill R, Lingappa J. Effect of mutations in Gag on assembly of immature human immunodeficiency virus type 1 capsids in a cell-free system. Virology 2001;279:257-70.
37. Zabransky A, Hunter E, Sakalian M. Identification of a minimal HIV-1 Gag domain sufficient for self-association. Virology 2002;294:141-50.
38. Khorchid A, Halwani R, Wainberg M, Kleiman L. Role of RNA facilitating Gag/Gag-Pol interaction. J Virol 2002;76:4131-7.
39. Gottlinger H, Dorfman T, Sodroski J, Haseltine W. Effect of mutations affecting the p6 Gag protein on human immunodeficiency virus type 1 particle release. Proc Natl Acad Sci USA 1991;88:3195-9.
40. Tritel M, Resh M. The late stage of human immunodeficiency virus type 1 assembly is an energy-dependent process. J Virol 2001;75:5473-81.
41. Parent L, Bennet R, Craven R, et al. Positionally independent and exchangeable late budding functions of the Rous sarcoma virus and human immunodeficiency virus type 1 Gag proteins. J Virol 1995;69:5455-60.
42. Huang M, Orenstein J, Martin M, Freed E. p6 Gag is required for particle production from full-length human immunodeficiency virus type 1 molecular clones expressing protease. J Virol 1995;69:6810-8.
43. Yuan B, Li X, Goff S. Mutations altering the moloney murine leukemia virus p12 Gag protein affect virion production and early events of the virus life cycle. EMBO J 1999;18:4700-10.
44. Puffer B, Parent L, Wills J, Montelaro R. Equine infectious anemia virus utilizes a YXXL motif within the late assembly domain of the Gag p9 protein. J Virol 1998;71:6541-6.
45. Li F, Chen C, Puffer B, Montelaro R. Functional replacement of homologous and heterologous L domains in equine infectious anemia virus replication. J Virol 2002;76:1569-77.
46. Yuan B, Campbell S, Bachrach E, Rein A, Goff S. Infectivity of moloney murine leukemia virus defective in late assembly events is restored by late assembly domains of other retroviruses. J Virol 2000;74:7250-60.
47. Dorfman T, Bukovsky A, Ohagen A, Hoglund S, Gottlinger H. Functional domains of the capsid protein of human immunodeficiency virus type 1. J Virol 68:8180-7.
48. Jowett J, Hockley D, Nermut M, Jones I. Distinct signals in human immunodeficiency virus type 1 Pr55 necessary for RNA binding and particle formation. J Gen Virol 1992;73:3079-86.
49. Reicin A, Ohagen A, Yin L, Hoglund S, Goff S. The role of Gag in human immunodeficiency virus type 1 virion morphogenesis and early steps of the viral life cycle. J Virol 70: 8645-52.
50. von Poblotzki A, Wagner R, Niedrig M, Wanner G, Wolf H, Modrow S. Identification of a region in the Pr55 Gag polyprotein essential for HIV-1 particle formation. Virology 1993;193:981-5.
51. Zhang W, Hockley D, Nermut M, Morikawa Y, Jones I. Gag-Gag interactions in the C-terminal domain of human immunodeficiency virus type 1 p24 capsid antigen are essential for Gag particle assembly. J Gen Virol 1996;77:743-51.
52. Mammano F, Ohagen A, Hoglund S, Gottlinger H. Role of the major homology region of human immunodeficiency virus type 1 in virion morphogenesis. J Virol 1994;68:4927-36.
53. Ebbets-Reed D, Scarlata S, Carter C. The major homology region of the HIV-1 gag precursor influences membrane affinity. Biochemistry 1996;35:14268-75.
54. Provitera P, Goff A, Harenberg A, Bouamr F, Carter C, Scarlata S. Role of the major homology region in assembly of HIV-1 Gag. Biochemistry 2001;40:5565-72.
55. Gitti R, Lee B, Walker J, Summers M, Yoo S, Sundquist W. Structure of the amino-terminal core domain of the HIV-1 capsid protein. Science 1996;273:231-5.

56. Pettit S, Moody M, Wehbie R, Kaplan A, Nantermet P, Klein C, Swanstrom R. The p2 domain of human immunodeficiency virus type 1 Gag regulates sequential proteolytic processing and is required to produce fully infectious virions. J Virol 1994; 68:8017-27.
57. Kaplan A, Zack J, Knigge M, Paul D, Kempf D, Norbeck D, et al. Partial inhibition of the human immunodeficiency virus type 1 protease results in aberrant virus assembly and the formation of noninfectious particles. J Virol 1993;67:4050-5.
58. Rey O, Canon J, Krogstad P. HIV-1 Gag protein associates with F-actin present in microfilaments. Virology 1996;220:530-4.
59. Ibarrondo F, Choi R, Geng Y, Canon J, Rey O, Baldwin G, et al. HIV type 1 Gag and nucleocapsid proteins: cytoskeletal localization and effects on cell motility. AIDS Res Hum Retroviruses 2001;17:1489-500.
60. Ott D, Coren L, Johnson D, et al. Actin-biling cellular proteins inside human immunodeficiency virus type 1. Virology 2000;266: 42-51.
61. Mariana R, Rasala B, Rutter G, et al. Mouse-human heterokaryons support efficient human immunodeficiency virus type 1 assembly. J Virol 2001;75:3141-51.
62. Quigley J, Rifkin D, Reich E. Pospholipid composition of Rous sarcoma virus, host cell membranes and other enveloped viruses. Virology 1971;46:106-16.
63. Aloia R, Tian H, Jensen F. Lipid composition and fluidity of the human immunodeficiency virus type 1 envelope and host cell plasma membranes. Proc Natl Acad Sci USA 1993;99:5181-5.
64. Owens R, Dubay J, Hunter E, Compans R. Human immunodeficiency virus type 1 envelope protein determines the site of virus release in polarized epithelial cells. Proc Natl Acad Sci USA 1991;88:3987-91.
65. Owens R, Compans R. Expression of human immunodeficiency virus type 1 envelope glycoprotein is restricted to basolateral surfaces of polarized epithelial cells. J Virol 1989;63:978-82.
66. Aloia R, Jensen F, Curtain C, Mobley P, Gordon L. Lipid composition and fluidity of the human immunodeficiency virus. Proc Natl Acad Sci USA 1988;85:900-4.
67. Gordon L, Jensen F, Curtain C, Mobley P, Aloia R. Thermotropic lipid phase separation in the human immunodeficiency virus. Biochim Biophys Acta 1988;943:331-42.
68. Simons K, Ikonen E. Functional rafts in cell membranes. Nature 1997;387:569-72.
69. Campbell S, Crowe S, Mak J. Lipid rafts and HIV-1: from viral entry to assembly of progeny virions. J Clin Virol 2001;22:217-27.
70. Brown D, Rose J. Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface Cell 1992;68:533-44.
71. Arni S, Hangumaran S, van Echten-Deckert G, et al. Differential regulation of Src- family protein tyrosine kinases in GPI domains of T lymphocytic plasma membranes. Biochem Biophys Res Commun 1996;225:801-7.
72. Sargiacomo M, Sudol M, Tang Z, Lisanti M. Signal transducing molecules and GPI-linked proteins form a caveolin-rich insoluble complex in MDCK cells. J Cell Biol 1993;122:789-807.
73. Stefanova L, Horejsi V, Ansotegui I, Knapp W, Stockinger H. GPI-anchored cell-surface markers complexed to protein tyrosine kinases. Science 1991;254:1016-19.
74. Nguyen D, Hildreth J. Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts. J Virol 2000;74:3264-72.
75. Lindwasser O, Resh M. Multimerization of human immunodeficiency virus type 1 Gag promotes its localization to barges, raft-like membrane microdomains. J Virol 2001;75:7913-24.
76. Ono A, Freed E. Plasma membrane rafts play a critical role in HIV-1 assembly and release. Proc Natl Acad Sci USA 2001;98:13925-30.
77. Wang J, Kiyokawa E, Verdin E, Trono D. The Nef protein of HIV-1 associates with rafts and primes T cells for activation. Proc Natl Acad Sci USA 2000;97:394-9.
78. VerPlank L, Bouamr F, LaGrassa T, Agresta B, Kikonyogo A, Leis J, et al. Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55 Gag. Proc Natl Acad Sci USA 2001;98:7724-9.
79. Garrus J, von Schwedler U, Pornillos O, et al. Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. Cell 2001;107:55-65.
80. Demirov D, Ono A, Orenstein J, Freed E. Overexpression of the N-terminal domain of Tsg101 inhibits HIV-1 budding by blocking late domain function. Proc Natl Acad Sci USA 2002;99:955-60.
81. Katzmann D, Babst M, Emr S. Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein-sorting complex, ESCRT-I. Cell 2001;106:145-55.